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Evaluation de l'efficacité des sutures avec triclosan sur les micro-organismes associés aux infections de site opératoire

Frédéric Daoud

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THÈSE PRÉSENTÉE

POUR OBTENIR LE GRADE DE

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L'UNIVERSITÉ DE BORDEAUX**

ÉCOLE DOCTORALE SP2

SPÉCIALITÉ : PHARMACOÉPIDÉMIOLOGIE

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**Evaluation de l'efficacité des sutures avec triclosan
sur les micro-organismes associés aux infections
de site opératoire**

Sous la codirection de

Professeur Nicholas MOORE et Professeur Anne-Marie ROGUES

Soutenue le 1^{er} juillet 2022 - Président du jury : Professeur Mathieu MOLIMART

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Titre : Evaluation de l'efficacité des sutures avec triclosan sur les micro-organismes associés aux infections de site opératoire

Résumé

Introduction : Les sutures résorbables avec triclosan (TS) visent à inhiber la colonisation microbienne pour réduire le risque d'infection de site opératoire (ISO). Cet effet a été testé dans des essais cliniques randomisés (RCTs) les comparant aux sutures sans triclosan (NTS). Le niveau de preuve en reste modéré avec une majorité de RCTs non-significatifs alors le risque relatif (RR) regroupé significatif des méta-analyses pourrait résulter de l'activité antimicrobienne du TS, d'un biais ou du hasard.

Objectifs : Ce travail évalue l'activité antimicrobienne de trois types de TS et son association avec les micro-organismes qui causent les ISO et le risque d'ISO.

Méthodes : Quatre études ont été menées. (1) Une comparaison standardisée *in vitro* de l'activité antimicrobienne des trois principaux TS sur un panel de micro-organismes retrouvés en chirurgie avec différents niveaux de sensibilité au triclosan. (2) L'analyse de la libération de triclosan des TS dans un modèle *in vitro* reproduisant la libération dans les tissus opérés. L'activité antistaphylococcique des sutures tressées en polyglactine après pré-immersion pendant des durées croissantes. (3) Une analyse conjointe de l'activité antimicrobienne des TS et de la concentration minimale inhibitrice (CMI) du triclosan sur un échantillon de micro-organismes cliniques isolés d'ISO du rachis et d'autres sources. (4) Une revue systématique de la littérature avec méta-analyse des RCTs prospectifs comparant la diversité microbienne dans les ISO après fermeture par TS vs. NTS. L'hypothèse était que l'association des TS au développement des ISO devrait s'accompagner d'une différence de diversité microbienne dans les ISOs après TS vs. NTS, suivant la sensibilité au triclosan. La méta-analyse du RR des ISOs confirmées par culture (ISOcc) entre TS et NTS

a été réalisée pour valider la similarité des RCTs inclus ici avec ceux des méta-analyses des ISO avec et sans confirmation par culture.

Résultats : (1) Les TS étaient initialement biocides pendant environ 8 heures avec toutes les souches microbiennes sauf le *Pseudomonas aeruginosa*. Aucune souche n'a été éradiquée et une croissance significative a repris dans la plupart des souches. La cinétique suggère que une biocidie par contact plutôt que par augmentation de la concentration de triclosan dans le milieu de culture. Aucune différence significative dans l'activité antimicrobienne entre les types de TS n'a été mesurée. (2) La biocidie initiale correspond à une pré-immersion des sutures jusqu'à 4 à 12 heures associée au taux élevé de libération du triclosan. L'activité antimicrobienne est indétectable la chute du taux libération. (3) La concentration de toutes les souches microbiennes cliniques avec une CMI de triclosan < 2-3 µg/mL a été réduite par les TS. Le *P. aeruginosa*, CMI > 128 µg/mL, n'a pas été inhibé. (4) Pas de diversité microbienne dans les ISOs n'a été retrouvée entre TS et NTS, mais la comparaison manquait de puissance. La fréquence des *Pseudomonas* a diminué non significativement dans le bras TS proportionnellement à l'ensemble des microorganismes, ce qui est incohérent avec la résistance au triclosan prédominante des *Pseudomonas* en chirurgie. Le RR significatif montre moins d'ISOcc avec TS, conformément aux méta-analyses générales. Le niveau de preuve reste modéré compte tenu du risque individuel de biais des RCTs et de la majorité des RCTs non significatif.

Conclusions : Les facteurs susceptibles d'expliquer les différences de RR des ISO entre RCTs sont : L'activité antimicrobienne de contact limité à la surface de la TS, la durée de la biocidie de 4 à 12 heures et l'exposition à des microorganismes résistants au triclosan comme le *P. aeruginosa*. La perte de biocidie du TS dans les 4 à 12 heures est associée à une réduction du taux marginal de libération de triclosan alors que l'essentiel de la charge initiale reste sur la suture. L'utilisation du TS selon les recommandations de l'OMS devrait être effectuée avec prudence.

Mots clés : triclosan, suture, infection de site opératoire, antimicrobien, efficacité, biocide de contact, taux de libération, diversité microbienne

Title : Assessment of the efficacy of sutures with triclosan on microorganisms associated with surgical site infections

Abstract :

Introduction: Absorbable sutures with triclosan are intended to inhibit microbial colonization and thus reduce surgical site infection (SSI) risk. This potential consequence has been tested in randomized clinical trials (RCTs) comparing triclosan sutures (TS) with non-triclosan sutures (NTS), but quality of evidence is moderate. Most RCTs in meta-analyses are non-significant, so the significant pooled relative risk (RR) could result from the antimicrobial activity of TS, bias or chance.

Objectives: The objective of this research is to assess the antimicrobial activity of three types of TS and the association with microorganisms that cause SSIs and, thereby, the risk of SSI.

Methods: Four studies were conducted. (1) A standardized *in vitro* comparison of the antimicrobial activity of the three main types of TS on a panel of microorganisms found in surgery with different levels of susceptibility to triclosan. (2) An analysis of the release of triclosan by the TS in an *in vitro* model reproducing the release in operated tissues. It was associated with a study of the antistaphylococcal activity of braided polyglactin sutures pre-immersed for increasing durations. (3) A joint analysis of the antimicrobial activity of TS and the minimum inhibitory concentration (MIC) of triclosan in a broad sample of clinical microorganisms isolated from spinal SSIs and other sources. (4) A systematic literature review with meta-analysis of prospective RCTs to compare microbial diversity in SSIs after closure by TS vs NTS. The underlying hypothesis was that if TS are associated with the development of SSIs, then a difference in microbial diversity should be found in SSIs after closure with TS vs NTS, which reflects microbial susceptibility to triclosan. The meta-analysis of the RR of culture-

confirmed SSIs between TS and NTS was performed to validate the similarity of the RCTs included here with those included in meta-analyses of SSIs with and without culture confirmation.

Results: (1) TS was initially biocidal for about 8 hours in all microbial strains except *Pseudomonas aeruginosa*. No strain was eradicated, and significant growth resumed afterwards in most strains. The kinetics suggest the antimicrobial activity is contact-killing rather than increasing triclosan concentration in the culture medium. No significant difference in antimicrobial activity between TS types was measured. (2) Triclosan release assays showed bactericidal antistaphylococcal activity providing sutures were pre-immersed no more than 4 to 12 hours, corresponding with triclosan early high release rate. Undetectable antimicrobial activity was associated with a slow-release rate. (3) The concentration of all clinical strains with triclosan MIC < 2-3 µg/mL was reduced by TS. *P. aeruginosa*, MIC > 128 µg/mL, was not inhibited. (4) Microbial diversity in v SSIs reported in RCTs was not different between TS and NTS, but the comparison was statistically underpowered. The frequency of the *Pseudomonas* genus was non-significantly decreased in the TS arm proportionally to all other microorganisms, which is not consistent with the triclosan resistance of most species of this genus found in surgery. The significant RR showed fewer culture-confirmed SSIs with TS consistently with comprehensive meta-analyses. However, the level of evidence remains moderate given the individual risk of bias of the RCTs and the majority of studies with a non-significant RR.

Conclusions: The identified factors likely to explain the differences in the RR of SSIs across RCTs are: The antimicrobial contact activity of TS limited to its surface, its estimated biocidal duration of 4 to 12 hours, and to some extent, the exposure to triclosan-resistant microorganisms like *P. aeruginosa*. TS biocidal activity loss within 4 to 12 hours is associated with a reduction in marginal triclosan release rate while most of the initial load remains on the suture. The use of TS according to WHO guidelines should be performed cautiously.

Keywords: triclosan, suture, surgical site infection, antimicrobial, biocidal rate, contact-killing, efficacy, release rate, microbial diversity

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RÉSUMÉ EN FRANÇAIS

INTRODUCTION

Définitions officielles Europe et US

Les cliniciens diagnostiquent les infections du site opératoire (ISO) selon différents critères. L'Organisation mondiale de la santé (OMS) fait référence à deux définitions :

- La définition publiée par les Centers for Disease Control des États-Unis (US CDC) : « *une infection qui survient après une chirurgie dans la partie du corps où la chirurgie a eu lieu. Les infections de site opératoire peuvent parfois être des infections superficielles impliquant uniquement la peau. D'autres infections de site opératoire sont plus graves et peuvent impliquer des tissus sous-cutanés, des organes ou du matériel implanté.* »
- La définition publiée par le Centre européen de prévention et de contrôle des maladies (ECDC) est : « *une infection qui survient dans les 30 jours suivant l'opération et implique la peau et le tissu sous-cutané de l'incision (incisionnelle superficielle) et/ou les tissus mous profonds (par exemple, fascia, muscle) de l'incision (incisionnelle profonde) et/ou de toute partie de l'anatomie (par exemple, organes et espaces) autre que l'incision qui a été ouverte ou manipulée lors d'une opération (organe/ espace).* »

Certains pays ont allongé la durée de diagnostic à 90 jours en cas d'ISO sur implant et un suivi jusqu'à un an est recommandé dans certains pays (exemples : France, Angleterre).

Le taux d'incidence des ISOs est très variable entre 0,5% [0,2 à 2,7] en chirurgie prothétique du genou et jusqu'à 10,1% [4,1 à 16,9] en chirurgie ouverte du colon.

Le précurseur nécessaire aux ISOs est la contamination microbienne. La relation conceptuelle suggérée qui définit le risque d'ISO repose sur trois facteurs :

$$\text{Risque ISO} = \frac{\text{dose bactérienne} \times \text{virulence}}{\text{résistance du patient hôte}}$$

La dose bactérienne (mesurée en CFU) est rarement mesurée dans la pratique clinique, mais des preuves cliniques de la relation entre la dose et le risque d'ISO ont été établies dans des études cliniques. Un site chirurgical contaminé par plus de 10⁵ CFU/gramme

de tissu a un risque d'ISO significativement accru. Une charge microbienne beaucoup plus faible peut produire une ISO en présence de corps étrangers dans le site chirurgical, par exemple, les staphylocoques peuvent provoquer une ISO en présence d'une suture de soie avec une dose de 100 CFU/gramme de tissu.

Il a été rapporté que les micro-organismes impliqués dans les ISO proviennent principalement de la peau, des tissus environnants de l'incision ou des organes opérés avec une flore microbienne comme l'intestin. Les micro-organismes qui causent les ISO peuvent également avoir une source éloignée et être transportés vers le site chirurgical par l'approvisionnement en sang (ISO hémotogène). L'ensemencement hémotogène est toujours possible mais peut être la cause la plus probable en cas de chirurgie propre, d'ISO retardée ou lorsque le germe est présent dans une infection concomitante.

Le CDC américain a établi dans les années 1990 le *National Nosocomial Infections Surveillance System* (NNIS) aux États-Unis, y compris des lignes directrices, avec une formation et une surveillance pour prévenir les ISO dans la pratique de routine à l'échelle nationale.

Les critères NNIS définissent trois catégories d'ISO :

- Incisionnelle superficielle : « *L'infection survient dans les 30 jours suivant l'opération et l'infection ne concerne que la peau ou le tissu sous-cutané de l'incision et au moins un des (...)* » quatre critères.

- Incisionnelle profonde : « *L'infection survient dans les 30 jours suivant l'opération si aucun implant n'est laissé en place ou dans l'année si l'implant est en place et que l'infection semble être liée à l'opération et que l'infection implique des tissus mous profonds (p. couches musculaires) de l'incision et au moins un des (...)* » quatre critères.

- Organe/Espace : « *L'infection survient dans les 30 jours suivant l'opération si aucun implant n'est laissé en place ou dans l'année si l'implant est en place et que l'infection semble être liée à l'opération et que l'infection implique une partie de l'anatomie (par exemple, organes ou espaces), autre que l'incision, qui a été ouverte ou manipulée au cours d'une opération, et au moins un des (...)* » quatre critères.

L'énoncé sur les quatre critères dans chaque catégorie comprend :

- "Drainage purulent" ET/OU
- autres critères objectifs, ET/OU

- des critères subjectifs avec notamment « *Diagnostic... par le chirurgien ou le médecin en service* » sans plus de précision.

Sutures résorbables étudiées dans cette recherche :

Le but des sutures chirurgicales résorbables est d'éviter de réinciser pour les retirer après la cicatrisation chirurgicale. Les trois sutures résorbables pertinentes dans cette recherche sont en polymère et sont utilisées depuis des décennies :

- **sutures tressées en polyglactine 910 enrobées** (Ethicon, Vicryl®), introduites en 1979 (abréviation : V)
- **les sutures en polydioxanone simple brin** (Ethicon, PDS II®), introduites en 1982 (abréviation : P)
- **les sutures simple brin poliglecaprone 25**, (Ethicon, Monocryl®), introduites en 1993 (abréviation : M).

Les sutures sont fournies avec une large gamme de diamètres et d'aiguilles afin de fournir une grande variété de résistance à la traction et de durée de traction jusqu'à leur fragmentation due à la résorption. Elles offrent également des propriétés de manipulation très différentes facilitant leur utilisation dans différents tissus et circonstances opératoires.

L'étiquette du fabricant des sutures tressées V et V+ mentionne que la résistance résiduelle à la traction à 28 jours est de 25 % et que l'absorption complète est atteinte entre les 56ème et 70ème jours.

Les versions de ces sutures avec du triclosan ont été introduites entre 2003 et 2006 :

- **sutures de polyglactine 910 enrobées tressées avec 472µg/m de triclosan** (Ethicon, Vicryl PLUS®) (abréviation : V+)
- **sutures monofilament en polydioxanone avec 2360µg/m de triclosan** (Ethicon, PDS PLUS®) (abréviation : P+)
- **sutures monofilament en poliglecaprone 25 sutures avec 2360µg/m de triclosan** (Ethicon, Monocryl PLUS®) (abréviation : M+)

Les six sutures sont produites dans la même gamme de longueurs de diamètres et d'aiguilles. Le triclosan modifie les propriétés mécaniques des sutures. De plus, une suture avec triclosan ne peut pas être distinguée (sauf marqueur de couleur) d'une

suture similaire sans triclosan car le triclosan ne modifie pas la sensation, l'aspect ou l'odeur de la suture. Cela rend possible les études avec mise en aveugle de l'opérateur.

Triclosan

Le triclosan (C₁₂H₇Cl₃O₂ désigné 2,4-dichlorophénoxyphénol, CAS : 3380-34-5) utilisé dans les sutures V+, M+ et P+ est produit par BASF Irgacare MP®.

Le triclosan est un composé aromatique avec deux cycles benzéniques liés par une liaison covalente éther. Cette structure chimique a une prédominance hydrophobe ce qui conduit à une solubilité faible dans l'eau (10 µg/mL) et plus élevée dans les solvants organiques tels l'éthanol. Le triclosan a une activité antimicrobienne topique à large spectre. L'imprégnation des sutures avec du triclosan (TS) vise à leur conférer cette activité antimicrobienne.

Recommandations d'utilisation de l'OMS

Des études *in vitro* et *in vivo* chez plusieurs espèces animales ont démontré l'activité antimicrobienne et l'innocuité des TS. Les méta-analyses d'un nombre important d'essais cliniques ont aussi montré une association entre la fermeture des plaies chirurgicales avec des TS et un taux d'incidence des ISOs significativement plus faible qu'avec des sutures sans triclosan (NTS).

La mise à jour des recommandations mondiales 2018 de l'OMS préconise de manière conditionnelle : *« l'utilisation de sutures enduites de triclosan dans le but de réduire le risque d'ISO, quel que soit le type de chirurgie... »*. Le niveau de qualité des preuves sous-jacentes est modéré.

PROLEMATIQUE DE RECHERCHE : DEMONSTRATION

Les revues systématiques de la littérature menées avec méta-analyses des essais randomisés homogènes ont le potentiel de fournir le niveau de preuve clinique le plus élevé.

Les essais randomisés prospectifs à bras parallèles (RCTs) évalués par un comité de relecture et publiés sont considérés comme étant les sources les plus fiables de preuves pour comparer la prévention des ISOs avec usage des sutures avec triclosan *versus* sans triclosan.

Les faiblesses des méta-analyses actuelles sur les TS sont les suivantes :

- Une majorité de RCTs non significatifs (plus de 80%) : faiblesse objective,
- Le risque de biais des RCTs individuels : Faiblesse subjective car ce risque dépend de jugement de l'évaluateur,
- Le caractère indirect (« indirectness ») de la relation entre l'activité antimicrobienne des TS et le développement d'ISO : Faiblesse objective car le développement d'ISO dépend de différents facteurs.

Ces faiblesses sont détaillées dans une analyse préliminaire et accessoire des RCT cliniquement pertinents, avec et sans détails microbiologiques, identifiés lors de l'extraction lors de la revue systématique de la littérature complétée en août 2021 et décrite dans le diagramme de flux PRISMA de la quatrième étude (voir 4., article publié).

La recherche a identifié trente-trois RCT cliniquement pertinents. Leur méta-analyse livre les résultats suivants :

- La distribution du taux d'incidence (IR) de référence des ISO, c'est-à-dire dans le groupe contrôle (NTS), couvre un intervalle avec des bornes inférieure à 1 % et supérieure à 35 % avec une moyenne de 10 % [7 %, 13 %]. On peut émettre l'hypothèse que cette distribution résulte de

l'hétérogénéité clinique entre les RCT car les patients inclus et les opérations réalisées comportent des risques différents d'ISO.

- En revanche, le diagramme de Forest de la distribution des RR présente peu d'hétérogénéité (Indice $I^2 = 24,8 \%$, Q-test non significatif, $p = 0,10$). De plus, le RR moyen groupé de 0,76 est significatif avec un intervalle de confiance à 95% (IC 95%) de [0,66 ; 0,88].
- Cette stabilité du RR entre les RCT malgré des IR très différents est confirmée par la méta-régression non significative sur l'ensemble des RCTs cliniquement pertinents (Figure 3). Le coefficient de détermination R^2 de 0 % montre l'absence d'association significative entre les variations importantes de l'IR et le RR stable, dont la pente de méta-régression est non significative (-0,88 [-2,55, 0,79]).
- L'absence d'impact de l'hétérogénéité clinique sur l'efficacité des TS.
- L'IR et le RR ci-dessus montrent une différence moyenne groupée entre sutures sans et avec triclosan de 2,4 % [0,84 %, 4,42 %], soit un IC 95% proche de la non-significativité.
- De plus, le diagramme de Forest montre la manière dont le RR moyen regroupé s'appuie sur 5 études significatives (15%) parmi les 33.

Le mécanisme des méta-analyses permet d'obtenir un résultat regroupé significatif même avec des études non-significatives seulement, dès lors qu'une majorité partage une tendance d'un côté du point d'équilibre (la seuil de 1), par réduction de la largeur de l'IC95% même si l'effet moyen regroupé est proche de 1. La tendance majoritaire peut résulter d'une efficacité de faible amplitude, ou de biais, ou du hasard.

Cette situation nécessitent une analyse approfondie des mécanisme de l'effet antimicrobien des TS et de leur rôle dans la microbiologie des ISOs.

QUESTION POSEE DANS CETTE RECHERCHE :

Les sutures au triclosan réduisent-elles le risque d'infection de site opératoire ?

Cette question en implique quatre autres :

Question 1 : Quel est le niveau d'activité antimicrobienne des sutures avec du triclosan ? Est-il similaire pour les trois types de sutures ?

Question 2 : Combien de temps les sutures avec du triclosan inhibent-elles le *Staphylococcus aureus* une fois implantées en conditions chirurgicales ?

Question 3 : Quels micro-organismes isolés dans les cultures d'ISO sont inhibés par les sutures avec triclosan ?

Question 4 : Les sutures avec triclosan modifient-elles la diversité microbienne des ISO de manière cohérente avec la sensibilité microbienne au triclosan ?

Une étude spécifique a été réalisée pour répondre à chacune de ces questions. La vraisemblance de la réduction du taux d'incidence des ISOs par l'utilisation des sutures à triclosan est estimée avec l'analyse jointe des résultats de ces quatre études.

ETUDE 1

Dynamique du mécanisme de prévention des infections de site opératoire par les sutures avec triclosan.

Objectifs : Les sutures résorbables en polyglactine-910 tressée, polydioxanone monofilament et poliglecaprone-25 monofilament sont des disponibles avec ou sans triclosan en tant qu'antimicrobien. Les quantités de triclosan par unité de longueur et les cinétiques de libération des trois types de sutures sont différentes. 88 % des essais cliniques contrôlés randomisés comparant suture avec triclosan (TS) et sans (NTS) ne sont non-significatifs. La dissection du mécanisme de l'activité antimicrobienne est nécessaire pourrait aider à comprendre les résultats cliniques.

Méthodes : Les 3 TS et NTS ont été testés sur 9 micro-organismes avec des concentrations minimales inhibitrices (CMI) du triclosan entre 0,03 et 512 µg/mL et des mesures de concentration microbienne à t0, 4, 8 et 24 heures, selon un protocole de biocidie normé. Les segments de TS contenaient suffisamment de triclosan pour que son entière dissolution dépasse la CMI de toutes les souches sauf le *Pseudomonas aeruginosa*.

Résultats : Aucune suture n'a inhibé le *P. aeruginosa*. Les trois TS ont montré une activité biocide initiale chez toutes les autres espèces. Les NTS n'ont montré aucune activité antimicrobienne. La TS a réduit les concentrations des souches de *S. aureus*, *S. epidermidis*, *E. coli* et *C. albicans* avec un minimum de concentration atteint entre 4 et 8 heures d'exposition. Une croissance significative a repris par la suite dans la majorité des souches. L'analyse de régression n'a montré aucune différence significative entre les types de suture indépendamment des micro-organismes.

Conclusions : Cette étude montre une biocidie dynamique similaire entre espèces microbiennes sensibles au triclosan avec les trois types de TS. Le *P. aeruginosa* se développe sur toutes les sutures. Les courbes de biocidie ont forme en J liée à la reprise de la croissance microbienne après 8 heures. La biocidie par contact est le mécanisme antimicrobien prédominant. Une inhibition prolongée de la colonisation microbienne des sutures n'est pas détectée mais n'est pas exclue. Les résultats devraient résulter en une inhibition du développement des ISO dans les tissus opérés.

ETUDE 2

Combien de temps les sutures avec triclosan inhibent-elles *Staphylococcus aureus* une fois implantées en conditions chirurgicales ? Un modèle pharmacologique.

Contexte :

Les sutures avec triclosan (TS) sont utilisées par les chirurgiens pour réduire le risque d'infection de site opératoire (ISO), mais la plupart des essais cliniques ne sont pas concluants. L'activité antimicrobienne des TS a été établie avec différentes études dont le critère de jugement était les zones d'inhibitions. Cette technique ne mesure pas les concentrations microbiennes et ne peut pas réaliser de comparaisons d'activité dans le temps. En outre, les études publiées établissent des modèles expérimentaux mais ne font pas appel à des situations chirurgicales avec des tissus opérés semblables à la chirurgie humaine. Enfin, aucune étude n'a été identifiée concernant la libération et la quantité résiduelle d'antimicrobien sur les sutures dans le temps. Les éléments permettant de prédire la durée d'activité antimicrobienne dans la chirurgie humaine des TS ne sont pas disponibles.

Objectifs :

Cette étude visait à prédire l'activité antistaphylococcique des sutures avec triclosan dans le temps, dans les tissus opérés.

Matériels et Méthodes :

Trois types de TS ont été immergées dans de l'eau statique pendant 30 jours : les sutures en polyglactine 910 tressée (V+), polyglecaprone monofilament (M+), polydioxanone monofilament (P+), et les taux de libération marginaux du triclosan échelonnés dans le temps ont été enregistrés afin d'établir un référentiel. Des segments de TS en polyglactine 910 (V+) intramusculaires et sous-cutanés ont été explantés d'ovins, de manière opportuniste, le septième jour post-opératoire après une chirurgie cardiaque expérimentale selon le principe des 3R.

L'utilisation de sources opportunistes provenant d'une chirurgie préclinique dans un modèle translationnel de chirurgie humaine (dans le cas présent, la chirurgie cardiaque ovine en tant que modèle de chirurgie cardiaque humaine) résulte de l'impossibilité d'obtenir des fragments de sutures chez des patients et de l'obligation d'optimiser l'utilisation de ressources de recherche animale (3R).

Les taux de libération marginaux de triclosan ont été mesurés et comparés aux taux de libération des mêmes sutures immergées sept jours dans de l'eau statique. Leur comparaison a fourni des repères *ex vivo* servant de spécifications de modèles *in vitro* reproduisant la libération dans les deux types de tissus opérés. Le modèle devait accélérer la libération du triclosan à partir des TS par rapport au référentiel en eau statique, afin que le taux de libération marginal des sutures après 7 jours d'immersion dans le modèle soit égal à celui des sutures explantées. Les paramètres pouvant être modifiés dans le modèle étaient indiqués par la relation de Noyes-Whitney:

$$\frac{dM}{dt}(t) = \frac{S \cdot D}{d} \cdot (C_s - C_t)$$

Où C_s est la solubilité du triclosan dans le solvant qui peut être augmentée avec un cosolvant organique, et d est l'épaisseur du gradient de dissolution qui peut être réduite par agitation.

Ceci a été réalisé en augmentant la solubilité du triclosan avec un cosolvant organique miscible dans l'eau et avec une agitation continue. Le taux de libération marginal du triclosan des sutures V+ dans le modèle a été mesuré à 4h, 8h puis toutes 12 heures jusqu'à 7 jours. Une fonction prédictive du taux de libération en fonction du temps a été ajustée aux mesures.

La charge initiale de triclosan par unité de longueur des trois types de sutures a été estimée par une immersion pendant 6 jours dans une cosolution éthanol/eau 100% *w/w*. Ces mesures associées aux mesures de libération marginale visaient à estimer la quantité de triclosan résiduelle au cours du temps sur les sutures implantées dans les tissus opérés.

L'activité antistaphylococcique a été mesurée par analyse de biocidie sur 24 heures selon les modalités décrites dans l'étude 1. Des cultures de *S. aureus* ont été exposées à des TS de type V+ ayant été immergées dans le modèle pendant des durées croissantes, jusqu'à ce que l'activité antistaphylococcique s'estompe. Etant donné les

durées d'immersion des sutures immédiatement avant et après l'arrêt de l'activité antistaphylococcique, l'intervalle du taux de libération marginal du triclosan à partir des TS a pu être calculé au moyen de la fonction de prédiction.

Résultats :

Le taux de libération marginal de triclosan des fragments de suture sous-cutanés explantés le 7^{ème} jour était 13,6 fois plus faible [IC 95%: 8,8 ; 18,5] que le référentiel en eau statique, et celui des fragments intramusculaires explantés était 64,2 fois plus faibles [52,5 ; 75,9] que le référentiel en eau statique. En outre, le taux de libération marginal des fragments intramusculaires était 3,13 fois plus faible [1,92 ; 4,35] que celui des fragments sous-cutanés.

Le taux de libération marginal des fragments intramusculaires a été reproduit en visant un facteur 60, au moyen d'une agitation par rotation continue à 24 tours par minute réalisant une accélération facteur 6 et une cosolution éthanol/eau 13,3% *m/w* réalisant une accélération facteur 10. L'accélération facteur 15 visée pour reproduire la libération des fragments sous-cutanés était réalisée également avec une agitation rotative à 24 tours par minute (facteur 6) et une cosolution éthanol/eau 3,3% *m/w* (accélération facteur 2,5).

Tous les modèles de dissolution en eau statique (V+ USP 2-0, USP 0 et USP 1, P+ USP 2-0) et en conditions accélérées facteurs 15 et 60 (V+ USP 0 et USP 1) étaient ajustés à une fonction mathématique de type double décroissance exponentielle à quatre paramètres :

$$R_{predicted}(t) = R_1 \cdot e^{\frac{-t \cdot \ln 2}{L_1}} + R_2 \cdot e^{\frac{-t \cdot \ln 2}{L_2}}$$

- R1 la composante prédominante du taux de libération initial et L1 la demi-vie correspondante. Cette partie modélisait la partie précoce de la libération de triclosan avec un taux initial élevé et une décroissance rapide sur environ 24 heures
- R2 la composante secondaire du taux de libération initial et L2 la demi-vie correspondante. Cette partie modélisait la partie tardive de la libération de triclosan avec un plateau prolongé avec un taux de libération lent en décroissance lente de 6 à 7 jours.

Les mesures des quatre modèles d'accélération facteur 60 et facteur 15 avec des fils calibres V+ USP 0 et USP 1 permettaient de s'approcher de très près des taux observés dans les modèles explantés.

Les sutures V+ USP 0 présentaient une activité biocide sur le *S. aureus* quand elles étaient immergées dans les conditions facteur 60 et facteur 15 pendant 1 heure et 4 heures. Après une immersion de 12 heures, la croissance du *S. aureus* était observée. Les fonctions de décroissance montrent qu'à l'intervalle de temps [4 h ; 12 h] correspond un intervalle de libération de triclosan de [0,175 µg/m/h ; 0.287 µg/m/h].

L'examen des quatre courbes du taux de libération accélérée en fonction du temps montre que la transition de la phase précoce de libération rapide vers le plateau prolongé avec un taux de libération lent se situe entre 6 et 12 heures.

L'extraction de la charge initiale des trois types de sutures était d'environ :

- Pour V+ : 50 µg/m soit environ 10% du plafond de 472 µg/m.
- Pour P+ : 550 µg/m soit environ 23% du plafond de 2360 µg/m.
- Pour M+ : 1070 µg/m soit environ 45% du plafond de 2360 µg/m.

En outre, la charge résiduelle calculée par rapport à la tentative d'extraction totale sur V+ était de 95% au moment de la disparition de l'effet bactéricide sur le *S. aureus* et de 62% après 7 jours d'immersion.

Discussion :

La relation sous forme de décroissance double-exponentielle s'applique à toutes les combinaisons suture-milieu de dissolution et fournit une validation interne à l'application de ce type de relation mathématique à la libération du triclosan par les sutures. Chaque combinaison est caractérisée par les quatre paramètres.

Cela devrait être le cas des études microbiologiques en milieu aqueux statique publiées jusqu'à présent, qui ressemblent au référentiel eau et qui sont susceptibles de surestimer la durée d'activité antimicrobienne *in vivo* des sutures à triclosan.

Le référentiel en eau statique et le modèle avec agitation continue et augmentation de la solubilité du triclosan ont pour but d'inclure les conditions de libération du triclosan dans les modèles d'utilisation des sutures des études microbiologiques publiées jusqu'à présent.

Cela devrait être également le cas des études sur rongeur vraisemblablement plus proches des modèles *in vitro* accélérés.

Une validation externe avec ces études n'est pas réalisable car aucune n'analyse l'association entre l'activité antimicrobienne et la biodisponibilité du triclosan libéré par les sutures.

Les limites principales de cette étude doivent être prises en compte dans l'interprétation des résultats : 1) les spécifications *ex vivo* démarrent le 7^{ème} jour post-opératoire, si bien que le modèle de prédiction mathématique n'a pas d'indications pour l'évolution précoce alors que c'est la période avec l'activité antimicrobienne la plus élevée et avec les changements de régime les plus rapides. 2) Le modèle de chirurgie cardiaque ovin est anatomiquement validé pour sa correspondance avec la chirurgie cardiaque humaine mais l'écart entre la libération de triclosan sous-cutané et intramusculaire ovin par rapport aux mêmes tissus humains n'est pas connu. 3) Les modèles simulant les tissus reproduisent les taux de libération du triclosan mais ne reproduisent pas l'hydrolyse du polymère qui semble stocker la majeure partie du triclosan. 4) Les fragments de sutures précliniques ne concernaient pas les sutures de type M+ et P+. 5) Les tests d'activité antimicrobienne ne sont réalisés que sur une souche de référence. Ces limites imposent des hypothèses relatives à la généralisation des résultats aux tissus humains. D'autres limites ont été identifiées mais leur rôle ne peut être discuté que conjointement avec les cinq limites décrites précédemment.

Conclusions :

Les sutures tressées en polyglactine 910 avec triclosan ont un effet antistaphylococcique de niveau bactéricide dans les tissus opérés estimé à 8 heures (entre 4 et 12 heures). Cet effet est associé au flux élevé de libération du triclosan et disparaît lorsque ce flux tombe sous 0,175 µg/m/h. La charge en triclosan initiale n'a pas pu être mesurée avec précision et le pourcentage libéré était d'environ 10% du plafond prévu par le fabricant. Cependant, la relation observée avec le flux de libération montre que plus de 95% de la charge initiale mesurée est encore présente sur la suture lorsque l'effet bactéricide s'arrête. La quantité résiduelle importante de triclosan est donc prisonnière du polymère de la suture et libérée progressivement dans les tissus avec l'hydrolyse du polymère. Ce processus étant lent (environ 20% de

perte de résistance mécanique au bout de deux semaines), nous émettons l'hypothèse que l'activité antimicrobienne résiduelle est biostatique ou infra-efficace.

Etant donné la sensibilité au triclosan de la majorité des espèces microbiennes retrouvées habituellement dans les ISO, il semble que l'effet bactéricide observé avec le *S. aureus* soit généralisable, à l'exception des espèces et souches résistantes au triclosan telles le *P. aeruginosa*.

Etant donné les flux élevés de libération de triclosan observés avec M+ et P+ sur des durées similaires à V+, il est raisonnable d'envisager que l'activité antimicrobienne *in vivo* des trois sutures soit similaire en termes de spectre et de durée.

Les différences significatives entre les fragments sous-cutanés et intramusculaires explantés montrent qu'un seul modèle ne peut pas prédire toutes les situations susceptibles d'être rencontrées en chirurgie humaine.

ETUDE 3

Les micro-organismes isolés dans les infections de site opératoire du rachis sont-ils sensibles aux sutures avec triclosan ?

Contexte : Le taux d'incidence des infections de site opératoire rachidiennes (ISO) varie d'environ 1 % à 16 % selon les facteurs de risque et les sources.

Une étude rétrospective a rapporté un risque relatif de 0,13 après fermeture avec des sutures de polyglactine 910 avec triclosan (TS) par rapport à des témoins sans triclosan (NTS). Quelle est la sensibilité des micro-organismes associés aux ISO rachidiennes aux sutures avec triclosan ?

Méthodes : Trois centres de chirurgie du rachis à Bordeaux ont fourni des micro-organismes à partir d'échantillons prospectifs et rétrospectifs consécutifs d'ISO du rachis. La concentration minimale inhibitrice (CMI) du triclosan dans chaque souche a été mesurée, et ces souches ont été mises à l'épreuve d'un segment de TS dans un test de biocidie standardisé sur 24 heures. Des souches cliniques supplémentaires d'orthopédie et des souches de référence ont été testées également.

Résultats : La CMI a été déterminée dans 105 souches, dont 46 souches collectées à partir d'ISO rachidiennes ; la CMI variait de 0,03 µg/mL à 512 µg/mL. La CMI était inférieure ou égale à 0,5 µg/mL dans 92,38 % des souches, dont la plupart était *S. aureus* (37 %), *E. coli* (12 %) et *E. faecalis* (9 %). Elle était égale à 3 µg/mL dans les deux souches de *C. albicans* et comprise entre 128 et 512 µg/mL dans les 5 souches de *P. aeruginosa* (3 % dans les ISO du rachis). Les tests de destruction temporelle ont montré une réduction précoce et importante de toutes les souches, avec une éradication dans quelques cas. La concentration de *C. albicans* était légèrement réduite ou statique. *P. aeruginosa* a grandi normalement. Les sutures étaient clairement efficaces avec des CMI inférieures à 2 µg/mL.

Conclusions : Les sutures au triclosan réduisent la charge microbienne de la plupart des espèces présentes dans les ISOs rachidiennes.

ETUDE 4

Les sutures au triclosan modifient-elles la diversité microbienne des infections du site opératoire ? Une revue systématique et une méta-analyse.

Contexte : Des essais cliniques contrôlés randomisés (RCT) rapportent un taux d'incidence plus faible d'infections du site opératoire (ISO) avec des sutures avec triclosan (TS) par rapport aux sutures sans triclosan (NTS). Les TS modifient-elles la diversité microbienne des ISO confirmées par culture (ISOcc) ? Si tel est le cas, cela appuierait l'association entre l'activité antimicrobienne des TS et le taux d'incidence des ISO. Dans le cas contraire, cela suggèrerait une absence d'association.

Méthodes : Cette revue systématique prospective de la littérature (protocole enregistré dans le répertoire PROSPERO sous l'identifiant CRD42019125099) a été réalisée selon la méthodologie PRISMA. La définition de la question a été définie selon le référentiel PICO: PATIENTS : Sujets ayant bénéficié d'une intervention chirurgicale quelle qu'elle soit, INTERVENTION : Fermeture de la plaie chirurgicale par suture avec triclosan (TS), COMPARATEUR : Fermeture de la plaie chirurgicale par suture sans triclosan (TNS), CRITERE de comparaison (« OUTCOME ») : Dénombrement de chaque micro-organisme identifié dans les ISOcc par groupe de suture. Les RCTs qui comparaient l'incidence des ISO avec le TS et le NTS (pertinence clinique) et rapportaient les numérations microbiennes des cultures de ces ISOs par groupe de suture (pertinence microbiologique) étaient éligibles. Les espèces microbiennes ont été tabulées telles que décrites dans les études puis regroupées par genre dans un tableau de contingence. L'association entre genres et sutures a été testée. Le risque relatif (RR) regroupé des ISOcc a également été calculé pour tester la validité externe, c'est-à-dire la représentativité des RCT éligibles ici par rapport à l'ensemble des RCT éligibles dans les méta-analyses.

L'association entre genres et sutures a été testée par test d'indépendance du Chi² (script STATA 17). Le niveau d'association entre les deux variables a été estimé par le V de Cramér.

Le risque relatif (RR) groupé des ISOcc a également été calculé par méta-analyse de RR avec un modèle à effets aléatoires selon la méthodologie proposée par la Cochrane Collaboration (dans Review Manager 5.4).

La puissance *post hoc* de chaque test était calculée afin d'interpréter le niveau de confiance dans le non-rejet éventuel de l'hypothèse nulle.

Résultats : Les bases bibliographiques Pubmed, Embase, Cochrane Library (dont CENTRAL), Web of Science ont été interrogées avec la même formule de requête répétée une fois en août 2021, sans critère d'exclusion (par ex. type de chirurgie, comorbidité particulière, année de publication, pays, ou autre).

Sur un total de 419 références bibliographiques identifiées et sélectionnées par deux relecteurs (MC et FD) et un troisième pour juger les désaccords (NM), 33 études RCTs étaient cliniquement pertinentes, et 12 d'entre elles étaient éligibles car elles fournissaient les détails microbiologiques des ISO. Aucun biais de publication n'a été identifié par rapport à la distribution des RR de ISOcc par RCT ni par le test de Harbor.

Le nombre de micro-organismes était de 180 dans 124 ISOcc avec TS *versus* 246 dans 199 ISOcc avec NTS, soit un nombre total de 426 micro-organismes pour 322 ISO : Rapport micro-organisme par ISO de 1,32 [1,25 ; 1,40] globalement, 1,45 [1,32 ; 1,62] après fermeture par TS et 1,24 [1,17 ; 1,33] après NTS.

Le tableau de contingence en résultant comportait 375 micro-organismes après exclusion de 51 enregistrements (12%), avec 8 genres sur deux colonnes (tableau 8 × 2) et toutes les cellules comptaient 5 observations au minimum. La diversité microbienne était non-significative ($p = 0.72$) avec un indice V d'association de Cramér faible entre variables ($V = 0,11$). La puissance statistique *post hoc* était de $1 - \beta = 0,28$, c'est-à-dire faible. De plus, les rapports du nombre microbien total de la colonne TS sur NTS était de 0,64 et les genres *Staphylococcus*, *Escherichia*, *Klebsiella* et *Pseudomonas* y ont tous contribué avec des rapports de 0,65 , 0,42 , 0,64 et 0,65 respectivement.

L'évaluation du risque de biais individuel des RCT, montrait un risque de biais hétérogène et globalement élevé. Le rapport de risque du RR groupé des ISOcc était de 0,62 [0,47 ; 0,85] et significatif.

L'hétérogénéité entre les RR des études était de $I^2 = 30\%$ attribuable à des différences de caractéristiques des études et non pas au hasard avec un test Q

d'hétérogénéité non significatif. La puissance *post hoc* de ce test de signification du RR regroupé était de $1-\beta = 0.98$, c'est-à-dire élevée. La majorité des RCT inclus (3/12 = 75%) présentaient un RR non-significatif.

Les analyses de sensibilité du tableau de contingence et du RR regroupé des ISOcc étaient robustes à l'analyse de sensibilité démontrant qu'aucune étude isolée ne modifiait le résultat regroupé.

Conclusions : L'hypothèse H0 d'absence de différence de diversité microbienne n'est pas rejetée mais le manque de puissance conduit à ne pas conclure en faveur de l'une ou l'autre hypothèse. Le RR des ISOcc significatif, robuste et cohérent avec les résultats des méta-analyses de tous les RCTs cliniquement pertinents, montre que les RCTs avec microbiologie sont un échantillon valide bien qu'en nombre insuffisant. Le niveau de certitude/qualité des données sous-jacentes au RR regroupé selon les critères GRADE était modéré.

COMMENTAIRE :

Le nombre d'observations avec les proportions de genres microbiens actuels dans les deux groupes de sutures aurait été significatif si toutes les cellules avaient eu un effectif 3,5 plus important. Cependant une significativité dans de telles conditions n'aurait fait que confirmer la différence de la fréquence du *P. aeruginosa* dans les deux groupes.

Une augmentation du nombre d'observations serait nécessaire pour augmenter la puissance du test, mais aussi pour confirmer si le différentiel actuel de fréquence du *P. aeruginosa*, se confirme, ou se rapproche significativement du point d'équilibre (un rapport de 1 entre les deux groupes de sutures).

INTERPRÉTATION ET DISCUSSION DES ETUDES

Les trois études *in vitro* ont produit des résultats cohérents et ont apporté les conclusions suivantes :

- L'étude 1 montre que le mécanisme de l'activité antimicrobienne des TS est la biocidie de contact à la surface de la suture plutôt qu'une augmentation de concentration dans le milieu environnant. Le rapport entre la surface de suture et le volume du site opératoire exposé est une des limites de la réduction du risque d'ISO par biocidie de contact par les TS.
- Les études 1 et 2 estiment l'activité biocide des TS pendant les 8 premières heures après implantation dans les tissus musculaires et sous-cutanés (entre 4 et 12 heures, sans détection d'inhibition microbienne au-delà). Cette durée est beaucoup plus courte que les estimations publiées par le passé. Les publications antérieures n'ont pas fourni de données permettant de comparer la libération de triclosan par les sutures au cours du temps dans des conditions reproduisant celle des tissus opérés ni sa relation avec l'activité antimicrobienne. Cependant, la concordance entre les études de bactéricidie de l'étude 1 et le taux de libération marginale du triclosan dans l'étude 2, qui examinent différents critères d'évaluation avec différentes méthodes, sont des preuves qui se soutiennent mutuellement. Le rapport du temps d'exposition microbienne à celui de la biocidie du TS entre 4 et 12 heures est une autre limite dans la réduction du risque d'ISO par les TS.
- Les études 1 et 3 suggèrent, compte tenu de l'analyse fragmentaire de l'échantillon microbien issu des ISOs, que l'activité biocide initiale du TS s'applique à la plupart des micro-organismes retrouvés en chirurgie. La plupart des souches inhibées présentent une CMI de triclosan inférieure à 1 µg/mL. Les TS sont inactifs sur les micro-organismes avec une CMI ≥ 128 µg/mL, tels que le *P. aeruginosa*. Une zone de transition de CMI entre 5 et 127 µg/mL, sépare les micro-organismes sensibles des non-sensibles aux TS. La plupart des micro-organismes testés dans les études 1 et 3 se trouvent du côté sensible de la zone. La réalisation de mesures microbiologiques manquantes sur les échantillons provenant d'ISOs devrait permettre d'estimer plus précisément les limites de la

zone de transition, de déterminer la proportion microbienne de chaque côté et d'identifier les micro-organismes susceptibles de changer de sensibilité après exposition au triclosan. Les publications antérieures n'ont pas fourni de preuves permettant de comparer les résultats ci-dessus. La proportion de micro-organismes isolés dans des échantillons chirurgicaux qui ne sont pas tués au cours de la phase de biocidie initiale des TS est la troisième limite dans la réduction du risque d'ISO par les TS.

- Les limites de l'activité antimicrobienne suggérées par les études 1, 2 et 3 doivent être interprétées avec les résultats d'études antérieures.
 - o Les sutures augmentent localement le risque d'ISO en facilitant la prolifération microbienne à leur surface.
 - o Le triclosan inhibe ce phénomène et empêche le développement de biofilm.
- Si le risque d'ISO est à son maximum en fin d'opération chez la plupart des patients et diminue progressivement avec le temps, à moins d'être localement facilités par les sutures, alors les TS pourraient offrir une fenêtre d'opportunité pour que les défenses naturelles contrôlent le développement d'ISO dans le reste du site opératoire.
- Après la phase biocide des TS, des facteurs de risque résiduels peuvent persister, notamment un retard de cicatrisation, un déficit immunitaire, des corps étrangers, des tissus endommagés, des hématomes et des sources d'infection hématogènes.
- L'étude 4 visait à rapprocher les conclusions sur l'activité antimicrobienne des TS des études 1, 2 et 3 avec le RR groupé d'ISO de la méta-analyse des RCTs comparant TS *versus* NTS. L'étude 4 ne montre pas de différence de diversité microbienne entre TS et NTS. Elle montre aussi un rapport du nombre de *P. aeruginosa* entre TS et NTS (rapport = 0,65) proche du rapport de l'effectif microbien total des deux sutures (rapport = 0,63). Ces résultats suggèrent que l'activité antimicrobienne du TS n'est pas associée au RR des ISOs. Cependant, ce test manque de puissance statistique pour conclure si les diversités microbiennes après TS et NTS sont similaires ou différentes. L'échantillon des RCTs avec ISOcc est testé valide car le RR groupé des ISOcc est cohérent avec

celui de la méta-analyse comportant le plus grand nombre de RCTs publiés jusqu'à présent, avec un chevauchement des deux IC95%. Cet écart pourrait s'expliquer en partie par le nombre d'observations qui fournit suffisamment de puissance pour tester une variable dépendante à 2 catégories (ISO / pas d'ISO) par groupe de suture, mais insuffisant pour tester une variable à huit catégories (les 8 genres microbiens) entre les deux mêmes groupes de sutures. Cependant, en regroupant les 7 genres autres que les *Pseudomonas*, le tableau de contingence devient une distribution binomiale, et le test demeure non significatif avec une faible puissance statistique (moins de 50%). Ceci montre que davantage d'observations sont nécessaires pour augmenter la puissance et pour déterminer si le RR des microorganismes résistants au triclosan tels le *P. aeruginosa* se déplace vers la non-infériorité par rapport à 1.

- Une fois qu'une puissance suffisante est atteinte, les résultats soutiendront ou contesteront l'association entre l'activité antimicrobienne et la diversité microbienne des ISO et donc le développement des ISO. Le calcul de la taille de l'échantillon montre que le nombre total d'observations devrait être multiplié par environ 3,5 pour que le test du chi carré du tableau de contingence avec les fréquences relatives actuelles atteigne la signification ($p = 0.03$) avec une puissance de 84%. Les publications précédentes n'ont pas décrit cette relation, de sorte que ces résultats ne peuvent pas être comparés.
- Les preuves issues des quatre études suggèrent un modèle schématique de la relation entre l'activité antimicrobienne et le risque d'ISO (Figure 15, dont seules deux configurations sont représentées dans l'intervalle possible). L'activité antimicrobienne des TS réduit le risque global d'ISO au fil du temps proportionnellement à la surface de suture et à la durée de biocidie. Ce modèle préliminaire ignore l'impact potentiel des microorganismes résistants au triclosan, mais un modèle plus avancé devrait le prendre en compte.
- Les deux modèles de risque, A et B, supposent que les TS réduisent temporairement le risque d'ISO en raison de leur biocidie de contact initiale *in vivo*. Étant donné que 85 % des RCT (28/33) sont non significatifs, le modèle B s'appliquerait dans 85 % des études.

- Les modèles A et B tentent de rapprocher les résultats *in vitro* des études 1 et 3 avec le RR combiné des ISO des méta-analyses. Cependant, cette recherche ne peut pas exclure les biais, étant donné :
 - o le test d'association non significatif et peu puissant entre le type de suture et la diversité microbienne.
 - o le risque local accru d'ISO près de la suture et le potentiel du triclosan d'inhiber le développement de biofilm.

LIMITES DES TRAVAUX

Ce programme de recherche comporte diverses limites et sources potentielles de biais.

Les principales limites à prendre en compte sont les suivantes :

- La charge initiale totale de triclosan par mètre de suture a été significativement sous-estimée car l'immersion des sutures dans une solution eau/éthanol à 100 % *w/w* avec agitation continue n'a pas permis d'extraire tout le triclosan. Définir des méthodes pour éroder la surface du polymère structurel sans dégrader le triclosan dépassait la portée de ce programme de recherche.
- L'extraction incomplète de la charge initiale de triclosan des trois sutures résorbables sous-estime la quantité résiduelle de triclosan dans les sutures au cours du temps.
- La perte d'activité biocide du TS dans les 4 à 12 heures est associée à une réduction du taux de libération marginale de triclosan alors que la majeure partie de la charge initiale de triclosan reste sur la suture.
- L'analyse de biocidie pourrait manquer de sensibilité pour détecter une inhibition microbienne prolongée après la phase de biocidie.
- Les analyses de CMI et de biocidie des TS n'ont été mesurées que dans certaines des souches microbiennes acquises. Par conséquent, l'estimation de la zone de transition d'efficacité TS, actuellement entre MIC 5 et 127 µg/mL, est moins précise que ne devrait permettre l'échantillon collecté. Les proportions de

souches cliniques au-dessous et au-dessus de la zone de transition ne peuvent pas être précisément estimées pour cette même raison.

- L'indisponibilité de repères provenant de fragments de sutures monofilament explantés de manière opportuniste pour estimer la libération de triclosan et la durée d'activité antimicrobienne de ces TS dans les tissus opérés.
- La puissance statistique du test de comparaison de la diversité microbienne entre TS et NTS est trop faible.
- Le risque élevé de biais dans les RCT individuels et la majorité des RCT non significatifs limite la qualité des preuves cliniques à un niveau modéré.

CONCLUSIONS

Les sutures au triclosan présentent une biocidie initiale sur la plupart des espèces microbiennes en chirurgie. Le mécanisme principal de l'activité antimicrobienne des sutures au triclosan est la biocidie de contact.

Les trois limites identifiées des sutures avec triclosan qui pourraient contribuer à la différence non significative du taux d'incidence des infections du site opératoire dans la majorité des essais cliniques randomisés sont :

- la faible surface d'une suture par rapport au volume cible d'un site opératoire exposé aux microorganismes,
- la durée maximale de l'activité biocide depuis l'immersion des sutures dans les tissus jusqu'à 4 à 12 heures,
- la rencontre occasionnelle avec des micro-organismes résistants au triclosan qui peuvent coloniser ces sutures, tel le *P. aeruginosa*.

La comparaison de la diversité microbienne dans les infections de site opératoire après fermeture avec des sutures avec triclosan *versus* sans triclosan n'a pas révélé de différence significative de la diversité microbienne cohérente avec la sensibilité microbienne au triclosan.

La puissance statistique insuffisante du test d'indépendance entre genres microbiens et types de sutures ne permet pas de conclure si le triclosan modifie ou non la diversité microbienne des infections du site opératoire. L'une des raisons est la fréquence plus faible, bien que non significative, du genre *Pseudomonas* après fermeture avec des sutures au triclosan par rapport aux sutures sans triclosan.

Davantage d'observations sont nécessaires pour augmenter la puissance statistique et déterminer si la fréquence plus faible des *Pseudomonas* après la fermeture avec des sutures au triclosan est confirmée.

La méta-analyse d'essais cliniques randomisés montre un risque relatif groupé significatif des infections de site opératoire confirmé par culture en faveur des sutures avec triclosan. Cependant, la qualité des preuves est modérée, compte tenu du risque de biais des études individuelles et de la minorité d'études significatives. Ces conclusions sont cohérentes avec celles des méta-analyses avec davantage d'essais cliniques randomisés et d'infections de site opératoire, avec ou sans confirmation microbiologique.

Ces résultats sont conformes aux recommandations conditionnelles de l'Organisation Mondiale de la Santé d'inclure les sutures au triclosan dans la prévention des infections du site opératoire. Cependant, ils suggèrent une mise en œuvre prudente de cette recommandation et de continuer le recueil de données cliniques et microbiologiques lorsque des infections de site opératoire surviennent pour évaluer l'association entre l'activité antimicrobienne des sutures avec triclosan et le risque d'infection de site opératoire.

De plus une analyse approfondie du triclosan résiduel après la phase de biocidie et du risque de développement de résistance microbienne devraient d'être envisagées.

COVER PAGE IN ENGLISH

ASSESSMENT OF THE EFFICACY OF SUTURES WITH TRICLOSAN
ON MICROORGANISMS ASSOCIATED WITH SURGICAL SITE
INFECTIONS

THESIS

PRESENTED FOR THE FINAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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DEFENDED BY

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BEFORE THE THESIS COMMITTEE

CHAired BY PR. MATHIEU MOLIMART

Table 1 Abbreviations

cc-SSI	Culture confirmed-SSI
CDC	Center for Disease Control
CI []	Confidence interval (95% unless specified otherwise)
Colorec.	Colorectal
ECDC	European Center for Disease Prevention and Control
EFTA	EU, Iceland, Liechtenstein, Norway, Switzerland
Elect.	Elective
EU / Euro	European Union / Europe
IR	Incidence rate
IQR	Interquartile Range
M/M+	Monofilament poliglecaprone 25 sutures without/with triclosan
MIC	Minimum inhibitory concentration
N.I.	Not identified
N.P.	Not published
N.S.	Non-significant
NNIS	National Nosocomial Infections Surveillance System
NTS	Non-triclosan suture
Obs.	Observational
OR	Odds ratio
P/P+	Monofilament polydioxanone sutures without/with triclosan
PK-PD	Pharmacokinetics - Pharmacodynamics
POA	Post-operative antibiotics
pp-RCT	prospective parallel arm RCT
RCT	Randomized clinical trial
Retro.	Retrospective
RR	Risk ratio
SLR	Systematic literature review
SSI	Surgical site infection
Surg.	Surgery
TS	Triclosan suture
UK	United Kingdom of England, Wales, Scotland, Northern Ireland
US	United States of America
USP	United States Pharmacopeia
V/V+	Braided polyglactin 910 sutures without/with triclosan
WHO	World Health Organiz(s)ation
w/w & v/v	weight/weight and volume/volume

1. INTRODUCTION

This introduction describes the background, objectives, strategy and organization of this research program.

1.1. SURGICAL SITE INFECTIONS

1.1.1. Definitions

Clinicians diagnose surgical site infections (SSI) according to different criteria. The World Health Organization (WHO) refers to two definitions:¹

The definition published by the United States Centers for Disease Control (US CDC): *“an infection that occurs after surgery in the part of the body where the surgery took place. Surgical site infections can sometimes be superficial infections involving the skin only. Other surgical site infections are more serious and can involve tissues under the skin, organs, or implanted material.”*²

The definition published by the European Center for Disease Prevention and Control (ECDC) is: *“an infection that occurs within 30 days after the operation and involves the skin and subcutaneous tissue of the incision (superficial incisional) and/ or the deep soft tissue (for example, fascia, muscle) of the incision (deep incisional) and/ or any part of the anatomy (for example, organs and spaces) other than the incision that was opened or manipulated during an operation (organ/ space).”*³

Several countries have extended the definition to 90 days in certain conditions, e.g.:

US CDC: 90 days where day-1 is the operation day in, organ/ space surgery concerning breast, cardiac, coronary, pacemaker surgery, craniotomy, spinal fusion, open fracture reduction, hip prosthesis, knee prosthesis, herniorrhaphy, ventricular surgery.⁴

France: 90 days in surgery with implants and up to one year in surgery with prosthetic material.^{5,6}

England: A minimum of 90 days and up to one year in surgery with prosthetic material.^{7,8}

Health authorities across the world issue SSI prevention guidelines for routine practice, with criteria to diagnose SSI as early as possible to minimize consequences.

The US CDC established in the 1990s the National Nosocomial Infections Surveillance System (NNIS) in the United States, including guidelines, with training and monitoring to prevent SSIs in routine practice nationwide.⁹⁻¹¹

The NNIS criteria define three SSI categories:

- Superficial Incisional: *“Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of (...)” four criteria.*
- Deep incisional: *“Infection occurs within 30 days after the operation if no implant is left in place or within 1 year if implant is in place and the infection appears to be related to the operation and infection involves deep soft tissues (e.g., fascial and muscle layers) of the incision and at least one of (...)” four criteria.*
- Organ/Space: *“Infection occurs within 30 days after the operation if no implant is left in place or within 1 year if implant is in place and the infection appears to be related to the operation and infection involves any part of the anatomy (e.g., organs or spaces), other than the incision, which was opened or manipulated during an operation “and at least one of (...)” four criteria.*

The statement about four criteria in each category includes:

- “Purulent drainage”, AND/OR
- other objective criteria, AND/OR
- subjective criteria including “Diagnosis...by the surgeon or attending physician” without further detail.

1.1.2. Incidence of SSIs

The WHO states that the burden of health-care associated infections (HCAI) is unknown due to the difficulty to gather reliable data. It also states that

SSI is among the most frequent type of HCAI and that its incidence rate varies considerably according to a country's income.¹

The European Center for Disease Prevention and Control (ECDC) monitors SSI statistics from certain Members States of the European Union and the European Economic Area. The 2019 annual report compiled data from 2017 collected in twelve Member States concerning nine types of surgery (Table II).¹² SSI incidence rates ranged from a 0.5% [0.2 to 2.7] low in knee prosthetic surgery to a 10.1% [4.1 to 16.9] high in open colon surgery.

Table II Percentage of SSIs by type of surgical procedure, EU/EEA, 2017

Type of surgical procedure	% of SSIs [intercountry range]
Coronary artery bypass graft	2.6 [0.0 to 5.5]
Laparoscopic cholecystectomy	1.5 [0.4 to 3.1]
Open cholecystectomy	3.9 [1.1 to 10.9]
Laparoscopic colon surgery	6.4 [0.0 to 12.5]
Open colon surgery	10.1 [4.1 to 16.9]
Caesarean section	1.8 [0.5 to 5.3]
Hip prosthesis surgery	1.0 [0.4 to 2.2]
Knee prosthesis surgery	0.5 [0.2 to 2.7]
Laminectomy	0.8 [0.2 to 2.7]

National levels are consistent with European ones. For example in France, 0.9% [0.7, 1.1] in primary knee prosthetic surgery and 7.0% [6.1, 8.0] in colon surgery.⁵ In England, 0.5% [0.4, 0.5] and 8.3% [7.9, 8.7] respectively.⁸

The incidence rates reported by broad types of surgery often pool difference rates in subcategories of the surgery related to depth, anatomic location, surgical method, clinical indication, prophylactic methods and other risk factors. For instance, a meta-analysis of 27 studies and 22,475 patients who underwent spinal surgery presented a pooled SSI incidence rate of 3.1% CI [2.3, 4.3%]. Stratification of the same patients stratified according to different risk factors for SSI leads to very different incidence rates (Table III). In that example, stratification by clinical indication and approach reveals very different levels of risks.

Table III Stratified SSI incidence rate by type of spinal surgery

Subgroups	number of SSIs	number of patients	pooled incidence rate 95 % CI
Categories			
Superficial	282	22475	1.4 [1.0 ; 1.9]
Deep	309	22475	1.7 [1.1 ; 2.5]
Indication			
Degeneration	63	1448	6.0 [3.4 ; 10.3]
Trauma	24	757	5.2 [1.7 ; 14.7]
Tumor	23	496	5.1 [3.4 ; 7.5]
Idiopathic scoliosis	23	929	2.6 [1.8 ; 3.9]
Neuromuscular scoliosis	62	477	13.0 [10.3 ; 16.4]
Surgical site			
Cervical	32	1329	3.4 [1.3 ; 8.6]
Thoracic	17	639	3.7 [2.4 ; 5.7]
Lumbar	99	5362	2.7 [1.6 ; 4.8]
Approach			
Anterior	21	1033	2.3 [1.5 ; 3.5]
Posterior	333	7832	5.0 [3.3 ; 7.6]
Procedure			
Non-instrument	20	2121	1.4 [0.4 ; 5.2]
Instrument	269	7662	4.4 [2.8 ; 6.8]
Minimally invasive			
No	152	4683	3.8 [1.9 ; 7.4]
Yes	9	1491	1.5 [0.2 ; 10.5]
Powder use			
No	55	2007	4.8 [1.5 ; 14.1]
Yes	22	1519	1.9 [0.7 ; 5.5]
Age (years)			
<60	494	15615	3.6 [2.5 ; 5.1]
≥60	53	2497	2.5 [1.3 ; 4.8]
BMI (kg/m²)			
<25	58	1898	3.2 [2.4 ; 4.1]
≥25	179	6516	2.9 [2.3 ; 3.8]
Surgery time (h)			
<3	91	4933	1.3 [0.5 ; 3.3]
≥3	203	4234	4.7 [3.0 ; 7.3]
Blood loss (ml)			
<500	85	4309	1.9 [0.9 ; 3.9]
≥500	221	6513	3.8 [2.3 ; 6.1]

1.1.3. Burden of illness

The burden of illness of SSI is regularly estimated in Member States. A 2016 European meta-analysis showed that the driving indicators were health-related Quality-of-life (HRQoL) and cost to the healthcare system.¹³ In medium to low-income countries, mortality remains a driving concern along with HRQoL and costs. Summarizing that data is a challenge because of differences in survey

methods. The WHO attempted to summarize the burden of illness by country but most quoted sources were outdated by about a decade.¹⁴

The impact of SSI on hospital costs and postoperative length of stay (between 3 and 20 additional days) is high.¹⁵ The cost of SSI varies considerably from less than \$400 per case in superficial SSI to more than \$30,000 per case in serious organ or space SSI.¹⁶

Countries that have not yet been able to implement effective SSI prevention and surveillance endure direct negative health consequences and costs, and countries that have could not take the risk of interrupting them.^{17,18}

Minimizing harm to patients, maintaining healthcare service performance, managing health care costs, and addressing new infectious threats as microorganisms evolve require continued optimization and improvement in SSI monitoring and prevention.

1.1.4. Pathogenesis

Microbial contamination of the surgical site is a necessary precursor of SSIs.¹¹ The risk of SSI has been suggested to result from the relationship between three fundamental factors:

$$\text{SSI risk} = \frac{\text{bacterial dose} \times \text{virulence}}{\text{resistance of the host patient}}$$

Factors that reduce patient resistance include individual factors such as age, nutritional status, smoking, diabetes, smoking, obesity, altered immunity, coexistent infections and other comorbidities, and transient factors such as hypothermia and poor glycemic control and length of stay.^{19,20}

Virulence has different definitions but most refer to the severity of disease manifestation associated with a microorganism: “*Virulence refers to the proportion of*

clinically apparent cases that are severe or fatal”²¹. Virulence varies across microbial species as they are the phenotypic expression of certain genes that result in the production of virulence factors (VF).²²⁻²⁷ VF determine mechanisms, including adherence, invasion, antiphagocytosis, and toxin secretion, that enable the microorganisms to grow, reproduce, spread, and cause damage to the host.^{24,28} The production of VF may be immunogenic (i.e., antibodies are produced against certain VF), and external factors may modify their production. Resistance to antibiotics is not part of VF, but the development of microbial multiresistance can facilitate virulence.²⁹

Microbial dose, measured in colony-forming units (CFU), is rarely measured in clinical practice, but clinical evidence of the relationship between dose and the risk of SSI has been established in clinical studies.³⁰⁻³² A surgical site contaminated with more than 10⁵ CFU/gram of tissue has a significantly increased risk of SSI.³³ A much lower microbial load can produce an SSI in the presence of foreign material in the surgical site, e.g., in the presence of silk suture, *Staphylococci* may cause an SSI with 100 CFU/gram of tissue.³⁴⁻³⁶

The US CDC’s clinical qualitative approach to microbial dose classifies surgical wounds into four categories (Table IV).¹¹

Table IV Surgical wound classification

Class I/Clean	An uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital, or uninfected urinary tract is not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow nonpenetrating (blunt) trauma should be included in this category if they meet the criteria
Class II/Clean-Contaminated	An operative wound in which the respiratory, alimentary, genital, or urinary tracts are entered under controlled conditions and without unusual contamination. Specifically, operations involving the biliary tract, appendix, vagina, and oropharynx are included in this category, provided no evidence of infection or major break in technique is encountered.
Class III/Contaminated	Open, fresh, accidental wounds. In addition, operations with major breaks in sterile technique (e.g., open cardiac massage) or gross spillage from the gastrointestinal tract, and incisions in which acute, nonpurulent inflammation is encountered are included in this category.
Class IV/Dirty-Infected	Old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation

Microorganisms involved in SSIs have been reported to originate mainly from the skin, surrounding tissues of the incision, or operated organs with a

microbial flora such as the bowel.¹⁹ The microorganisms that cause SSIs may also have a distant source and be transported to the surgical site by the blood supply (hematogenous SSI).³⁷ Hematogenous seeding related to a distance microbial source is always possible and can cause delayed SSIs.

Operative factors that facilitate SSIs include: Duration of surgical scrub, skin antiseptics, preoperative shaving (or clipping), preoperative skin preparation duration of operation, antimicrobial prophylaxis, operating room ventilation, inadequate sterilization of instrument, foreign material in the surgical site, surgical drains, surgical technique, poor hemostasis, failure to obliterate dead space, tissue trauma.¹¹ These operative factors impact mainly microbial dose and/or patient resistance.

1.1.5. Microorganisms associated with SSIs

The distribution of microbial species by surgical type across ECDC member States in 2017 (Table V) shows that *S. aureus* (21.5%) and *E. coli* are the most frequent microorganisms associated with SSI.¹² *P. aeruginosa* was detected in 4.7% of SSIs.

Table V Microbial percentages per surgery type – source ECDC 2017 SSI surveillance report

Microorganisms	CABG	Lap CHOL	Open CHOL	Lap COLO	Open COLO	CSEC	HPRO	KPRO	LAM	Total
Gram-positive cocci	50.6	30.7	38.5	26.7	31.4	52.5	67.1	72	66.2	51.6
Staphylococcus aureus	16.4	5.3	3.1	2.1	4.2	30.7	31.9	38.7	38.2	21.5
Coagulase-negative staphylococci	26.4	2.7	4.6	1.3	2.4	3.5	18.9	17.6	15.4	11.0
Enterococcus species	3.7	14.0	27.7	16.7	21.5	8.3	7.7	7.1	3.7	11.9
Streptococcus species	1.5	8.0	3.1	5.6	2.6	9.0	5.0	6.4	2.9	4.9
Other gram-positive cocci	2.6	0.7	0	1.1	0.7	1.0	3.7	2.9	5.9	2.2
Gram-positive bacilli	2.2	2.0	0	0.5	0.5	1.0	4.1	4.8	0.7	2.3
Gram-negative bacilli, Enterobacteriaceae	32.3	44.7	50.8	50.8	46.6	25.7	19.3	15.5	17.6	30.7
Escherichia coli	5.2	25.3	23.1	31.7	22.5	13.7	6.9	4.6	5.1	13.9
Citrobacter species	1.9	2.7	6.2	3.2	1.8	0.7	0.6	0.7	1.5	1.4
Enterobacter species	5.6	4.7	4.6	6.3	7.2	3.0	3.0	2.4	1.5	4.4
Klebsiella species	6.7	7.3	10.8	5.6	7.2	2.9	2.3	2.4	2.9	4.4
Proteus species	5.6	2.7	1.5	2.4	2.4	3.9	4.0	2.2	4.4	3.3
Serratia species	3.7	0.7	1.5	0.3	0.8	0.3	1.5	1.5	0.7	1.2
Other Enterobacteriaceae	3.7	1.3	3.1	1.3	4.7	1.2	1.0	1.5	1.5	2.2
Gram-negative non- fermentative bacilli	9.3	4.0	0	6.6	11.2	3.9	5.0	2.1	6.6	6.3
Acinetobacter species	1.1	0	0	0.3	0.2	0.2	0.4	0	0	0.3
Haemophilus species	0	0	0	0	0	0.3	0	0	0	0
Pseudomonas aeruginosa	6.7	3.3	0	5.8	8.8	1.0	3.6	1.9	6.6	4.7
Pseudomonadaceae family, other	1.1	0	0	0	1.8	1.2	0.9	0.2	0	1.0
Stenotrophomonas maltophilia	0	0.7	0	0	0	0.2	0	0	0	0
Other gram-negative non- fermentative bacilli	0.4	0	0	0.5	0.3	1.0	0.1	0	0	0.3
Anaerobes	0.7	9.3	1.5	8.7	4.4	13.5	2.9	3.1	5.1	5.2
Bacteroides species	0	1.3	1.5	6.9	3.2	1.2	0.2	0.3	0	1.7
Other anaerobes	0.7	8.0	0	1.9	1.1	12.3	2.7	2.8	5.1	3.6
Other bacteria	1.9	8.7	4.6	4.5	3.0	1.4	0.8	1.7	0.7	2.2
Fungi, parasites	2.6	0.7	4.6	2.1	2.7	1.5	0.5	0.2	2.2	1.5
Candida species	2.2	0.7	4.6	2.1	2.7	1.2	0.5	0	2.2	1.4
Other fungi or parasites	0.4	0	0	0	0	0.3	0	0.2	0	0.1

Table V shows the relative distribution of germs reported by the European countries that contributed to the ECDC 2017 SSI surveillance. The table provides fewer details about species than its source national reports, such as ISO-RAISIN in France. Labels and sample sizes are:

CABG: coronary artery bypass surgery, n=269

Lap CHOL: Laparoscopic cholecystectomy, n=151

Open CHOL: Open cholecystectomy, n=65

Lap COLO: Laparoscopic colon surgery, n=378

Open COLO: Open colon surgery, n=1 146

CSEC: Caesarian section, n=592

HPRO: Hip prosthetic surgery, n=1 409

KPRO: Kneed prosthetic surgery, n=581

LAM: Laminectomy, n=136

Total, n=4 727

Data from France, showed that diagnosis was microbiological in 67.8% (1057/1540 cases), based on local signs in 14.3% (220/1534), based on purulent incisions in 11.7% and surgeon diagnosis in 5.3%.⁵

The median time to SSI diagnosis was 14 days IQR 7 to 22 days, and 49.2% required a re-operation (Figure 1).

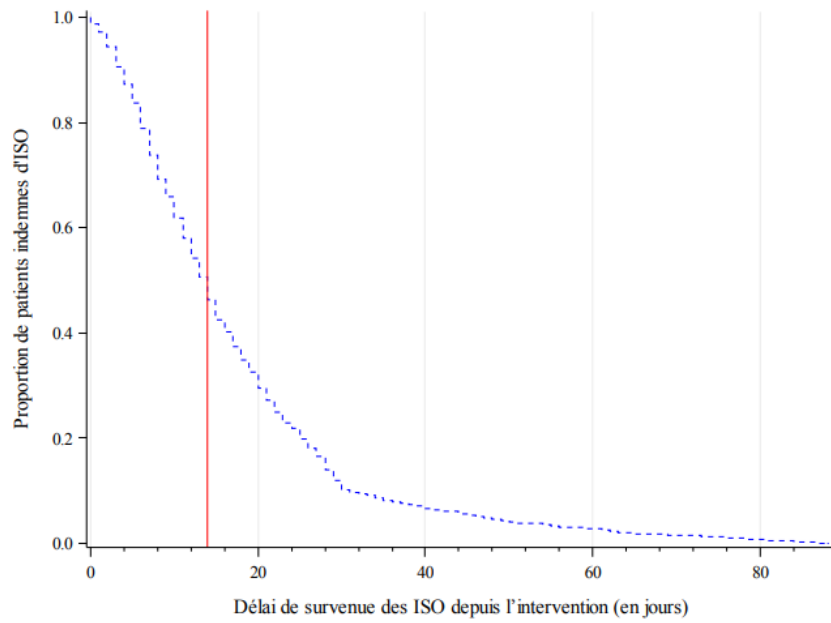


Figure 1 Days from any surgery & SSI diagnosis (ISO-RAISIN 2018 France)

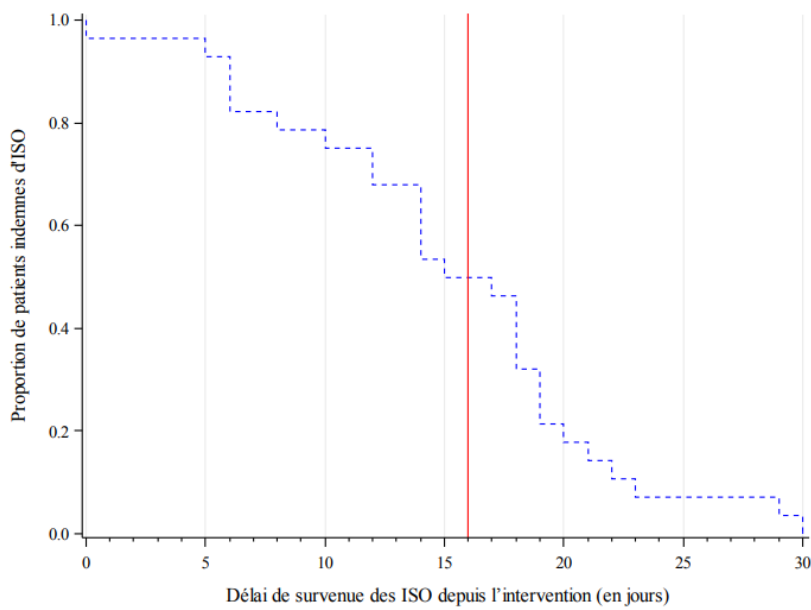


Figure 2 Days from neuro/spinal surgery & SSI diagnosis (ISO-RAISIN 2018 France)

1.1.6. Prevention

Microbial circulation is a worldwide issue given people, animals, plants, and goods travel extensively. International consensus has been reached to optimize SSI prevention methods. Most countries and/or country groups work on that topic and exchange with the WHO to consolidate global guidelines for health care providers to optimize their methods given patient and surgical risk factors, given each country's resources.

The WHO 2016 outline lists 30 recommendations:

- 9 strong recommendations, with a high-level evidence base, that should apply to most surgeries in all countries, and
- 21 conditional recommendations, with a high or a moderate evidence basis, should be implemented if deemed relevant and feasible.

WHO recommendations were updated in 2018.¹⁴

The 9 strong recommendations are:

*1- Patients with known nasal carriage of *S. aureus* should receive intranasal applications of mupirocin 2% ointment with or without a combination of chlorhexidine gluconate body wash.*

2- Mechanical bowel preparation alone (without the administration of oral antibiotics) should NOT be used in adult patients undergoing elective colorectal surgery.

3- In patients undergoing any surgical procedure, hair should either NOT be removed or, if absolutely necessary, should only be removed with a clipper. Shaving is strongly discouraged at all times, whether preoperatively or in the operating room.

4- Surgical antibiotic prophylaxis (SAP) should be administered before surgical incision, when indicated.

5- SAP should be administered within 120 min before incision, while considering the half-life of the antibiotic.

6- Surgical hand preparation should be performed either by scrubbing with a suitable antimicrobial soap and water or using a suitable alcohol-based handrub before donning sterile gloves.

7- Alcohol-based antiseptic solutions based on CHG for surgical site skin preparation should be used in patients undergoing surgical procedures.

8- *Adult patients undergoing general anaesthesia with endotracheal intubation for surgical procedures should receive 80% fraction of inspired oxygen intraoperatively and, if feasible, in the immediate postoperative period for 2–6 h.*

9- *Surgical antibiotic prophylaxis administration should not be prolonged after completion of the operation.*

The 21 conditional recommendations are:

1- *Immunosuppressive medication: Immunosuppressive medication should not be discontinued prior to surgery for the purpose of preventing SSI.*

2- *Nutritional formulas: Consider the administration of oral or enteral multiple nutrient-enhanced nutritional formulas for the purpose of preventing SSI in underweight patients who undergo major surgical operations.*

3- *Bathing before surgery: It is good clinical practice for patients to bathe or shower before surgery. Either a plain soap or an antiseptic soap could be used for this purpose.*

4- *Intranasal mupirocin: Consider treating patients with known nasal carriage of *S. aureus* undergoing other types of surgery with perioperative intranasal applications of mupirocin 2% ointment with or without a combination of CHG body wash.*

5- *Antibiotics & MBP: Preoperative oral antibiotics combined with MBP (i.e., Mechanical Bowel Preparation) should be used to reduce the risk of SSI in adult patients undergoing elective colorectal surgery.*

6- *Antimicrobial sealants: Antimicrobial sealants should not be used after surgical site skin preparation for the purpose of reducing SSI.*

7- *Warming devices: Warming devices should be used in the operating room and during the surgical procedure for patient body warming with the purpose of reducing SSI.*

8- *Blood glucose control: Protocols for intensive perioperative blood glucose control should be used for both diabetic and non-diabetic adult patients undergoing surgical procedures.*

9- *Fluid therapy: Goal-directed fluid therapy should be used intraoperatively for the purpose of reducing SSI.*

10- *Drapes and gowns: Either sterile disposable non-woven or sterile reusable woven drapes and surgical gowns can be used during surgical operations for the purpose of preventing SSI.*

11- *Adhesive drapes: Plastic adhesive incise drapes with or without antimicrobial properties should **not** be used for the purpose of preventing SSI.*

12- *Wound protectors: Consider the use of wound protector devices in clean-contaminated, contaminated and dirty abdominal surgical procedures for the purpose of reducing the rate of SSI*

13- *Saline wound irrigation: There is insufficient evidence to recommend for or against saline irrigation of incisional wounds for the purpose*

14- *Povidone iodine: Consider the use of irrigation of the incisional wound with an aqueous povidone iodine solution before closure for the purpose of preventing SSI, particularly in clean and clean-contaminated wounds.*

15- *Antibiotic irrigation/ Antibiotic incisional wound irrigation before closure should not be used for the purpose of preventing SSI.*

16- *Neg pressure: Prophylactic negative pressure wound therapy may be used on primarily closed surgical incisions in high-risk wounds and, taking resources into account, for the purpose of preventing SSI.*

17- Coated sutures: Triclosan-coated sutures may be used for the purpose of reducing the risk of SSI, independent of the type of surgery.

18- *Laminar airflow ventilation: Laminar airflow ventilation systems should not be used to reduce the risk of SSI for patients undergoing total arthroplasty surgery.*

19- *Peri-op antibiotics: Perioperative surgical antibiotic prophylaxis should not be continued due to the presence of a wound drain for the purpose of preventing SSI.*

20- *Wound drains: The wound drain should be removed when clinically indicated. No evidence was found to allow making a recommendation on the optimal timing of wound drain removal for the purpose of preventing SSI.*

21- *Advanced dressings: Advanced dressing of any type should not be used over a standard dressing on primarily closed surgical wounds for the purpose of preventing SSI.*

Each recommendation covers a specific aspect of SSI risk management, with some to reduce contamination sources and others to maintain the patient's physiological ability to eliminate microorganisms from tissues. For example:

- Strong items 1, 3, 6, 7, conditional items 4, 11, 12, and 18 aim to minimize germ penetration risk from the body surface or the surrounding air through the surgical incision.
- Strong item 2 and conditional item 5 aim to minimize the amount of germ penetration from within the digestive tract during bowel surgery.
- Conditional items 13, 14, and 16 aim to topically eliminate germs that penetrated the surgical wound during the operation.
- Strong items 4 and 5 aim to minimize germs that have penetrated the body, through whatever the source during the operation.
- Strong item 8, conditional items 2, 7, 8 and 9 aim to maintain the patient's natural defences to eliminate germs once inside the body.
- Strong item 9 and conditional items 6, 15, 19, 20 and 21 aim at mitigating the risk of adverse events related to prolonged prophylactic antibiotics and antimicrobials and the end of the surgery and postoperatively. It also aims at minimizing the development of germ resistance to those drugs.
- Conditional item 1 is specific to balancing SSI and adverse events risks due to immune response, e.g., in transplanted patients.

The above list does not cover the risk of introducing germs related to the sterility of surgical instruments, consumables and implants. It also does not cover risks related to surgical team hygiene and dressing. Those topics are dealt with in separate regulations and guidelines.

Conditional guideline ***17- Coated sutures: Triclosan-coated sutures may be used for the purpose of reducing the risk of SSI, independent of the type of surgery.***

The intended purpose is to minimize the risk of microbial colonization, in turn to avoid the initiation or facilitation of SSI development.

That concept could be regarded as paradoxical in the WHO global guideline context.

First, triclosan sutures are medical devices subject to laws and regulations that overrule guidelines, and triclosan sutures are the only specific devices recommended in WHO global guidelines without consideration for the legal and regulatory status.

Secondly, triclosan sutures are intended to maintain sufficient antimicrobial surgical wound closure. This could biologically be inconsistent with strong item 9 and conditional items 6, 15, 19, 20 and 21, because triclosan operates like a local antibiotic (i.e., action upon bacterial receptors), although it has not been, so far, regulated as a medicinal product.

Thirdly, the WHO 2018 global guideline update describes the level of evidence in item 17 as follows¹⁴: “*the panel suggests the use of triclosan-coated sutures for the purpose of reducing the risk of SSI, independent of the type of surgery. Strength: Conditional. Quality of evidence: Moderate.*” Item 17 statement is inconsistent. Its scientific basis was a meta-analysis published by Wu *et al.* in 2017.³⁸ The key results were the odds ratio (OR) of the frequency of SSI after wound closure with triclosan sutures (TS) over non-triclosan sutures (NTS). The OR with data from randomized clinical trials (RCT) was 0.72 [0.59, 0.88], and 0.58 [0.40, 0.83] with data from observational studies.

The conclusions of the Wu *et al.* 2017 review are consistent with the most comprehensive meta-analysis of prospective RCTs published to date with 25 studies and n = 11957 patients.³⁹ One major objective cause of uncertainty in the significant pooled effect is that 88% of the included RCTs (22/25) are non-significant.

1.2. ABSORBABLE SURGICAL SUTURES WITH TRICLOSAN

1.2.1. Sutures

1.2.1.1. Overview of suture selection criteria

There is no international consensus on the choice of surgical sutures. One published overview from 2009 provides a comprehensive description of suture characteristics surgeons may consider when choosing a suture for a given purpose.⁴⁰ These include intrinsic features, such as tensile strength, tissue absorption (a factor of the duration of tensile strength over time), cross-sectional diameter, coefficient of friction, knot security, handling (related to elasticity, plasticity, and memory), tissue reactivity (should be nonelectrolytic, noncapillary, nonallergenic, and noncarcinogenic), origin (natural, synthetic, etc.), physical configuration (monofilament, braided, twisted, etc.), capillarity, fluid absorption, ease of removal, color, needle characteristics.

The author mentions, *“The final decision concerning the method and material used in closure is highly dependent on the length and anatomic location of the wound”*.

1.2.1.2. Absorbable sutures studied in this research

Absorbable surgical sutures avoid re-incising to remove them after surgical wound healing. The three absorbable sutures relevant in this research are made of polymer and have been in use for decades:

- **braided coated polyglactin 910 sutures** (Ethicon, Vicryl®), was introduced in 1979 (short used here: V)
- **single-stranded polydioxanone sutures** (Ethicon, PDS II®) was introduced in 1982 (short used here: P)

- **single-stranded sutures poliglecaprone 25 sutures** (Ethicon, Monocryl®) was introduced in 1993, (short used here: M)

The sutures are provided with a range of calibers and various needles to provide a wide variety of tensile strength and duration until their fragmentation due to bioabsorption. They also offer different handling properties that facilitate suturing in different tissues and operative circumstances.

The manufacturer's label of V and V+ braided sutures mentions that the remaining tensile strength at 28 days is 25%, and complete bioabsorption is reached between 56 and 70 days.⁴¹

Versions of those sutures with triclosan were introduced between 2003 and 2006:

- **braided coated polyglactin 910 sutures with 472µg/m of triclosan** (Ethicon, Vicryl PLUS®)⁴¹ (short used here: V+)

- **single-stranded polydioxanone sutures with 2360µg/m of triclosan** (Ethicon, PDS PLUS®)⁴² (short used here: P+)

- **single-stranded sutures poliglecaprone 25 sutures with 2360µg/m of triclosan** (Ethicon, Monocryl PLUS®)⁴³ (short used here: M+).

The six sutures are produced in the same calibers, lengths and variety of needles. Triclosan does not alter the mechanical properties of the sutures. Moreover, a triclosan suture cannot be distinguished from its non-triclosan counterpart because triclosan does not change the suture feel, aspect, or smell, which enables operator-blinded studies.

1.2.1.3. Triclosan

1.2.1.3.1. Chemical characteristics

Triclosan (C₁₂H₇Cl₃O₂ designated 2,4-dichlorophenoxyphenol, CAS: 3380-34-5) is an aromatic compound with two benzene rings linked through an ether covalent bond (Figure 3).⁴⁴⁻⁴⁶

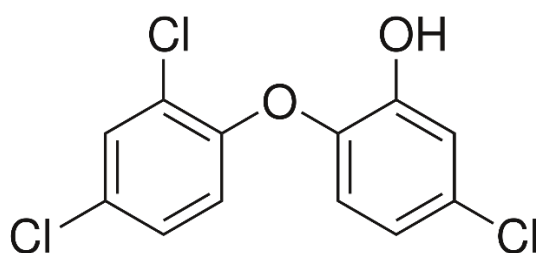


Figure 3 Representation of a molecule of triclosan

It is moderately soluble in water with a solubility (i.e., saturation concentration) $C_s = 10 \text{ mg/L} = 10 \text{ }\mu\text{g/mL}$ at 20°C ; It is a solid at temperatures compatible with human life (melting point: 54 to 57.3°C) and thus a solid on sutures ready for use.

1.2.1.3.2. Antimicrobial properties

Triclosan antimicrobial properties have various enzymatic pathways.⁴⁷ One of the most documented mechanisms is the inhibition of the synthesis of fatty acids at the enoyl-acyl carrier protein reductase (FabI) step.⁴⁸ This causes the disruption of microbial membranes and of other physiological pathways.

The germ *P. aeruginosa* presents extreme resistance to triclosan through various mechanisms, including an efflux pump complex that reduces the triclosan concentration in those bacteria, as well as a different fatty acid synthesis enzymatic pathway that involves gene FabV rather than FabI, that triclosan does not inhibit.^{48,49} The development of resistance to triclosan in *E. coli* has been observed in the presence of mutations of the FabI gene and the acquisition of the FabV gene. Other pathways and related genes have also been identified.

The minimum inhibitory concentration (MIC expressed in $\mu\text{g/mL} = \text{mg/L}$) of triclosan has been determined in a variety of microbial species, including *E. coli* $0.1 \text{ }\mu\text{g/mL}$, *K. Pneumoniae* $0.12\text{-}2$, methicilline sensitive *S. aureus* (MSSA) 0.016 to >0.25 , same range with methicilline resistant strains of *S. aureus* (MRSA) although some strains reach $2\text{-}4$, *S. epidermidis* $0.12\text{-}8$, *P. aeruginosa* assumed MIC is >1000 as is the case with mutant *E. coli*.⁵⁰⁻⁵³ MICs reported above the solubility of triclosan in water are assumed given triclosan precipitates in solid particles the saturation

concentration and solutions compatible with bacterial growth very moderately increase triclosan solubility compared to water.

1.2.1.3.3. Intended performance of triclosan sutures

The triclosan production used in V+, M+, and P+ is BASF Irgacare MP®.⁵⁴ The intended performance related to triclosan is regulated information on sutures labels.

Polyglactin 910 braided TS ‘:

“no more than 472 µg/m”...

*“Using zone of inhibition studies, Irgacare MP (triclosan) in Coated VICRYL Plus Antibacterial Suture has been shown to inhibit colonization of the suture by Staphylococcus aureus, Staphylococcus epidermidis, Methicillin Resistant S. aureus and Methicillin Resistant S. epidermidis, which are microorganisms known to contribute to surgical site infections. Animal studies have demonstrated that **VICRYL Plus inhibits bacterial colonization of suture after direct in vivo challenge with bacteria. The clinical significance of this finding is unknown.**”⁴¹*

Monofilament Poliglecaprone 25 TS:

“no more than 2360 µg/m”...

*“Using zone of inhibition studies, Irgacare MP (triclosan) in PDS Plus Antibacterial Suture has been shown to inhibit colonization of the suture by Staphylococcus aureus, Staphylococcus epidermidis, Methicillin Resistant S. aureus, Methicillin Resistant S. epidermidis, Escherichia Coli, and Klebsiella pneumoniae which are microorganisms known to contribute to surgical site infections. Animal studies have demonstrated that **PDS Plus Antibacterial Suture inhibits bacterial colonization of suture after direct in vivo challenge with bacteria.**”⁴³*

Monofilament Polydioxanone TS:

“no more than 2360 µg/m”...

*“Using zone of inhibition studies, Irgacare MP (triclosan) in PDS Plus Antibacterial Suture has been shown to inhibit colonization of the suture by Staphylococcus aureus, Staphylococcus epidermidis, Methicillin Resistant S. aureus, Methicillin Resistant S. epidermidis, Escherichia Coli, and Klebsiella pneumoniae which are microorganisms known to contribute to surgical site infections. Animal studies have demonstrated that **PDS Plus Antibacterial Suture inhibits bacterial colonization of suture after direct in vivo challenge with bacteria.**”⁴²*

1.2.1.3.4. Amount and bioavailability of triclosan on sutures

The efficacy of any antimicrobial drug is dependent on both the strain’s sensitivity to that drug and the exposure to that drug, both in terms of concentration and duration of residual triclosan and its release rate. Those pharmacokinetic concepts should, in theory, influence the bioavailability of the triclosan of implanted sutures and, in turn, determine the clinical efficacy.

Despite best efforts in searching literature databases and manufacturer documentation, no study reporting the release of triclosan and its residual amount on a suture over time has been identified. Furthermore, no study has reported the actual amount of triclosan per unit of length.

One publication mentions that the triclosan concentration per meter of V+ at implantation would be 2.7 µg/cm, i.e., 270 µg/m, and quotes two other references, but those do report any amount of triclosan per unit of length at all, nor the methods for its determination.⁵⁵⁻⁵⁷ The only information available is the maximum amount of triclosan planned by the manufacturer presented previously.⁴¹⁻⁴³

Published information about sutures with triclosan does not enable checking longitudinal microbiological study results against triclosan bioavailability.

1.2.1.4. Clinical evidence

The number of published randomized clinical trials (RCTs) reporting the incidence of SSIs in wounds triclosan sutures (TS) *versus* non-triclosan sutures (NTS) has gradually built up since 2005, and so has the number of meta-analyses about those RCTs.^{58,59}

Table VI provides a summary status of clinical evidence. It provides a comparison of the published SLRs between 2012 and 2021 (columns) with the corresponding list of RCTs (top rows) they included in the pooled measure of efficacy, usually the risk ratios (RR) or the odds ratio (OR). It also compares the *post hoc* quest for potential predictors of the RR (or the OR) conducted with those meta-analyses (bottom rows).

The table shows that the number of published RCTs gradually increased from six with 836 patients in 2012 to 33 with 14,877 in the update of this research program's SLR performed in August 2021. (see **Erreur ! Source du renvoi introuvable..** PRISMA flow chart, figure 1 of the submitted manuscript)

From 2014 until 2019, comprehensive meta-analyses reported significant pooled risk ratios (RR) with a lower incidence of SSIs in the TS arm than in the NTS arm and pooled analyses.^{38,39,60-63} Pooled RR changed little over the years from 0.70 [0.57, 0.85] (Wang *et al.* in 2013) to RR 0.73 [0.65, 0.82] (Ahmed *et al.* in 2019) and RR 0.76 [0.66, 0.88] in this research program's SLR update in August 2021 (summary of comprehensive meta-analyses in Table VI).

Abbreviations in Table VI below:

pp-RCT: prospective parallel arm-RCT, Retro.: Retrospective, Obs.: Observational, Retro.: Retrospective.
N.P.: Not published, N.I.: not identified, Surg.: surgery, Colorec.: Colorectal, Elect.: Elective

Table VI Clinical evidence status – summary table of comprehensive meta-analyses

SSIs TS vs NTS	Study type	Daoud 2021 N.P.	Jiang 2021	Ahmed 2019	Uchino 2018	Wu (WHO) 2017	de Jonge 2017	Konstantelias 2017	Sandini 2016	Apisarnthanarak 2015	Daoud 2014	Edmiston 2013	Wang 2013	Chang 2012
Ford 2005	pp-RCT	x	0	x	0	x	x	x	0	x	x	x	x	x
Defazio 2005	pp-RCT poster	0	0	0	0	0	x	0	0	x	0	0	x	x
Fleck 2007	Retro.	0	0	0	0	0	0	x	0	0	0	0	0	0
Rozzelle 2008	pp-RCT	x	0	0	0	0	x	x	0	x	x	x	x	x
Deliaert 2009	bilateral RCT	0	0	0	0	0	0	0	0	x	0	0	x	x
Justinger 2009	Obs.	0	0	0	0	0	0	0	0	x	0	0	0	0
Zhuang 2009	pp-RCT	x	0	0	0	0	0	0	0	x	x	x	x	0
Mingmalairak 2009	pp-RCT	x	0	x	x	x	x	x	0	x	x	x	x	x
Singh 2010	RCT	N.I.	0	0	0	0	x	0	0	x		0	x	0
Baracs 2011	pp-RCT	x	0	x	x	x	x	x	x	x	x	x	x	x
Huszar 2012	pp-RCT	x	0	x	0	x	x	x	0	x	x	N.I.	0	0
Chen 2011	pp-RCT	x	0	x	0	x	x	x	0	x	x	N.I.	0	0
Galal 2011	pp-RCT	x	0	x	0	x	x	x	0	x	x	x	x	0
Justinger 2011	Non-RCT	0	0	0	0	0	0	0	0	x	0	0	0	0
Khachatryan 2011	RCT	N.I.	0	0	0	0	x	0	0	x	N.I.	N.I.	x	0
Rasic 2011	pp-RCT	x	0	x	x	x	x	x	x	x	x	x	x	0
Matsumoto 2011	Obs.	0	0	0	x	0	0	0	0	0	0	0	0	0
Mattavelli 2011	Abstract	0	0	0	0	0	0	0	0	x	0	0	x	0
Stadler 2011	Retro.	0	0	0	0	0	0	x	0	0	0	0	0	0
Williams 2011	pp-RCT	x	0	x	0	x	x	x	0	x	x	x	x	0
Zhang 2011	pp-RCT	x	0	x	0	0	0	x	0	x	x	x	x	x
Seim 2012	pp-RCT	x	0	x	0	x	x	x	0	x	x	x	x	N.P.
Turtiainen 2012	pp-RCT	x	0	x	0	x	x	x	0	x	x	x	x	N.P.
Isik 2012	pp-RCT	x	0	x	0	x	x	x	0	x	x	x	x	N.P.
Laas 2012	Non-RCT	0	0	0	x	x	0	0	0	x	0	0	0	N.P.
Justinger 2013	pp-RCT	x	0	x	x	x	x	x	0	x	x	N.P.	0	N.P.
Nakamura 2013	pp-RCT	x	0	x	x	x	x	x	x	x	x	x	0	N.P.
Thimour-B. 2013	pp-RCT	x	0	x	0	x	x	x	0	x	x	N.P.	0	N.P.
Hoshino 2013	Retro.	0	0	0	x	x	0	x	0	x	0	0	0	N.P.
Ueno 2013	Retro.	0	x	0		x		x	0	x	0	0	0	N.P.
Yam 2013	RCT	N.I.	0	0	0	0	x	0	0	0	0	N.P.	N.P.	N.P.
Arslan 2014	pp-RCT poster	0	0	0	0	0	x	0	0	0	0	0	N.P.	N.P.
Diener 2014	pp-RCT	x	0	x	x	x	x	x	x	x	N.P.	N.P.	N.P.	N.P.
Fracalvieri 2014	Obs.	0	0	0	0	0	0	x	0	0	0	N.P.	N.P.	N.P.
Itatsu 2014	Non-RCT	0	0	0	0	0	0	x	0	0	0	0	0	N.P.
Okada 2014	Non-RCT	0	0	0	x	x		x	0	x	0	N.P.	N.P.	N.P.
Olmez 2019	pp-RCT	x	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
Steingrimsen 2015	pp-RCT	x	0	0	0	0	0	x	0	N.P.	N.P.	N.P.	N.P.	N.P.
Mattavelli 2015	pp-RCT	x	0	x	x	0	x	x	x	N.P.	N.P.	N.P.	N.P.	N.P.
Ruiz-Tovar 2015	pp-RCT	x	0	x	x	0	0	x	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
Karip 2016	pp-RCT	x	0	x	0	0	0	x	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
Nakamura 2016	Obs.		0	0	x	0	0	x	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
Umemura 2016	RCT		0	0	x	0	0	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
Renko 2017	pp-RCT	x	0	x	x	0	0	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.

SSIs TS vs NTS	Study type	Daoud 2021 N.P.	Jiang 2021	Ahmed 2019	Uchino 2018	Wu (WHO) 2017	de Jonge 2017	Konstantelias 2017	Sandini 2016	Apisarntharak 2015	Daoud 2014	Edmiston 2013	Wang 2013	Chang 2012	
Soomro 2017	pp-RCT	x	0	0	0	0	0	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Sprowson 2018	pp-RCT	x	x	x	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Arslan 2018	pp-RCT	x	0	x	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Ichida 2018	pp-RCT	x	0	x	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Lin 2018	pp-RCT	x	x	x	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Sun 2018	Retro.	0	x	0	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Sukeik 2019	pp-RCT	x	x	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Tabrizi 2019	pp-RCT	x	0	x	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Roy 2019	pp-RCT	x	0	x	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Santos 2019	pp-RCT	x	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Ruiz-Tovar 2020	pp-RCT	x	0	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	
Focus		All surg.	Orthopedics	All surg.	Digestive surg.	All surg.	All surg.	All surg.	Elect. colorec.	All surg.	All surg.	All surg.	All surg.	All surg.	
Sutures types		All	V / V+	All	All	All	All	All	All	All	All	All	All	All	
TS SSI/n NTS SSI/n All SSI/n		519/7372 678/7505	24/1793 50/1715	420/6008 581/5949	160/1798 RCTs 205/1690	267/2661 RCTs 345/2685	330/3208 450/3254		129/1102 143/1166		180/2323 272/2477	126/1654 190/1914	149/1726 227/1994		
pooled result		RR 0.76 [0.66, 0.88]	OR 0.44 [0.14, 1.39]	RR 0.73 [0.65, 0.82]	RCTs RR 0.67 [0.48, 0.94]	RCTs OR 0.72 [0.59-0.88]	RR 0.72 [0.60, 0.86]		RR 0.68 [0.57, 0.81]	OR 0.81 [0.58,1.13]	RR 0.65 [0.55, 0.77]	RR 0.67 [0.54, 0.84]	RR 0.69 [0.53, 0.92]	RR 0.70 [0.57, 0.85]	OR 0.77 [0.40, 1.51]
Total patients		14877	3528	11957	5188	7458	6462	15385	2168	11942	4800	3568	3720	836	
Studies		pp-RCTs	pp-RCTs + Obs.	pp-RCTs	pp-RCTs + Obs.	pp-RCTs + Obs.	pp-RCTs	pp-RCTs + Obs.	pp-RCTs	pp-RCTs + Obs.	pp-RCTs	pp-RCTs	pp & bilat-RCT	pp & bilat-RCT	
Assessment of potential predictors:															
SSI diagnostic method		no	no	no	no	no	no	yes (SSI definition)	no	yes (definition)	yes: NS	no	no	no	
blinding		no	no	no	no	no	no	yes	yes: NS	yes: NS	yes: NS	no	no	no	
surgery type		no	yes: spinal surgery	no	yes: colorec surg (p=0,04)	yes: NS	no	yes: colorectal	no	yes: abdominal (p=0.006)	yes: NS	no	yes: abdominal (p=0.003)	no	
suture type		no	no	no	yes: polyfilament (p=0.004)	yes: NS	yes: V/V+ (p<0,001)	yes	yes: NS	yes: NS	yes: NS	no	yes: V/V+ (p=0.016)	no	
wound classification (NNIS)		no	no	yes: p=0.005	yes: III/infected p<0,01	yes: NS	yes: I p=0.003 >I p=0.01	yes: significant in I, II, III	no	yes: NS	yes: NS	no	yes: I (p=0.021) and II (p=0.026)	yes: NS	
incidence rate		yes: NS	no	no	no	no	no	no	no	no	yes: NS	no	no	no	
depth		no	no	yes: NS	no	no	no	yes	no	no	no	no	no	no	
risk of bias		no	no	no	no	no	no	no	no	no	no	no	yes: low-risk p=0.015	no	
follow-up		no	no	no	no	no	no	no	no	no	no	no	yes:1mo p=0.037, >1mo p=0.021	no	
Study design		no	no	no	no	no	no	no	no	yes: NS	no	no	no	no	
Other age ; centers		no	no	no	no	no	no	no	n yes: single- center (p=0.003)	no	no	no	yes: adult p<0,001	no	

Those RRs represent a reduction of about one-quarter of all SSIs throughout surgeries.

Several comprehensive SLR concluded agreed that the quality and the certainty of the pooled evidence is moderate.^{38,39}

The eight criteria for assessing the quality of evidence of Cochrane's GRADE system show underlying reasons for the "moderate" rating of evidence and the impact on the strength of guidelines.⁶⁴

The **risk of bias of individual RCTs**: That term refers to the risk that one RCT overestimates or underestimates the RR because something went wrong during the study planning or its conduct. Two categories of factors can cause bias and may occur independently:

- **Criterion 1: Rating methodological bias** is related to the organization, conduct, reporting, and analysis of an RCT. Asserting methodological bias can be performed in all clinical diseases based on logic and statistics (helped by methods guidelines)
 - o Of the individual RCTs is the focus of Cochrane's RoB-2 risk of bias analysis method in SLRs.⁶⁵ Regarding RCTs comparing TS and NTS, many RCTs present that kind of risk related to:
 - Study design and randomization procedures
 - Operator or observer blinding
 - Inaccurate or incomplete or selective reporting, without patient screening log or description of patient disposal as defined in CONSORT guidelines.⁶⁶
 - o Assessing the risk of methodological bias of source evidence (RCTs in this context) is one of the four dimensions used in the GRADE method to assess the quality of evidence.^{67,68}
 - o In the situation analyzed here, individual methodological risks of bias of several RCTs were rated differently by the authors of several SLRs. That shows the subjective component of the risk of bias assessment.

- **Criterion 2: Rating the inconsistency of Results:** This refers to the *statistical heterogeneity* of the measures of outcome to be pooled according to meta-analysis. This is about estimating whether the estimates of the different outcomes are likely to be samples of a common distribution. Cochran Q-test and the I-squared are used to assess statistical heterogeneity of the statistical estimates assessed for pooling.⁶⁹⁻⁷¹
 - In the situation analyzed here, the statistical risk of bias was often non-significant or low or moderate. However, that component requires joint interpretation with the other quality items, and the absence of heterogeneity does not compensate for weaknesses in the dimensions of evidence quality.
- **Criterion 3: Rating indirectness.** This refers to the various factors related to *clinical specificities that may cause bias or heterogeneity*. Users of evidence need *direct evidence*, that (1)directly compares (2)the intended interventions (3)used in patients from the population of interest, (4)using a relevant measure of outcomes.⁷² The corresponding four risks of indirectness are:
 - (1) *indirect comparisons*. This takes place when deducting the comparison of A to C based on the comparison of A to B, and of B to C.
 - (2) *differences in compared interventions* where the tested interventions are significantly different from those that conclusion will apply to,
 - (3) *differences in the tested patients* and the population of interest,
 - (4) *differences in the measure of outcome*. When the measure concerns an intermediate effect or a consequence of the intended effect, or a mix of the intended effects and collateral factors. This includes “surrogate endpoints.”

- **Criterion 4: Rating imprecision of the evidence:** This refers to enough data to calculate outcomes locations and dispersions (e.g., point estimates of probabilities and confidence intervals) with enough precision to establish a significant difference between the compared treatment arm. This requires enough statistical power for confidence intervals to be sufficiently narrow, given the central value of each arm. The number of observations drives the precision of comparisons. In the present situation, a sufficient number of observed SSIs with TS *versus* NTS is required for a comparison to be meaningful.
- **Criterion 5: Publication bias:** Publication bias is bias caused by the unavailability of information, in particular when it results in modifying the conclusion of comparing two treatments. The probability of publication bias can be assessed by comparing the dispersion and symmetry of reported outcomes intended to be pooled. ⁷³⁻⁷⁵

Indirectness is an issue in the evaluation of RCTs of TS *versus* NTS in several ways.

The three other criteria are:

- Large magnitude of effect,
- Dose-response relationship,
- Confounding factors.

All criteria of GRADE apply when assessing the quality of evidence here. A closer look at the various aspects of indirectness of the evidence of RCTs :

- **Indirectness in the measure of outcome:** This is the case in all the reviewed meta-analyses summarized in Table VI. The patient-level primary endpoint is the occurrence of SSI, and the study endpoint is the comparison of SSI incidence rates between suture arms. SSI reduction is a wanted clinical consequence of TS antimicrobial activity. Whether closing surgical wounds with TS is associated with reducing the incidence of SSIs compared with NTS is the question addressed in the meta-analyses

described in Table VI. The measure of the efficacy of TS compared to NTS, according to GRADE, is “a consequence of the intended effect, or a mix of the intended effects and collateral factors.” The incidence rate of SSI is an indirect measure of TS efficacy in achieving its intended performance with an unknown and variable diagnostic accuracy across the RCTs.

- ***Indirectness in compared interventions:*** The review compared TS *versus* NTS where sutures were selected according to the investigator’s practice for approximating each tissue layer. Moreover, most SSI prevention measures used in each trial were those defined in the center’s procedures or were compatible with those procedures. This applies to the use of antibiotics.
- ***Indirectness in tested patients compared to the target populations:*** Several trials included multiple surgical procedures without stratifying outcomes per surgical type. The results of those RCTs can be used in the overall pooled analysis but do not contribute to stratification according to potential predictors of RRs such as operation type or the causal microbial species.⁷⁶⁻⁷⁸ One cannot infer pooled conclusions to any specific patient profile cannot be achieved with certainty.

NOTE: One additional characteristic related to the overall risk of bias and the precision of evidence is the proportion of the pooled evidence reported in significant RCTs.

1.3. FORMAL DEMONSTRATION OF THE PROBLEM

Meta-analysis of homogenous RCTs identified through SLRs is intended to provide the highest level of clinical evidence.^{79,80} Peer-reviewed parallel-arm prospective RCTs are therefore assumed to be the most reliable source of evidence to compare SSI prevention using sutures without *versus* with triclosan.

An ancillary analysis of the clinically-relevant RCTs, with and without microbiological details retrieved by the SLR extraction in August 2021 and described in the PRISMA flowchart of the fourth study (see: published Study 4, figure 1) identified thirty-three clinically relevant RCTs. The meta-analysis of those studies has three outputs interpreted jointly:

- The distribution and pooled value of the incidence rate (IR) of SSIs in the control arms without triclosan (NTS) (Figure 4),
- The distribution and pooled value of the risk ratio (RR) of the incidence IR SSIs in the triclosan arms (TS) divided by the same in NTS (Figure 4)
- The meta-regression study RRs is dependent on IR (Figure 5).

SSI incidence rates in the NTS arms range from less than 1% to more than 35%, with a pooled average of 10% [7%, 13%].

This distribution is assumed to result from the clinical heterogeneity of the RCTs. Indeed, the patients enrolled, and operations performed carry very different risks of SSI.

The Forest plot of the distribution of RR displays little heterogeneity (I-squared = 24.8%, non-significant Q-test, $p = 0.10$). That Forest plot also shows a significant pooled average RR of 0.76 [0.66, 0.88].

The non-significant meta-regression confirms this stability of the RR throughout RCTs with a different IR. The 0% R-squared shows that the broad changes in IR have no significant association with the RR, which is very stable given the non-significant slope (-0.88 [-2.55, 0.79]).

Considering SSI risk varies significantly across surgical operations, the independence of IR and RR suggests analysing the relationship between triclosan sutures' antimicrobial activity and the risk of SSI.

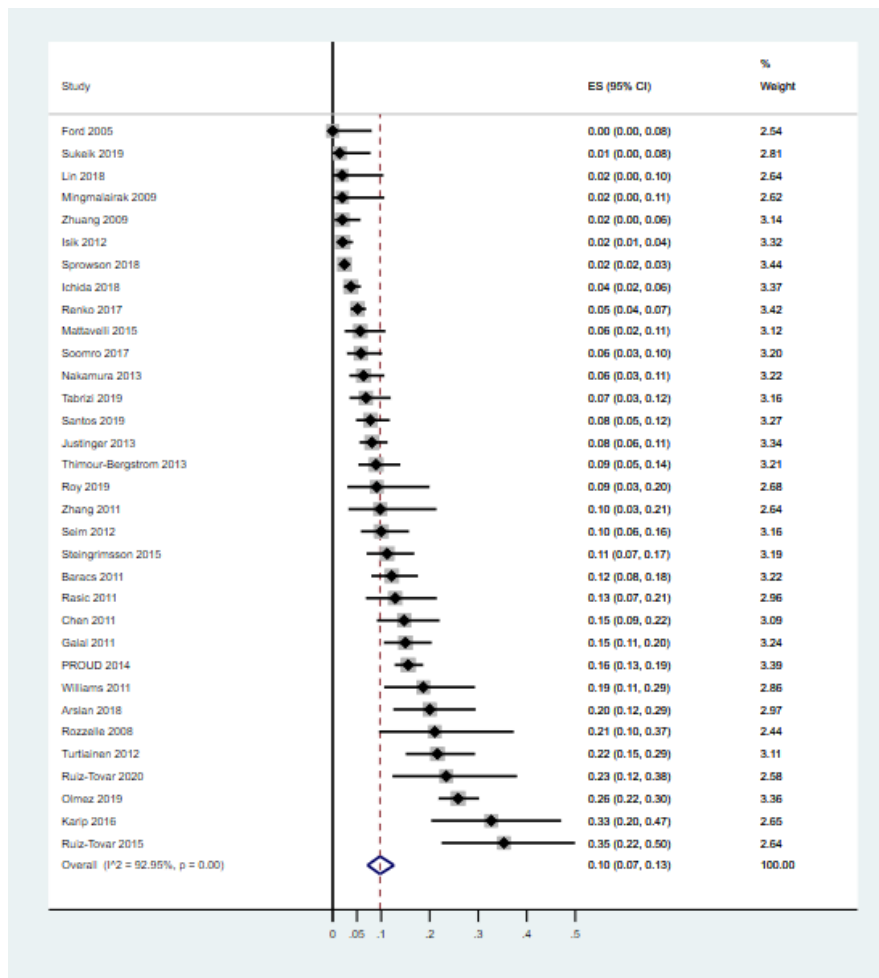
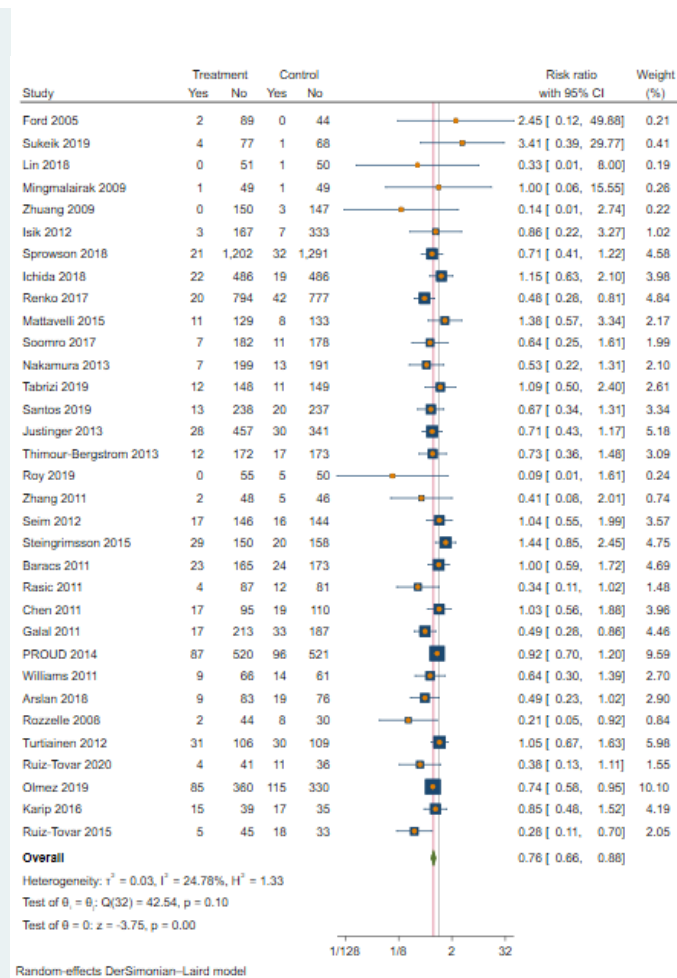


Figure 4 Forest plot of Incidence Rates of SSI with NTS



Forest plot of Risk Ratio of SSI with TS (left) over NTS (right)

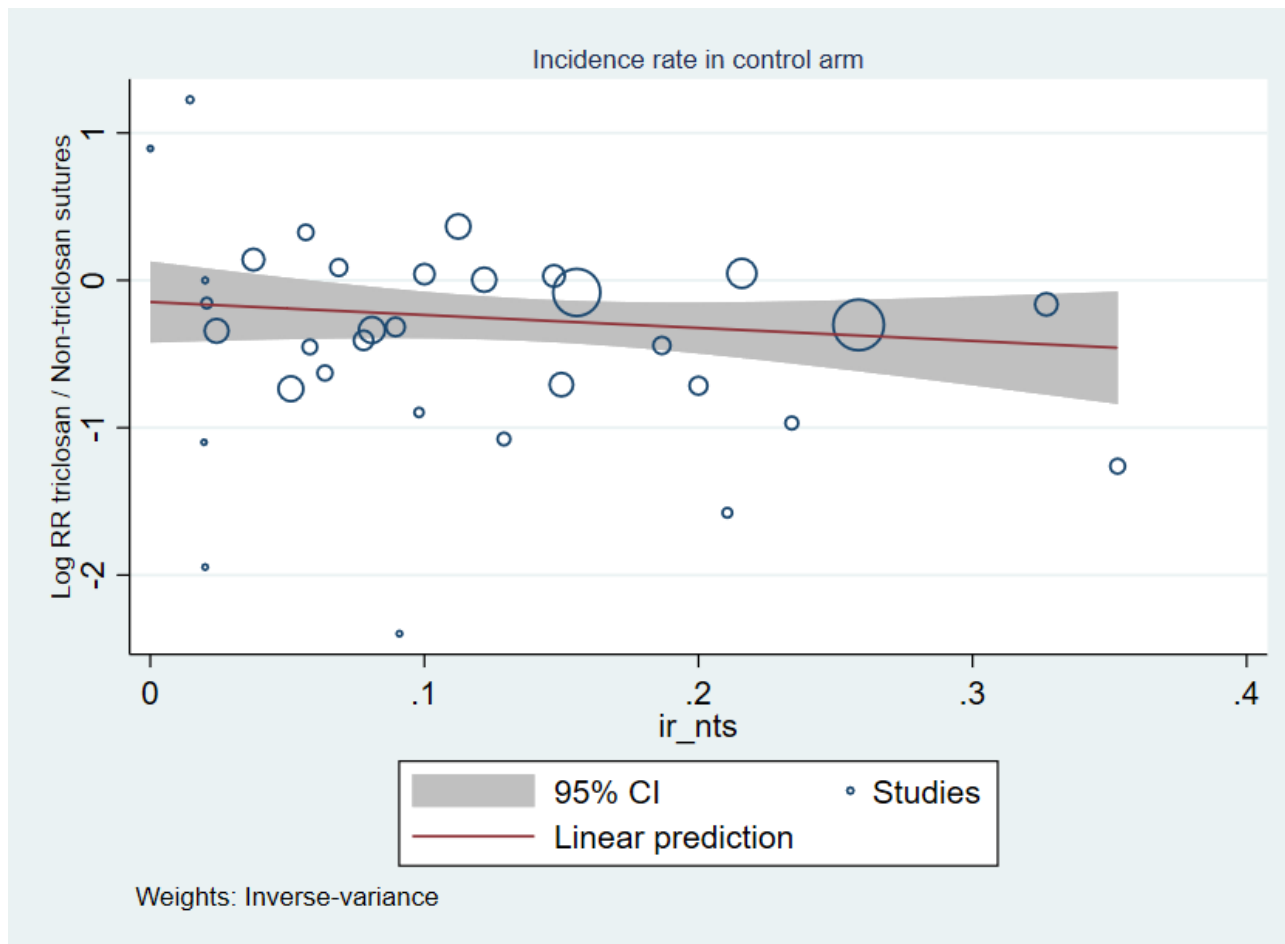


Figure 5 Meta-regression of study RR TS/NTS depending on study IR with NTS

Table VII Meta-regression of study RR TS/NTS depending on study IR with NTS

Random-effects meta-regression	Number of obs = 33
Method: DerSimonian-Laird	Residual heterogeneity:
	tau2 = .03867
	I2 (%) = 25.65
	H2 = 1.34
	R-squared (%) = 0.00
	Wald chi2(1) = 1.07
	Prob > chi2 = 0.3013

_meta_es	Coefficient	Std. err.	z	P> z	[95% conf. interval]	
ir_nts	-.8795276	.8509696	-1.03	0.301	-2.547397	.7883421
_cons	-.1470006	.1404734	-1.05	0.295	-.4223235	.1283223

Test of residual homogeneity: Q_res = chi2(31) = 41.69 Prob > Q_res = 0.0951

The statistical independence of IR and RR enables estimating the average risk reduction of SSI between TS and NTS by multiplying IR by 1-RR with sensitivity analysis, taking into account their 95% CIs. The estimated average IR reduction is 2.4% [0.84%, 4.42%], close to non-significance.

One source of uncertainty regarding the pooled RR is that only 5 among 33 individual RCTs RRs (i.e., 15%) are significant. Therefore, considering the indirectness of SSI as an endpoint of TS antimicrobial activity, it is unclear whether the pooled RR results from 85% non-significant efficacy or bias or chance.

The three potential weaknesses of current meta-analyses are:

- the majority of non-significant RCTs (more than 80%): objective weakness
- the risk of bias in individual RCTs: subjective weakness given the rater's judgement
- the indirectness of the relationship between TS antimicrobial activity and SSI development: Objective weakness given SSI development depends on several factors.

Therefore, the association between the pooled RR of SSI favouring TS and the random allocation to patients of TS or NTS could result from the clinical efficacy of TS antimicrobial activity in operated tissues or bias or chance.

Those weaknesses are described graphically (Figure 1 & Figure 7).

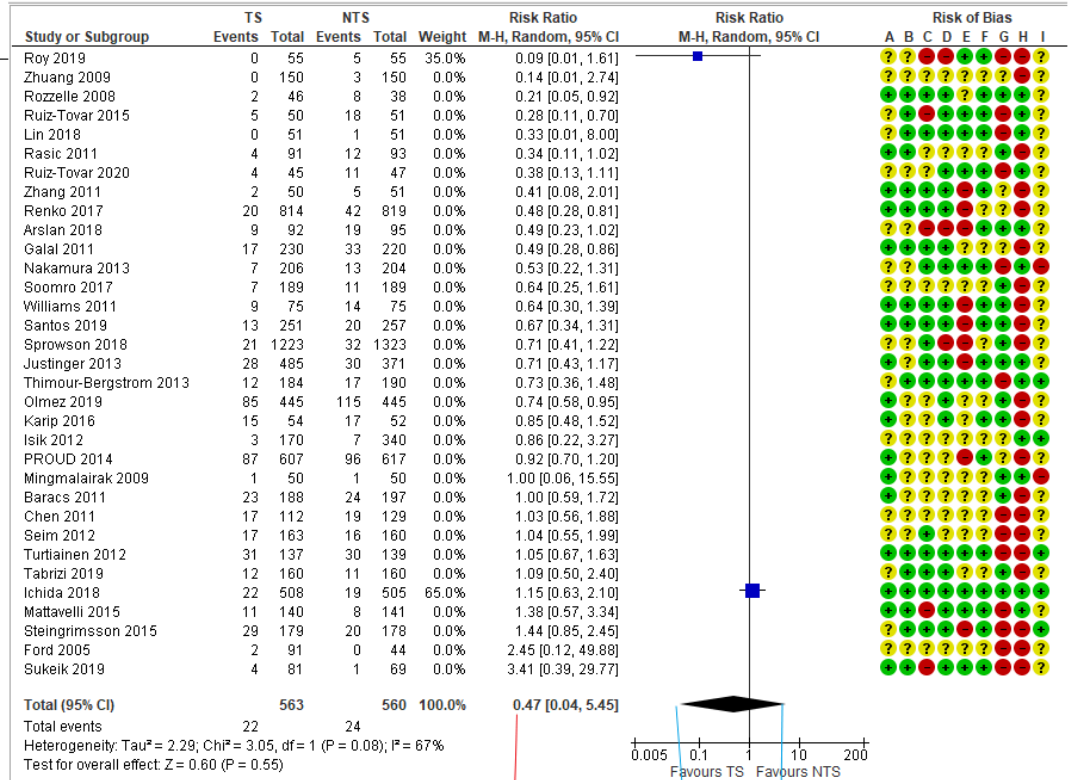
Les essais randomisés cliniques comparant le taux d'incidence des ISO après fermeture par TS contre NTS actuellement:
Les trois causes de l'incertitude concernant la validité de la comparaison combinée (pooled RR)



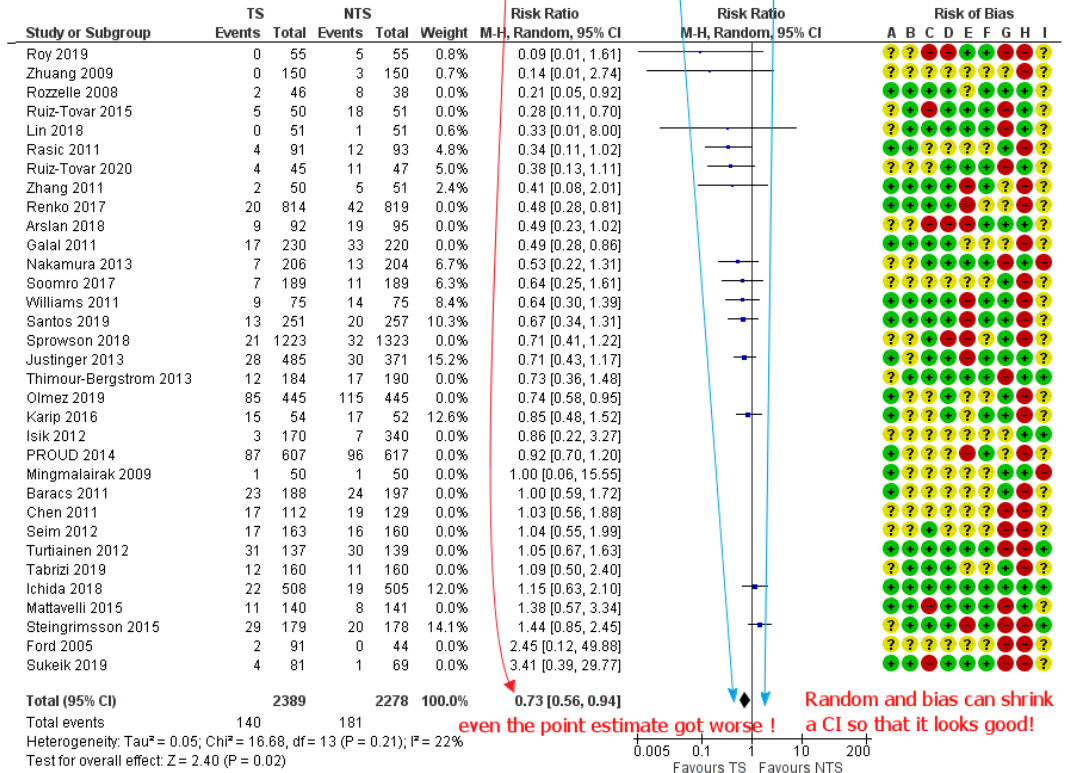
Figure 6 Meta-analysis: the three reasons for the uncertainty

Pooling many non-significant studies with a point estimate on the same side of the 1-line may result in a significant pooled estimate by shrinking the confidence interval whether the individual studies are on the same side out of minimal efficacy or random or bias (Figure 7).

TWO non-significant trial, with a randomly favorable point estimate



14 non-significant trials with totally random point estimates, including 2 unfavorable: 12 favorable shrank the CI to significance. What if bias (all the yellow and red lights) explained the 12 non-significant favorable trends?



Random and bias can shrink a CI so that it looks good! even the point estimate got worse!

Figure 7 How adding up error or bias can resemble compiled non-significant efficacy

1.4. DEFINITION OF THE RESEARCH QUESTION

Do sutures with triclosan reduce the risk of surgical site infection?

This involves the following four questions:

Question 1: What is the level of antimicrobial activity of sutures with triclosan? Is it similar across the three types of sutures?

Question 2: How long do implanted sutures with triclosan inhibit *Staphylococcus aureus* in surgical conditions?

Question 3: Which microorganisms isolated in SSI cultures are inhibited by sutures with triclosan?

Question 4: Do sutures with triclosan modify the microbial diversity of SSIs consistently with microbial susceptibility to triclosan?

1.5. RESEARCH PROGRAM

Does the pooled relative risk of SSI in meta-analyses of RCTs that compare TS versus NTS, measure the clinical efficacy of sutures with triclosan, or a bias or the result of chance? The question is relevant given the majority of non-significant RCTs.

The proposed approach is to address this question is:

- To describe as accurately as possible the antimicrobial activity of sutures with triclosan with its mechanism.
- And examine if evidence from RCTs is consistent with TS antimicrobial activity.

1.5.1. Objectives and approach

The four questions to be addressed drove the planning and conduct of dedicated studies (flowchart in Figure 8).

Question 1: What is the level of antimicrobial activity of sutures with triclosan? Is it similar across the three types of sutures?

A standardized *in vitro* time-kill assay was developed to accurately measure and to compare the performance of the sutures with a panel of microorganisms found in surgical site infections.

Question 2: How long do implanted sutures with triclosan inhibit *Staphylococcus aureus* in surgical conditions?

An *in vitro* pharmacokinetic assay was developed to estimate triclosan release in conditions that reproduce the release rate in human tissues. It was specified with benchmarks obtained by similar sutures opportunistically explanted from large animals that will take part in preclinical surgery for other purposes. Once the duration of antimicrobial release was estimated, the residual antimicrobial performance of sutures was measured on sutures after pre-exposure to the conditions that reproduce triclosan release in operated tissues. That was performed by using the time-kill assay developed to address Question 1.

Question 3: Which microorganisms isolated in SSI cultures are inhibited by sutures with triclosan?

Consecutive spinal SSI specimens were collected prospectively, and the microorganisms were isolated. Conserved microorganisms that had been isolated from previous consecutive spinal SSIs in another hospital were added. The antimicrobial activity of TS and minimum inhibitory concentration of triclosan was measured on each strain. That was performed by using the time-kill assay developed to address Question 1.

Question 4: Do sutures with triclosan modify the microbial diversity of SSIs consistently with microbial susceptibility to triclosan?

A difference will support the association between TS antimicrobial activity and reduced SSI incidence. No difference will challenge that association.

The validation subquestion is: Is the pooled relative of culture-confirmed SSIs consistent with the pooled relative risk of comprehensive meta-analyses?

This question is addressed with a systematic literature review and a meta-analysis of prospective parallel arm RCTs that report the microorganisms isolated in SSI cultures per suture arm. In addition, the similarity of microbial diversity between culture-confirmed SSIs after TS and NTS is tested.

The clinical efficacy of TS in reducing the risk of SSI is estimated from the joint interpretation of the above study results.

Triclosan sutures (TS: types V+, M+, P+) are designed to inhibit microbial colonization of the suture itself. The targeted consequence is a reduction in the incidence of surgical site infections (SSIs). The meta-analysis (Aug. 2021) with 33 RCTs & 14,877 patients: shows significantly fewer SSI with TS than non-TS (NTS): pooled RR = 0.76 [0.66, 0.88]

BUT

- clinical heterogeneity across trials
- risk of indirectness
- a minority 15% (5/33) of RCTs are significant
- pooling of non-significant RRs can result in a significant RR can be significant if they share a common trend (non-significant effect in the same direction) whether it results from a biological effect or bias.

PURPOSE of this research: Does evidence support the association between the antimicrobial activity of sutures with triclosan and the pooled reduced incidence of SSI observed with those suture in RCTs?

Question 1: What level of antimicrobial activity is displayed by sutures with triclosan? Is it similar across the three types of sutures?

Addressed in Study 1 (submitted):
24-hour Time-kill comparisons of V, V+ P+, M, M+ on a panel of microorganisms with a broad range of susceptibility to triclosan (MIC < 0.01 µg/mL up to MIC > 128 µg/mL): *S. aureus*, *S. epidermidis*, *E. Coli*, *P. aeruginosa*, *C. albicans*

Question 2: How long do implanted sutures with triclosan inhibit *Staphylococcus aureus* in surgical conditions?

Addressed in Study 2 (published):
In vitro pharmacological model of triclosan release from V+, P+, M+, in conditions that reproduce a large animal model of human cardiac surgery: *Ex vivo* benchmarks with opportunistically retrieved TS after a pre-clinical cardiac surgery study. Model estimates triclosan release rate and residual amount over time in relation, to residual antistaphylococcal activity.

Question 3: Which microorganisms isolated in SSI cultures are inhibited by sutures with triclosan?

Addressed in Study 3 (interim results to be submitted):
A broad sample of microbial species, mainly isolated from spinal SSIs, underwent 24-hour time-kill analysis of triclosan suture (P+)
Subquestion: Does microbial susceptibility to triclosan (triclosan MIC) predict the sutures antimicrobial activity on the panel of microorganisms?

Question 4: Do sutures with triclosan modify the microbial diversity of SSIs consistently with microbial susceptibility to triclosan?
If they do, this would support the association between TS antimicrobial activity and reduced SSI incidence. Otherwise, it would challenge that association.

Addressed in Study 4 (accepted for publication):
A systematic literature review and meta-analysis of microbial distribution between TS and NTS.
Validation subquestion: Is the pooled RR of culture-confirmed SSIs consistent with the pooled RR of comprehensive meta-analyses?

Answers to Q1, Q2, Q3, Q4

- Are the answers to the 4 questions consistent?
- Are they consistent with published evidence?
- Does the overall evidence support the use of TS as a cause of reduced risk of SSI ?

Figure 8 Flowchart of the research program

1.5.2. Organization and teamwork

Conducting the above studies required organizing teams by objective:

Bordeaux University's experimental microbiology laboratory (Aquitaine Microbiologie with FM, AZ) was appointed to develop the microbiological assay to address Questions 1, 2 and 3. This team worked with the core team (FD, AMR and NM).

A team of spinal surgeons (JCL, SB, MP, JMC) and microbiologists (VD, AMR, AZ) was solicited to provide specimens of spinal surgical site infection or microbial strains previously isolated from spinal SSI. They cooperated with the experimental microbiology team and the core team (FD, AMR, NM).

A public departmental toxicology laboratory (LaboPL with ML, FM *et al.*) was appointed to determine triclosan concentrations released by sutures. A preclinical research laboratory (IMMR with NB, AE *et al.*) was solicited to opportunistically use triclosan sutures instead of their non-triclosan versions in large animals involved in experimental surgery modelling human surgery, with planned sacrifice after a few days. The implanted triclosan sutures were retrieved to provide benchmark triclosan concentrations. The two teams worked with the core team (FD and NM) supported by a pharmacologist-toxicologist (RG), and a support biologist (NP) to plan and prepare the samples according to the protocol and the staff of the hired toxicology laboratory. This team addressed Question 3.

A literature review team consisting of the core research team and another independent reviewer conducted the systematic literature review. This team addressed Question 4.

2.
**WHAT IS THE LEVEL OF
ANTIMICROBIAL ACTIVITY OF
SUTURES WITH TRICLOSAN? IS
IT SIMILAR ACROSS THE THREE
TYPES OF SUTURES?**

Manuscript: **Mechanism dynamics of triclosan sutures prevention of surgical site infections.** Frederic C. Daoud, Fatima M'Zali, Arnaud Zabala, Nicholas Moore, and Anne-Marie Rogues.

Currently resubmitted to the **European Journal of Clinical Microbiology & Infectious Diseases**

Manuscript identifier: **pending**

The manuscript is copied below.

Figure 9 shows photographs of key practical aspects of the study.

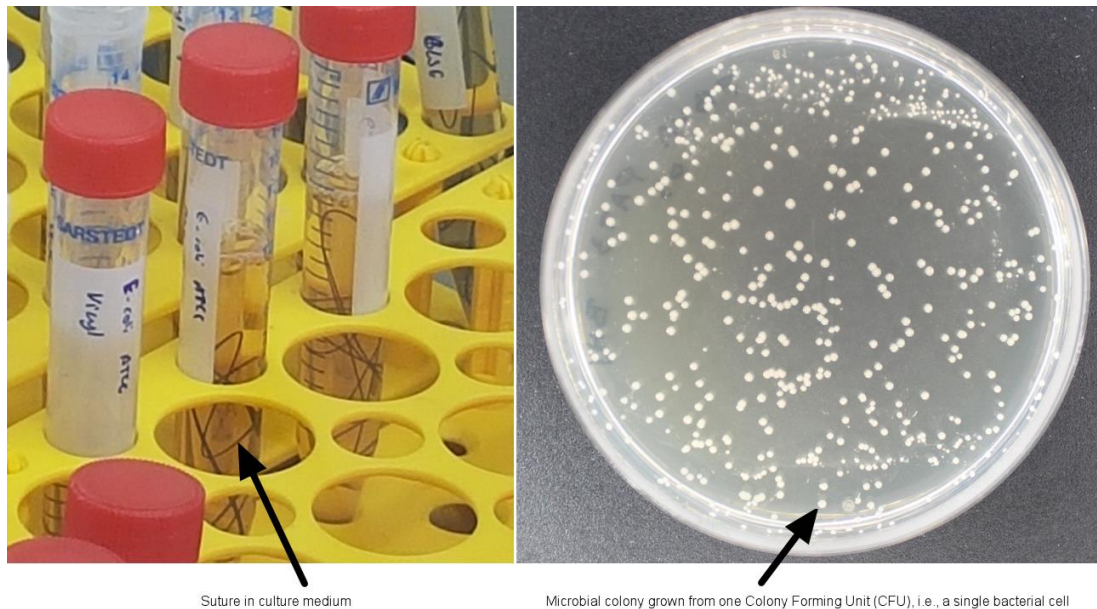


Figure 9 Study 1 time-kill analysis methods

Dynamics of triclosan sutures prevention of surgical site infections.

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ABSTRACT

Purpose: Braided polyglactin-910, monofilament polydioxanone, and monofilament poliglecaprone-25 are absorbable sutures optionally available with antimicrobial triclosan. Triclosan amounts per length unit and release kinetics vary across suture types. Up to 88% of randomised controlled clinical trials comparing those sutures with triclosan (TS) and without (NTS) are non-significant. Dissecting the antimicrobial activity could help to understand clinical outcomes.

Methods: The three TS and NTS challenged nine microorganisms with triclosan minimal inhibitory concentrations (MIC) ranging from 0.03 to 512 µg/mL. Time-kill assays microbial concentrations after 4, 8, and 24 hours. The length of TS carried enough triclosan for its complete dissolution to result in a concentration exceeding triclosan MICs of all species, except for *P. aeruginosa*.

Results: No suture inhibited *P. aeruginosa*. All TS displayed an initial biocidal activity in all other species. NTS showed no antimicrobial activity. TS reduced *S. aureus*, *S. epidermidis*, *E. coli* and *C. albicans* concentrations to a minimum between 4 and 8 hours. Significant growth resumed afterwards in most strains. Regression analysis showed no significant difference between the suture types independently of microorganisms.

Conclusions: This study shows similar dynamic biocidal activity with all tested triclosan susceptible microbial species challenged by the three TS types. *P. aeruginosa* grows on all sutures. The time-kill plots are J-shaped due to microbial growth resumption after eight hours. Contact killing is the predominant antimicrobial mechanism. Extended inhibition of suture microbial colonisation is not detected but not ruled out. The results should translate into operated tissues to inhibit SSI development.

Keywords: suture; triclosan; antimicrobial; time-kill; contact killing.

INTRODUCTION

Triclosan is an adjuvant, broad-spectrum antimicrobial used in absorbable sutures to inhibit microbial colonisation and prevent surgical site infections (SSI) [1].

Three sutures are available with triclosan (TS) or without (NTS): Braided polyglactin-910 TS with a maximum triclosan amount of 472 $\mu\text{g}/\text{m}$ (V+) vs NTS (V), monofilament polydioxanone TS with a maximum 2,360 $\mu\text{g}/\text{m}$ TS (P+) vs NTS (P), and monofilament poliglecaprone-25 with a maximum 2,360 $\mu\text{g}/\text{m}$ TS (M+) vs NTS (M) [2-4]. Those sutures have a five-fold difference in initial triclosan load and display different triclosan release kinetics [5].

Microbial susceptibility to triclosan, measured with minimal inhibitory concentrations (MIC) in microorganisms isolated from surgical samples, ranges from less than 0.02 $\mu\text{g}/\text{mL}$ in *Staphylococcus aureus* to more than 1,000 $\mu\text{g}/\text{mL}$ in *Pseudomonas aeruginosa* and mutant strains of otherwise susceptible species such as *Escherichia coli* [6-10].

In vitro studies using zone of inhibition (ZOI) measurements have estimated the antimicrobial activities of TS vs NTS controls. One study has shown that V+ inhibits *S. aureus* and *S. epidermidis* growth after 24-hour exposure, while V causes no inhibition [11]. Other studies have shown similar results after comparing P+ with P and M+ with M [12, 13]. Sequential ZOI studies report TS antimicrobial activity durations of several days or weeks [14]. Electron microscopy shows microorganisms and biofilm inhibition on TS surface [14, 15].

More than thirty-five randomised controlled clinical trials (RCTs) have measured the relative risk of SSI with TS vs NTS. The most comprehensive meta-analysis of RCTs published to date (25 RCTs and 11,957 patients) reports a significant pooled relative risk (RR) of 0.73 [0.65, 0.82] with fewer SSIs when using TS. Still, 88% (22/25) of RCTs are non-significant [16]. The World

Health Organisation (WHO) has conditionally recommended using triclosan-coated sutures to reduce SSI risk, independently of surgery type, because the quality of evidence is rated moderate [17, 18]. Therefore, the pooled RR could result from mild TS efficacy, bias or chance.

Many factors impact SSI risk and possibly the RR measured in RCTs [19]. A previous meta-analysis stratified RCTs according to surgery and suture types, and no association was significant between those factors and pooled RRs [20]. However, *post hoc* statistical power was insufficient and currently remains so. Therefore, further understanding of TS antimicrobial activity could provide additional information to understand observed RRs in RCTs.

The objective of this *in vitro* study (AD16-174/AST2016-181/IIS15-216/2016-11-09) is to measure the dynamics of TS antimicrobial mechanisms taking into account differences between sutures and microbial susceptibility to triclosan to prevent SSIs.

MATERIALS AND METHODS

Microbiology

A panel of nine microbial strains was selected: *E. coli* ATCC 25922 (MIC 0.03 µg/mL), *E. coli* ESBL producer collection clinical strain (MIC 0.03 µg/mL), *S. epidermidis* CIP 8155T (MIC 0.03 µg/mL), *S. aureus* ATCC 29213 (MIC 0.03 µg/mL), Methicillin-resistant *S. aureus* (MRSA) ATCC 33592 (MIC 0.03 µg/mL), MRSA collection clinical strain, *Candida albicans* ATCC 10231 (MIC 4 µg/mL), *C. albicans* collection clinical strain, *Pseudomonas aeruginosa* collection clinical strain (MIC 256 µg/mL). Each strain was challenged with a segment of each suture. All segments had a diameter between 0.35 and 0.399

mm (United States Pharmacopeia USP 2-0), and a 35 cm length, thus a 0.03 to 0.04 cm³ volume.

A time-kill assay protocol was specified according to CLSI M26-A standard [21]. Sutures and microbial cultures were handled in a safety cabinet. Sutures were unpackaged and immediately cut into segments. Suture sterility was checked by incubation on Agar plates for 18 hours at 37°C.

The purity of each microorganism was checked by culture on an Agar plate. A single colony was then inoculated in a tube containing 10 mL sterile tryptic soy culture broth (TSB; Difco, BD Diagnostics, Sparks, MD, USA). The liquid suspension cultures were incubated at 37°C for 4 hours with continuous shaking to attain the exponential growth phase and obtain a culture with 0.5 McFarland turbidity (approximately 1.5×10^8 colony-forming unit CFU/mL). An inoculum of the culture was extracted with densitometric control. The inoculum was added to 10 mL of TSB suspension culture medium in a sterile 50 mL Eppendorf tube containing 10 mL TSB to set the baseline microbial concentration to approximately 10^6 CFU/mL. After stepwise calibration tests, 35 cm suture segments were determined to be the optimal length to measure differences in antimicrobial activity across timeslots and suture types and microbial strains. A single segment was immersed per culture, and tubes were incubated at 37°C for 24 hours. Microbial concentrations in the cultures were determined at various timeslots, baseline, 4 hours, 8 hours and 24 hours (T0, T4, T8, T24).

Each determination started with 48 Mhz sonication of culture tubes for 4 minutes to detach viable microorganisms from the tube walls and suture, then a 100 µL culture sample was drawn. The sample underwent five serial 1/10 v/v dilutions. The original sample and each dilution were spread on separate Agar plates with Mueller Hinton (MH) culture medium. The plates were incubated at

37°C for 24 hours. Only plates enabling individual non-overlapping colony count were used.

The microbial concentration in the source culture at the timeslot measured in CFU/mL was obtained by multiplying the plate's colony count by the dilution factor, thus ensuring the proportionality of colony count with the microbial concentration in the culture. The six sutures challenged with the nine strains resulted in 54 suture × strain combinations. The assay of each combination was repeated six times.

Data analysis

For each combination, the \log_{10} of viable microbial concentrations in CFU/mL were related to the corresponding timeslots (T0 through T24) as were plotted as time-kill function. Changes in viable CFU concentration were considered biocidal if a significant reduction was observed. At 24 hours, a reduction factor of 1,000 (i.e., 99.9% kill) compared to baseline (i.e., T24-T0: -3 on the \log_{10} scale) was considered biocidal. A lesser reduction was considered bacteriostatic. Intermediate changes at T4 and T8 were also examined. A multivariate longitudinal statistical model tested the significance of the associations of microbial concentration (in CFU/mL) as the dependent variable, with timeslot, triclosan, suture material, microbial strain, and as the independent variables. The model also estimated the effect size of each independent variable on CFU concentration. A random-effects (RE) panel regression model and a pooled ordinary least square (OLS) model were considered the most suitable models given the data structure [22]. A pooled OLS model would apply if the variance of the residues of microbial concentration in the model was 0 (i.e., stable variance across independent variables, i.e., no heteroskedasticity). RE model would be applicable otherwise. Heteroskedasticity was tested with the Breusch-Pagan Lagrange multiplier test

(LM). The null hypothesis (H0) is no heteroskedasticity and supports a pooled OLS model. The rejection H0 ($p < 0.05$) supports an RE model.

Data management and computations were performed with the xt module in Stata 17, StataCorps LLC, College Station, TX, USA

RESULTS

Time-kill analyses

The time-kill plots with NTS showed rapid growth with all microbial strains. The plots of the three materials overlapped. The time-kill plots with TS showed a fast reduction in microbial concentration from T0 to T4 or T8 with all TS \times strain combinations, except for *P. aeruginosa* concentration that increased 100-fold ($+2 \log_{10}$) from T0 to T24. Plots of the three TS materials followed the same pattern with a spread at each timeslot less than $1 \log_{10}$ (Figure 1, Figure 2, and Figure 3).

Microbial concentrations in triclosan susceptible strains reached their minimum between T4 and T8, with reductions ranging from $-1.5 \log_{10}$ to $-2.8 \log_{10}$, depending on the TS \times strain combination. The maximum mean reduction ratio, all TS \times strain combinations combined, was maximum between T0 and T8 with $-2.2 \log_{10}$ [$-2.0 \log_{10}$; $-2.3 \log_{10}$], $p < 0.0001$. Those reductions led to minimum residual microbial concentrations between $3.1 \log_{10}$ and $5.7 \log_{10}$.

There was growth resumption in triclosan susceptible strains between the T8 and T24 with the three TS and most triclosan susceptible strains. The maximum growth was $+1 \log_{10}$ with *E. coli* ESBL producer. Mean growth ratio, all TS \times strain combinations combined, was $+0.2 \log_{10}$ [$+0.1 \log_{10}$; $+0.3 \log_{10}$], $p < 0.0001$.

Multivariate time analysis and comparison of the sutures

Statistical analysis did not reject the hypothesis of the absence of significant heteroskedasticity ($p = 0.2085$). Therefore, data were analysed with a pooled OLS multivariate regression (Table 1).

The regression coefficients of each suture material and triclosan susceptible microorganisms were non-significant compared with their respective reference levels, with all other factors being equal. That reflected no difference in microbial concentrations between those levels. The three significantly associated predictive factors were the use of triclosan, exposure duration, and *P. aeruginosa*.

DISCUSSION

This work measured the dynamic antimicrobial activity with repeated microbial concentration measurements of nine strains with triclosan MICs ranging from 0.015 µg/mL to 256 µg/mL and exposure to V, V+, P, P+, M and M+. Measurements were at baseline, 4, 8 and 24 hours. In addition, each combination was repeated six times to enable statistical comparisons of microbial concentrations.

The panel of tested microbial species spanned across the triclosan MIC spectrum found in SSIs and species with significant frequencies in SSI annual surveillance, such as ECDC 2017: *S. aureus* in 21.5% of SSIs, *E. coli* in 13.9%, coagulase-negative staphylococci in 11% and *P. aeruginosa* in 4.7% [23].

Antimicrobial activity changes and predominant mechanism analysis

The antimicrobial activity plots of the three TS with triclosan susceptible strains were J-shaped with an initial biocidal phase, i.e., a reduction ratio greater than 2 log₁₀ between baseline and 8 hours of exposure. A minimum microbial concentration was reached, followed by significant microbial growth resumption. *P. aeruginosa* was not inhibited by TS, and the presumed cause was intrinsic triclosan resistance [24].

The multivariate longitudinal regression of microbial concentrations showed no difference in the antimicrobial activities of the three TS, in all

triclosan-sensitive microorganisms, given random fluctuation can produce differences between repeated measurements up to 1 log₁₀. The three significantly associated predictive factors were the use of triclosan, exposure duration, and *P. aeruginosa*.

Had the baseline triclosan amount on the 35 cm suture segments fully dissolved in the 10 mL culture media, triclosan concentration would have reached 16.5 µg/mL with V+, and 82.6 µg/mL with P+ and M+. Such concentrations are 4 to 20-folds the triclosan MIC in *C. albicans* ATCC 10231 and 550 to 2750-folds the MIC in *E. coli* ATCC 25922. Those concentrations would have been biocidal and eradicated all strains except for *P. aeruginosa*.

Moreover, complete dissolution of the maximum baseline amount would have exceeded triclosan's 10 µg/mL solubility in water and caused triclosan to form visible flakes that settle at the bottom of the tube [25-27]. Given triclosan sediments were not observed, and 10 mL culture tube content was not modified except for drawing four 100 µL samples during the 24-hour assays, the logical explanation is that most of the initial triclosan load remained on the suture segments.

The above findings suggest TS primary antimicrobial mechanism is contact killing, as described with other antimicrobial solids [28]. Furthermore, given the volume ratio of a suture segment to the culture medium is between 0.3% and 0.4%, TS contact killing microorganisms is a probabilistic phenomenon that balances contact killing and surviving microorganism growth [29].

The J-shaped time-kill plots suggest an initially predominant contact killing that results in the biocidal and a decline in contact killing leading to the predominance of microbial growth. The minimum concentration should be the moment contact killing and growth are equal.

Triclosan release kinetics shows the bactericidal activity of V+ on *S. aureus* in conditions designed to reproduce triclosan dissolution in muscle and subcutaneous tissue [5]. V+ is bactericidal when the triclosan release rate is between 1.40 and 0.29 $\mu\text{g}/\text{m}/\text{h}$, from immersion onset to 4 hours. Antistaphylococcal activity is no longer detected when the release rate falls below 0.17 $\mu\text{g}/\text{m}/\text{h}$ after 12 hours of immersion. The transition from 0.29 $\mu\text{g}/\text{m}/\text{h}$ to 0.17 $\mu\text{g}/\text{m}/\text{h}$ is between 4 and 12 hours. The release rate and its changes are slower when V+ is immersed in static water. V+, P+ and M+ display similar triclosan release curves in static water with an initially high release rate that exponentially declines, followed by a slow-release rate plateau extending several days. V+ and P+ display higher initial release rates than V+. These triclosan release kinetics in static water are consistent with the J-shaped antimicrobial activities over 24 hours observed in the time-kill assays of the three TS with all triclosan susceptible microorganisms.

The initial biocidal activities with *S. aureus*, *S. epidermidis*, and *E. coli* are compatible with inhibitions estimated in previous ZOI assays [11-15, 30-32]. But time-kill assays detect a relatively short biocidal phase followed by growth resumption in the surrounding medium and do not detect extended inhibition.

In contrast, ZOI assays detect an extended inhibition without identifying the initial biocidal phase. One study with sequential ZOI assays using the same suture segments until hydrolysis detected the inhibition of *E. coli*, *S. pseudintermedius* and *S. aureus* up to 5 days with V+ and up to 30 days with P+ and M+ [14]. Corresponding triclosan release kinetics were the slow plateau in static water conditions, i.e., 0.75 to 0.5 $\mu\text{g}/\text{m}/\text{h}$ with V+ and 0.75 to 1 $\mu\text{g}/\text{m}/\text{h}$ with P+ and M+, supported by more than 90% of the initial triclosan load remaining on the sutures when the biocidal phase stops [5]. Unlike the relatively uniform biocidal activity across the three TS and the tested triclosan sensitive strains, the reported extended inhibition with sequential ZOI assays displays

different durations depending on the suture material and the challenged microorganism.

Impact of assay specifications - Statistical methods – Strengths and weaknesses

The strength of this time-kill study is the microbial concentration determination through proportionality with discrete colony counts on plates using the same culture throughout the assay. This enables accurate quantification of the magnitude and timing of antimicrobial activity, including increases and decreases. In addition, method properties facilitate reproducibility to perform repetitions with the same inputs and comparison with multiple parameter differences, here suture type, microorganism, triclosan use, and timeslot [33].

One set of limitations of this time-kill study is the upper and lower limits determined by the volume of the culture samples and the sequential dilution factors. The 10^8 CFU/mL upper limit cannot distinguish concentrations equal to or greater than that level. The 0 CFU/mL lower limit (no colonies on the plates with samples without dilution) cannot distinguish low concentrations from eradication. In the present case, microbial concentrations exceeded the upper limit in the NTS arm and with *P. aeruginosa*, but without consequence on results interpretation. In contrast, they did not exceed the lower limit, thus enabling results interpretation.

Another limitation is the lack of sensitivity to detect inhibition with surrogate endpoints when culture concentration is below the lower limit of detection.

The accuracy ranges of time-kill, ZOI and microscopic methods enable dissecting various aspects of antimicrobial activity with possibly different impacts on SSI prevention.

Translation of results to clinical settings

The ability to translate these results to clinical settings is based on the observation that contact killing mechanism depends on the bioavailability of triclosan at the suture surface, and microbial concentration surrounding it, independently of the contaminated volume, especially once the suture is anchored in the tissues.

The initial biocidal phase could explain TS ability to minimise high microbial concentration on the tissue surface it is in direct contact with. The difference in the risk of SSI developing on the suture between TS and NTS would be marked in clean-contaminated or contaminated wounds [34]. One RCT concerning the treatment of faecal peritonitis with a relatively low sample size reports a significant RR in contaminated wounds [35]. Microbial concentration in clean surgery, such as in hip or knee arthroplasty, is low, so contact killing as rare targets and detecting a difference in SSI incidence rate is hard to achieve even with a large sample size [36]. The biocidal period is limited in time, but it should support natural defences while SSI risk is high. Variability across patients in the duration of microbial persistence inside the surgical wound and the body's ability to defend itself may also explain discrepancies between the biocidal activity measured *in vitro* and clinical observations.

The extended microbial inhibition reported in other studies could also reduce SSI development in contact with the suture after the biocidal phase due to residual microorganisms or hematogenous seeding [37, 38].

The dynamic aspects of TS antimicrobial activity should be taken into account with the other factors that influence surgical suture selection [39].

CONCLUSION

This study shows braided polyglactin-910, monofilament polydioxanone, and monofilament poliglecaprone-25 with triclosan are biocidal in triclosan susceptible microbial species common in surgical site infections. The duration of that activity is about eight hours and results in a J-shaped curve as microbial growth overtakes microbial kills. Sutures with triclosan do not inhibit *P. aeruginosa*, which is triclosan-resistant. Contact killing is the predominant antimicrobial mechanism, rather than increasing triclosan concentration in the surrounding medium. Extended inhibition of the sutures' microbial colonisation was not detected but is not ruled out. This biocidal contact killing should translate into operated tissues and reduce SSI development risk in contact with the suture. It should thus support natural defences while SSI risk is high.

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COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

AUTHOR CONTRIBUTION

The project was supervised by Anne-Marie Rogues and Nicholas Moore.

Study objectives and deliverables were defined by Frederic Daoud, Anne-Marie Rogues, Nicholas Moore and Fatima M'Zali.

Study methods, quality compliance, data acquisition and verification were defined by Fatima M'Zali, Arnaud Zabala, and Frederic Daoud.

Material preparation, microbiological assay execution and data collection were performed by Fatima M'Zali, and Arnaud Zabala.

Statistical analysis plan, data auditing, statistical script programming, and statistical result interpretation were performed by Frederic Daoud.

The first draft of the manuscript was written by Frederic Daoud with inputs from all authors.

All authors commented on previous versions of the manuscript.

All authors read and approved the final manuscript.

DATA AVAILABILITY

The electronic copy of the raw database is available at:

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Source data will be available upon written request after approval by Université de Bordeaux.

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TABLES AND FIGURES

Table 1. Multivariate regression of logCFU - Pooled ordinary least square regression model

log(CFU)	Coef.	St.Err.	t-value	p-value	[95% Conf Interval]	Sig
<i>S. epidermidis</i> CIP 8155T	0	Reference level for microorganisms				
<i>C. albicans</i> ATCC 10231	-0.097	0.124	-0.78	0.436	-0.339 0.147	
<i>C. albicans</i> clinical	-0.205	0.124	-1.66	0.098	-0.448 0.038	
<i>E. coli</i> ATCC 25929	0.052	0.124	0.42	0.674	-0.191 0.295	
<i>E. coli</i> ESBL clinical	0.183	0.124	1.48	0.140	-0.060 0.426	
MRSA ATCC 33592	0.152	0.124	1.23	0.221	-0.195 0.395	
MRSA clinical strain	0.048	0.124	0.38	0.700	-0.205 0.291	
<i>P. aeruginosa</i> clinical	1.306	0.124	10.55	0.000	1.063 1.549	*
<i>S. aureus</i> ATCC 29213	0.170	0.124	1.37	0.170	-0.073 0.413	
Polyglactone 25	0	Reference level for suture materials				
Polyglactin 910	0.035	0.072	0.47	0.637	-0.105 0.175	
polydioxanone	-0.015	0.072	-0.21	0.835	-0.156 0.125	
No triclosan	0	Reference level for use of triclosan				
triclosan	-2.256	0.058	-38.64	0.000	-2.370 -2.141	*
0 hours	0	Reference level for hours				
4	-0.379	0.083	-4.66	0.000	-0.541 -0.217	*
8	-0.237	0.083	-2.90	0.005	-0.399 -0.075	**
24	0.416	0.083	5.11	0.000	0.254 0.578	*
Overall model intercept	6.656	0.113	58.88	0.000	6.434 6.878	*
Number of obs		1296				
F-test		df=14, 1282	p-value	<0.001		
R-squared		0.5857	Adjusted R-squared	0.5802		

* $p < 0.001$, ** $p < 0.005$,

Random effects panel data regression not used given non-significance of Breusch and Pagan Lagrangian multiplier test for random effects:

$$\text{logcfu}[\text{groupid},t] = Xb + u[\text{groupid}] + e[\text{groupid},t]$$

Chi-bar-squared(df = 1) = 0.66, p-value = 0.2085

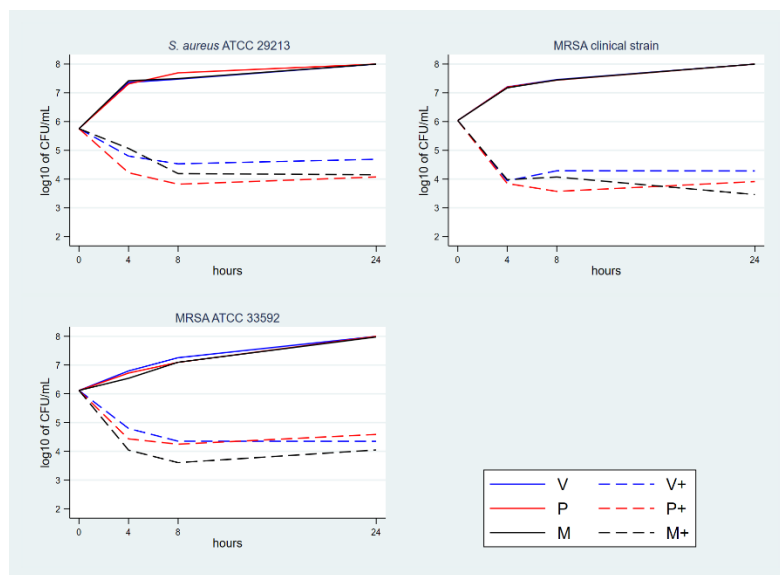


Figure 1 Time-kill plots – *S. aureus* and MRSA

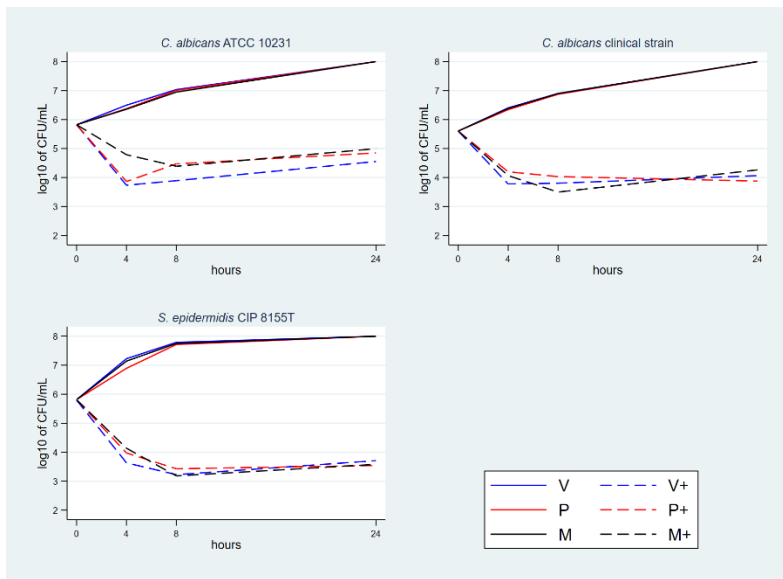


Figure 2 Time-kill plots – *S. epidermidis* – *C. albicans*

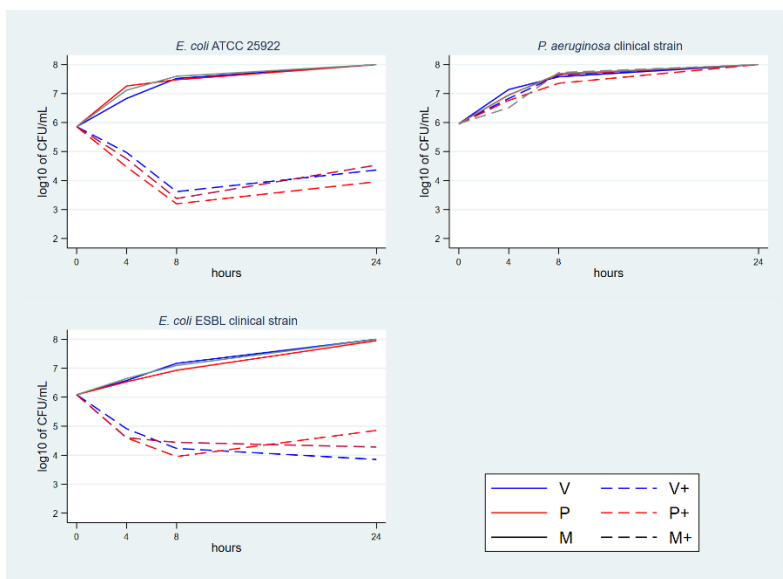


Figure 3 Time-kill plots – *E. coli* – *P. aeruginosa*

**3.
HOW LONG DO IMPLANTED
SUTURES WITH TRICLOSAN
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AUREUS IN SURGICAL
CONDITIONS?**

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The article is copied below and the supplemental material is in Appendix 1.

Figure 10 shows photographs of key practical aspects of the study.

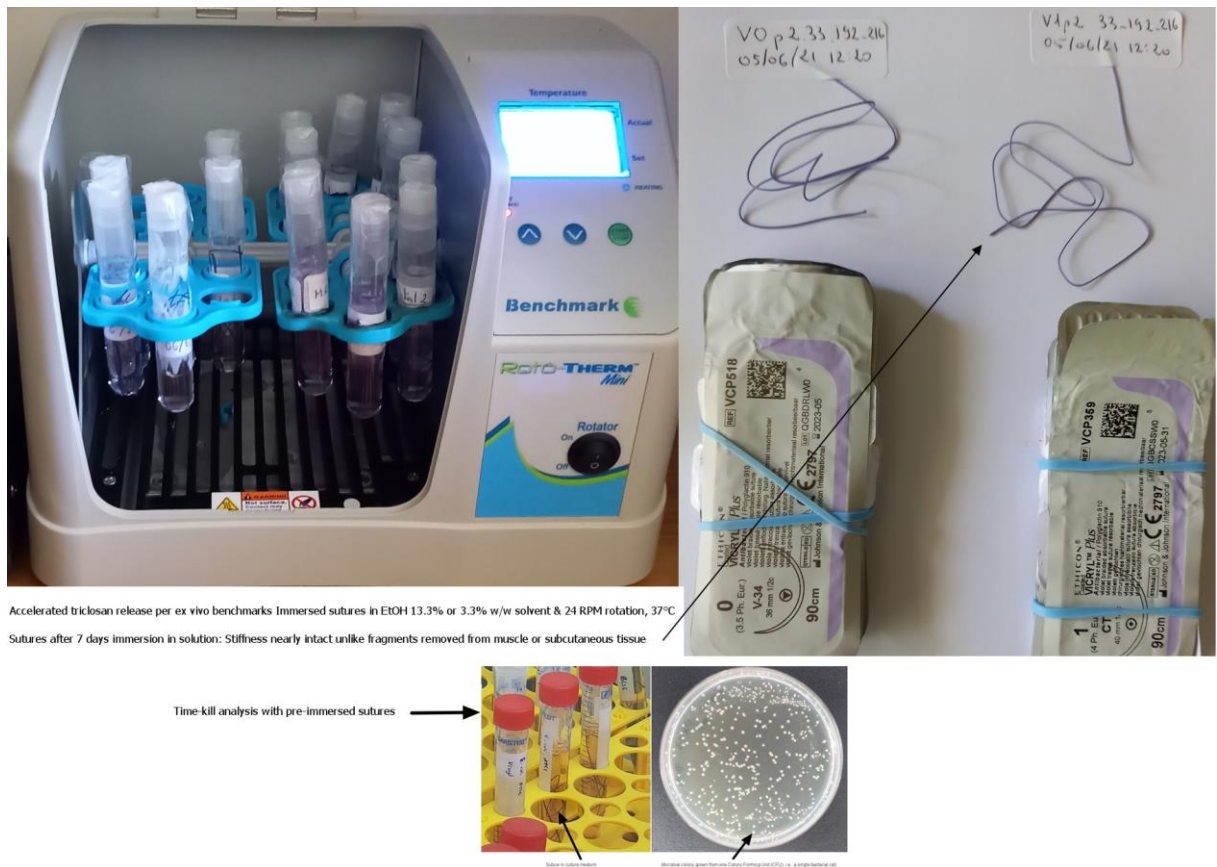




Figure 10 Study 2 - Methods

Article

How Long Do Implanted Triclosan Sutures Inhibit *Staphylococcus aureus* in Surgical Conditions? A Pharmacological Model

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Abstract: (1) Background: Sutures with triclosan (TS) are used to reduce the risk of surgical site infections (SSI), but most clinical trials are inconclusive. The traceability of SSI risk to antimicrobial activity in operated tissues is needed. (2) Objectives: This study aimed to predict triclosan anti-staphylococcal activity in operated tissues. (3) Methods: Three TS were exposed to static water for 30 days, and triclosan release was recorded. Polyglactin TS explanted from sheep seven days after cardiac surgery according to 3Rs provided ex vivo acceleration benchmarks. TS immersion up to 7 days in ethanol-water cosolvency and stirring simulated tissue implantation. Controls were 30-day immersion in static water. The release rate over time was measured and fitted to a predictive function. Antistaphylococcal activity and duration were measured by time-kill analysis with pre-immersed polyglactin TS. (4) Fifteen to 60-fold accelerated in vitro conditions reproduced the benchmarks. The rate prediction with double-exponential decay was validated. The antistaphylococcal activity was bactericidal, with TS pre-immersed for less than 12 h before then *S. aureus* began to grow. The residual triclosan level was more than 95% and played no detectable role. (5) Conclusions: Polyglactin, poliglecacprone, and polydioxanone TS share similar triclosan release functions with parametric differences. Polyglactin TS is antistaphylococcal in surgical conditions for 4 to 12 h.

Keywords: sutures; triclosan; 3Rs; in vitro model; release kinetics



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1. Introduction

Triclosan (2,4-dichlorophenoxyphenol) is a broad-spectrum antimicrobial that inhibits bacterial fatty acid synthesis. The target receptor in various Gram-positive and negative species such as *Staphylococcus aureus* and *Escherichia coli* is NADH-dependent enoyl-[acyl carrier protein] reductase (FabI) [1–3].

Triclosan is an optional adjuvant in absorbable surgical sutures such as polyglactin 910 (Vicryl PLUS “V+”), polydioxanone (PDS PLUS “P+”), and poliglecacprone 25 (Monocryl PLUS “M+”) [4–6]. The structural polymers provide different mechanical and absorption properties that drive suture selection given the surgical circumstances [7]. The intended effect from adding triclosan is to inhibit suture microbial colonisation.

In vitro and animal experiments have shown that triclosan sutures (TS) exhibit antimicrobial activity in microorganisms commonly associated with surgical site infections (SSI) such as *S. aureus*, *S. epidermidis* and *E. coli* [8–12].

The expected clinical efficacy is a reduced incidence of surgical site infections (SSIs). Comprehensive meta-analyses of clinical trials have consistently shown a significantly lower incidence of SSIs after wound closure with TS as compared to non-triclosan sutures (NTS) [13,14]. The meta-analysis with the most randomised controlled trials (RCTs) reported a pooled relative risk (RR) of 0.73 [0.65, 0.82], but individual study RRs ranged

from 0.09 to 2.45, and only 12% of them were significant. Considering the moderate level of evidence, the WHO made a conditional recommendation to use TS “for the purpose of reducing the risk of SSI, independent of the type of surgery” [15].

Pooling can result in a statistical synergy of non-significant study RRs that are in the same direction, whether the cause bias or efficacy. Therefore, the tracing of individual study measures of efficacy to its cause, i.e., exposure to germs *versus* strength and duration of the antimicrobial activity in real-life conditions, is needed.

In vitro studies and experiments in rodents have shown TS antimicrobial activity lasting several days [8–12,16]. However, a literature review has shown that animal models of human surgery often translate poorly to clinical conditions [17]. Moreover, published studies do not relate the antimicrobial activity to triclosan bioavailability on TS.

A review of publication databases found articles on the range of triclosan load on a TS to meet antimicrobial efficacy and non-toxicity objectives, on TS surgical properties, microbiological assays and clinical trial outcomes [8–14,16,18]. No studies described triclosan bioavailability and pharmacodynamics in the three TS. Product labels indicate the maximum initial triclosan amount (472 µg/m in V+ and 2360 µg/m in P+ and M+), but the average initial amount and variability and the expected duration of bioavailability once implanted are not mentioned. No clinical claims are made [4–6].

One literature review reported the initial triclosan load on V+ to be 2.7 µg/cm, but the sources of that value and the methods used to determine it is published [19–21].

The use of translational models is unavoidable, given that absorbable sutures cannot be removed from patients for research purposes. Exploring the triclosan bioavailability of triclosan on sutures in animal studies would be scientifically acceptable only if the protocol were accurately specified with in vitro study results, had a significant benefit for patients, and was in compliance with animal ethics, compatible with 3Rs principles [22,23].

Therefore, this study developed an empirical in vitro model specified with ex vivo benchmarks using opportunistically explanted TS fragments from a valid large animal translational model of human surgery in the course of an independent preclinical study according to the *reduction* and *refinement alternatives* of the 3Rs and published approaches to predict drug dissolution in vitro [22–25].

The critical assumption of this study is that in vivo TS antimicrobial activity reduces the risk of SSI depending on activity duration, with two clinical period phases:

- Intraoperative: the objective is to avoid germ inoculation by the suture.
- Postoperative: the objective is to prevent suture colonisation while natural defences kill residual microorganisms.

2. Materials and Methods

2.1. Quantitative Methods

The marginal release rate $R(t)$ in µg/m/h was defined as the amount of triclosan released per unit of suture length and per unit of time (1):

$$R(t) = [\text{triclosan}]_{t+dt} \times \frac{V}{L \cdot dt} \quad (1)$$

All rates were derived from the triclosan concentration $[\text{triclosan}]_{t+dt}$ measured after immersing a segment of TS of length L in meters, in a volume of solvent V in litres for a duration dt in hours, starting at time t .

If the initial amount of triclosan per suture length unit was A_0 , the residual amount at time t since the beginning of the immersion was $A(t)$, calculated as the initial amount less the amount released between immersion start and t (2):

$$A(t) = A_0 - \int_{t=0}^t R_{\text{model}}(t) dt \quad (2)$$

Triclosan is a solid at temperatures below 55 °C. It is moderately soluble in water ($C_s = 0.001$ g triclosan/100 g of water, i.e., 10 µg/mL) and highly soluble in ethanol ($C_s > 100$ g triclosan/100 g ethanol) [1,26,27]. Triclosan was assumed to dissolve in liquids according to the Noyes & Whitney relationship (3) [28,29]:

$$\frac{dM}{dt}(t) = \frac{S \cdot D}{d} \cdot (C_s - C_t) \quad (3)$$

$\frac{dM}{dt}(t)$ is the dissolution rate of the solute at a given time t , S is the dissolution surface (m^2), D is the diffusion coefficient ($m \cdot s^{-1}$), C_s is the solubility of the solute in the solvent ($Kg \cdot L^{-1}$ or $moles \cdot L^{-1}$), i.e., the maximum concentration of solute the solvent can hold, above which the solute precipitates. C_t ($Kg \cdot L^{-1}$ or $moles \cdot L^{-1}$) is the solute concentration in the bulk of the solvent at time t (s), and d (m) is the thickness of the solute's diffusion layer equal to the concentration gradient. Assuming the dissolution surface was constant for each suture during the assay, solubility and diffusion layer thickness were the two independent parameters to modify the dissolution rate.

2.2. Standardised Sample Preparation and Triclosan Concentration Determination

The studied sutures were V+, P+, and M+ calibre USP 2-0 (diameter: 0.35 to 0.4 mm), USP 0 (diameter: 0.4 to 0.5 mm) and USP 1 (diameter: 0.5 to 0.6 mm). All test sutures were unpackaged and cut as 35 cm long segments and immersed in 10 mL solvent. All immersions were in glass tubes and incubated at 37 °C in the dark to avoid photodegradation [30]. The tested solvents were pure water obtained by distillation or water plus absolute ethanol as a cosolvent. Immersions were either static or continuously stirred with a tube rotator/incubator (Roto-Therm™ model H2020, Benchmark Scientific, Sayreville, NJ, USA). An independent laboratory accredited for compliance with the EN ISO 17025 standard determined the triclosan concentrations [31,32]. The determinations were performed by liquid chromatography-mass spectrometry using direct sample injection into a high-performance liquid chromatography device (HPLC Shimadzu Nexera X2, Kyoto, Japan) with liquid chromatography-tandem mass spectrometry (LC-MS/MS Sciex 5500, AB Sciex Pte. Ltd., Singapur). The targeted quantification limit was 0.25 µg/L, but the validated limit was 0.01 µg/L.

The samples were prepared at Bordeaux University, Bordeaux, France, and concentrations were determined at Laboratoires des Pyrénées et des Landes (LPL), Mont De Marsan, France, who provided the test tubes and distilled water. Two copies of each in vitro sample were prepared in order to have a pair of determinations. Six copies of each ex vivo sample type and corresponding in vitro controls were prepared.

2.3. Opportunistic Explanted TS Fragments and Ex Vivo Triclosan Release Benchmarking

The government authorised the preclinical cardiac surgical trial involving three sheep with scheduled sacrifice on postoperative day-seven (DDPP Paris approval number: APAFIS 12534-2017121118586862 v3) and conducted at the IMMR laboratory, Paris, France. Bordeaux University's clinical pharmacology department solicited IMMR to piggyback that study. The plan consisted in replacing standard NTS used to close the surgical wounds of the animals by TS and to retrieve TS fragments immediately after animal sacrifice. The piggybacking was agreed because suture replacement did not cause bias to the cardiac surgery study, did not cause additional harm to the animals, and met the *reduction* and *refinement alternatives* of the 3Rs. Moreover, this cardiac surgery involved a validated translational model of human cardiac surgery [22,23,33].

The animals were operated on in conditions similar to human surgery. The surgeons closed the deep muscle layer with V+ 1 and the subcutaneous tissue with V+ 0. The explanted suture fragments were immediately packaged in biohazard bags, one per animal and tissue, labelled, deep-frozen, and forwarded to Bordeaux University, Bordeaux, France.

The explanted intramuscular V+ 1 fragments were long and allowed for the cutting of six 35 cm fragments to be immersed in 10 mL of water. The explants had lost much of their stiffness compared to new sutures.

The explanted subcutaneous V+ 0 fragments were much shorter and were grouped to form pairs of equal length: four groups reached 22 cm and were immersed in 6.3 mL of water, while the two others reached 17.25 cm and were immersed in 4.95 mL, thus ensuring the same length to volume ratio in all samples.

The immersion was static and lasted 12 h. Fragments were then removed, and the tubes with the solvent were forwarded for triclosan concentration determination.

2.4. In Vitro Triclosan Release Controls

Segments of V+ 2-0, P+ 2-0, and M+ 2-0 were unpackaged and immersed in static water. Early marginal release rates were calculated with triclosan concentrations determined after segment immersion in a single tube at 4, 8, and 12 h. Subsequent marginal release rates were estimated after segment pre-immersion in tubes during 1, 2, 3, 4, 5, 10, 15, 20, 25, and 30 days. After pre-immersion, each segment was transferred to a tube for the final 12-hour static water immersion used to calculate the marginal triclosan release rate. Segments V+ 0 and V+ 1 were pre-immersed for seven days, then transferred to the final tube for 12 h. Segments were removed from the final tubes before forwarding the tubes for triclosan concentration determination. After 30 days in water, sutures remained stiffer than sutures explanted on day-seven from animal muscle.

2.5. Benchmark-Driven Accelerated In Vitro Triclosan Release Models

In vitro conditions were modified targeting a 15-fold to a 60-fold acceleration in triclosan dissolution rate given the two parameters identified with the Noyes & Whitney formula [24]. It was assumed that minimising diffusion layer thickness (d) and increasing the difference between solubility and bulk concentration ($C_s - C_t$) would have multiplicative effects. The thickness was minimised by continuous rotation at 24 rounds-per-minute (rpm) and 37 °C incubation targeting a six-fold acceleration. The rotation speed was selected based on approaches described previously and was confirmed by checking that stirring dispersed triclosan flakes temporarily when triclosan concentration was equal to 60 µg/mL, i.e., six times triclosan solubility in water at 20 °C [26,27,34,35]. Triclosan solubility was increased by adding 1.2 g of absolute ethanol (EtOH) as a cosolvent to 9 g of pure water (i.e., a 13.3% w/w solvent). That ratio targeted a 10-fold increase in solubility because preparatory stepwise gravimetric titration showed that that ratio dissolved precipitated flakes of triclosan in a 100 µg/mL saturated solution. A parallel 2.5-fold increase in solubility was targeted with one-quarter of the amount of EtOH (0.3 g) in 9 g of water, i.e., a 3.3% w/w solvent. The choice of EtOH, among other organic solvents with high triclosan solubility such as acetone, was guided by published experience and by EtOH compatibility with the suture material [36,37]. Another effect of the organic solvent was stabilising triclosan flake dispersion by rotation.

V+ 0 and V+ 1 segments were immersed in parallel sequences of 11 tubes filled with 13.3% w/w solvent in the rotator/incubator. Immersions were from baseline to 4 h, then 4 to 12, 12 to 24, and then every 12 h until tube 11 from 168 to 192 h. Thus, the total immersion time was eight days. Parallel assays were conducted with similar segments in the 3.3% w/w solvent.

2.6. Estimation of the Initial Triclosan Load

The initial amount of triclosan on a suture segment was estimated with the assumption that it would completely dissolve in a 100% w/w EtOH/water solvent (1 g EtOH + 1 g water) at 37 °C with continuous stirring for six days. The released concentrations were determined with V+ 0, V+ 1, V+ 2-0, P+ 2-0, and M+ 2-0.

2.7. Release Kinetics—Pharmacodynamics Matching Tests

In vitro time-kill analyses were performed at Université de Bordeaux, Aquitaine Microbiologie, UMR 5234 CNRS, 33000 Bordeaux, France, according to CLSI standard M26-A [38]. Suture segments were pre-immersed in the accelerated conditions described previously to establish the relationship between the marginal triclosan release rate and the corresponding antimicrobial activity on triclosan-sensitive bacteria [39]. The tests were conducted at Bordeaux University's experimental microbiology laboratory. All tests were performed in a safety cabinet in sterile conditions. V+0 suture segments of the same batch as previously underwent accelerated triclosan release in 3.3% and 13.3% *w/w* solvents, respectively. Pre-immersion durations were 1, 4, 12, 24, and 48 h. At the end of the immersion period, segments were rinsed in distilled sterile water with vortex and then left to dry in a sterile environment. Each segment was then transferred into an Eppendorf tube filled with 10 mL of tryptic casein soy broth (TSB, Difco, BD Diagnostics, Sparks, MD, USA) for a suspension culture with a 0.5 McFarland microbial inoculum (i.e., 10^5 to 10^6 colony-forming units CFU/mL) of reference *S. aureus* ATCC 29213 incubated 24 h at 37 °C. Microbial concentration (CFU/mL) in the broth at 24 hours was compared with baseline. Culture tubes were vortexed with the segment inside; next, a 100 µL broth sample was drawn with an aliquot. The sample underwent five serial 1/10 *v/v* dilutions. The original broth and each dilution were each spread on an Agar plate with a Mueller Hinton (MH) culture medium and then incubated at 37 °C for 24 h. Viable colonies were counted on the plates at the end of the incubation. The plate selected for determining CFU concentration in the broth was the one where colonies were large enough to be identifiable and individualised. The microbial concentration (CFU/mL) in the culture at each time slot was calculated by multiplying the colony count on the plate by the dilution factor. Two copies of the time-kill assays were performed per solvent and pre-immersion duration.

2.8. Data Management and Statistical Analysis

The determined concentrations with sample identifiers and preparation details were recorded in a database. Each pair of copies provided a minimum, a maximum, and a mean. Groups of six copies provided 95% confidence intervals (CI). Statistical analysis computed the time plots of the marginal release rates for each type of suture and solvent. Marginal *ex vivo* release rates and their controls were computed with CI and tested against their respective controls with two-sided t-tests and a type-I error of 5%. *Ex vivo* benchmark release rates from subcutaneous and intramuscular explanted fragments were compared.

A mathematical model was fitted to in vitro marginal release rates measured in accelerated conditions and the *ex vivo* benchmark release rates and residual triclosan over time. The purpose of this fitting was to predict the marginal release rate at a given moment, such as when the antimicrobial activity stopped. Model fitting was performed by single and double exponential decay functions for each series of in vitro triclosan marginal release rates with V+0 and V+1 after immersion in 13.3% *w/w* and 3.3% *w/w* solutions, respectively. A similar fitting was performed on 30-day water immersed reference sutures to find out if the model type varied across sutures and triclosan dissolution conditions. The model parameters were solved for each suture/solvent combination. The model with the adjusted coefficient of determination ($R^2_{adjusted}$) closest to 1 was selected as the best fit (4) [40]:

$$R^2_{adjusted} = 1 - \left(1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \right) \frac{n-1}{n-(k+1)} \quad (4)$$

The single-decay model estimated the release rate at time *t* since baseline $R(t)$ with R_0 is the baseline release rate and L is the half-life (5):

$$R_{predicted}(t) = R_0 \cdot e^{-\frac{t \cdot \ln 2}{L}} \quad (5)$$

The double-decay model decomposed the single decay baseline rate and half-life into an early decay with a partial baseline rate related to a short half-life overlapping with a later decay with another partial baseline related to a long half-life (6) [41]:

$$R_{predicted}(t) = R_1 \cdot e^{-\frac{t \ln 2}{L_1}} + R_2 \cdot e^{-\frac{t \ln 2}{L_2}} \quad (6)$$

The models that best predicted the animal ex vivo benchmarks were selected.

The observed residual amount of triclosan on the suture at time t was the extracted initial amount less the sum of the observed released amounts at each time slot since immersion started to time (7):

$$A_{observed}(t) = A_{0_{observed}} - \sum_{t=0}^t A_{observed}(t) \quad (7)$$

The predicted residual amount at time t was the extracted initial amount less the integral of the fitted model from time 0 to t (8):

$$A_{predicted}(t) = A_{0_{observed}} - \int_{t=0}^t R_{predicted}(t) dt \quad (8)$$

Data management and computations were performed in Stata 17, StataCorps LLC, College Station, TX, USA. Mathematical modelling was performed in Maple 2021.1, Maple Inc., Waterloo, ON, Canada.

3. Results

3.1. Marginal Release Rate in Water Control

The initial rate was high and declined during the first day. It was followed by a slow plateau up to 30 days in all suture types (Figures 1–3). The function with the best fit for all three sutures was a double exponential decay. The adjusted R-squared ranged from 0.88 to 0.99.

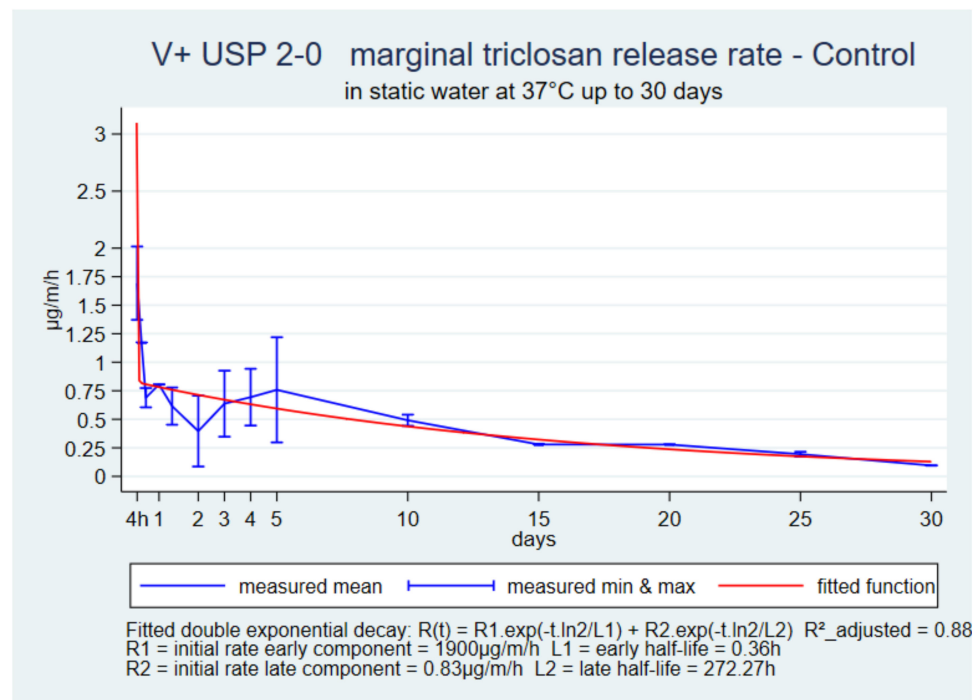


Figure 1. V+ USP 2-0, marginal release rate in static up to 30 days.

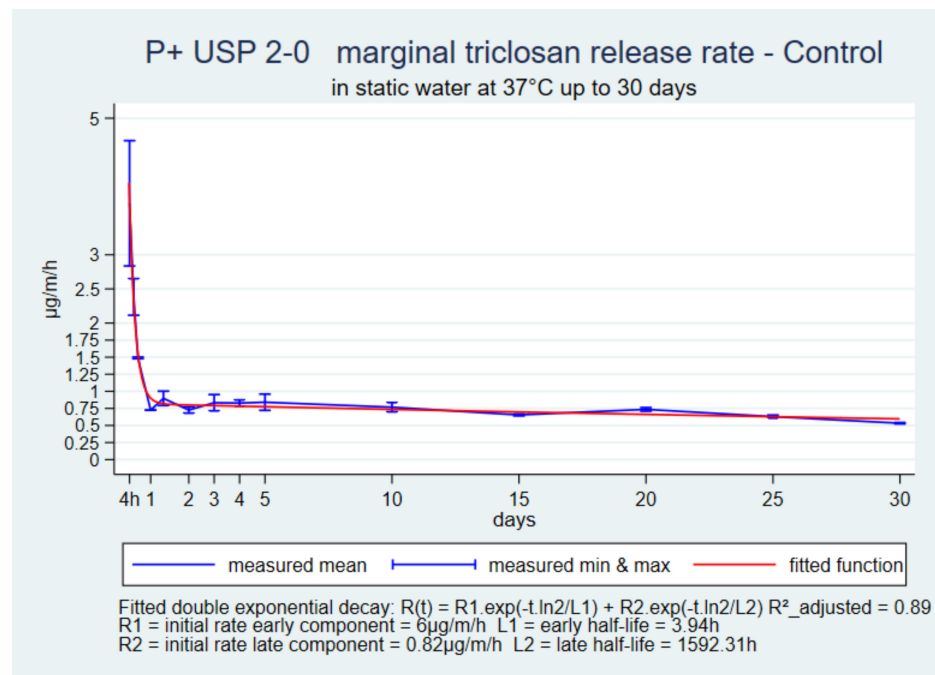


Figure 2. P+ USP 2-0, marginal release rate in static water up to 30 days.

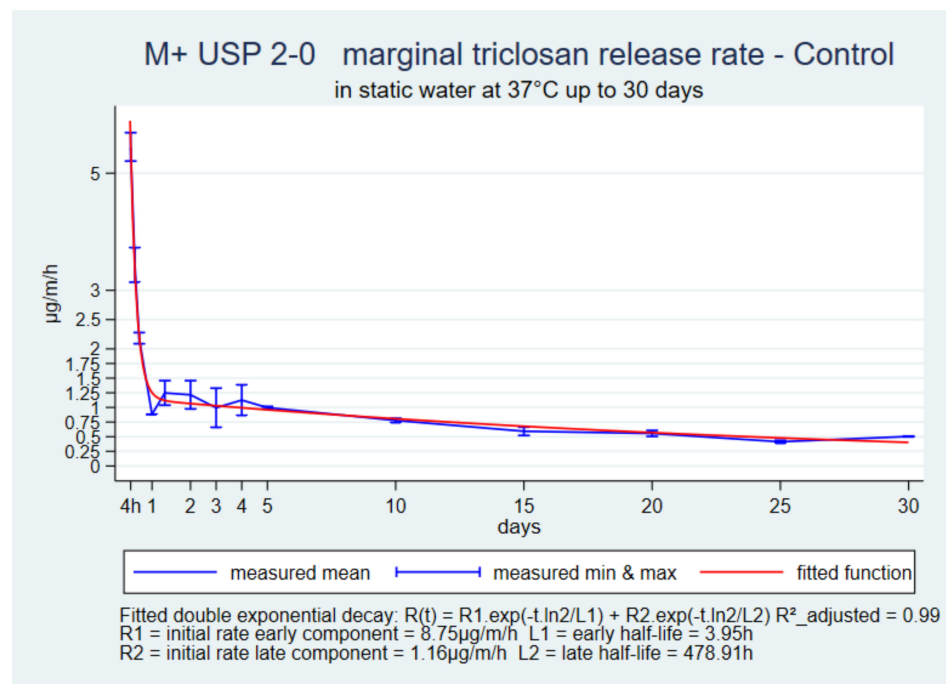


Figure 3. M+ USP 2-0, marginal release rate in static water up to 30 days.

3.2. Marginal Release Rate in Ex Vivo Animal Benchmarks

Ex vivo release rates with V+ on day seven were significantly lower than their controls (Figure 4). The ex vivo intramuscular V+ 1 rate was $0.005 \mu\text{g/m/h}$ [0.003, 0.006] versus $0.30 \mu\text{g/m/h}$ [0.26, 0.35] in control, thus a significant control/explant ratio of 64.2 was found [52.5, 75.9]. The ex vivo subcutaneous V+ 0 rate was $0.015 \mu\text{g/m/h}$ [0.009, 0.025] versus $0.203 \mu\text{g/m/h}$ [0.179, 0.220] in control, thus a significant control/explant ratio of 13.6 [8.8, 18.5] was realized. The subcutaneous V+ 0 over intramuscular V+ 1 ratio was also significant with 3.13 [1.92, 4.35].

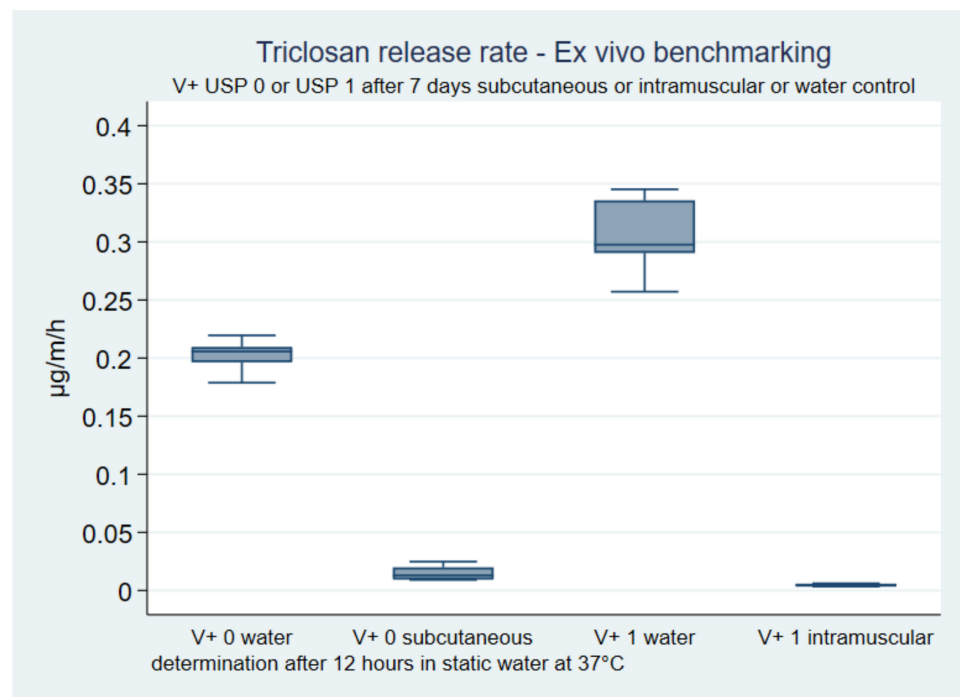


Figure 4. Ex vivo marginal release rate benchmarks *versus* in vitro controls.

3.3. Marginal Release Rate at Accelerated In Vitro Conditions

The shapes of the marginal release rate curves up to seven days of V+ USP 0 and USP 1, in 13.3% and 3.3% solutions, were similar to the shape in water with V+ USP 2-0 up to 30 days. The functions with the best fit were double-exponential decays as well (Figure 5). The initial rates with the 3.3% solution were about 50% lower than with the 13.3% solution, but the two rate functions converged during day-one, and the seven-day rates came close to the ex vivo benchmarks. (Figures for each other suture and solvent combination are in the Supplementary Materials).

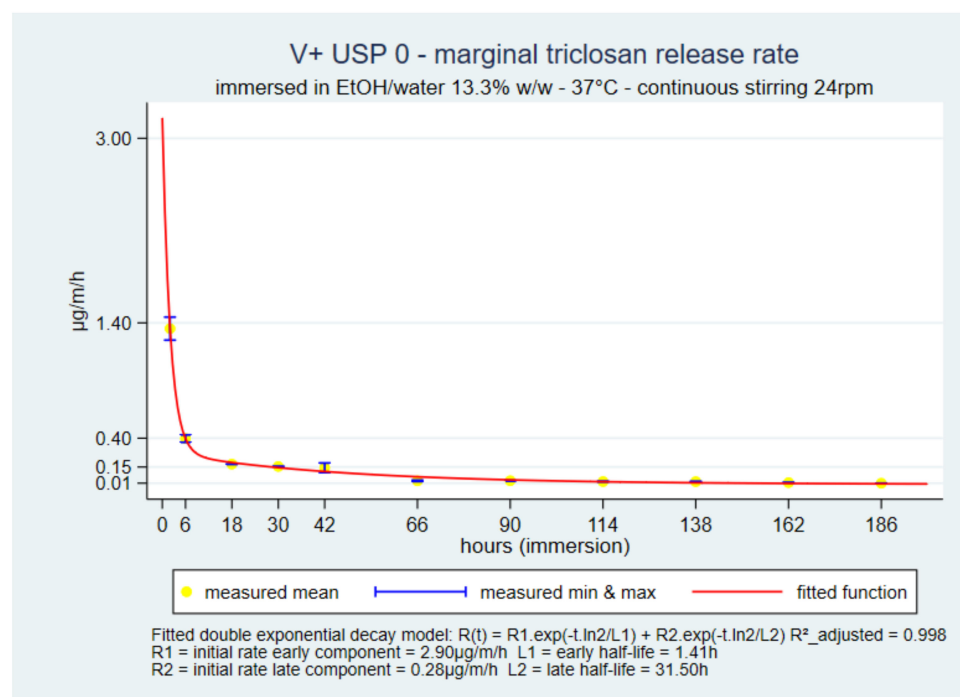


Figure 5. V+ 0 in 13.3% w/w marginal release rates up to 7 days.

3.4. Initial Amount of Triclosan on a Suture

The extraction with the three calibres of braided sutures (V+ 2-0, V+ 0, V+ 1) yielded between 10.1% and 11.1% of the 472 $\mu\text{g}/\text{m}$ specified maximum amount. In the case of monofilament sutures with a common 2360 $\mu\text{g}/\text{m}$ maximum amount, the extraction was 23% of the maximum with P+ 2-0, and 45% with M+ 2-0 (Table 1).

Table 1. Estimate of initial amount $\mu\text{g}/\text{m}$.

Release in $\mu\text{g}/\text{m}$	Suture	Days Immersed	N	Min	Mean	Max
Solvent: EtOH/water 100% w/w	V+ 2-0	6	2	42	47.6	53.1
	V+ 0	6	2	36.3	48.1	60
	V+ 1	6	2	40.3	52.3	64.3
	P+ 2-0	6	2	471.4	551.4	631.4
	M+ 2-0	6	2	971.4	1071.4	1171.4

3.5. Residual Amount of Triclosan on the Suture over Time

The residual triclosan amount in V+ 0 was close to 30 $\mu\text{g}/\text{m}$ on day-7, i.e., 62% of the extracted initial amount. The residual amount was estimated by subtracting the cumulative release sum from the initial amount. The predicted cumulative release was also used as an alternate method (Figure 6). Overall, the residual amount on day-seven was approximately 93% of the maximum initial load. (Figures for each other suture and solvent combination are in the Supplementary Materials).

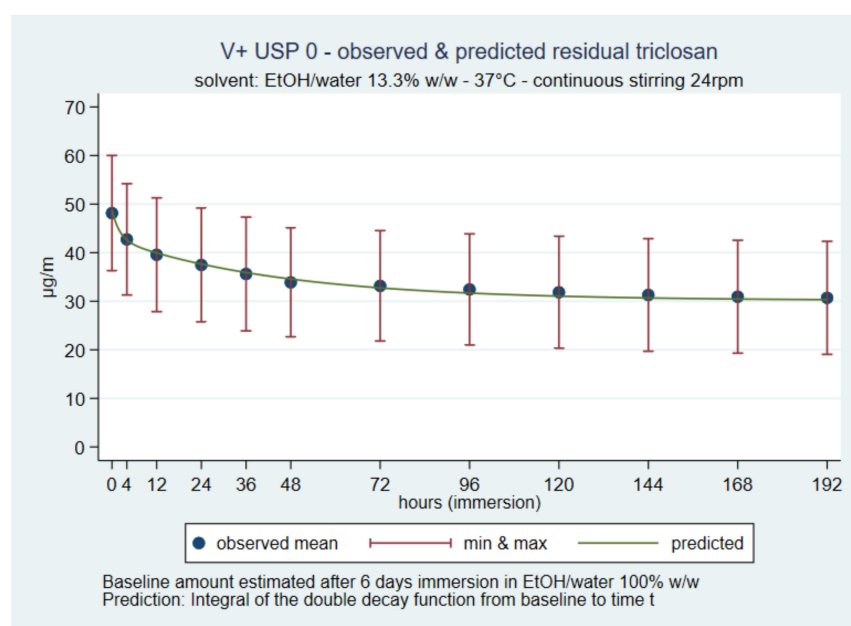


Figure 6. V+ 0 in 13.3% w/w predicted residual triclosan over time.

3.6. Release Kinetics—Pharmacodynamics Matching Results

S. aureus grew on the full surface of control plates after spreading samples of cultures without exposure to TS. No colonies grew on plates after spreading samples of cultures exposed to V+ 0 immersed in EtOH/water 13.3% w/w or 3.3% w/w during 1 h and 4 h, thus demonstrating the biocidal effect of the triclosan on the suture segment.

Viable colonies of *S. aureus* grew on plates after spreading samples of cultures exposed to suture segments immersed in 13.3% w/w or 3.3% w/w solvents between 12 h and 48 hours (Figure 7). These results demonstrated that microbial growth rate increased as the marginal triclosan release rate decreased, as predicted. Moreover, the growth rate was

faster in cultures exposed to segments pre-immersed in 13.3% *w/w* than in 3.3% *w/w*. That difference in rates was consistent with the steeper drop in triclosan marginal release rate with 13.3% *w/w* than 3.3% *w/w*, at equal immersion duration.

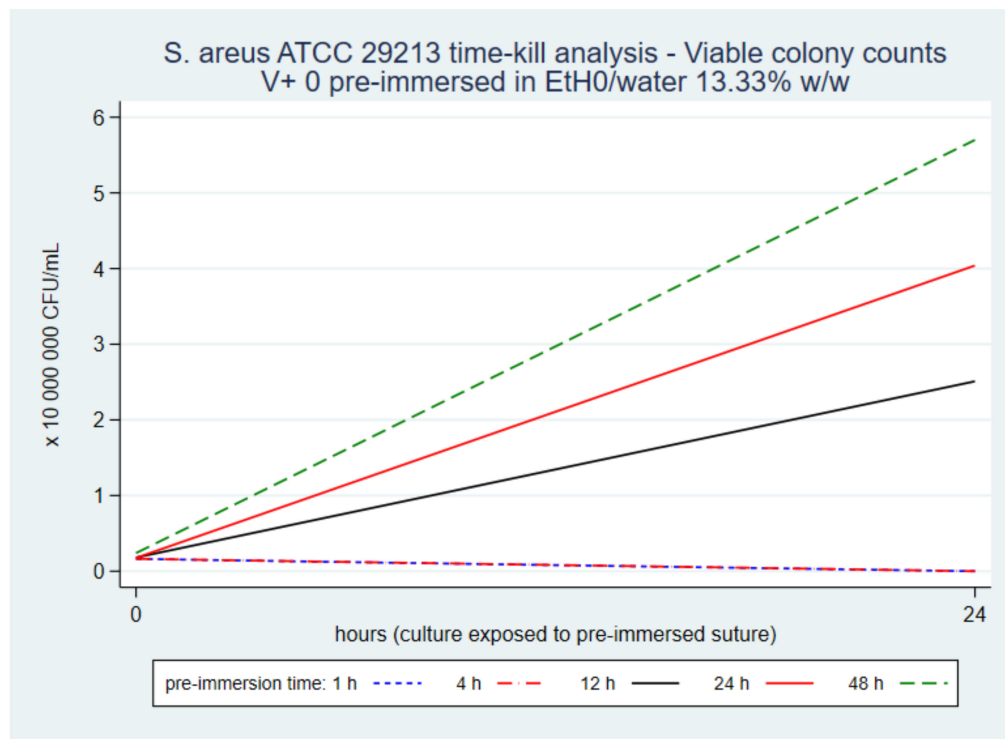


Figure 7. V+ 0 in 13.3% *w/w* 24-h time-kill analysis.

4. Discussion

This study achieved most of its goals. The *in vitro* dissolution model was defined according to approaches to predict drug dissolution *in vitro*, and they agree with the *ex vivo* benchmarks from TS [24]. Piggybacking of the planned preclinical study according to the 3Rs was achieved, and the opportunistic suture fragments were the source of the benchmarks as planned [22,23,33]. IMMR confirmed that piggybacking was rarely achieved, either because of confidentiality or incompatibilities between the protocol and the piggybacking objectives.

The common shapes and double-exponential decay functions of the 30-day references in static water with the three sutures V+, P+ and M+ USP 2-0 presented an excellent fit and showed that the release kinetics were very similar. The differences between suture types were in the four parameters of the prediction functions that described the initial high release rate, its rapid decline over one to two days and then a low-release rate plateau for up to 30 days. M+ and V+ had a higher initial rate than P+ and then a more horizontal plateau, which could be related to their higher initial load. The sutures were still reasonably stiff on day-30, and had a uniform surface and a marginal release rate still above 0.5 $\mu\text{g}/\text{m}/\text{h}$, which was incompatible with the known hydrolysis of the structural polymers once implanted. M+ loses 50 to 60% tensile strength during the first week, and P+ and V+ lose 20% to 25% within two weeks [4–6]. Accelerating triclosan release *in vitro* in proportions indicated by the ratio of the benchmarks and water controls was necessary to estimate the release kinetics as they occur in operated tissues.

The combination of continuous stirring and increased solubility with ethanol as an organic cosolvent were tested methods, and adapting those methods to the needs of this research was straightforward [24,26,27,34–37]. The expertise of LPL was instrumental in the success of this study because handling the considerable range of triclosan concentrations in the samples required proven procedures to ensure ad hoc dilution and conservation [31,32].

The similar shapes of the marginal release rate curves and the common double-exponential decay forms between V+ 2-0 in static water and V+ 0 and V+ 1 in EtOH/water solutions with stirring were an important interim internal validation test of the accelerated dissolution models.

One possible explanation for the shape of the triclosan release curves could be the rapid release of surface triclosan and the slow release of most of the triclosan embedded in the polymer and released with the progressive hydrolysis of the biomaterial [42,43].

This study also enables qualitative comparisons with prior studies. In vitro static culture medium models should have triclosan release-kinetics similar to the 30-day water reference in this study. In vivo studies performed with subcutaneous sutures in rodents should have release-kinetics with V+ closer to those of our accelerated models [8–12,16]. The main difficulty in interpreting those studies is translating their results to surgery because of the unavailability of triclosan release data. Planning an external validation of all those studies would have been possible if they had reported triclosan release data along with the observed TS antimicrobial activity.

The prediction functions suggested that the antistaphylococcal activity of V+ was lost when the marginal triclosan release rate was in a transition range between 0.175 $\mu\text{g}/\text{m}/\text{h}$ and 0.287 $\mu\text{g}/\text{m}/\text{h}$. That change occurred when exposing cultures to sutures pre-immersed between 4 and 12 h, with 13.3% or 3.3% solutions. The end of the antistaphylococcal activity coincided with the end of the rapid decline in curves and the transition into the low-level plateau. The total triclosan released was between 1.7% and 5.3% of the extracted amount and between 0.2% and 0.4% of the maximum amount in V+ (472 $\mu\text{g}/\text{m}$) [5]. These results suggest that V+ has an estimated antistaphylococcal activity duration with bactericidal strength for 4 to 12 h postoperatively; covering the intraoperative and early postoperative risks. The residual amount of triclosan after that period is more than 95% of the actually extracted triclosan and more than 99% of the maximum authorised amount on V+.

According to the determinations with the explants from muscle and subcutaneous tissue, most triclosan remaining after that period is released progressively for at least seven days with either bacteriostatic activity or a non-lethal release.

The minimum inhibitory concentration (MIC) of triclosan in *S aureus* is close to the MIC in other common species found in SSIs, including *E. coli* and *S. epidermidis*. Therefore, the antistaphylococcal activity duration measured in the time-kill analyses should predict the antimicrobial activity duration in several other triclosan-sensitive microorganisms often isolated in SSIs with triclosan MIC < 1 $\mu\text{g}/\text{mL}$. Intrinsically, triclosan-resistant species such as *P. aeruginosa*, where the triclosan MIC ranges from 128 $\mu\text{g}/\text{mL}$ to more than 1000 $\mu\text{g}/\text{mL}$, can colonise TS intraoperatively [8–12,18,39,44].

The intensive 4 to 12-h antistaphylococcal biocidal-level activity could explain significant differences in efficacy between trials. Differences could depend on the microbial load, microbial nesting at a distance from the suture (e.g., haematoma, devitalised tissue, implant), and the time the patient's natural defences required in order to kill residual microorganisms.

This study was executed according to the protocol, with some deviations. The main deviation was that ex vivo benchmarks were available only with V+ sutures. Regarding P+ and M+, the study yielded only water controls. In addition, the preparation of a few samples failed, resulting in outliers or missing concentrations. However, those failures were compensated by the backups (at least two copies of each determination) and the small repercussion of an error at a time slot on the next ones.

One potential bias concerning the ex vivo benchmarks was the risk of mechanical removal of triclosan during suture dissection. However, the visual examination of sutures when preparing them for immersion showed that most dissection damages concerned the subcutaneous fragments because of dense adhesion to tissue. Thus, subcutaneous dissection retrieved sutures incompletely and fragmented the available material into segments less than 5 cm long. The same type of suture in muscle had less adhesion, and several fragments were 35 cm long. Determinations showed that ex vivo triclosan marginal release rates in

subcutaneous explants were significantly higher than in intramuscular explants. Therefore, dissection in this study had little effect on the triclosan load compared with the differences between muscle and subcutaneous tissue over seven days [17].

The main limitations of this study were the incomplete extraction of the initial triclosan load, no benchmarks before seven days, no explanted fragments with polydioxanone and poliglecaprone 25 sutures, the uncertain of translating sheep benchmarks to humans, and not reproducing suture polymer erosion. The *in vitro* model focused on triclosan dissolution and did not release the bulk of triclosan that is assumed to be embedded in the polymer. If polymer hydrolysis had been reproduced, the time-kill analysis might have detected antistaphylococcal activity after 12-h of segment pre-immersion.

The difference between subcutaneous and intramuscular implantations show that different models are required to predict TS antimicrobial activity in various clinical situations.

Future studies should explore the amount of residual triclosan.

5. Conclusions

This study determined triclosan release rate kinetics and the antistaphylococcal activity duration of polyglactin 910 absorbable sutures. An *in vitro* dissolution model was developed and specified using *ex vivo* benchmarks. The dissolution model was continuous, stirring in 3.3% and 13.3% ethanol/water solvents at 37 °C. The benchmarks were the triclosan release rates measured in suture fragments opportunistically retrieved from sheep muscle and subcutaneous tissue after independently planned experimental cardiac surgery.

The exposure of similar sutures to the dissolution model showed an early high release rate that rapidly declined and transitioned to a long-lasting slow plateau after the second day.

A time-kill analysis showed antistaphylococcal activity with bactericidal intensity for 4 to 12 h, and staphylococcal growth with sutures exposed for more than 12 h, with both solvents. The end of the antistaphylococcal activity coincided with the end of the rapid decline in the marginal release rate. Therefore, the residual amount of triclosan was probably more than 99% of the maximum authorised amount on polyglactin 910 braided plus triclosan sutures.

This study showed that polyglactin 910, polydioxanone, and poliglecaprone 25 have similar triclosan release profiles. It also showed a similar profile between polyglactin 910 sutures in static water and an accelerated dissolution model. Differences between suture types and dissolution models seem to be mainly in the maximum initial release rate and the half-life of the decline of that rate.

Sheep are a valid translational model for human cardiac surgery. Polyglactin 910 sutures with triclosan exhibit an intense antistaphylococcal activity for a duration of four to twelve postoperative hours. This activity logically applies to microbial species with a minimal inhibitory concentration of less than 1 µg/mL.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pharmaceutics14030539/s1>. Table S1. *In vitro* water and *ex vivo* sample preparation schedule; Table S2. Modeling phase–Sample preparation–EtOH/water with stirring; Table S3. Estimate of marginal release rate µg/m/h—in static water; Table S4. *Ex vivo* marginal release rate benchmarks versus *in vitro* controls (µg/m/h); Table S5. Cumulative and marginal release rates calculated from raw data with EtOH/water solvent; Table S6. Residual amount of triclosan over time with in EtOH/water 13.3% *w/w*. Figure S1. V+ USP 0 in 3.3% *w/w* release rates and double exponential fitting; Figure S2. V+ USP 0 in 3.3% *w/w* predicted residual triclosan; Figure S3. V+ USP 1 in 13.3% *w/w* release rates and double exponential fitting; Figure S4. V+ USP 1 in 13.3% *w/w* predicted residual triclosan; Figure S5. V+ USP 1 in 3.3% *w/w* release rates and double exponential fitting; Figure S6. V+ USP 1 in 3.3% *w/w* predicted residual triclosan; Figure S7. V+ USP 0 in 3.3% *w/w* time-kill assay.

Author Contributions: F.C.D.: Designed and analysed the study, prepared the samples and participated in the article. R.G.: Reviewed the work and participated in the article. N.M.: Approved

and supervised the project and participated in the article. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was in vitro and used explants of animals that had participated in another preclinical cardiac surgical study and were sacrificed for reasons other than this study. That study had been conducted in Paris and was authorised by government DDPP Paris approval number: APAFIS#12534-2017121118586862 v3.

Informed Consent Statement: Not applicable.

Data Availability Statement: The complete datasets used in this study are available in the tables within the Supplementary Materials file: *TriclosanSutures_Pharmaceutics.SupplementaryMaterial.odt*.

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Conflicts of Interest: The authors declare that they have no conflicts of interest. This study was carried in academic stings out of scientific interest only.

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**4.
WHICH MICROORGANISMS
ISOLATED IN SSI CULTURES ARE
INHIBITED BY SUTURES WITH
TRICLOSAN?**

This *in vitro* study has not yet been submitted for peer review because the microbiological analysis of collected microbial samples and strains is yet to be completed.

The draft manuscript below presents the interim results available to date.

Figure 11 shows photographs of key practical aspects of the study.

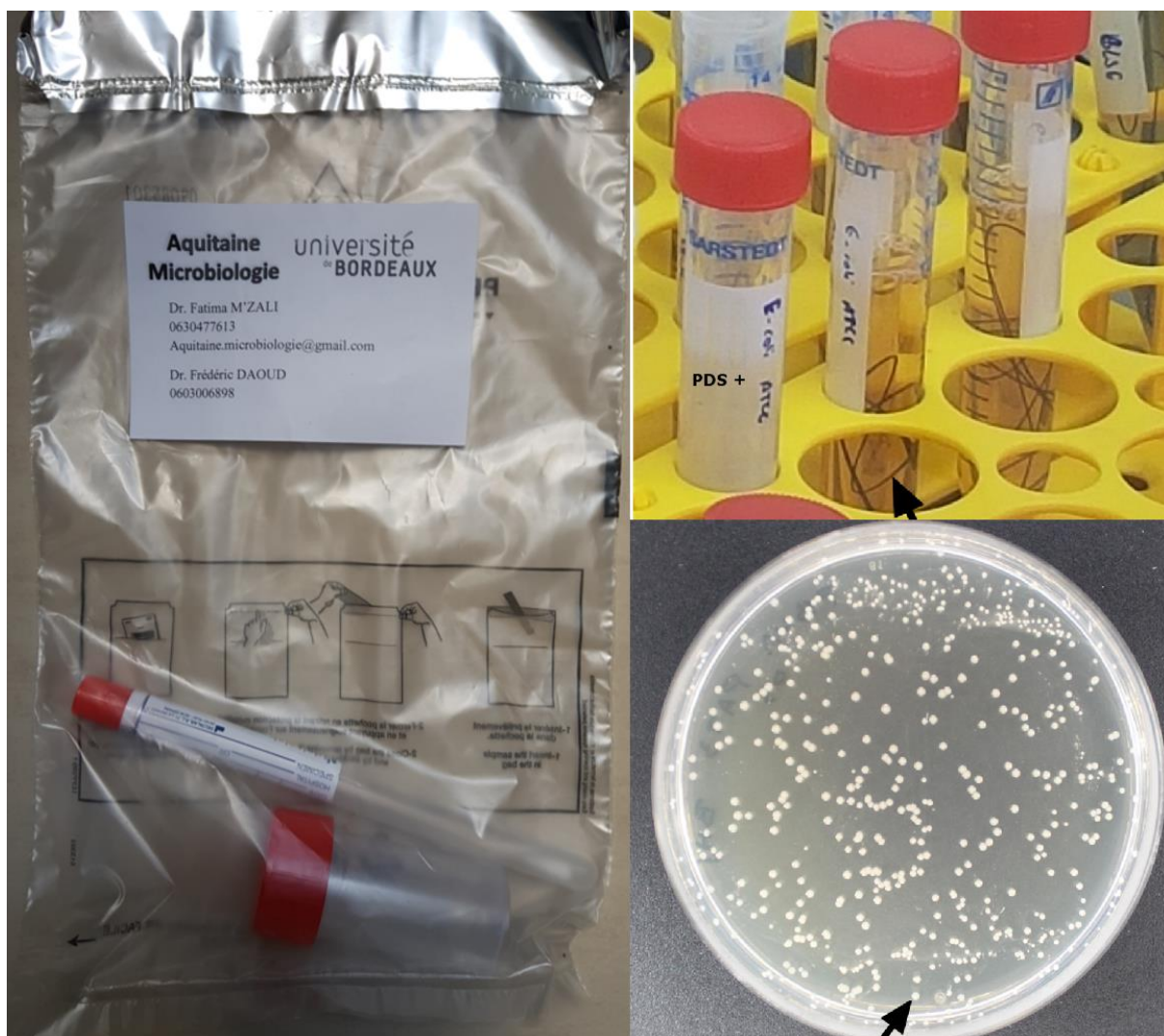


Figure 11 Study 3 - Methods

Are microorganisms in spinal surgical site infections sensitive to triclosan sutures? A real-life study.

INTERIM RESULTS OF ONGOING STUDY (raw data in Appendix 2)

Short title: Microorganisms in spinal SSIs and sutures with triclosan.

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Abstract

Background: Microbial species found isolated in surgical site infections (SSIs) present a wide range of triclosan minimal inhibitory concentration (MIC). Which ones and what proportion of could be inhibited by triclosan sutures?

Methods: This observational study microbial strains isolated from consecutive reoperations for deep spinal surgical site infections. The resulting panel was supplemented with additional clinical strains from spinal and orthopedic surgery and reference strains. MICs were calculated for all strains, and 24-hour time-kill determinations were performed with triclosan polydioxanone sutures.

Results: Strains could be classified into two groups: those with a MIC between 0.03 µg/mL and 4 µg/mL, which were all killed by the sutures' biocidal effect. Those above 128 µg/mL were resistant. The transition zone of MIC where the biocidal effect is lost, between 4 and 128 µg/mL could not be determined accurately.

Conclusions: Most clinical species have a low MIC and are killed by triclosan sutures, so differences in sensitivity to triclosan are unlikely to contribute much to heterogeneity in results between RCTs. *Pseudomonas aeruginosa* with its high MIC could contribute to heterogeneity in Clinical settings where its prevalence is high.

Keywords: surgical site infection; spinal surgery; sutures; triclosan; bacterial sensitivity.

Introduction

Triclosan (2,4-dichlorophenoxyphenol) is a broad-spectrum antimicrobial added to absorbable surgical sutures to minimize microbial colonization and thus reduces the risk of surgical site infections (SSI).¹ The structural biomaterials of the main three types of triclosan sutures (TS) are braided polyglactin 910 in Vicryl PLUS (“V+”), monofilament polydioxanone in PDS PLUS (“P+”), and monofilament poliglecaprone 25 in Monocryl PLUS (“M+”). They provide different mechanical and absorption characteristics. Product labels report a maximum initial triclosan amount of 472 µg/m in V+ and 2360 µg/m in P+ and M+.²⁻⁴

The World Health Organization recommends “*the use of triclosan-coated sutures to reduce the risk of SSI, independent of the type of surgery*” although acknowledging that the quality of the evidence is moderate.⁵⁻⁸ A recent meta-analysis comparing the incidence of SSIs after closure with TS *vs.* NTS in non-spinal surgery shows a significantly lower risk ratio (RR) of SSI with TS but the RRs of individual RCTs are heterogeneous.⁹ Moreover, a minority of the individual RCTs are significant. Several factors could contribute to that heterogeneity, including differences in sensitivity to triclosan between microbial strains. The minimal inhibitory concentration (MIC) of triclosan has been determined in various species such as *Escherichia coli* 0.1 µg/mL, methicillin-sensitive *Staphylococcus aureus* (MSSA) 0.016 to >0.25, with some methicillin-resistant strains (MRSA) reaching 2 to 4, *Staphylococcus epidermidis* 0.12 to 8, *Candida albicans* 0.06 to 4, *Pseudomonas aeruginosa* and some mutant strains of *E. coli* >1000.¹⁰⁻¹⁴

At present, there has been no study on a broad panel of clinical microbial strains isolated from human SSIs with a measurement of the MIC and the corresponding antimicrobial effect of triclosan sutures. This question is relevant in all surgical fields, including spinal surgery, where no RCT comparing the risk of SSI with TS *vs.* NTS has been published so far.

One retrospective comparative clinical record study compared the incidence rate of spinal SSI after closure with braided polyglactin 910 TS *vs.* similar NTS in 405 patients. The reported incidence rate was 0.5% [0.01, 0.28%] (1 case / 200 patients) with TS *vs.* 3.9% [0.17%, 0.75%] (8 / 205) with NTS.¹⁵ The odds ratio was significant with a lower SSI rate in the TS arm (OR 0.12 [0.003, 0.941], $p = 0.02$). Seven of the overall nine SSIs were deep, all in the NTS arm. The responsible bacteria were mainly *Escherichia coli*, methicillin-sensitive or resistant *Staphylococcus aureus* (MSSA or MRSA), *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.

A meta-analysis of 27 studies about spinal surgery, published between 2008 and 2018, showed a pooled incidence rate of surgical site infections (SSI) of 3.1%, 95% confidence interval (CI) [2.3, 4.3] (603 SSIs / 22,475 patients). The depth split was 1.25% superficial (282 / 22,475) and 1.7% (309 / 22475) deep. The pooled rates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *methicillin-resistant Staphylococci* were 37.9%, 22.7%, and 23.1%, respectively.¹⁶ A review of hospital records from 2015 through 2017 at Bordeaux University reported an incidence rate of 1.031% [0.645, 1.557] (22 SSIs / 2133 operation).¹⁷ Depth and associated germs were not reported. Several general and specialized practice guidelines are proposed to prevent and manage spinal SSI.^{18,19} Those take into account the fact given the

presence of implanted material, SSIs may occur long after the operation. However, assuming a significant proportion of SSIs develop early, TS could be relevant if it inhibits most involved strains.

Objective

The objective of this observational study (AD16-278/AST2018-379/IIS15-216/InsermTransfert161449A20/IIS 15-216) is to establish the map of the sensitivity to triclosan of microbial strains that may cause deep spinal SSIs in real-life clinical practice along with the antimicrobial effect of triclosan sutures.

Material and Methods

Three spinal surgery departments of the Bordeaux urban area, France, contributed to a panel of germs isolated from consecutive deep spinal SSIs after surgical wound closures without TS. The germs underwent *in vitro* analyses. One center recruited all reoperations prospectively to treat spinal SSIs between November 28, 2019, through March 15, 2021. The second center performed the same from August 06, 2020, through February 01, 2021. After SSI intraoperative samples were collected for the clinical microbiology laboratory, additional samples were collected (swabs, soft tissue or bone fragments) and forwarded in biohazard bags to Bordeaux university experimental microbiology laboratory with a card mentioning the operation type, anatomic location and initial operation date and reoperation date, use of antibiotics the previous days and triclosan sutures. The third center, Bordeaux University hospital, enabled the retrospective identification

of spinal SSIs between August 02, 2019, through June 29, 2020, through the ledger of the clinical microbiology laboratory and access to surgical reports. The ledger indicated the list and data of each microbial strains isolation per patient and mentioned the strains that were preserved in deep freeze. The experimental microbiology laboratory cultured the prospective samples in aerobically and anaerobically and isolated all microbial strains. It also sampled the spinal SSI strains preserved at the university hospital's clinical microbiology laboratory and additional preserved species that had been isolated in the same hospital over the same period from non-SSI spinal surgery and orthopedic surgery. This resulted in a broad panel of clinical strains, to which were added reference strains (ATCC, CIP, or local collection) for benchmarking. All strains underwent identification with MALDI-TOF mass spectrometry (Microflex® LRF, Bruker Corporation, Billerica, MA, USA) and a baseline antibiogram. Each strain was stored in Brain Heart Infusion (BHI) broth with glycerol at -80°C.

The MIC of each strain's triclosan was then determined according to the following procedure:

A 10^6 CFU/mL inoculum of each target strain was prepared in Mueller Hinton (MH) culture medium. A baseline triclosan solution was prepared by dissolving 5.12 mg of pure powder triclosan (Sigma-Aldrich PHR1338-1G) in 5 mL of absolute ethanol. Fifteen (15) 1/2 v/v absolute ethanol serial dilutions of the baseline triclosan solution were conducted so as to produce a set of 16 test triclosan concentrations (in $\mu\text{g/L}$) of 1024, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 and 0.03125. A 2 mL sample of each triclosan solution was

mixed with 18 mL liquid Agar and laid on a plate until the mix solidified. For each strain, a 200 μ L inoculum was placed in each well. A 96-dot Steers replicator sampled the inoculum to deliver a standard 10^4 CFU per spot on each Agar/triclosan plate. Each MIC analysis started with a triclosan-free control baseline inoculum. The plates were incubated at 37°C for 24 hours, and MICs were read. The MIC was defined as the minimum triclosan concentration that significantly reduced the baseline inoculum, i.e., the CFU concentration with some surviving CFU/colonies next to the concentration where no presence of germs was detected.

The time-kill assays were performed on the selected strains to establish the match between MIC levels and the antimicrobial effect of the sutures immediately after removal from their package. The assays consisted in exposing, in a sterile tube with 10 mL liquid suspension culture medium (sterile tryptic soy culture broth, TSB; Difco, BD Diagnostics, Sparks, MD, USA), a 0.5 McFarland inoculum (i.e., 10^5 to 10^6 CFU/mL) of one selected strain with a 35 cm long segment, 2,360 μ g/m triclosan loaded USP 2-0 polydioxanone monofilament suture (Ethicon PDS PLUS). The culture and suture were incubated at 37°C with continuous shaking for 24 hours with CFU concentration determinations at baseline (t_0), 4, 8, and 24 hours. Each determination involved 48 Mhz sonication of culture tubes for 4 minutes to detach viable microorganisms then draw a 100 μ L broth sample. The sample underwent five serial 1/10 v/v dilutions. The original sample and dilutions were spread on separate Agar plates with MH culture medium and then incubated at 37°C for 24 hours. Viable microbial colonies were counted on the plates where

they could be individualized. The number of CFU/mL at the time slot in the source culture was obtained by multiplying the count with the dilution factor and then multiplying by ten.

Data analysis: The general characteristics of the spinal SSI sample were summarized to enable comparisons with other studies. The number of days between initial surgery and sampling to define an SSI was set to 90 days because most operations required implanted prosthetic material. The MICs interval of the entire microbial panel were reported on a scale with the solubility of triclosan in water as reference point. The time-kill analyses were reported alongside the corresponding strains and results were expressed in percentage of the baseline CFU/mL determination at each time slot. The transition zone of the MIC range from a biocidal effect of TS to no effect was estimated. The proportion of deep spinal SSIs with at least one germ probably resistant to the biocidal effect of TS was determined.

Data management and computations were performed in Stata 17, StataCorps LLC, College Station, TX, USA.

Results

Altogether, 38 patients with spinal SSIs and microbiological were identified, and cultures were positive in 37 patients, 16 prospectively and 21 retrospectively. Recruitment was consecutive with the exception of two prospective cases that could not be reached in time. The culture was negative in one sample. Forty-six percent were polymicrobial (Table 1).

The duration between the initial operation and reoperation with a sampling of infected material collection ranged from 10 to 1365 with four cases beyond 90 days (98, 177, 227 and 1365 days, respectively).

The distribution from post-operative time to sampling, when excluding the 4 delayed cases, could be fitted a Poisson distribution with a mean of $\lambda = 24$ days, a median of 20.5 days and an interquartile interval (IQR) of 13 to 35 days (Figure 1).

Sixty-five strains from 20 species were isolated or obtained from the preserved collection (Table 2). Sixty-three strains from spinal non-SSIs and orthopedic surgery were added so that the panel had a total of 128 strains representing 41 species.

The antibacterial activity of TS was determined on 17 strains (INTERIM STATUS), 3 from spinal SSIs, 4 from defined orthopedic surgery, 1 from spinal non-SSI, and 9 from references species.

The MIC of triclosan was determined in 105 strains (INTERIM STATUS), 46 strains collected from spinal SSIs, 34 from orthopedic surgery, 5 from spinal non-SSI, 5 from undefined orthopedic surgery, and 14 from references species (Table 3). MIC ranged from 0.03 $\mu\text{g}/\text{mL}$ to 512 $\mu\text{g}/\text{mL}$ (Figure 3, Figure 4). The MIC less than 4 $\mu\text{g}/\text{mL}$ in 96% of strains and less than or equal to 0.5 $\mu\text{g}/\text{mL}$ in 92.38%. It was equal to 3 $\mu\text{g}/\text{mL}$ in the two *C. albicans* strains and between 128 and 512 $\mu\text{g}/\text{mL}$ in the 5 strains of *P. aeruginosa*.

The MIC of *Candida albicans* was between 2 and 4 $\mu\text{g}/\text{mL}$ and sensitive to the biocidal effect of the suture. The limits of the transition area could not be accurately determined because of the large MIC gap between *Candida albicans* and *Pseudomonas aeruginosa* and are expected to be between 4 and 128 $\mu\text{g}/\text{mL}$.

TS was biocidal in all species except *Pseudomonas aeruginosa*, i.e. 88% of tested strains (Table 3, Figure 2); that was all strains with MIC < 4 $\mu\text{g}/\text{mL}$ (Table 4).

Discussion

This study achieved the collection of a sample of clinical microbial strains from consecutive spinal SSI supplemented with selected clinical strains and reference strains. Compared with the French national surveillance (ISO-RAISIN) in spinal and neurosurgery in 2018, which reports cases up 90 days in prosthetic surgery, time to reoperation and sampling was on average (median) 5 days later than time to diagnosis (ISO-RASIN 2018: mean 15 days, median 16 days, IQR 11 to 19 days).²⁰ The relative frequency of microbial species was similar with *Staphylococcus aureus*, *Escherichia coli* and *E. Enterococcus faecalis* in the top three and *Pseudomonas aeruginosa* was more frequent in the national surveillance with a frequency of about 6%.

The evidence level of the retrospective comparative study in spinal surgery is low, but the distribution of MICs in the NTS arm is consistent with the findings of this observational study.¹⁵ The species isolated in SSIs in the NTS group have a MIC expected to be < 4 µg/mL except for the single case of *Pseudomonas aeruginosa* (1/205 patients) 0.49% [0.01%, 2.69%], i.e., (1/8) 12.5% [3.16%, 52.65%] of SSIs. This observational study isolated two strains with high MICs, *Pseudomonas aeruginosa* and *balearica*, i.e., (2/54) 3.70% [0.045%, 12.75%]. Other species that can be found in clinical practice, such as *Clostridium perfringens*, have been reported with a MIC of 521 µg/mL, placing them in the same area of resistance as *Pseudomonas aeruginosa*, but none was observed in this pannel.²¹

The microbial species isolated in RCTs comparing TS vs. NTS can be compared to this panel, and that includes RCTs in abdominal/digestive surgery, total knee arthroplasty, neuro-surgery and vein harvesting during coronary artery bypass surgery.²²⁻³² Out of 33 species reported by authors, 14 were in this panel, and eleven were of the same genus (*Pseudomonas spp*, *Enterobacter spp*, *Klebsiella spp*, *Staphylococcus spp*, *Coagulase-negative Staphylococcus*, *Enterococcus spp*, *Koxyzitoca*, *Proteus vulgaris*, *Corynebacterium spp*, *Citrobacter freundii*, *Streptococcus mutans*) and eight belonged to other genera (mainly *Bacteroides spp*, *fragilis*, *ovatus*, *tetaiotaomicron*, *Morganella morganii*, *Peptostreptocoque magnus*, *Moraxella catarrhalis*, *Serratia marcescens*).

The first limitation is the absence of microbial species with a MIC between 4 and 128 µg/mL. As a result, the MIC transition zone where P+ biocidal effect reduces from high to null cannot be accurately estimated, and neither can the incidence rate of the clinical species in that gap zone. Some species such as *Haemophilus influenzae* have been reported to have a MIC of 32µg/mL²¹. This panel has one strain of *Haemophilus parainfluenzae* that might have enabled to explore the gap, but the MIC and time-kill analysis were not performed. The exposure of some species to sub-lethal triclosan concentrations has been demonstrated to shift the MIC gap zone with MICs reaching 16 to 21 µg/mL.¹⁰ Secondly, only time-kill assays and MIC determinations have not been performed on the complete sample so that the agreement between MICs, a surrogate endpoint, and TS antimicrobial activity could not be statistically estimated. The third was the use of P+ only to perform time-kill analyses because of its high triclosan load and the expected potential to kill strains in the upper end of the MIC range. V+ would have been more appropriate from a spinal surgery viewpoint, but our calibration studies did not detect major differences in time-kill curves between the two sutures types.

Conclusion

This observational study on consecutive reoperations for deep spinal surgical site infections isolated all identified microbial strains and additional clinical and reference strains provided a panel of minimal inhibitory concentration ranging from 0.03 µg/mL to 512 µg/mL. Altogether 128 strains were collected, including 65 from consecutive spinal SSIs, and time-kill analyses showed that polydioxanone monofilament sutures with triclosan was biocidal in all strains except *Pseudomonas aeruginosa*, i.e., 88% of challenged strains. The strains could be classified into two categories, those with a minimal inhibitory concentration below 4 µg/m, which concerned all strains except *Pseudomonas aeruginosa* and *balearica*. All strains in that group were killed by triclosan polydioxanone sutures in a 24-hour *in vitro* time-kill assay. The species of the other group, *Pseudomonas aeruginosa* and potentially *balearica*, were not inhibited and grew. This microbial panel analysis shows the

differences in minimal inhibitory concentration within the 0.03 to 4 µg/mL range should not contribute to the heterogeneity in the risk ratio the incidence rate of surgical site infections across randomized clinical trials. *Pseudomonas aeruginosa* could contribute to that heterogeneity in surgical settings where its prevalence is high. The minimal inhibitory concentration transition zone where biocidal activity is lost is between 4 and 128 µg/mL, but given no strain was identified in that range, the limits could not be accurately determined.

List of tables

Table 1 Number of strains per culture-confirmed spinal SSI

Number of germs	n of patients	%
1	18	54.1
2	8	24.3
3	6	16.2
4	1	2.7
5	1	2.7
Total	37	100.00

Table 2 Distribution of species isolated in spinal SSIs

Species in spinal SSI	Times identified	%
<i>S. aureus</i>	24	36.92
<i>Escherichia coli</i>	8	12.31
<i>Enterococcus faecalis</i>	6	9.23
<i>Enterococcus faecium</i>	5	7.69
<i>Bacillus pumilus</i>	3	4.62
<i>Propionibacterium acnes</i>	3	4.62
<i>Pseudomonas aeruginosa</i>	2	3.08
<i>S. epidermidis</i>	2	3.08
<i>Citrobacter koseri</i>	1	1.54
<i>Corynebacterium striatum</i>	1	1.54
<i>Enterobacter aerogenes</i>	1	1.54
<i>Enterobacter cloacae</i>	1	1.54
<i>Lactobacillus spp</i>	1	1.54
<i>Proteus mirabilis</i>	1	1.54
<i>Staphylococcus haemolyticus</i>	1	1.54
<i>Staphylococcus hominis</i>	1	1.54
<i>Staphylococcus saprophyticus</i>	1	1.54
<i>Haemophilus parainfluenzae</i>	1	1.54
<i>Brevibacillus spp</i>	1	1.54
<i>Actinomyces radingae</i>	1	1.54
Total	65	100

Table 3 MIC of triclosan and 24-hour time-kill analyses in a panel of microbial species

species	MIC µg/mL		% of baseline CFU/mL			
	min	max	baseline	4 h	8 h	24 h
<i>Achromobacter</i>	0.03	0.03				
<i>Bacillus cereus</i>	0.03	0.03				
<i>Citrobacter braaki</i>	0.03	0.03				
<i>Enterobacter bugandensis</i>	0.03	0.03				
<i>Enterococcus avium</i>	0.03	0.03				
<i>Enterococcus faecalis</i>	0.03	0.03	100	0.18	0.00	0.00
<i>Enterococcus faecium</i>	0.03	0.03				
<i>Enterococcus hirae</i>	0.03	0.03				
<i>Lactobacillus spp</i>	0.03	0.03				
<i>Staphylococcus capitis</i>	0.03	0.03				
<i>Staphylococcus haemolyticus</i>	0.03	0.03				
<i>Staphylococcus saprophyticus</i>	0.03	0.03				
<i>Staphylococcus aureus</i> MRS.A	0.03	0.03	100	2.07	1.35	2.95
			100	0.64	0.34	0.75
<i>Propionibacterium acnes</i>	0.03	0.03	100	48.43	4.16	0.01
			100	52.73	3.83	0.01
<i>Bacillus pumilus</i>	0.03	0.06				
<i>Brevibacillus spp</i>	-					
<i>Haemophilus parainfluenzae</i>	-					
<i>Klebsiella pneumoniae</i>	0.03	0.06				
<i>Staphylococcus hominis</i>	0.03	0.06				
<i>Escherichia coli</i> ESBL producer	0.03	0.06	100	3.24	0.74	58.51
			100	0.00	0.00	0.00
<i>Escherichia coli</i>	0.03	0.25	100	2.61	0.00	0.00
			100	0.00	0.00	0.00
<i>Enterobacter cloacae</i>	0.03	0.25				
<i>Staphylococcus aureus</i>	0.03	0.25	100	0.25	0.00	0.00
			100	2.93	1.17	2.09
<i>Staphylococcus epidermidis</i>	0.03	0.5	100	1.45	0.41	0.54
			100	3.64	0.38	0.00
<i>Staphylococcus warneri</i>	0.06					
<i>Enterobacter aerogenes</i>	0.06	0.06				
<i>Streptococcus canis</i>	0.06	0.06				
<i>Actinomyces radingae</i>	0.06	0.06				
<i>Proteus mirabilis</i>	0.06	0.125				
<i>Citrobacter koseri</i>	0.125	0.125				
<i>Clostridium sporogenes</i>	0.125	0.125				
<i>Staphylococcus lugdunensis</i>	0.125	0.125				
<i>Corynebacterium striatum</i>	0.125	0.25	100	40.43	4.46	0.00
<i>Hafnia alvei</i>	0.25	0.25				
<i>Streptococcus spp</i>	0.25	0.25				
<i>Streptococcus constellatus</i>	0.25	0.25				
<i>Streptococcus dysgalactiae</i>	0.25	0.25				
<i>Streptococcus anginosus</i>	0.5	0.5				
<i>Candida albicans</i>	2	4	100	0.00	0.00	0.00
			100	0.00	0.00	0.06
triclosan solubility in water	10 µg/mL					
<i>Pseudomonas aeruginosa</i>	128	512	100	136.13	250.97	5567.74
			100	664.39	2582.48	11376.56
<i>Pseudomonas balearica</i>	256	256	100			

Table 4 Biocidal activity of triclosan sutures and MIC

MIC µg/mL	biocidal			
	no	yes	pending	Total
0.03	0	10	56	66
0.06	0	0	17	17
0.125	0	1	4	5
0.25	0	0	7	7
0.5	0	0	2	2
3	0	1	1	2
128	1	0	0	1
256	0	0	4	4
512	0	0	1	1
pending	2	2	19	23
Total	3	14	111	128

Note: biocidal if 24-hour CFU concentration reduction is a factor 100 ($\log=-2$)

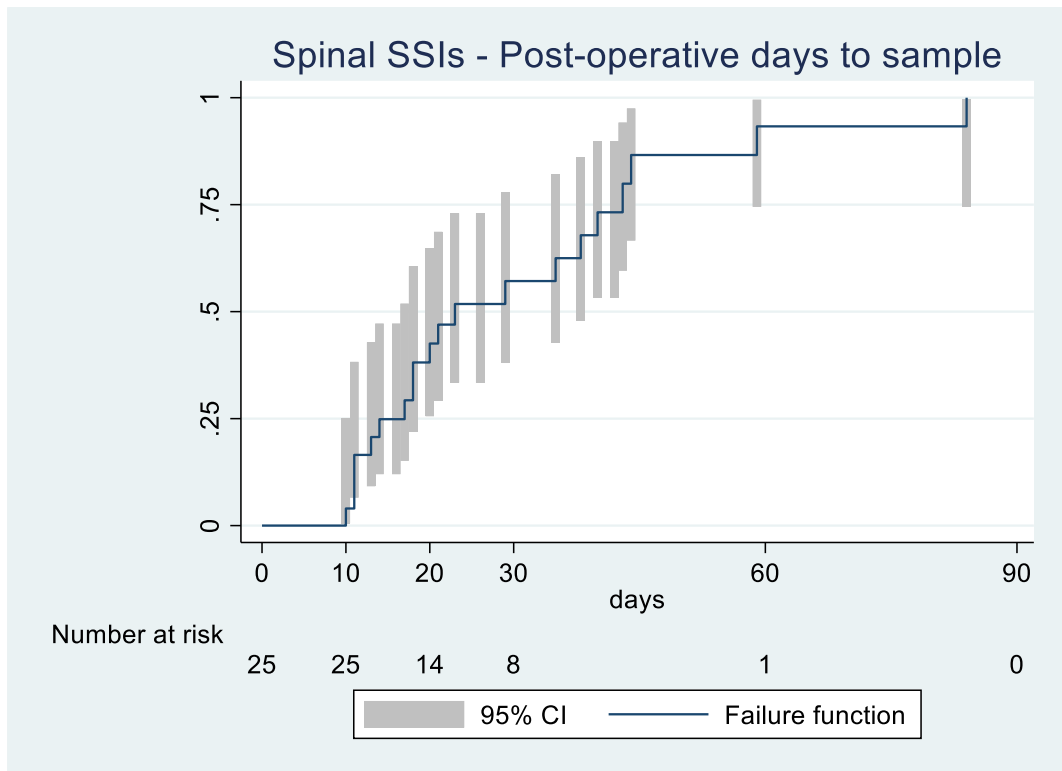


Figure 1 Kaplan-Meier analysis of time to spinal SSI sample up to 90 day follow-up after initial operation

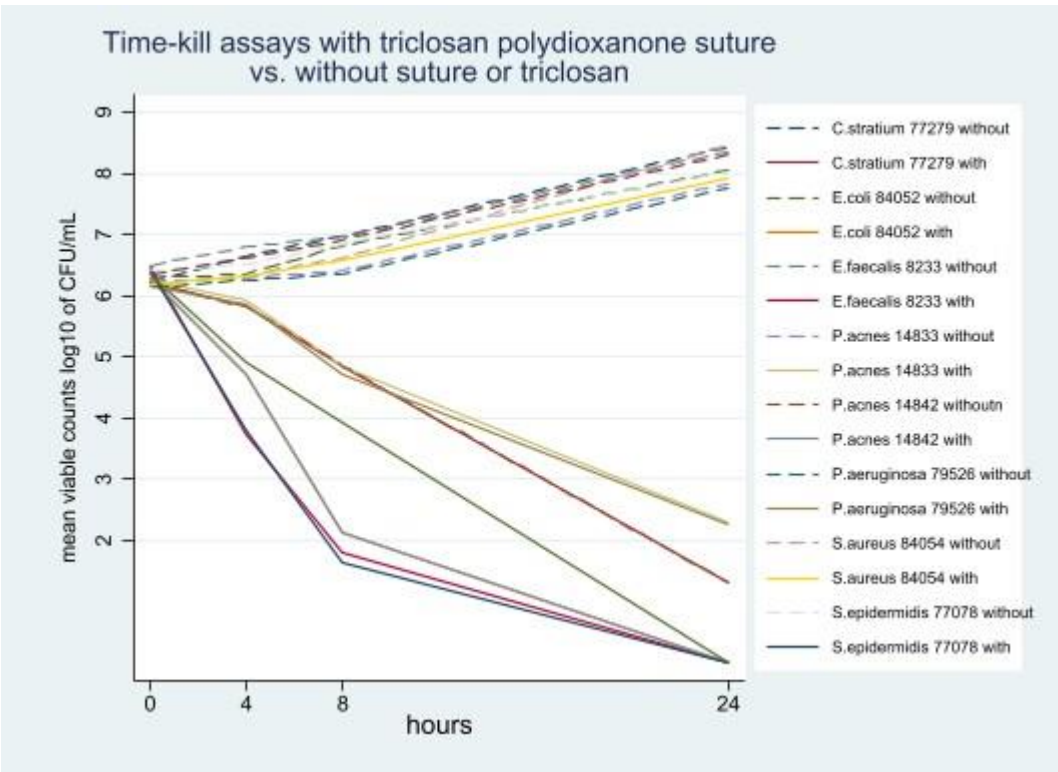


Figure 2 Time-kill analyses of microbial cultures exposed to polydioxanone sutures

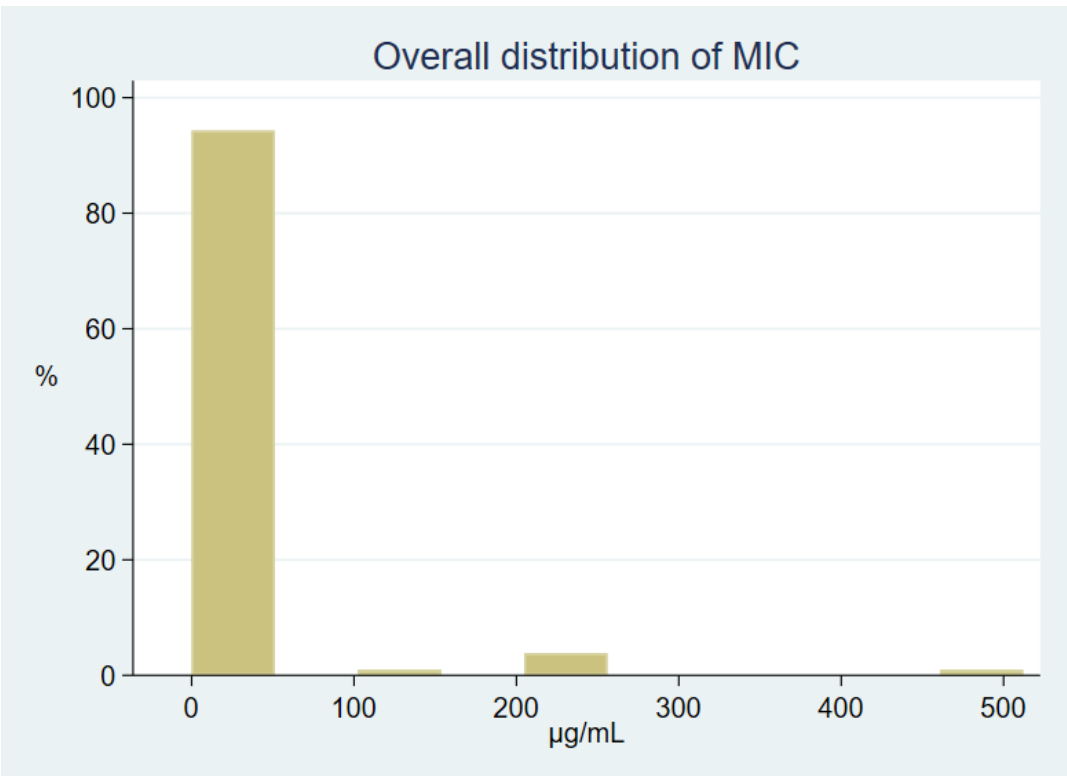


Figure 3 MIC Distribution – All strains

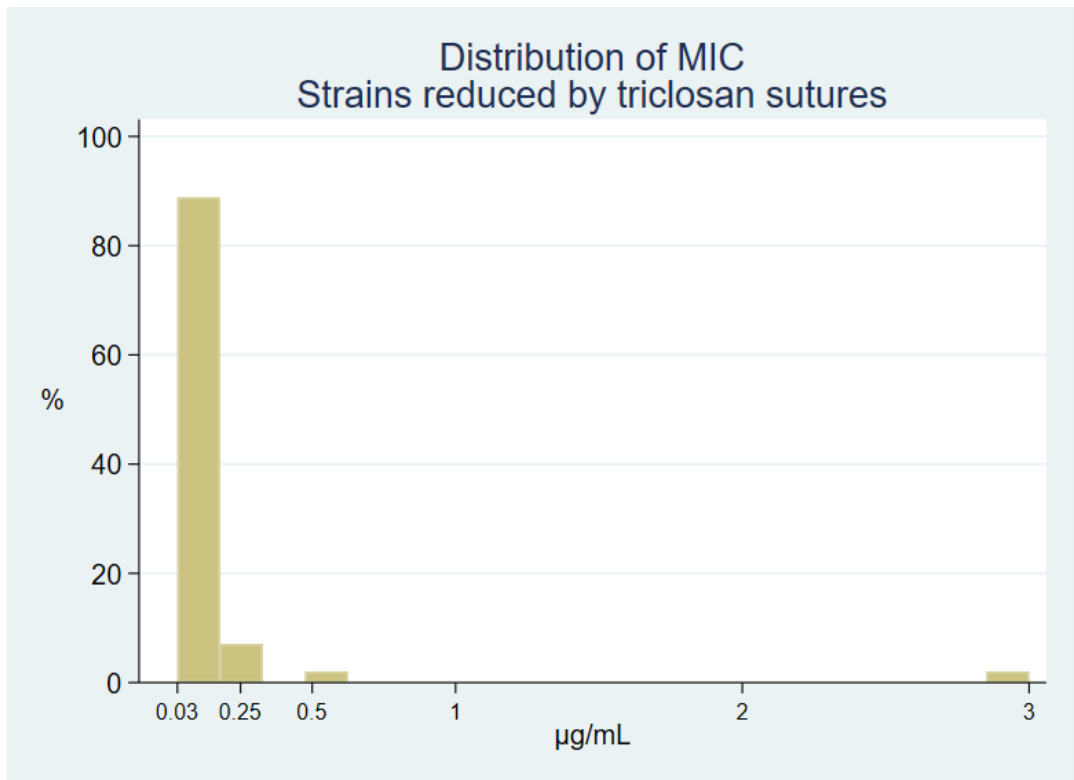


Figure 3 MIC Distribution – Zoom on strains reduced by triclosan sutures

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AUTHOR CONTRIBUTION

Frederic Daoud: Defined the study objectives, approved the microbiological protocol, verified source data, analyzed the data, interpreted the results and wrote the article.

Fatima M'Zali: Microbiologist, specified and supervised the microbiological assays and and managed the collected microbial strains.

Jean-Charles Le Huec : Surgeon. Provided samples.

Stéphane Bourret : Surgeon. Provided samples.

Morad Pedram : Surgeon. Provided samples.

Jean-Marc Vital : Surgeon. Provided samples.

Véronique Dubois : Microbiologist. Identified strains from the University Hospitals.

Nicholas Moore: Defined the study objectives approved the microbiological protocol.

Anne-Marie Rogues: Defined the study objectives approved the microbiological protocol.

AUTHOR DISCLOSURE STATEMENT

Disclosures

Frederic Daoud: No competing interest.

Jean-Charles Le Huec: No competing interest.

Jean-Marc Vittal: No competing interest.

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**5.
DO SUTURES WITH TRICLOSAN
MODIFY THE MICROBIAL
DIVERSITY OF SSIS
CONSISTENTLY WITH
MICROBIAL SUSCEPTIBILITY TO
TRICLOSAN?**

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The published supplemental material file is available in Appendix 3.



Review

Do Triclosan Sutures Modify the Microbial Diversity of Surgical Site Infections? A Systematic Review and Meta-Analysis

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Abstract: Randomised controlled clinical trials (RCTs) report a lower incidence rate of surgical site infections (SSIs) with triclosan sutures (TSs) compared with non-triclosan sutures (NTSs). Do triclosan sutures modify the microbial diversity of culture-confirmed SSIs (ccSSIs)? If so, this would support the association between TS antimicrobial activity and the SSI incidence rate. This prospective systematic literature review (PROSPERO CRD42019125099) was conducted according to PRISMA. RCTs that compared the incidence of SSIs with TSs and NTSs and reported microbial counts from SSI cultures per suture group were eligible. The microbial species were grouped by genus, and the association between genera and sutures was tested. The pooled relative risk (RR) of ccSSIs was also calculated. Twelve RCTs were eligible. No publication bias was identified. The microorganism count was 180 in 124 SSIs with TSs versus 246 in 199 SSIs with NTSs. No significant difference in microbial diversity was found, but statistical power was low for test results to support or challenge the association between the antimicrobial activity of TSs and the reduced rate of SSIs. The RR of the ccSSIs was significant and consistent with comprehensive meta-analyses. The certainty of the pooled RR was moderate.

Keywords: surgical site infection; microorganisms; diversity; sutures; triclosan; meta-analysis



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1. Introduction

Surgical site infections (SSI) are diagnosed up to 30 days postoperatively, although some guidelines extend the duration up to one year in prosthetic surgery. SSIs are superficial incisional (skin and subcutaneous tissue), deep incisional (fascia and muscle), and organ/space [1,2]. SSIs may extend across the three domains. SSI surveillance networks report a wide range of incidence rates across operations; e.g., from 0.5% [0.2, 2.7] in prosthetic knee surgery to 10.1% [4.1, 16.9] in laparotomic colon surgery [3].

The precursor of SSI is microbial contamination, and the conceptual relationship of SSI risk has three factors (Formula (1)) [4]:

$$\text{SSI risk} = \frac{\text{bacterial dose} \times \text{virulence}}{\text{resistance of the host patient}} \quad (1)$$

Virulence refers to disease severity associated with a microorganism. One proposed definition is “the proportion of clinically apparent cases that are severe or fatal” [5]. Virulence varies across microorganisms [6–8]. Microorganisms involved in SSI have been reported to originate mainly from the skin, surrounding tissues of the incision, or operated organs with microbial flora such as the bowel [9]. Concerning the bacterial dose, surgical sites contaminated with more than 10^5 /grammes of tissue have a significantly increased risk of SSI [10]. Much lower doses can produce an SSI when foreign material is inside the

surgical site, such as sutures; e.g., 100/g of tissue in the case of staphylococci when silk sutures were used [11–13].

The guidelines of the World Health Organization (WHO) for SSI prevention conditionally recommend “the use of triclosan-coated sutures to reduce the risk of SSI, independent of the type of surgery” because the quality of the evidence is moderate [14,15]. Triclosan is a broad-spectrum antimicrobial, and in vitro and animal studies have shown that it inhibits microbial colonisation in TSs [16–21]. Once implanted, TSs are estimated to display biocidal-level antistaphylococcal activity during the first 4 to 12 h [22]. Therefore, TSs potentially reduce SSI development through the early decrease in bacterial load at the suture surface and the inhibition of suture colonisation.

Prospective randomised controlled clinical trials (RCTs) since 2005 have compared SSI incidence rates with TSs versus NTSs. The most frequently studied TSs have been braided polyglactin 910, with a maximum triclosan load of 472 µg/m; and monofilament polydioxanone and monofilament polyglycaprone, with up to 2360 µg/m [23–25].

The pooled relative risks (RRs) and odds ratios (ORs) of comprehensive meta-analyses of RCTs have shown a significantly lower SSI rate with TSs than with NTSs, but most included RCTs were nonsignificant [26–32]. The meta-analysis with the most data (25 RCTs and 11,957 patients) reported a significant pooled RR of 0.73 [0.65, 0.82] with 88% (22/25) of nonsignificant RCTs [32]. It is unclear whether the significant pooled RR reflected the consequence of TS antimicrobial activity or chance or bias, given the many risk factors of SSIs and the variability in diagnostic criteria [33].

Identifying an expected effect of TS antimicrobial activity on SSIs’ characteristics independent of the pooled RR of the SSIs would support or challenge the association between the use of TSs and the pooled RR.

Microbial susceptibility to triclosan varies by more than 60,000-fold, with a minimum inhibitory concentration (MIC) of 0.016 µg/mL in *Staphylococcus aureus* to more than 1000 µg/mL in *Pseudomonas aeruginosa* and mutant strains of otherwise susceptible species such as *Escherichia coli* or *Klebsiella pneumoniae* [34–43]. Therefore, one could expect TSs to inhibit microorganisms associated with SSIs in different proportions according to microbial susceptibility to triclosan. A significant difference in microbial diversity of culture-confirmed SSIs (ccSSIs) between TSs and NTSs would be the supportive evidence. One could expect fewer triclosan-susceptible species with TSs, no frequency difference for triclosan-resistant species, or an increase in triclosan-resistant species with TSs due to reduced competition with other species. This systematic literature review (SLR) was performed to test the null hypothesis H0: SSI microbial diversity is not different between TSs and NTSs versus the alternative HA: SSI microbial diversity is different between TSs and NTSs.

2. Materials and Methods

2.1. Question Framing and Eligibility Criteria

This prospective SLR (PROSPERO CRD42019125099) was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [44,45]. The research question was specified according to the Patient, Intervention, Comparison, and Outcome (PICO) framework (Table 1) [46].

Table 1. PICO specification of the research question.

Item	Specification
Patients	Surgically operated patients
Intervention	Surgical wound closure with any TS
Comparator	Surgical wound closure with any NTS
Outcome	Count of each microorganism isolated in ccSSIs

2.2. Search Strategy

PubMed, Embase, Web of Science, and the Cochrane Library (including CENTRAL) were searched using the following string: “triclosan AND (suture OR sutures OR ligation OR ligations) AND (surgery OR surgeries OR surgical OR operation OR operations) AND ((systematic AND review) OR random* OR RCT OR guide* OR recom* OR meta-analy* OR metaanaly*)” [29,30]. No exclusion filter was applied. The extraction was up to date on 18 August 2021. Appendix A displays the search strategy as implemented in each database (Tables A1–A4).

2.3. Eligibility Criteria

Prospective parallel-group RCTs that met the PICO specifications were eligible. Posters, abstracts, communications, and studies that did not report institutional review board or ethics committee approval and patient informed consent were excluded.

2.4. Study Selection, Data Extraction, and Risk of Bias Assessment

Two reviewers (F.D. and M.C.) independently conducted the three steps, and the differences were adjudicated by a third reviewer (N.M.). All references were imported into a repository (EndNote X8, Clarivate Analytics, Philadelphia, PA, USA). Eligibility was determined by reading titles, abstracts, and full text. Duplicates were flagged, and multiple publications about the same study were grouped for joint review. Additional studies were identified from the references of RCTs and previous SLRs. Automated queries were used for post hoc verification.

Potentially relevant RCTs were exported to Review Manager (RevMan) 5.4 software (The Cochrane Collaboration, 2020). The individual RCT risk of bias was assessed using the seven items of the built-in risk of bias (RoB) tables [47].

2.5. Extracted Data

Data were extracted in standardised tables:

1. Study characteristics: Design, committee approval and informed consent, study registration, statistical methods including power calculation, screening methods, treatment allocation and blinding details, sponsor details, enrollment period and sites, inclusion sites, patient inclusion and exclusion criteria, patient demographics, clinical indication, type of surgery, suture material by suture group, SSI prevention details, and additional patient groups.
2. Detailed patient disposition.
3. Number of patients with a ccSSI by suture type and list of microorganisms per culture or the aggregate count of each microbial designation. When microbial percentages were reported, counts were calculated using the corresponding total number.

2.6. Microbial Data Analysis

For descriptive analysis, microbial counts were summed in a spreadsheet according to designation and suture group. The relative frequency of each cell was calculated.

The counts by original designation were then summed according to genus and suture group in a contingency table. Microorganisms that could not be traced to their genus and genera with an expected count of less than $n = 5$ per cell were excluded from the analysis.

The independence of genera and sutures was tested with Pearson’s chi-squared and Fisher’s exact tests. The significance threshold was $p < 0.05$. The measure of association between genera and sutures was Cramér’s V (0–0.29 weak association, 0.3–0.59 moderate, 0.6–1 strong) [48].

The robustness to sensitivity analysis was tested by iteratively repeating the contingency table analysis with the data of one study removed.

2.7. Consistency with Clinical Outcomes

The consistency of microbiological findings with clinical outcomes was assessed by comparing results with the eligible studies' RRs of ccSSIs (TSs over NTSs). A risk of publication bias was suspected if the funnel plot of the RR was asymmetrical or if Harbord's test for binary variables was significant (i.e., $p < 0.05$) [49,50].

The heterogeneity of the distribution of the RCTs' RRs was tested with Cochran's Q-test (threshold: $p \geq 0.05$) and the I^2 statistic, the percentage of variation across the RCTs' RRs due to heterogeneity rather than chance. The heterogeneity was considered high if $I^2 > 25\%$ [51–56]. The robustness of test results was assessed with a sensitivity analysis.

The contingency table analysis, sensitivity analysis, power calculation, and Harbord's tests were computed in STATA 17 (StataCorp LLC, College Station, TX, USA). The overall bias summary, stratified pooling of RR, heterogeneity analysis, and figure creations were performed in Review Manager 5.3. The risk of bias of the individual RCTs was summarised graphically with Review Manager's automated table coupled with a forest plot of the RRs. The level of certainty of the pooled RR of the ccSSIs was rated according to GRADE [57].

3. Results

3.1. Study Identification and Selection

A total of 49 records concerning 33 RCTs were in the clinical scope; 20 of them concerning 12 RCTs fulfilled the PICO specifications and were included in the pooled analysis (Figure 1).

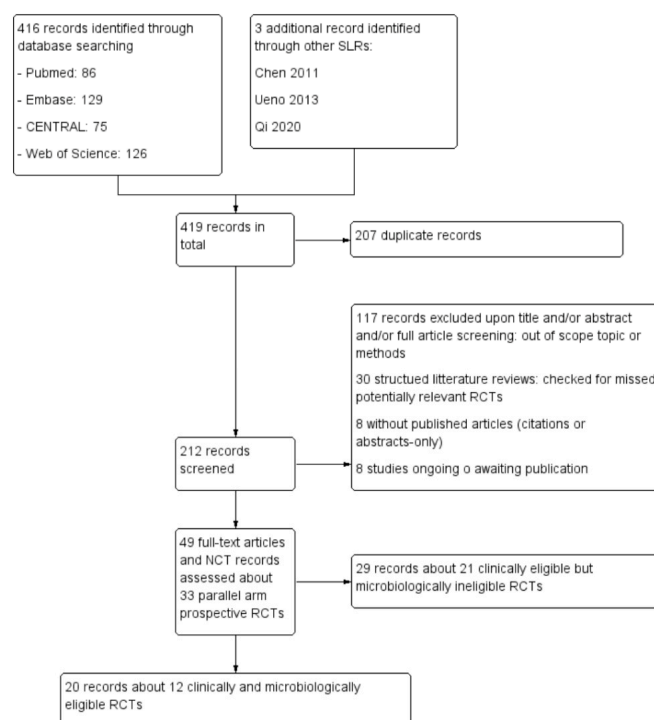


Figure 1. PRISMA flow chart.

3.2. Characteristics of Eligible Studies and Risk of Bias

The 12 included studies represented 36% (12/33) of clinically relevant RCTs and included 27% (322/1197) of all SSIs; 25% (3/12) were significant.

The summary of characteristics of the eligible studies showed that half of them were about abdominal surgery (mainly digestive, but also pilonidal and others). The others focused on cardiovascular operations, knee arthroplasty, and neurosurgery (Table 2). Polyglactin sutures were the most frequently compared (83% of the studies), followed by polydioxanone (33%) and polyglecaprone 25 (once). One-third of studies compared associations of TSs.

Table 2. Characteristics of eligible studies.

Study	Patients TS, NTS	Enrollment	Type of Surgery	Sutures TS/NTS	Diagnostic Criteria and Follow-Up	ccSSIs/Microorganisms TS, NTS
Ruiz-Tovar 2020 [58]	45 and 50 BTS), 47	4 centers, Spain, 2018–2019	Midline laparotomy, acute abdomen	PDS+ and Stratafix), PDS II	CDC + culture, 30 days	4/4, 11/22
Arslan 2018 [59]	86, 91	1 center, Turkey, 2011–2013	Excision of pilonidal disease	Vicryl+ and PDS+, Vicryl & polypropylene	CDC + culture, 30 days	9/11, 19/22
Ichida 2018 [60]	508, 505	1 center, Japan, 2009–2011	Digestive tract surgery	Vicryl+ and PDS+, Vicryl & PDS II	CDC + culture, 30 days	22/72, 19/59
Lin 2018 [61]	51, 51	1 center, ROC, 2011–2012	Total knee arthroplasty	Vicryl+, Vicryl	Own rules + cultures, 6 months	0/0, 1/1
Mattavelli 2015 [62]	140, 141	4 centers, Italy, 2010–2013	Elective colorectal resection	Vicryl+ and PDS+, Vicryl and PDS II	CDC + culture, 30 days	11/18, 8/13
Ruiz-Tovar 2015 [63]	50, 51	2 centers, Spain, 2007–2013	Fecal peritonitis	Vicryl+, Vicryl	CDC + culture, 60 days	5/5, 18/35
Nakamura 2013 [64]	206, 204	1 center, Japan, 2009–2011	Elective colorectal	Vicryl+, Vicryl	CDC + culture, 30 days	7/12, 13/17
Jüstinger 2013 [65]	485, 371	1 center, Germany, 2009–2011	Laparotomy for various causes	PDS+, PDS II	CDC + culture, 30 days	28/28, 30/30
Thimour-Bergström 2013 [66]	184, 190	1 center, Sweden, 2009–2012	Saphenous vein harvesting, CABG	Vicryl+ and Monocryl+, Vicryl and Monocryl	CDC + culture, 60 days	14/22, 23/29
Isik 2012 [67]	170, 340	1 center, Turkey, 2008–2009	Sternal and saphenous vein harvesting, CABG	Vicryl+, Vicryl	CDC + culture, 30 days	5/5, 9/9
Mingmalairak 2009 [68]	50, 50	1 center, Thailand, 2006–2007	Appendectomy	Vicryl+, Vicryl	Criteria not reported + culture, 30 days	1/1, 1/1
Rozelle 2008 [69]	46, 38	1 center, USA, 2005–2006	CSF shunt in children	Vicryl+, Vicryl	Criteria not reported + culture, 6 months	2/2, 8/8

The counting of microorganisms was straightforward in all but two studies. In Jüstinger 2013, counts were calculated by multiplying the number of ccSSIs by the corresponding percentages of the microorganisms and then rounding decimals to the nearest integer. In Isik 2012, the random allocation ratio was 1 TS to 2 NTSs, thus unbalancing the microbial and SSI counts.

The risk of bias varied significantly across RCTs. The RoB tables of the included RCTs with the supportive information used to rate each item are displayed in Appendix B (Tables A5–A16).

3.3. Microbial Diversity

Microbial diversity consisted of 34 reported species, including remarkable strains (e.g., MRSA) and genera (e.g., *Staphylococcus* spp.) (Table 3). The individual counts were too low to compare the relative frequencies between TSs and NTSs. *E. coli* was the most frequent species, and the only one with a significant RR of 0.58 [0.37, 0.92], with fewer cases in TSs.

Table 3. Count of microbial species in culture-confirmed SSIs from the 12 RCTs.

Microbial Designations	TS n	TS %	NTS n	NTS %	Total n	Total %
<i>Staphylococcus aureus</i>	10	5.6%	26	10.6%	36	8.5%
MRSA	1	0.6%	2	0.8%	3	0.7%
Coagulase-negative <i>Staphylococcus</i>	4	2.2%	7	2.8%	11	2.6%
<i>Staphylococcus epidermidis</i>	5	2.8%	5	2.0%	10	2.3%
<i>Staphylococcus</i> spp.	25	13.9%	29	11.8%	54	12.7%
<i>Escherichia coli</i>	22	12.2%	52	21.1%	74	17.4%
<i>Enterococcus</i> spp.	18	10.0%	16	6.5%	34	8.0%
<i>Enterococcus faecalis</i>	8	4.4%	12	4.9%	20	4.7%
<i>Enterococcus fecium</i>	0	0.0%	2	0.8%	2	0.5%
<i>Enterococcus avium</i>	1	0.6%	0	0.0%	1	0.2%
<i>Klebsiella pneumoniae</i>	13	7.2%	17	6.9%	30	7.0%
<i>Klebsiella</i> spp.	4	2.2%	11	4.5%	15	3.5%
<i>Koxytoca</i>	1	0.6%	0	0.0%	1	0.2%
<i>Pseudomonas aeruginosa</i>	7	3.9%	17	6.9%	24	5.6%
<i>Pseudomonas</i> spp.	6	3.3%	3	1.2%	9	2.1%
<i>Enterobacter</i> spp.	5	2.8%	7	2.8%	12	2.8%
<i>Enterobacter cloacae</i>	4	2.2%	5	2.0%	9	2.1%
<i>Streptococcus mutans</i>	2	1.1%	7	2.8%	9	2.1%
<i>Streptococcus</i> spp.	3	1.7%	2	0.8%	5	1.2%
<i>Streptococcus anginosus</i>	1	0.6%	0	0.0%	1	0.2%
<i>Bacteroides fragilis</i>	4	2.2%	6	2.4%	10	2.3%
<i>Bacteroides</i> spp.	2	1.1%	1	0.4%	3	0.7%
<i>Bacteroides ovatus</i>	0	0.0%	1	0.4%	1	0.2%
<i>Bacteroides thetaiotaomicron</i>	0	0.0%	1	0.4%	1	0.2%
<i>Proteus mirabilis</i>	2	1.1%	0	0.0%	2	0.5%
<i>Proteus vulgaris</i>	2	1.1%	0	0.0%	2	0.5%
<i>Citrobacter freundii</i>	0	0.0%	1	0.4%	1	0.2%
<i>Citrobacter koseri</i>	1	0.6%	0	0.0%	1	0.2%
<i>Morganella morganii</i>	1	0.6%	1	0.4%	2	0.5%
<i>Peptostreptococcus magnus</i> (*)	1	0.6%	0	0.0%	1	0.2%
<i>Corynebacterium</i> spp.	0	0.0%	1	0.4%	1	0.2%
<i>Moraxella catarrhalis</i>	1	0.6%	0	0.0%	1	0.2%
<i>Serratia marcescens</i>	0	0.0%	1	0.4%	1	0.2%

Table 3. Cont.

Microbial Designations	TS n	TS %	NTS n	NTS %	Total n	Total %
Other bacteria	14	7.8%	11	4.5%	25	5.9%
Polymicrobial	12	6.7%	0	0.0%	12	2.8%
Fungus: <i>C. Albicans</i>	0	0.0%	2	0.8%	2	0.5%
TOTAL microorganism count	180	100%	246	100%	426	100%
Culture-confirmed SSIs	124		198		322	
Patients included by authors	2021		2079		4100	

(*) *Finegoldia magna*.

The microorganisms were grouped in the contingency table according to eight phylogenetic genera (Table 3). The genera that were excluded due to an expected count of less than five per cell were *Proteus*, *Citrobacter*, *Morganella*, *Corynebacterium*, *Moraxella*, *Serratia*, and *Peptostreptococcus*. Thirty cases designated as polymicrobial or “other bacteria” were also excluded.

The 2-by-8 contingency table had 375 microorganisms, 39% in the TS arm and 61% in NTS (Table 4). The association between genera and sutures was weak (Cramér’s $V = 0.11$) and nonsignificant (chi-squared $p = 0.72$). The power calculated post hoc was low ($1 - \beta = 0.28$). The sensitivity analysis (Supplementary Materials) did not change the conclusions of the overall table and the subtables, so no RCT was identified as a significant cause of bias in the microbial diversity analysis.

The null hypothesis was not rejected.

Table 4. Count of microbial species in culture-confirmed SSIs from the 12 RCTs.

Genus, n (%)	TS	NTS	Total
<i>Staphylococcus</i>	45 (39.47)	69 (60.53)	114 (30.40)
<i>Escherichia</i>	22 (29.73)	52 (70.27)	74 (19.73)
<i>Enterococcus</i>	27 (47.37)	30 (52.63)	57 (15.20)
<i>Klebsiella</i>	18 (39.13)	28 (60.87)	46 (12.27)
<i>Pseudomonas</i>	13 (39.39)	20 (60.61)	33 (8.80)
<i>Enterobacter</i>	9 (42.86)	12 (57.14)	21 (5.60)
<i>Streptococcus</i>	6 (40.00)	9 (60.00)	15 (4.00)
<i>Bacteroides</i>	6 (40.00)	9 (60.00)	15 (4.00)
Total	146 (38.93)	229 (61.07)	375 (100)

3.4. Clinical Outcomes

The funnel plot (Figure 2) showed moderate asymmetry, and Harbord’s test was nonsignificant ($p = 0.27$). Therefore, no publication bias was detected.

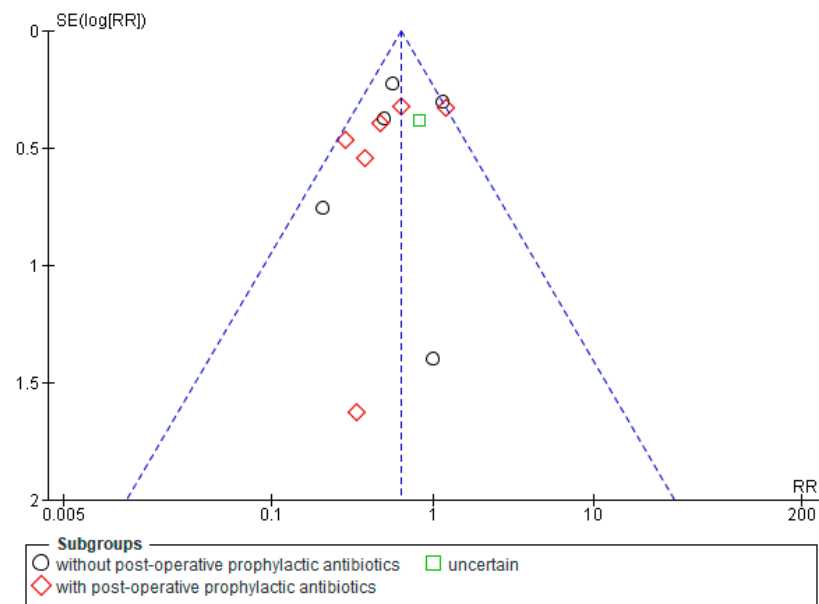
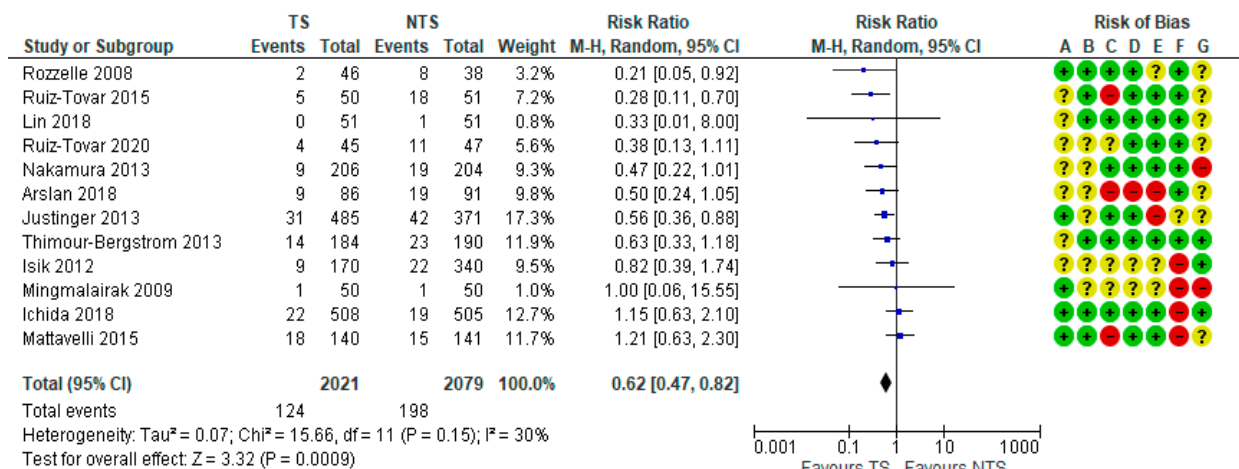


Figure 2. Publication bias analysis—funnel plot.

The meta-analysis of ccSSIs showed a significant RR of 0.62 [0.47, 0.82] favouring TSs. The power calculated *post hoc* was high ($1 - \beta = 0.98$), and the overall heterogeneity was moderate ($I^2 = 30\%$, Q-test $p = 0.15$) (Figure 3).

The visual display of RoB for each item and each included RCT is next to the forest plot of the pooled RR (Figure 3).



Risk of bias legend

- (A) Random sequence generation (selection bias)
- (B) Allocation concealment (selection bias)
- (C) Blinding of participants and personnel (performance bias)
- (D) Blinding of outcome assessment (detection bias)
- (E) Incomplete outcome data (attrition bias)
- (F) Selective reporting (reporting bias)
- (G) Other bias

Figure 3. Forest plot—pooled relative risk of ccSSIs and RCTs’ risk of bias [58–69].

The average RoB of each item across the included RCTs was low in about half the studies and items combined, and unclear or high in the other half (Figure 4).

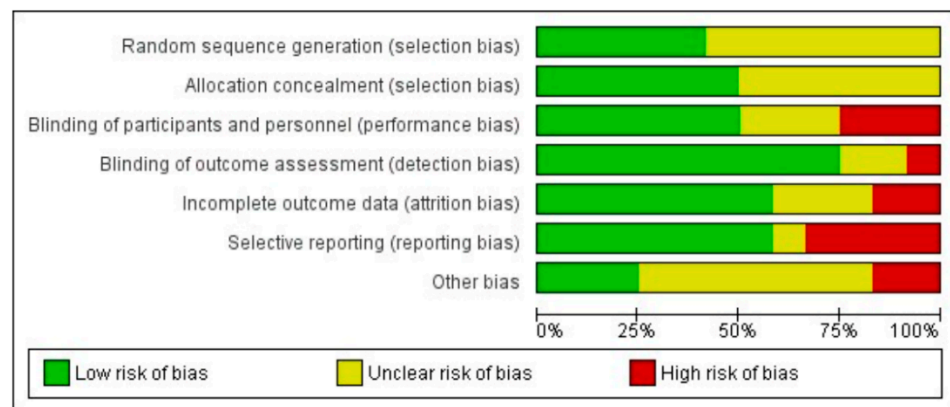


Figure 4. Risk of bias summary of each RoB item as percentages across all included studies.

The overall RR was robust to the sensitivity analysis (Supplementary Materials).

The level of certainty of the evidence underlying the overall pooled RR of the culture-confirmed SSIs was rated moderate according to GRADE (Table 5).

Table 5. GRADE rating of the level of certainty of the evidence supporting the pooled RR of culture-confirmed SSIs.

Certainty Assessment							Summary of Findings				
	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Overall Certainty of Evidence	Study Event Rates (%)		Relative Effect (95% CI)	Anticipated Absolute Effects	
							With Sutures without Triclosan	With Sutures with Triclosan		Risk with Sutures without Triclosan	Risk Difference with Sutures with Triclosan
New outcome (follow up: range 30 days to 365 days; assessed with: clinically and positive culture)											
4100 (12 RCTs)	Serious ^a	Not serious ^b	Not serious ^c	Serious ^d	None observed	⊕⊕⊕○ Moderate	198/2079 (9.5%)	124/2021 (6.1%)	RR 0.62 [0.47; 0.82]	95 per 1000	36 fewer per 1000 (5 to 17 fewer)

CI: confidence interval; RR: relative risk. Explanations: ^a Seven studies had insufficient information about random sequence generation and concealment. ^b The overall I² was 30%, and heterogeneity assessment with Q-test $p = 0.15$ ^c All RCTs had included relevant patients treated who underwent the same type of surgery in the two treatments arms with the compared treatments (TSs versus NTSs). SSIs were culture-confirmed. SSI occurrence was a consequence of multiple factors, but it was the intended clinical effect of TS antimicrobial activity. ^d With $n_{TS} = 124$ $N1 = 2021$ and $n2 = 198$ $N1 = 2079$, overall power was 98%, which was reasonable to compare the two suture arms. Moreover, only 25% of trials (3/12) were significant.

4. Discussion

This review tested if SSI microbial diversity differed between the TS and NTS groups. The protocol assumed that if the TS antimicrobial activity reduced the incidence of SSIs, then SSI cultures' microbial counts would reflect the microorganism's triclosan susceptibility.

The contingency table's independence test was nonsignificant because all eight genera (one per row) reduced the TS column's total count compared with the NTS column. The ratio of the total microbial count in TSs over NTSs was 0.64. The ratio was 0.65 in *Staphylococcus* (MIC 0.015 to 8 µg/mL), 0.42 in *Escherichia* (0.1 to 0.5 µg/mL), 0.9 in *Enterococcus* (MIC 0.5 to 128 µg/mL; NOTE: MIC > 32 µg/mL is rare), and 0.64 in *Klebsiella* (0.1 to 1 µg/mL), which are usually triclosan-susceptible. The ratio was 0.65 in *Pseudomonas* despite the usual triclosan resistance of most species in human surgery (MIC 100 µg/mL up to ≥1000 µg/mL) [34–43,70–74]. The sensitivity analysis showed that no RCT contributed enough to the overall dataset for its removal to change the conclusions. That applied to Isik 2012 with a 1:2 allocation ratio; and Jüstinger 2013 with potential inaccuracies in the microbial count.

The absence of a significant difference in the SSIs' microbial diversity after TSs and NTSs should challenge the association between the difference in the incidence rate of SSIs

after TSs and NTSs. However, the statistical power of the chi-squared was low (28%), so the test results could have resulted from chance, and both hypotheses remain plausible.

The power calculation showed that multiplying all cells of the contingency table by 3.5 with the observed proportions would result in a significant chi-squared test result, with $p = 0.03$ and a power of 84%. Such an increase would require a total microbial count of $n = 1309$. However, such a scenario would still challenge the association between TS and SSI incidence reduction, because the contribution of *Pseudomonas* to the overall lower microbial count in the TS column, with a 0.64 ratio, would be confirmed. Therefore, adding more microbial counts from RCTs would need to show a significant shift of the *Pseudomonas* ratio towards one to demonstrate that *Pseudomonas* were equally frequent in the SSIs of the TS and NTS arms, whereas triclosan-susceptible species remained fewer.

The 12% of excluded culture results were insufficient to bias the contingency table significantly. The designated species are usually intrinsically triclosan-susceptible, and the unspecified cases had an expected 10% triclosan-resistant microorganisms.

No similar study was previously published, so the differences in microbial diversity between the TS and NTS groups of this study could not be compared with other sources.

However, the overall microbial diversity in this study was consistent with the European 2017 SSI surveillance report, in which *Staphylococcus* and *Escherichia* were the most frequent genera, and *P. aeruginosa* represented 4.7% of microorganisms [3]. The microbial diversity was also reasonably consistent with a study of retrieved sutures from SSIs in which *Staphylococcus* was the most frequent genus, and *P. aeruginosa* represented about 5% of microorganisms [75].

The CI of the overall pooled RR of ccSSIs overlapped with the CI of the most comprehensive meta-analysis of RCTs published (Ahmed 2019) [32]. The two studies also agreed in rating the level of evidence as moderate. These similarities suggested that the evidence used here represented the evidence used in Ahmed 2019.

The two limitations of the quality of the evidence in the 12 pooled RCTs; i.e., (1) the minority of significant studies and (2) the uncertain or high risk of bias in about half of the rated points, along with the nonconclusive test of the primary criterion, suggested implementing the WHO conditional guideline with caution. One approach could be making TSs available in routine surgeries for patients with a high risk of SSI or severe SSI complications. Systematically collecting SSI culture details in priority patient groups operated with TSs or NTSs with a minimal clinical dataset incorporated in current surveillance programs would enable an analysis of real-life practice data with evidence from RCTs. That would give those patients a chance to reduce SSI risk with an acceptable risk of adverse suture effects and enable the gathering of evidence to assess the impact of TSs on SSI microbial diversity and ecology. Close monitoring of triclosan-resistant microorganisms such as the *Pseudomonas* genus and mutant strains of usually triclosan-susceptible genera require specific focus.

5. Conclusions

This systematic literature review of randomised controlled clinical trials did not show a significant difference in the microbial diversities of surgical site infections after closure using sutures with or without triclosan. However, the amount of evidence was insufficient to support or challenge the relationship between the antimicrobial activities of sutures with triclosan and the incidence rate of surgical site infections.

The meta-analysis of the relative risk of culture-confirmed surgical site infections favoured sutures with triclosan and was consistent with comprehensive meta-analyses. The certainty of the pooled RR was confirmed as moderate.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microorganisms10050927/s1>. U Bordeaux13042022. Supplement. List of excluded randomised clinical trials; Table S1: Source data—microbial count suture treatment arm and per study; Table S2. Sensitivity analysis of the relative risk of culture-confirmed SSIs; Table S3. Sensitivity analysis of the association between genera and suture types.

Author Contributions: Conceptualisation, F.C.D.; methodology, F.C.D. and A.-M.R.; Literature search, data extraction, and quality assessment, F.C.D., M.C. and N.M.; validation, A.-M.R. and N.M.; data analysis, F.C.D.; interpretation, F.C.D., M.C. and A.-M.R.; writing—original draft preparation, F.C.D.; writing—review and editing, M.C., A.-M.R. and N.M.; supervision, A.-M.R. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board: Not applicable.

Informed Consent: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. Executed Search Strategies

All tables below were set with the same string with the wildcard "*" meaning that the search engine should also retrieve words that are followed with any character. For example: "random*" sets the search engine to retrieve: random, randomised, randomized, randomly, etc. This applies to all four search engines. In Table A1, Pubmed has replaced the "*" with all available explicit words. In Tables A2–A4, the search engine did not.

Table A1. PubMed.

Query
("triclosan"[MeSH Terms] OR "triclosan"[All Fields]) AND ("suturability"[All Fields] OR "suture s"[All Fields] OR "sutured"[All Fields] OR "sutures"[MeSH Terms] OR "sutures"[All Fields] OR "suture"[All Fields] OR "suturing"[All Fields] OR ("suturability"[All Fields] OR "suture s"[All Fields] OR "sutured"[All Fields] OR "sutures"[MeSH Terms] OR "sutures"[All Fields] OR "suture"[All Fields] OR "suturing"[All Fields]) OR ("ligate"[All Fields] OR "ligated"[All Fields] OR "ligates"[All Fields] OR "ligating"[All Fields] OR "ligation"[MeSH Terms] OR "ligation"[All Fields] OR "ligations"[All Fields]) OR ("ligate"[All Fields] OR "ligated"[All Fields] OR "ligates"[All Fields] OR "ligating"[All Fields] OR "ligation"[MeSH Terms] OR "ligation"[All Fields] OR "ligations"[All Fields])) AND ("surgery"[MeSH Subheading] OR "surgery"[All Fields] OR "surgical procedures, operative"[MeSH Terms] OR "surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "general surgery"[MeSH Terms] OR ("general"[All Fields] AND "surgery"[All Fields]) OR "general surgery"[All Fields] OR "surgery s"[All Fields] OR "surgerys"[All Fields] OR "surgeries"[All Fields] OR ("surgery"[MeSH Subheading] OR "surgery"[All Fields] OR "surgical procedures, operative"[MeSH Terms] OR "surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "general surgery"[MeSH Terms] OR ("general"[All Fields] AND "surgery"[All Fields]) OR "general surgery"[All Fields] OR "surgery s"[All Fields] OR "surgerys"[All Fields] OR "surgeries"[All Fields] OR ("surgical procedures, operative"[MeSH Terms] OR "surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "surgical"[All Fields] OR "surgically"[All Fields] OR "surgicals"[All Fields] OR "operability"[All Fields] OR "operable"[All Fields] OR "operate"[All Fields] OR "operated"[All Fields] OR "operates"[All Fields] OR "operating"[All Fields] OR "operation s"[All Fields] OR "operational"[All Fields] OR "operative"[All Fields] OR "operatively"[All Fields] OR "operatives"[All Fields] OR "operator"[All Fields] OR "operator s"[All Fields] OR "operators"[All Fields] OR "surgery"[MeSH Subheading] OR "surgery"[All Fields] OR "operations"[All Fields] OR "surgical procedures, operative"[MeSH Terms] OR ("surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "operation"[All Fields] OR ("operability"[All Fields] OR "operable"[All Fields] OR "operate"[All Fields] OR "operated"[All Fields] OR "operates"[All Fields] OR "operating"[All Fields] OR "operation s"[All Fields] OR "operational"[All Fields] OR "operative"[All Fields] OR "operatively"[All Fields] OR "operatives"[All Fields] OR "operator"[All Fields] OR "operator s"[All Fields] OR "operators"[All Fields] OR "surgery"[MeSH Subheading] OR "surgery"[All Fields] OR "operations"[All Fields] OR "surgical procedures, operative"[MeSH Terms] OR ("surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields] AND "operation"[All Fields])) AND ("classification"[MeSH Terms] OR "classification"[All Fields] OR "systematic"[All Fields] OR "classification"[MeSH Subheading] OR "systematics"[All Fields] OR "systematical"[All Fields] OR "systematically"[All Fields] OR "systematisation"[All Fields] OR "systematise"[All Fields])

Table A1. Cont.

Query
OR "systematised"[All Fields] OR "systematization"[All Fields] OR "systematizations"[All Fields] OR "systematize"[All Fields] OR "systematized"[All Fields] OR "systematizes"[All Fields] OR "systematizing"[All Fields]) AND ("review"[Publication Type] OR "review literature as topic"[MeSH Terms] OR "review"[All Fields]) OR "random*" [All Fields] OR "RCT"[All Fields] OR "guide*" [All Fields] OR "recom*" [All Fields] OR "meta analy*" [All Fields] OR "metaanaly*" [All Fields])
Translations
triclosan: "triclosan"[MeSH Terms] OR "triclosan"[All Fields]
suture: "suturability"[All Fields] OR "sutable"[All Fields] OR "sutural"[All Fields] OR "suration"[All Fields] OR "suture's"[All Fields] OR "sutured"[All Fields] OR "sutures"[MeSH Terms] OR "sutures"[All Fields] OR "suture"[All Fields] OR "suturing"[All Fields]
sutures: "suturability"[All Fields] OR "sutable"[All Fields] OR "sutural"[All Fields] OR "suration"[All Fields] OR "suture's"[All Fields] OR "sutured"[All Fields] OR "sutures"[MeSH Terms] OR "sutures"[All Fields] OR "suture"[All Fields] OR "suturing"[All Fields]
ligation: "ligate"[All Fields] OR "ligated"[All Fields] OR "ligates"[All Fields] OR "ligating"[All Fields] OR "ligation"[MeSH Terms] OR "ligation"[All Fields] OR "ligations"[All Fields]
ligations: "ligate"[All Fields] OR "ligated"[All Fields] OR "ligates"[All Fields] OR "ligating"[All Fields] OR "ligation"[MeSH Terms] OR "ligation"[All Fields] OR "ligations"[All Fields]
surgery: "surgery"[Subheading] OR "surgery"[All Fields] OR "surgical procedures, operative"[MeSH Terms] OR ("surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "general surgery"[MeSH Terms] OR ("general"[All Fields] AND "surgery"[All Fields]) OR "general surgery"[All Fields] OR "surgery's"[All Fields] OR "surgeries"[All Fields] OR "surgeries"[All Fields]
surgeries: "surgery"[Subheading] OR "surgery"[All Fields] OR "surgical procedures, operative"[MeSH Terms] OR ("surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "general surgery"[MeSH Terms] OR ("general"[All Fields] AND "surgery"[All Fields]) OR "general surgery"[All Fields] OR "surgery's"[All Fields] OR "surgeries"[All Fields] OR "surgeries"[All Fields]
surgical: "surgical procedures, operative"[MeSH Terms] OR ("surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "surgical"[All Fields] OR "surgically"[All Fields] OR "surgicals"[All Fields]
operation: "operability"[All Fields] OR "operable"[All Fields] OR "operate"[All Fields] OR "operated"[All Fields] OR "operates"[All Fields] OR "operating"[All Fields] OR "operation's"[All Fields] OR "operational"[All Fields] OR "operative"[All Fields] OR "operatively"[All Fields] OR "operatives"[All Fields] OR "operator"[All Fields] OR "operator's"[All Fields] OR "operators"[All Fields] OR "surgery"[Subheading] OR "surgery"[All Fields] OR "operations"[All Fields] OR "surgical procedures, operative"[MeSH Terms] OR ("surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "operation"[All Fields]
operations: "operability"[All Fields] OR "operable"[All Fields] OR "operate"[All Fields] OR "operated"[All Fields] OR "operates"[All Fields] OR "operating"[All Fields] OR "operation's"[All Fields] OR "operational"[All Fields] OR "operative"[All Fields] OR "operatively"[All Fields] OR "operatives"[All Fields] OR "operator"[All Fields] OR "operator's"[All Fields] OR "operators"[All Fields] OR "surgery"[Subheading] OR "surgery"[All Fields] OR "operations"[All Fields] OR "surgical procedures, operative"[MeSH Terms] OR ("surgical"[All Fields] AND "procedures"[All Fields] AND "operative"[All Fields]) OR "operative surgical procedures"[All Fields] OR "operation"[All Fields]
systematic: "classification"[MeSH Terms] OR "classification"[All Fields] OR "systematic"[All Fields] OR "classification"[Subheading] OR "systematics"[All Fields] OR "systematical"[All Fields] OR "systematically"[All Fields] OR "systematisation"[All Fields] OR "systematise"[All Fields] OR "systematised"[All Fields] OR "systematization"[All Fields] OR "systematizations"[All Fields] OR "systematize"[All Fields] OR "systematized"[All Fields] OR "systematizes"[All Fields] OR "systematizing"[All Fields]
review: "review"[Publication Type]. or. "review literature as topic"[MeSH Terms]. or. "review"[All Fields]

Table A2. Embase.

Query
('triclosan'/exp OR triclosan) AND ('suture'/exp OR suture OR 'sutures'/exp OR sutures OR 'ligation'/exp OR ligation OR ligations) AND ('surgery'/exp OR surgery OR surgeries OR surgical OR 'operation'/exp OR operation OR operations) AND (systematic AND ('review'/exp OR review) OR random* OR rct OR guide* OR recom* OR 'meta analy*' OR metaanaly*)

Table A3. Web of Science.

Query
triclosan AND (suture OR sutures OR ligation OR ligations) AND (surgery OR surgeries OR surgical OR operation OR operations) AND ((systematic AND review) OR random* OR RCT OR guide* OR recom* OR meta-analy* OR metaanaly*) (All Fields)

Table A4. Cochrane Library.

Query
triclosan AND (suture OR sutures OR ligation OR ligations) AND (surgery OR surgeries OR surgical OR operation OR operations) AND ((systematic AND review) OR random* OR RCT OR guide* OR recom* OR meta-analy* OR metaanaly*) in Title Abstract Keyword

Appendix B. Risk of Bias (RoB) of Included Studies

Table A5. Arslan 2018.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Unclear risk	Not reported
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding of participants and personnel (performance bias)	High risk	No
Blinding of outcome assessment (detection bias)	High risk	No
Incomplete outcome data (attrition bias)	High risk	Patient disposition: no patient lost to follow-up reported. Excluded patients after randomisation and use of allocated sutures due to postoperative administration of antibiotics or use of drains caused a risk of bias.
Selective reporting (reporting bias)	Low risk	Not with respect to ccSSIs
Other bias	Unclear risk	Calculated sample size was not justified with respect to the primary endpoint.

Table A6. Ichida 2018.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Low risk	Permuted block (size 2) randomisation, although generation process was not described
Allocation concealment (selection bias)	Low risk	Envelope with randomisation code delivered the allocated sutures to the operating room
Blinding of participants and personnel (performance bias)	Low risk	Yes
Blinding of outcome assessment (detection bias)	Low risk	Yes
Incomplete outcome data (attrition bias)	Low risk	Patient disposition: no, as described in details of patient flow
Selective reporting (reporting bias)	High risk	Cultures collected in 22/35 and 9/30 SSIs
Other bias	Low risk	No

Table A7. Isik 2012.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Unclear risk	Not reported
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding of participants and personnel (performance bias)	Unclear risk	Reported as double blind, but procedures not described
Blinding of outcome assessment (detection bias)	Unclear risk	Reported as double blind, but procedures not described
Incomplete outcome data (attrition bias)	Unclear risk	Patient disposition: Insufficient details
Selective reporting (reporting bias)	High risk	Fewer data reported about cSSIs than about diagnosed SSIs: sternal TS = 4/170, NTS = 12/328 N.S. (bacteria reported in 4/4 and 8/12); leg TS = 5/142, NTS = 10/160 N.S. (bacteria reported in 2/5 and 2/10)
Other bias	Low risk	No

Table A8. Jüstinger 2013.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Low risk	Random block sizes of 50 to 100, although the generation process was not described
Allocation concealment (selection bias)	Unclear risk	Reported, but without description
Blinding of participants and personnel (performance bias)	Low risk	Yes
Blinding of outcome assessment (detection bias)	Low risk	Yes
Incomplete outcome data (attrition bias)	High risk	Patient disposition: number of patients excluded after randomisation was much larger than the number of SSIs (111 > 73), especially in the TS group, which had twice as many excluded than the NTS group
Selective reporting (reporting bias)	Unclear risk	The number of patients with culture results and isolated microorganisms compared to the number of SSIs was unclear
Other bias	Unclear risk	Identified bacteria reported as percentages that, when multiplied by the number of SSIs, resulted in numbers with a decimal instead of being integers

Table A9. Lin 2018.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Unclear risk	Suggested, but mechanisms were not reported
Allocation concealment (selection bias)	Low risk	Sealed envelopes
Blinding of participants and personnel (performance bias)	Low risk	Yes
Blinding of outcome assessment (detection bias)	Low risk	Yes
Incomplete outcome data (attrition bias)	Low risk	Patient disposition: all randomised patients completed study in their group and were included in the analysis
Selective reporting (reporting bias)	Low risk	Not with respect to cSSIs
Other bias	Unclear risk	Calculated sample size was not justified with respect to the primary endpoint

Table A10. Mattavelli 2015.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Low risk	Computer-generated list
Allocation concealment (selection bias)	Low risk	Sealed envelopes
Blinding of participants and personnel (performance bias)	High risk	Operators not blinded, although nonoperating staff and patients were blinded
Blinding of outcome assessment (detection bias)	Low risk	Assessor-blinded
Incomplete outcome data (attrition bias)	Low risk	Patient disposition: detailed. Discontinuations explained and not related to SSIs.
Selective reporting (reporting bias)	High risk	Number of cultures less than the number of diagnosed SSIs
Other bias	Unclear risk	Recruited sample size could not be checked against the calculated sample size

Table A11. Mingmalairak 2009.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Low risk	Random number tables
Allocation concealment (selection bias)	Unclear risk	Insufficiently described
Blinding of participants and personnel (performance bias)	Unclear risk	Insufficiently described
Blinding of outcome assessment (detection bias)	Unclear risk	Insufficiently described
Incomplete outcome data (attrition bias)	Unclear risk	Patient disposition: inconsistencies in flowchart
Selective reporting (reporting bias)	High risk	Inconsistencies in flowchart and ccSSI reporting
Other bias	High risk	Discontinuation after 7.4% of calculated sample size

Table A12. Nakamura 2013.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Unclear risk	Not reported
Allocation concealment (selection bias)	Unclear risk	Envelope method without further detail
Blinding of participants and personnel (performance bias)	Low risk	No
Blinding of outcome assessment (detection bias)	Low risk	Yes
Incomplete outcome data (attrition bias)	Low risk	Patient disposition: detailed. No losses to follow-up or dropouts
Selective reporting (reporting bias)	Low risk	Not with respect to ccSSIs
Other bias	High risk	Insufficient sample size to reach target power

Table A13. Rozzelle 2008.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Low risk	Described
Allocation concealment (selection bias)	Low risk	Described
Blinding of participants and personnel (performance bias)	Low risk	Described
Blinding of outcome assessment (detection bias)	Low risk	Described

Table A13. Cont.

Bias	Author's Judgement	Support for Judgement
Incomplete outcome data (attrition bias)	Unclear risk	Patient disposition: no flowchart, but no loss to follow-up reported
Selective reporting (reporting bias)	Low risk	Not with respect to ccSSIs
Other bias	Unclear risk	No sample-size calculation. 37.7% of patients (23/61) were included twice; i.e., 27.4% (23/84) of procedures. The distribution of those 23 dual-inclusions between the two suture groups was not accurately reported, and two observations in the same patient were not statistically independent.

Table A14. Ruiz-Tovar 2015.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Unclear risk	Not reported
Allocation concealment (selection bias)	Low risk	Sequentially numbered container method
Blinding of participants and personnel (performance bias)	High risk	Randomisation performed by the surgeon without blinding
Blinding of outcome assessment (detection bias)	Low risk	Nurse in charge of diagnosing SSIs was blinded
Incomplete outcome data (attrition bias)	Low risk	Patient disposition flowchart available showed no attrition. Exclusions from SSI incidence comparison were deaths before SSIs.
Selective reporting (reporting bias)	Low risk	Not with respect to ccSSIs
Other bias	Unclear risk	Insufficient information

Table A15. Ruiz-Tovar 2020.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Unclear risk	Not reported
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding of participants and personnel (performance bias)	Unclear risk	Operator blinded until the last minute. Operator should have been blinded to the presence of triclosan until the operation was completed.
Blinding of outcome assessment (detection bias)	Low risk	Nurse in charge of SSI diagnosis was blinded as well
Incomplete outcome data (attrition bias)	Low risk	Patient disposition CONSORT flowchart available. No patients lost to follow-up or dropout. Patients excluded due to reoperation or mortality within 30 days were counted. Their exclusions were explainable given the change in risk, and an analysis on an intention-to-treat basis was not performed
Selective reporting (reporting bias)	Low risk	Not with respect to incisional ccSSIs reported, for both incisional and organ/space
Other bias	Unclear risk	Uncertain whether deep and incisional SSIs were in the same patients or different patients. No culture report for deep SSIs.

Table A16. Thimour-Bergström 2013.

Bias	Author's Judgement	Support for Judgement
Random sequence generation (selection bias)	Unclear risk	Not reported, although some details were provided
Allocation concealment (selection bias)	Low risk	Yes
Blinding of participants and personnel (performance bias)	Low risk	Yes
Blinding of outcome assessment (detection bias)	Low risk	Yes
Incomplete outcome data (attrition bias)	Low risk	Patient disposition detailed flowchart showed a small number of patients lost to follow-up or unreachable minor compared to the number of SSIs
Selective reporting (reporting bias)	Low risk	Results reported for all outcome variables described in the methods
Other bias	Low risk	Assuming a one-sided test was planned

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6.
JOINT INTERPRETATION AND
DISCUSSION

6.1. OVERVIEW OF THE RESULTS

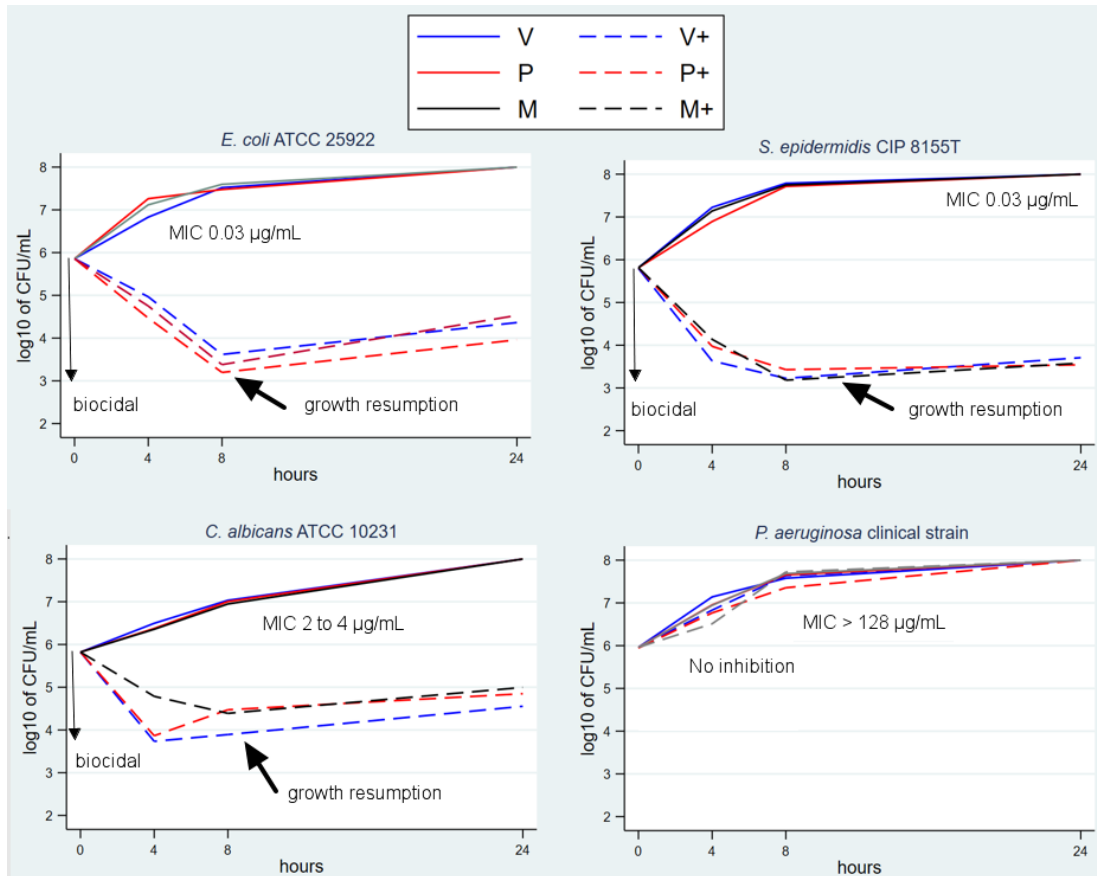
Question 1: What is the level of antimicrobial activity of sutures with triclosan? Is it similar across the three types of sutures?

Answer 1: Nine strains of *S. aureus*, *S. epidermidis*, *E. coli*, *C. albicans* and *P. aeruginosa* were challenged *in vitro* with TS and NTS controls. The sutures were made of braided polyglactin 910, monofilament polydioxanone, and monofilament poliglecaprone 25. The time-kill assays were performed with 35 cm suture segments of in 10 mL of culture medium and a 0.5 McFarland inoculum. All triclosan sutures reduced the baseline microbial concentration of all microorganisms except *P. aeruginosa*, which is resistant to triclosan. Concentration reduction of each strain was not significantly different across triclosan sutures. A minimum microbial concentration was reached and microbial growth resumed after 8 hours in most suture × strain combinations. The similar *in vitro* performance of the three suture types with each strain suggests the three sutures display similar early inhibition of microbial colonization in clinical settings (summary Figure 12). The antimicrobial differences between the types of TS are unlikely to significantly modify the risk of surgical site infections across RCTs.

The assay also showed that TS kill microorganisms by direct contact, rather than by increasing culture medium triclosan concentration above the minimum inhibitory concentration.

The ability to translate these *in vitro* results to clinical settings is based on the observation that the contact killing mechanism depends on the bioavailability of triclosan at the suture surface and microbial concentration surrounding it, independently of the contaminated volume. The initial biocidal phase could explain TS ability to minimise high microbial concentration on the tissue surface it is in direct contact with. The difference in the risk of SSI developing on the suture between TS and NTS would be marked in clean-contaminated or contaminated wounds as suggested in one RCT concerning the treatment of faecal peritonitis with a relatively low sample size.^{11,81} Microbial concentration in clean surgery, such as in hip or knee arthroplasty, is low, so contact killing as rare targets and detecting

a difference in SSI incidence rate is hard to achieve even with a large sample size.⁸² The biocidal period is limited in time, but it should support natural defences while SSI risk is high. Variability across patients in the duration of microbial persistence in the surgical wound and the body's ability to defend itself may also explain discrepancies between the biocidal activity measured *in vitro* and clinical observations. The extended microbial inhibition reported in studies with sequential ZOI assays and electron microscopy was not observed but cannot be ruled out. It could reduce SSI development after the biocidal phase given the residual amount of triclosan in the suture exceed 90% of the baseline amount.



- TS: Biocidal up to 8 hours in all species with MIC < 2 to 4 µg/mL but no eradication.
- Significant growth resumption after 8 hours: t-test of mean [+48,77; +21,081 CFU/mL], p = 0.0019)
- No significant difference between V+ P+ M+

Figure 12 Summary and comments of study 1 time-kill results

Question 2: How long do implanted sutures with triclosan inhibit *Staphylococcus aureus* in surgical conditions?

Answer 2: Two accelerated *in vitro* model assays reproduced *in vivo* triclosan release conditions from sutures guided by *ex vivo* benchmark marginal triclosan release rates derived from opportunistic explanted intramuscular and subcutaneous suture fragments. A fitted mathematical function predicted triclosan marginal release rate over time when polyglactin 910 braided sutures were immersed in the *in vitro* models of operated tissues. The model and function (a double exponential decay) reproduced the early high triclosan marginal release rate that rapidly declined according to a short half-life and agreed with *ex vivo* benchmarks. The rate transitioned after the first day to a long-lasting slow release plateau for several days. Time-kill analysis of *S. aureus* cultures exposed to artificially aged sutures by immersing them in the two model assays over increasing durations suggested the biocidal antistaphylococcal activity of braided polyglactin 910 with triclosan lasts between 4 and 12 hours after implantation. The fitted function estimated triclosan marginal release rate when the antistaphylococcal effect was no longer detected (summary Figure 13). This relatively short biocidal duration could contribute to differences in the relative risk of SSIs reported across RCTs.

The attempt to extract the entire initial triclosan initial load failed and the extracted amount underestimated the actual initial load given the maximum amount described in suture specifications. The marginal release rate was between 0.175 $\mu\text{g}/\text{m}/\text{h}$ and 0.287 $\mu\text{g}/\text{m}/\text{h}$ when it transitioned to the slow plateau. Released was $< 8 \mu\text{g}/\text{m}$ since the onset of immersion, so the estimated residual amount of triclosan on braided polyglactin 910 sutures was more than 95% of the estimated initial load and more than 99% of the specified maximum ceiling.

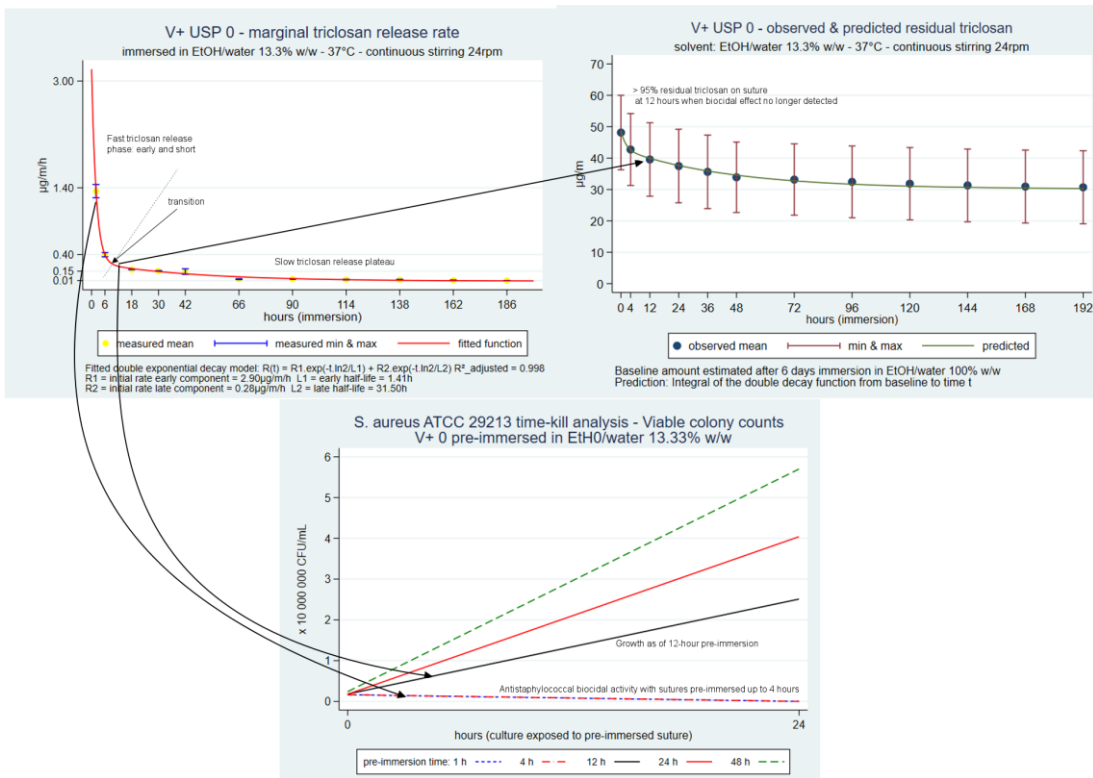


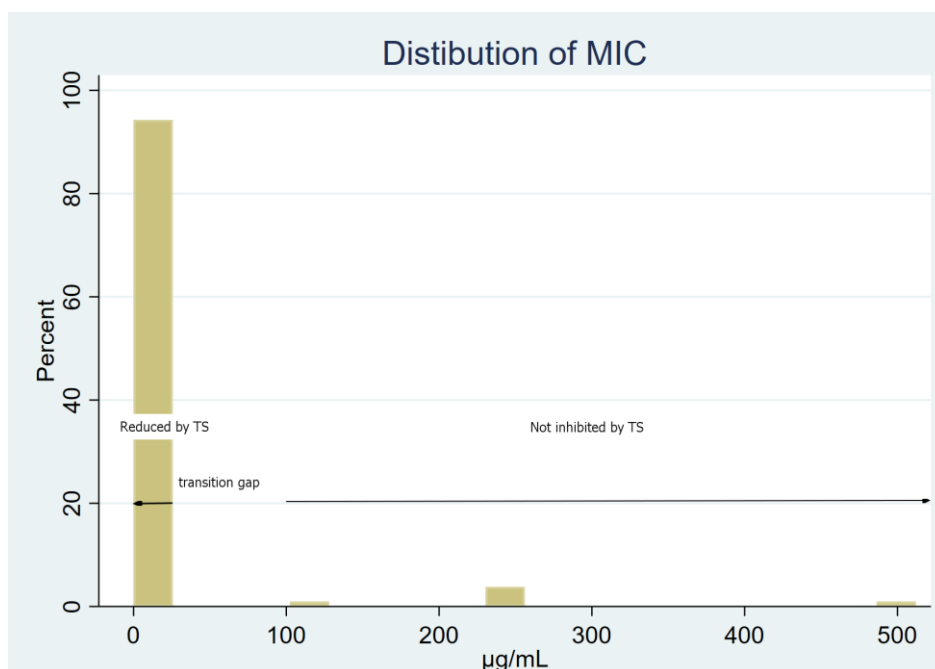
Figure 13 Summary and comments of study 2 - triclosan release & antimicrobial activity

Question 3: Which microorganisms isolated in SSI cultures are inhibited by sutures with triclosan?

Answer 3: The database gathered 128 microbial strains representing 41 species, including 65 strains representing 20 species isolated from spinal SSIs. Microbiological analyses were completed to date in a part of the species: Time-kill analysis with polydioxanone TS in 17/128 strains and triclosan MIC in 105/128 strains. TS was biocidal in all species except *Pseudomonas aeruginosa*, i.e., 88% of tested strains. The MIC ranged from 0.03 µg/mL to 512 µg/mL. *Pseudomonas aeruginosa* and *Pseudomonas balearica* MIC were greater than 128 µg/mL. *Pseudomonas aeruginosa* was not inhibited by TS.

All other microbial strains of all species have triclosan MIC between 0.03 µg/mL and 4 µg/mL (summary Figure 14). All time-kill analyses performed with TS in strains with a MIC less than 4 µg/mL had their concentration reduced with early biocidal levels. Therefore, the transition from TS sensitivity to resistance is to be confirmed with additional data.

The spinal SSI sample agreed with the distribution of species observed in other sources of microbiological data in spinal and neurological SSI, and the incidence rate of *Pseudomonas aeruginosa* in this study was less than 2%.



Question 4: Do triclosan sutures modify SSI microbial diversity?

The validation subquestion is: Is the pooled relative of culture-confirmed SSIs consistent with the pooled relative risk of comprehensive meta-analyses?

Answer 4: The SLR of RCTs shows no significant difference in the microbial relative distribution (i.e., the microbial diversity) in culture-confirmed SSIs after closure with TS compared with NTS (Table VIII). However, this analysis is underpowered to detect an impact of TS antimicrobial activity on SSI microbial diversity by that potential cannot rule out either. The qualitative diversity of the observed microorganisms is similar to that reported in other studies. The top frequencies of *Staphylococci* and *E. coli* and the approximately 5% frequency of *P. aeruginosa* are similar to those reported in national and European SSI surveillance networks.

The study shows a significantly lower risk of culture-confirmed SSI with TS than NTS, but the certainty of the supporting evidence is moderate given the minority of RCTs have a significant individual relative risk to support the pooled RR. This result is similar to those in comprehensive meta-analyses and suggests the RCTs included here are representative of the comprehensive RCT sets.

Table VIII Relation between the microbial relative distribution (diversity) difference and the pooled RR of ccSSI

Genus, n (%)	All studies		
	TS	NTS	Total
<i>Staphylococcus</i>	45 (39.47)	69 (60.53)	114 (100)
<i>Escherichia</i>	22 (29.73)	52 (70.27)	74 (100)
<i>Enterococcus</i>	27 (47.37)	30 (52.63)	57 (100)
<i>Klebsiella</i>	18 (39.13)	28 (60.87)	46 (100)
<i>Pseudomonas</i>	13 (39.39)	20 (60.61)	33 (100)
<i>Enterobacter</i>	9 (42.86)	12 (57.14)	21 (100)
<i>Streptococcus</i>	6 (40.00)	9 (60.00)	15 (100)
<i>Bacteroides</i>	6 (40.00)	9 (60.00)	15 (100)
Total	146 (38.93)	229 (61.07)	375 (100)

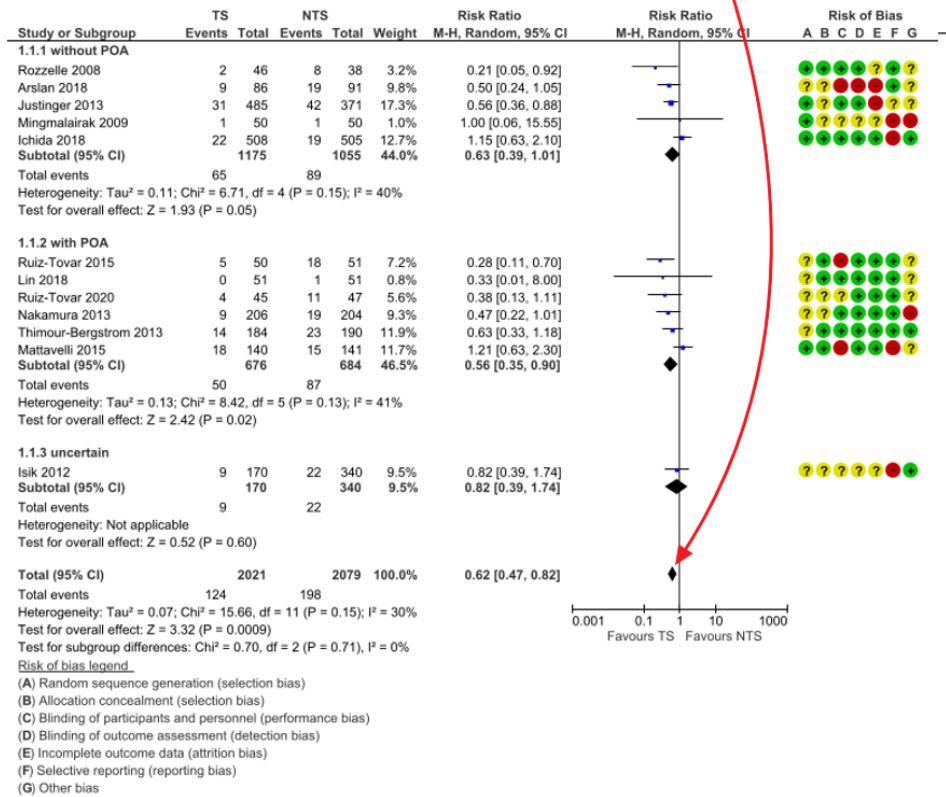
Weak degree of association: Cramér's V = 0.20

Non-significant Chi² p = 0.39 and, Fisher's exact test p = 0.39

Low statistical power 1-β = 0.43

No difference in SSI microbial diversity between TS and NTS despite differences in microbial susceptibility to triclosan.

This could challenge the relationship between TS antimicrobial activity and significant relative risk of SSI. However, the association cannot be ruled out because the test is underpowered.



6.2. DISCUSSION

The three *in vitro* studies produce consistent results and suggest the following:

- Study 1 shows the TS antimicrobial activity mechanism is contact-kill at the suture surface rather than increasing triclosan concentration in the surrounding medium. The surface ratio between the suture surface and exposed surgical site volume is a TS contact-kill limitation in reducing SSI risk.
- Study 1 and study 2 estimate TS antimicrobial activity is biocidal during the first 8 hours after implantation in muscle and subcutaneous tissues (range of 4 to 12 hours). Extended antimicrobial inhibition is not detected. The duration is significantly shorter than the measurements previously published estimates.⁸³⁻⁸⁵ Previously published have not provided data to compare triclosan release by sutures over time in conditions that reproduce operated tissues and its relationship with antimicrobial activity. However, the agreement between the time-kill assays of study 1 and the triclosan marginal release rate in study 2, which examine different endpoints with different methods, are mutually supportive evidence. The time ratio of exposure to microbial seeding and TS biocidal activity between 4 and 12 hours is another limitation in reducing SSI risk with TS.
- Study 1 and Study 3 suggest, given the fragmental analysis of the collected SSI microbial sample, that TS initial biocidal activity applies to most microorganisms found in surgery. Most inhibited strains exhibit a triclosan MIC below 1 µg/mL. TS are inactive on microorganisms with MIC ≥ 128 µg/mL, such as *P. aeruginosa*. A MIC transition zone between 4 and 128 µg/mL, separates TS susceptible from non-susceptible microorganisms. Most tested microorganisms in studies 1 and 3 are on the susceptible side of the zone. Completing microbiological measurements on the collected SSI samples should more accurately estimate the boundaries of the transition zone, determine the microbial proportion on each side, and identify microorganisms that could potentially change susceptibility after exposure to

triclosan. The proportion of microorganisms that are not killed TS during the initial biocidal phase is the third limitation in reducing SSI risk with TS.

- The limitations of antimicrobial activity suggested by studies 1, 2 and 3 must be interpreted with the results of previous studies:
 - Sutures increase SSI risk locally by facilitating microbial proliferation at their surface.³⁴⁻³⁶
 - Triclosan inhibits that phenomenon by preventing biofilm development.^{86,87}
- If SSI risk is maximum in most patients at the end of the operation and progressively declines over time unless locally facilitated by sutures. In that case, TS could minimize local SSI risk and provide a window of opportunity for natural defences to control SSI development in the rest of the surgical site.
- After the TS biocidal phase, residual SSI risk factors may persist, including delayed incision healing, immune deficiency, foreign bodies, damaged tissues, hematoma, and hematogenous microbial seeding.
- Study 4 aimed to reconcile TS antimicrobial activity conclusions from studies 1, 2 and 3 with the pooled RR of SSI from the meta-analysis of RCTs comparing TS *versus* NTS. Study 4 shows no difference in microbial diversity between TS and NTS. It also shows a ratio of the count of *P. aeruginosa* in TS *versus* NTS nearly equal to the ratio of overall microbial count in TS *versus* NTS (ratio = 0.63). Those results suggest that TS antimicrobial activity is not associated with the RR of SSIs. However, the amount of SSI culture results is insufficient to conclude whether microbial diversity in TS and NTS is similar or different (underpowered test). The sample of RCTs with ccSSIs is tested valid because its pooled RR of ccSSIs is consistent with the one produced by the most comprehensive meta-analysis of RCTs published so far, with an overlap of most of the width of the two 95% CIs. The discrepancy of the non-significant pooled microbial diversity test and the significant pooled RR test could be partially explained by the number of observations that provides

enough power to compare a 2-category outcome (SSI or no SSI) per suture arm in the case of the RR, but not enough power to compare an 8-category outcome (the 8 genera) between the same two sutures groups. However, when pooling the 7 genera other than *Pseudomonas*, the contingency table becomes a binomial distribution, and the test remains non-significant with low statistical power (less than 50%). This shows that more observations are required to build up power and determine if the RR of triclosan-resistant microorganisms such as *P. aeruginosa* shifts toward non-inferiority to 1.

- Once at least 80% power is reached, the results will either support or challenge the causal relationship between antimicrobial activity and SSI occurrence. Sample-size calculation shows the total number of observations should be multiplied by about 3.5 for the Chi-squared test of the current contingency table to reach significance. Previous publications have not described this relationship, so these results cannot be compared.
- The evidence from the four studies suggests a schematical model of the relationship between antimicrobial activity and SSI risk (Figure 15). TS antimicrobial activity reduces the overall risk of SSI over time proportionally to suture surface and biocidal duration. This preliminary model does not consider the potential impact of triclosan-resistant microorganisms, but a more advanced model should consider it.

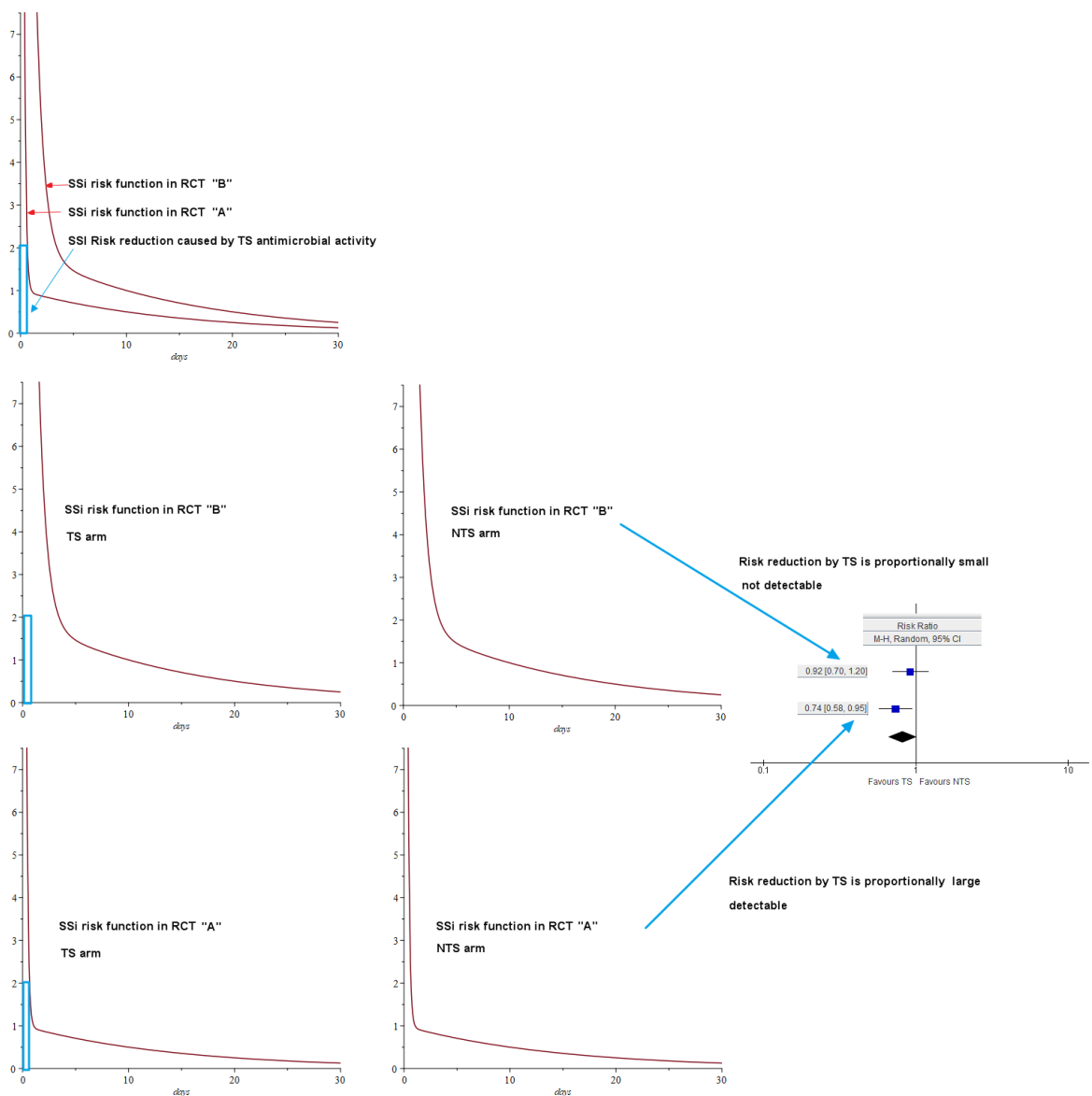


Figure 15 Schematic representation of the proposed effect of TS on SSI risk function

Note: This figure displays only two configurations in the range.

The two risk models, A and B, assume that TS temporarily reduces SSI risk due to its *in vivo* initial contact-killing effect. Given that 85% of RCTs (28/33) are non-significant, model B would apply in 85% of studies.

Models A and B attempt to reconcile *in vitro* findings of studies 1 and 3 with the pooled RR of SSIs in meta-analyses. However, this research cannot rule out bias, given:

- the non-significant and underpowered association test between suture type and microbial diversity.

- the locally increased risk of SSI near the suture and triclosan's potential to inhibit biofilm development.

6.3. LIMITATIONS OF THIS RESEARCH

This research program has various limitations and potential sources of bias.

The main limitations that should be taken into account are the following:

Total triclosan initial load per meter was significantly underestimated because immersion of the sutures in a water/ethanol 100% w/w colsoveny with continuous stirring several failed extracting all the triclosan. Defining methods to erode the structural polymer surface without degrading the triclosan was beyond this research program's scope.

The incomplete extraction of the initial triclosan load from the three absorbable sutures underestimates the residual triclosan amount in the sutures over time.

TS biocidal activity loss within 4 to 12 hours is associated with a reduction in marginal triclosan release rate while most of the initial triclosan load remains on the suture. The assay could lack sensitivity to detect extended microbial inhibition.

The MIC and time-kill analyses of TS were only measured in some of the acquired microbial strains. Therefore, the TS efficacy transition zone estimate, currently between MIC 5 and 127 $\mu\text{g}/\text{mL}$, is less accurate than the collected sample should enable. The proportions of clinical strains below and above the transition zone cannot be accurately estimated for the same reason.

- The unavailability of benchmarks from opportunistically explanted fragments of monofilament sutures to estimate triclosan release in operated tissues and antimicrobial duration with those TS.

- The statistical power to test the difference in microbial diversity between TS and NTS is too low.

- The high risk of bias in individual RCTs and the majority of non-significant RCTs cause the quality of clinical evidence to remain moderate.

7. CONCLUSIONS

Sutures with triclosan display initial biocidal activity on most microbial species in surgery. The main antimicrobial mechanism of action of sutures with triclosan is contact-killing.

The three identified limitations of sutures with triclosan that potentially contribute to the non-significant difference in the incidence rate of surgical site infections in the majority of randomized clinical trials are:

- the small surface of a suture compared to the target volume of a surgical site exposed to microorganisms,
- the maximum duration of the biocidal activity from the immersion of sutures in tissues up to 4 to 12 hours,
- the occasional encounter of microorganisms that are resistant to triclosan that can colonize those sutures, such as *P. aeruginosa*.

The comparison of the microbial diversity in surgical site infections after closure with sutures with *versus* without triclosan did not show a significant difference in microbial diversity consistent with microbial susceptibility to triclosan. The insufficient statistical power of the independence test between microbial genera and suture types prevents concluding whether triclosan modifies surgical site infections' microbial diversity or not. One of the reasons is the lower, although non-significant, frequency of the *Pseudomonas* genus after closure with sutures with triclosan compared to sutures without triclosan.

More observations are required to build up statistical power and determine if the lower frequency of *Pseudomonas* after closure with sutures with triclosan is confirmed.

The meta-analysis of randomized clinical trials shows a significant pooled relative risk of culture-confirmed surgical site infections favouring sutures with triclosan. However, the quality of evidence is moderate, given the risk of bias in individual trials and the minority of significant studies. These conclusions are consistent with those of meta-analyses with more randomized clinical trials and surgical site infections, with or without culture confirmation.

These results are consistent with the conditional recommendations of the World Health Organization to include sutures triclosan in the prevention of surgical site infections. However, they suggest a cautious implementation of that guideline and continue collecting clinical and microbiological data when surgical site infections occur to assess the association between the antimicrobial activity of sutures with triclosan and the risk of surgical site infection.

An in-depth assessment of the residual triclosan after the biocidal phase and and microbial resistance development risk should be considered.

8. APPENDICES

APPENDIX 1: SUPPLEMENTAL MATERIAL OF STUDY 2

Daoud, F.C.; Goncalves, R.; Moore, N. How Long Do Implanted Triclosan Sutures Inhibit Staphylococcus aureus in Surgical Conditions? A Pharmacological Model. *Pharmaceutics* 2022, 14, 539.

The published supplemental material file is copied below.

How Long Do Implanted Triclosan Sutures Inhibit *Staphylococcus aureus* in Surgical Conditions? A Pharmacological Model

Frederic C. Daoud, Ruben Goncalves and Nicholas Moore

1. Tested Sutures:

Vicryl PLUS USP 0. Part number VCP518H. Lot QGBDRLW0. Expiry date 2023-05
 Vicryl PLUS USP 1. Part number VCP359H. Lot QGBCSSW0. Expiry date 2023-05-31
 Vicryl PLUS USP 2-0. Part number VCP317H. Lot QHBBHJW0. Expiry date 2023-06
 PDS PLUS USP 2-0. Part number VCP317H. Lot QGMHDDH. Expiry date 2022-05
 Monocryl PLUS USP 2-0. Part number VCP317H. Lot QBMCT. Expiry date 2022-01
 Triclosan (powder) Sigma-Aldrich PHR 1338-1G. Lot LRAC4483. Expiry date 2023-10

Table S1. In vitro water and ex vivo sample preparation schedule.

Water without Stirring	Tube 1	Tube 2	Tube 3	Tube 4	Pre-Immersion	Marginal Immersion
P+, M+, V+ USP 2-0 (2 samples each)	4 h	-	-	-	-	4 h
P+, M+, V+ USP 2-0 (2 samples each)	8 h	-	-	-	-	8 h
P+, M+, V+ USP 2-0 (2 samples each)	12 h	-	-	-	-	12 h
P+, M+, V+ USP 2-0 (2 samples each)	1 d	12 h	-	-	1 d	12 h
P+, M+, V+ USP 2-0 (2 samples each)	2 d	12 h	-	-	2 d	12 h
P+, M+, V+ USP 2-0 (2 samples each)	3 d	12 h	-	-	3 d	12 h
P+, M+, V+ USP 2-0 (2 samples each)	4 d	12 h	-	-	4 d	12 h
P+, M+, V+ USP 2-0 (2 samples each)	5 d	12 h	-	-	5 d	12 h
P+, M+, V+ USP 2-0 (2 samples each)	10 d	-	12 h	-	10 d	12 h
P+, M+, V+ USP 2-0 (2 samples each)	10 d	5 d	12 h	-	15 d	12 h
P+, M+, V+ USP 2-0 (2 samples each)	10 d	10 d	12 h	-	20 d	12 h
P+, M+, V+ USP 2-0 (2 samples each)	10 d	10 d	5 d	12 h	25 d	12 h
P+, M+, V+ USP 2-0 (2 samples each)	10 d	10 d	10 d	12 h	30 d	12 h
V+ USP 0 (6 samples)	7 d	12 h	-	-	7 d	12 h
V+ USP 1 (6 samples)	7 d	12 h	-	-	7 d	12 h
V+ USP 0 explanted from subcutaneous tissue (6 samples)	7 d	12 h	-	-	7 d	12 h
V+ USP 1 explanted from muscle (6 samples)	7 d	12 h	-	-	7 d	12 h

h: hours, *d*: days.

Table S2. Modeling phase–Sample preparation–EtOH/water with stirring.

13.30% w/w V+ USP 0 (2 samples)	13.30% w/w V+ USP 1 (2 samples)	3.30% w/w V+ USP 0 (2 samples)	3.30% w/w V+ USP 1 (2 samples)	50.00% w/w V+ USP 0 (2 samples)	50.00% w/w V+ USP 1 (2 samples)
1: 0 to 4 h	1: 0 to 4 h	1: 0 to 4 h	1: 0 to 4 h	1: 0 to 144 h	1: 0 to 144 h
2: 4 to 12 h	2: 4 to 12 h	2: 4 to 12 h	2: 4 to 12 h		
3: 12 to 24 h	3: 12 to 24 h	3: 12 to 24 h	3: 12 to 24 h	50.00% w/w	50.00% w/w
4: 24 to 36 h	4: 24 to 36 h	4: 24 to 36 h	4: 24 to 36 h	V+ USP 2-0	P+ USP 2-0

5: 36 to 48 h	5: 36 to 48 h	5: 36 to 48 h	5: 36 to 48 h	(2 samples) 1: 0 to 144 h	(2 samples) 1: 0 to 144 h
6: 48 to 72 h	6: 48 to 72 h	6: 48 to 72 h	6: 48 to 72 h	50.00% w/w M+ USP 2-0 (2 samples) 1: 0 to 144 h	
7: 72 to 96 h	7: 72 to 96 h	7: 72 to 96 h	7: 72 to 96 h		
8: 96 to 120 h	8: 96 to 120 h	8: 96 to 120 h	8: 96 to 120 h		
9: 120 to 144 h	9: 120 to 144 h	9: 120 to 144 h	9: 120 to 144 h		
10: 144 to 168 h	10: 144 to 168 h	10: 144 to 168 h	10: 144 to 168 h		
11: 168 to 192 h	11: 168 to 192 h	11: 168 to 192 h	11: 168 to 192 h		

Table S3. Estimate of marginal release rate µg/m/h—in static water.

Medium	Suture	Pre-Immersion days	Immersion Hours	N	Min	Mean	Max
water	Vicryl+ 2-0	0	4	2	1.37143	1.69286	2.01429
		0	8	2	1.17143	1.17143	1.17143
		0	12	2	0.60476	0.68929	0.77381
		1	12	2	0.45238	0.61548	0.77857
		2	12	2	0.08595	0.39655	0.70714
		3	12	2	0.34762	0.63690	0.92619
		4	12	2	0.44524	0.69405	0.94286
		5	12	2	0.29762	0.75833	1.21905
		10	12	2	0.44286	0.49167	0.54048
		15	12	2	0.27381	0.27976	0.28571
		20	12	2	0.27619	0.27857	0.28095
		25	12	2	0.17333	0.19440	0.21548
		30	12	1	0.09500	0.09500	0.09500
		water	PDS+ 2-0	0	4	2	2.83571
0	8			2	2.11429	2.38214	2.65000
0	12			2	1.47857	1.49048	1.50238
1	12			2	0.79286	0.89762	1.00238
2	12			2	0.68095	0.72619	0.77143
3	12			2	0.71429	0.83333	0.95238
4	12			2	0.78333	0.82857	0.87381
5	12			2	0.72143	0.84048	0.95952
10	12			2	0.70000	0.76786	0.83571
15	12			2	0.64048	0.65476	0.66905
20	12			2	0.70952	0.73571	0.76190
25	12			2	0.60714	0.62976	0.65238
30	12			2	0.52381	0.53333	0.54286
water	Monocryl+ 2-0			0	4	2	5.20714
		0	8	2	3.13929	3.43393	3.72857
		0	12	2	2.08810	2.18333	2.27857
		1	12	2	1.03810	1.24762	1.45714
		2	12	2	0.97381	1.21548	1.45714
		3	12	2	0.65952	0.99405	1.32857
		4	12	2	0.86429	1.12500	1.38571
		5	12	2	0.97619	0.99643	1.01667
		10	12	2	0.74048	0.77738	0.81429
		15	12	2	0.52143	0.59286	0.66429
		20	12	2	0.50476	0.55595	0.60714
		25	12	2	0.38810	0.41310	0.43810

30 12 2 0.49762 0.50357 0.50952

Table S4. Ex vivo marginal release rate benchmarks versus in vitro controls (µg/m/h).

Medium/Solvent	Suture	Days Immersed	N	Min	Mean	Max	SD
Static water	V+ 0	7	6	0.17881	0.20262	0.21952	0.01388
Static water	V+ 1	7	6	0.25714	0.30397	0.34524	0.03229
subcutaneous	V+ 0	7	6	0.00913	0.01486	0.02482	0.00652
intramuscular	V+ 1	7	6	0.00345	0.00473	0.00612	0.00095

Ex Vivo Animal Benchmarking Data—Tests of Significance

Comparison of triclosan release rate of V + 0 after explantation from subcutaneous tissue on day-7 vs. immersion in pure water 7 days
 ttest microgpmph if (suture == 4), unequal by(medium)

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
Static water	6	0.2026	0.0057	0.0139	0.1881	0.2172
Subcutaneous	6	0.0149	0.0027	0.0065	0.0080	0.0217
Combined	12	0.1087	0.0285	0.0956	0.0541	0.1714
diff		0.1878	0.0062		0.2654	0.2017
diff = mean(water)-mean(subcutaneous)					t = 29.9901	
H0: diff = 0					Satterthwaite's dF = 10	
Ha: diff < 0			Ha: diff! = 0		Ha: diff > 0	
Pr(T < t) = 1.0000			Pr(T > t) = 0.0000		Pr(T > t) = 0.0000	

Ratio of marginal release in water/subcutaneous tissue

Mean	Coefficient	Std. Err.	z	P > z	[95% Conf.	Interval]
_nl_1	13.634	2.471	5.52	0.000	8.791	18.478

Comparison of triclosan release rate of V+1 after explantation from muscle on day-7 vs. immersion in pure water 7 days: t-test

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
Static water	6	0.3040	0.0132	0.0323	0.2701	0.3378
muscle	6	0.0047	0.0004	0.0010	0.0037	0.0057
Combined	12	0.1544	0.0455	0.1578	0.0541	0.2546
diff		0.2992	0.0132		0.2654	0.3331
diff = mean(water)-mean(muscle)					t = 22.6931	
H0: diff = 0					Satterthwaite's dF = 5.00874	
Ha: diff < 0			Ha: diff! = 0		Ha: diff > 0	
Pr(T < t) = 1.0000			Pr(T > t) = 0.0000		Pr(T > t) = 0.0000	

Ratio of marginal release in water/muscle

Mean	Coefficient	Std. Err.	z	P>z	[95% Conf.	Interval]
_nl_1	64.208	5.974	10.75	0.000	52.498	75.918

Ratio of marginal release V+1 subcutaneous/V + 0 intramuscular

Mean	Coefficient	Std. Err.	z	P > z	[95% Conf.	Interval]
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<u>_nl_1</u>	3.139	0.619	5.07	0.000	1.926	4.352
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Ratio of marginal release V+1/V+0 after 7 days in water both

Mean	Coefficient	Std. Err.	z	P > z	[95% Conf.	Interval]
<u>_nl_1</u>	0.667	0.034	19.38	0.000	0.599	0.734

Table S5. Cumulative and marginal release rates calculated from raw data with EtOH/water solvent.

Solvent/Medium	Suture Type	N	Cumulative Immersion Hours	Cumulative Release (µg/m)			Marginal Immersion Hours	Marginal Release Rate (µg/m/h)		
				Min	Mean	Max		Min	Mean	Max
EtHO/water 3.3% w/w	Vicryl+ USP 0	2	4	2.685714	2.814286	2.942857	4	0.6714286	0.7035714	0.7357143
		2	12	4.928571	5.114286	5.3	12	0.2803572	0.2875	0.2946429
		2	24	6.771429	6.792857	6.814286	24	0.122619	0.139881	0.1571429
		2	36	8.342857	8.385715	8.428572	36	0.1273809	0.1327381	0.1380952
		2	48	9.274286	9.522857	9.771428	48	0.0704762	0.0947619	0.1190476
		2	72	9.951428	10.24571	10.54	72	0.0282143	0.030119	0.0320238
		2	96	10.50286	10.78143	11.06	96	0.0216667	0.0223214	0.0229762
		2	120	10.96286	11.19286	11.42286	120	0.015119	0.0171429	0.0191667
		2	144	11.45143	11.57386	11.69629	144	0.0113929	0.015875	0.0203571
		2	168	11.91657	11.92543	11.93429	168	0.0091786	0.0146488	0.020119
2	192	12.12343	12.18171	12.24	192	0.008619	0.0106786	0.0127381		
2	216	12.27257	12.374	12.47543	216	0.0062143	0.0080119	0.0098095		
EtHO/water 3.3% w/w	Vicryl+ USP 1	2	4	3.6	3.7	3.8	4	0.9	0.925	0.95
		2	12	6.628572	7	7.371428	12	0.3785714	0.4125	0.4464286
		2	24	9	9.435714	9.871428	24	0.197619	0.2029762	0.2083333
		2	36	10.9	11.31429	11.72857	36	0.1547619	0.1565476	0.1583333
		2	48	12.64286	12.91429	13.18571	48	0.1214286	0.1333333	0.1452381
		2	72	13.51143	13.75857	14.00571	72	0.0341667	0.0351786	0.0361905
		2	96	14.12	14.30143	14.48286	96	0.019881	0.022619	0.0253571
		2	120	14.52857	14.70429	14.88	120	0.0165476	0.0167857	0.0170238
		2	144	15.05714	15.12429	15.19143	144	0.0129762	0.0175	0.0220238
		2	168	15.34571	15.38243	15.41914	168	0.0094881	0.010756	0.0120238
2	192	15.64571	15.74957	15.85343	192	0.0125	0.0152976	0.0180952		
2	216	15.912	16.00157	16.09114	216	0.0099048	0.0105	0.0110952		
EtHO/water 13.3% w/w	Vicryl+ USP 0	2	4	5	5.4	5.8	4	1.25	1.35	1.45
		2	12	8.428572	8.571429	8.714286	12	0.3642857	0.3964286	0.4285714
		2	24	10.52857	10.67143	10.81429	24	0.175	0.175	0.175
		2	36	12.38571	12.52857	12.67143	36	0.1547619	0.1547619	0.1547619
		2	48	13.61143	14.25143	14.89143	48	0.1021429	0.1435714	0.185
		2	72	14.48	14.97286	15.46571	72	0.0239286	0.0300595	0.0361905
		2	96	15.29714	15.71572	16.13429	96	0.0278571	0.0309524	0.0340476
		2	120	15.95429	16.29	16.62572	120	0.0204762	0.0239286	0.027381
		2	144	16.58571	16.85143	17.11714	144	0.0204762	0.0233929	0.0263095
		2	168	16.97714	17.21286	17.44857	168	0.0138095	0.0150595	0.0163095
2	192	17.22486	17.44243	17.66	192	0.0088095	0.0095655	0.0103214		
EtHO/water 13.3% w/w	Vicryl+ USP 1	2	4	6.8	6.871429	6.942857	4	1.7	1.717857	1.735714
		2	12	12.68571	13.05714	13.42857	12	0.7357143	0.7732143	0.8107143
		2	24	16.17143	16.42857	16.68571	24	0.2714286	0.2809524	0.2904762
		2	36	18.2	18.27143	18.34286	36	0.1380952	0.1535714	0.1690476
		2	48	19.39429	20.01	20.62572	48	0.087619	0.144881	0.2021429
		2	72	20.03429	20.72	21.40571	72	0.0266667	0.0295833	0.0325
2	96	20.59143	21.29714	22.00286	96	0.0232143	0.0240476	0.024881		

2	120	20.92571	21.71286	22.5	120	0.0139286	0.0173214	0.0207143
2	144	21.26	22.08143	22.90286	144	0.0139286	0.0153571	0.0167857
2	168	21.43714	22.39286	23.34857	168	0.007381	0.0129762	0.0185714
2	192	21.77143	22.65743	23.54343	192	0.008119	0.0110238	0.0139286

Table S6. Residual amount of triclosan over time with in EtOH/water 13.3% w/w.

Suture/Method	A0	4 h	12 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h	174 h
V+ 0 measured	48.1	42.7	39.5	37.4	33.8	33.1	32.4	31.8	31.2	30.9	30.8
V+ 0 predicted	48.1	42	39.3	37.0	33.9	32.1	31.1	30.4	30.1	29.8	29.8
V+ 1 measured	52.3	45.4	39.2	35.9	32.3	31.6	31.0	30.6	30.2	29.9	29.8
V+ 1 predicted	52.3	45.1	40.1	36.7	33.1	31.6	30.9	30.6	30.5	30.4	30.4

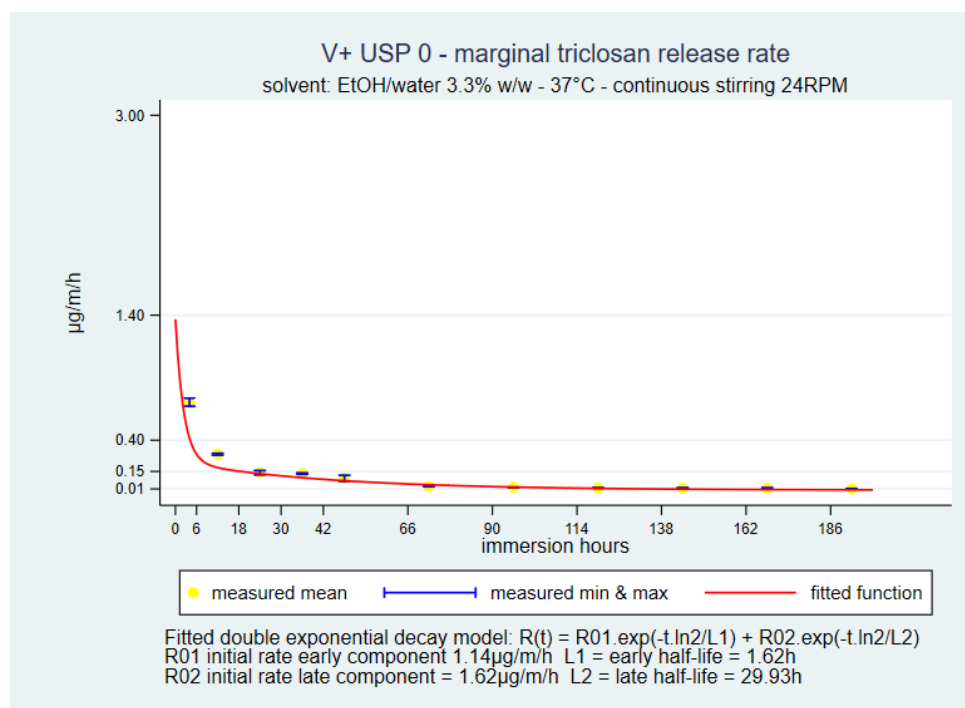


Figure S1. V+ USP 0 in 3.3% w/w release rates and double exponential fitting.

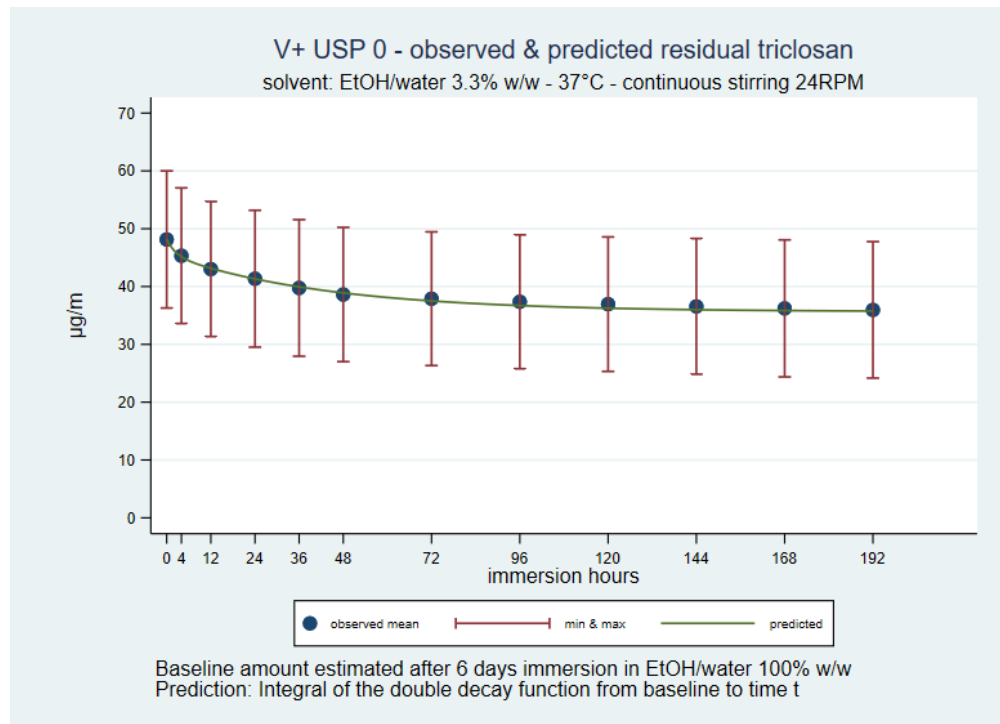


Figure S2. V+ USP 0 in 3.3% w/w predicted residual triclosan.

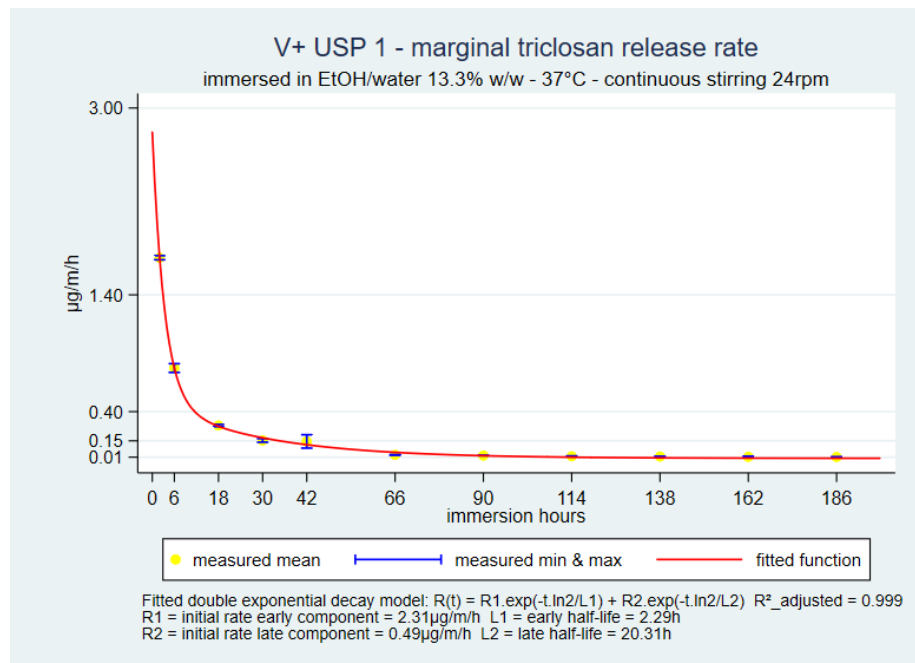


Figure S3. V+ USP 1 in 13.3% w/w release rates and double exponential fitting.

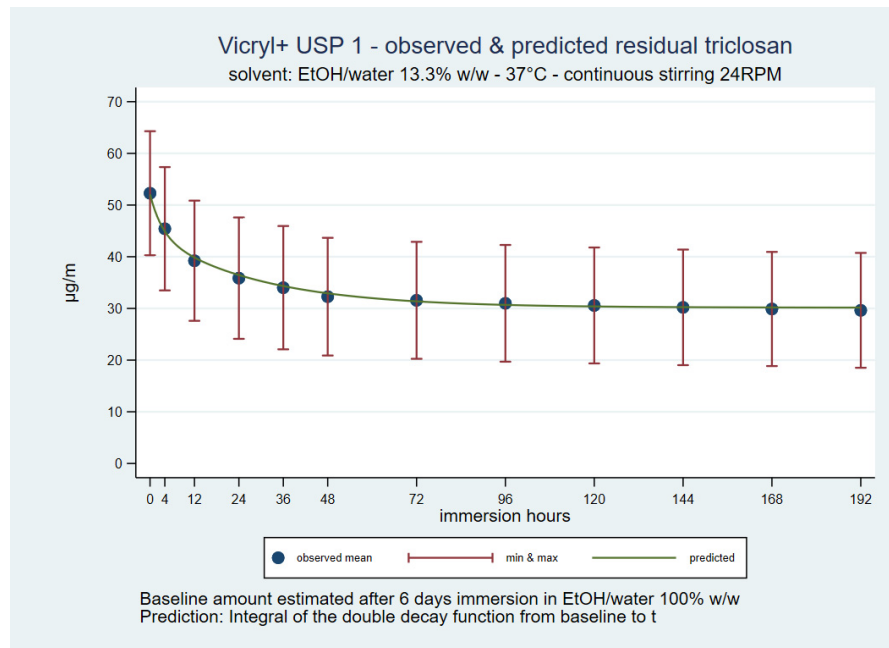


Figure S4. V+ USP 1 in 13.3% w/w predicted residual triclosan.

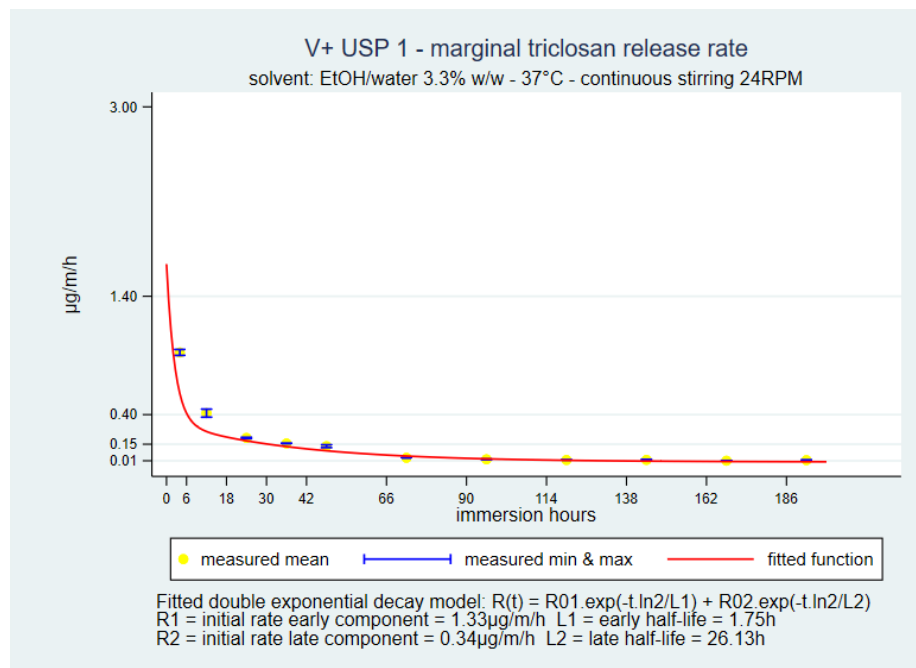


Figure S5. V+ USP 1 in 3.3% w/w release rates and double exponential fitting.

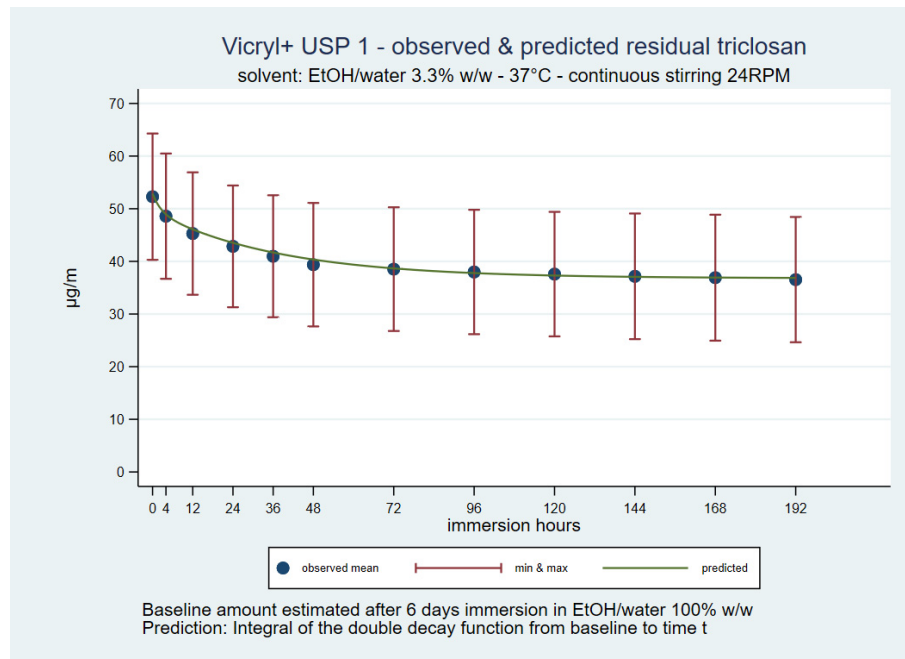


Figure S6. V+ USP 1 in 3.3% w/w predicted residual triclosan.

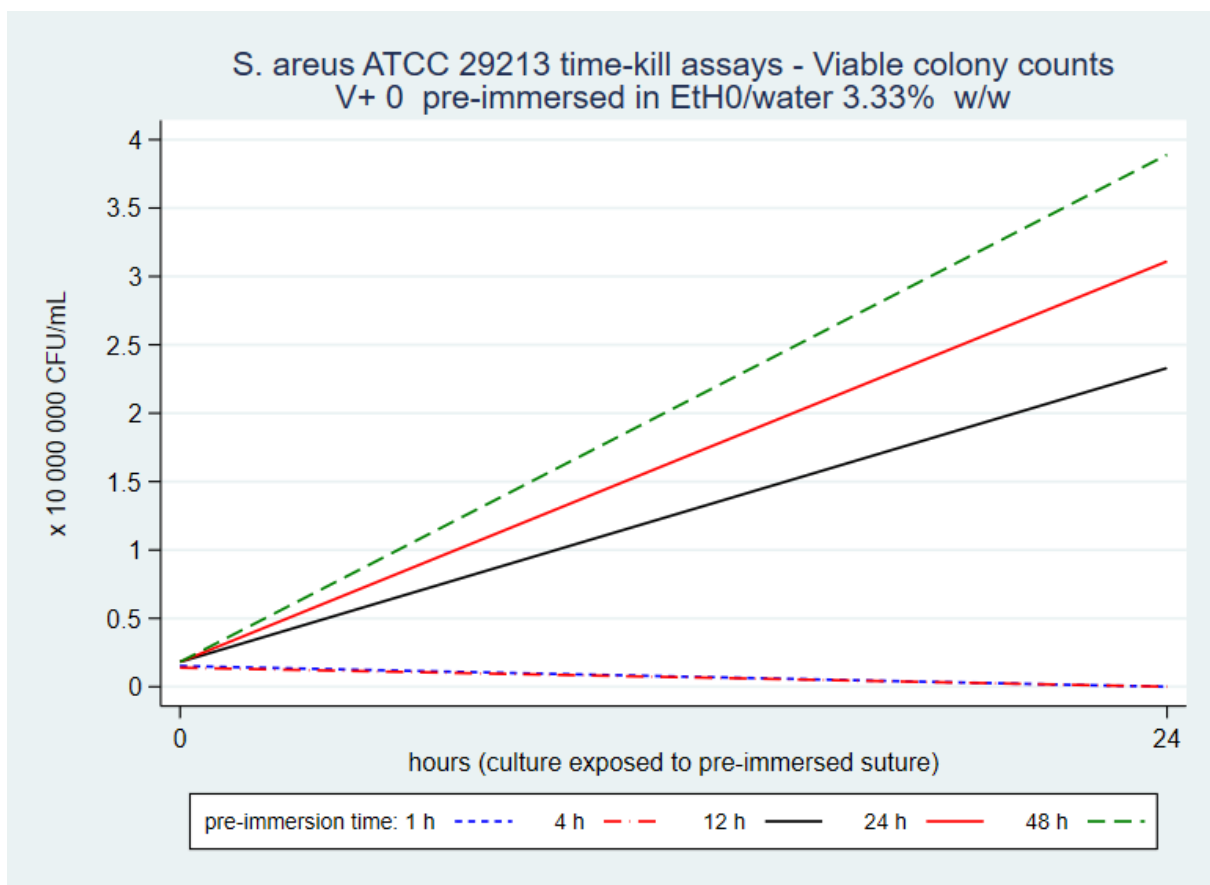


Figure S7. V+ USP 0 in 3.3% w/w time-kill assay.

Raw Data

Raw data-Exploratory phase: Static water immersion & animal explanted sutures

Sample Id	Tube	Suture Type & Caliber (USP)	Solvent/Medium	Solvent Volume (L)	Segment Length (m)	Immersion Time to Determination (hours)	Pre-Immersion Time (Hours)	Triclosan Concentration (µg/L)
19822	34 C Neg	control	water	0.01	0.35	0	0	0
19823	1 V+	Vicryl+ 2-0	water	0.01	0.35	4	0	192
19824	2 V+	Vicryl+ 2-0	water	0.01	0.35	4	0	282
19825	7 V+	Vicryl+ 2-0	water	0.01	0.35	8	0	328
19826	8 V+	Vicryl+ 2-0	water	0.01	0.35	8	0	328
19827	13 V+	Vicryl+ 2-0	water	0.01	0.35	12	0	325
19828	14 V+	Vicryl+ 2-0	water	0.01	0.35	12	0	254
19829	19B V+	Vicryl+ 2-0	water	0.01	0.35	12	24	327
19830	22B V+	Vicryl+ 2-0	water	0.01	0.35	12	48	297
19831	25B V+	Vicryl+ 2-0	water	0.01	0.35	12	72	389
19832	28B V+	Vicryl+ 2-0	water	0.01	0.35	12	96	396
19833	31B V+	Vicryl+ 2-0	water	0.01	0.35	12	120	512
19834	19A V+	Vicryl+ 2-0	water	0.01	0.35	24	0	676
19835	22A V+	Vicryl+ 2-0	water	0.01	0.35	48	0	612
19836	25A V+	Vicryl+ 2-0	water	0.01	0.35	72	0	900
19837	28A V+	Vicryl+ 2-0	water	0.01	0.35	96	0	672
19838	31A V+	Vicryl+ 2-0	water	0.01	0.35	120	0	776
19839	3 P+	PDS+ 2-0	water	0.01	0.35	4	0	397
19840	4 P+	PDS+ 2-0	water	0.01	0.35	4	0	654
19841	9 P+	PDS+ 2-0	water	0.01	0.35	8	0	742
19842	10 P+	PDS+ 2-0	water	0.01	0.35	8	0	592
19843	15 P+	PDS+ 2-0	water	0.01	0.35	12	0	631
19844	16 P+	PDS+ 2-0	water	0.01	0.35	12	0	621
19845	20B P+	PDS+ 2-0	water	0.01	0.35	12	24	421
19846	23B P+	PDS+ 2-0	water	0.01	0.35	12	48	324
19847	26B P+	PDS+ 2-0	water	0.01	0.35	12	72	400
19848	29B P+	PDS+ 2-0	water	0.01	0.35	12	96	367
19849	32B P+	PDS+ 2-0	water	0.01	0.35	12	120	403
19850	20A P+	PDS+ 2-0	water	0.01	0.35	24	0	608
19851	23A P+	PDS+ 2-0	water	0.01	0.35	48	0	575
19852	26A P+	PDS+ 2-0	water	0.01	0.35	72	0	465
19853	29A P+	PDS+ 2-0	water	0.01	0.35	96	0	507
19854	32A P+	PDS+ 2-0	water	0.01	0.35	120	0	474
19855	5 M+	Monocryl+ 2-0	water	0.01	0.35	4	0	797
19856	6 M+	Monocryl+ 2-0	water	0.01	0.35	4	0	729
19857	11 M+	Monocryl+ 2-0	water	0.01	0.35	8	0	879
19858	12 M+	Monocryl+ 2-0	water	0.01	0.35	8	0	1044
19859	17 M+	Monocryl+ 2-0	water	0.01	0.35	12	0	957
19860	18 M+	Monocryl+ 2-0	water	0.01	0.35	12	0	877
19861	21B M+	Monocryl+ 2-0	water	0.01	0.35	12	24	612
19862	24B M+	Monocryl+ 2-0	water	0.01	0.35	12	48	612
19863	27B M+	Monocryl+ 2-0	water	0.01	0.35	12	72	558
19864	30B M+	Monocryl+ 2-0	water	0.01	0.35	12	96	582
19865	33B M+	Monocryl+ 2-0	water	0.01	0.35	12	120	427
19866	21A M+	Monocryl+ 2-0	water	0.01	0.35	24	0	741
19867	24A M+	Monocryl+ 2-0	water	0.01	0.35	48	0	776
19868	27A M+	Monocryl+ 2-0	water	0.01	0.35	72	0	571
19869	30A M+	Monocryl+ 2-0	water	0.01	0.35	96	0	569
19870	33A M+	Monocryl+ 2-0	water	0.01	0.35	120	0	571

1322	150B NEGATIVE CON	control	water	0.01	0.35	0	0	0
1335	134B V+	Vicryl+ 2-0	water	0.01	0.35	12	24	190
1336	131B V+	Vicryl+ 2-0	water	0.01	0.35	12	48	36.1
1337	128B V+	Vicryl+ 2-0	water	0.01	0.35	12	72	146
1338	125B V+	Vicryl+ 2-0	water	0.01	0.35	12	96	187
1339	122B V+	Vicryl+ 2-0	water	0.01	0.35	12	120	125
1340	119B2 V+	Vicryl+ 2-0	water	0.01	0.35	12	240	186
1341	119B1 V+	Vicryl+ 2-0	water	0.01	0.35	12	240	227
1342	122B2 V+	Vicryl+ 2-0	water	0.01	0.35	12	360	120
1343	122B1 V+	Vicryl+ 2-0	water	0.01	0.35	12	360	115
1344	125B2 V+	Vicryl+ 2-0	water	0.01	0.35	12	480	118
1345	125B1 V+	Vicryl+ 2-0	water	0.01	0.35	12	480	116
1346	128B2 V+	Vicryl+ 2-0	water	0.01	0.35	12	600	72.8
1347	128B1 V+	Vicryl+ 2-0	water	0.01	0.35	12	600	90.5
1348	131B2 V+	Vicryl+ 2-0	water	0.01	0.35	12	720	39.9
1350	135B P+	PDS+ 2-0	water	0.01	0.35	12	24	333
1351	132B P+	PDS+ 2-0	water	0.01	0.35	12	48	286
1352	129B P+	PDS+ 2-0	water	0.01	0.35	12	72	300
1353	126B P+	PDS+ 2-0	water	0.01	0.35	12	96	329
1354	123B P+	PDS+ 2-0	water	0.01	0.35	12	120	303
1355	120B2 P+	PDS+ 2-0	water	0.01	0.35	12	240	351
1356	120B1 P+	PDS+ 2-0	water	0.01	0.35	12	240	294
1357	123B2 P+	PDS+ 2-0	water	0.01	0.35	12	360	269
1358	123B1 P+	PDS+ 2-0	water	0.01	0.35	12	360	281
1359	126B2 P+	PDS+ 2-0	water	0.01	0.35	12	480	320
1360	126B1 P+	PDS+ 2-0	water	0.01	0.35	12	480	298
1361	129B2 P+	PDS+ 2-0	water	0.01	0.35	12	600	255
1362	129B1 P+	PDS+ 2-0	water	0.01	0.35	12	600	274
1363	132B2 P+	PDS+ 2-0	water	0.01	0.35	12	720	228
1364	132B1 P+	PDS+ 2-0	water	0.01	0.35	12	720	220
1365	136B M+	Monocryl+ 2-0	water	0.01	0.35	12	24	436
1366	133B M+	Monocryl+ 2-0	water	0.01	0.35	12	48	409
1367	130B M+	Monocryl+ 2-0	water	0.01	0.35	12	72	277
1368	127B M+	Monocryl+ 2-0	water	0.01	0.35	12	96	363
1369	124B M+	Monocryl+ 2-0	water	0.01	0.35	12	120	410
1370	121B2 M+	Monocryl+ 2-0	water	0.01	0.35	12	240	311
1371	121B1 M+	Monocryl+ 2-0	water	0.01	0.35	12	240	342
1372	124B2 M+	Monocryl+ 2-0	water	0.01	0.35	12	360	279
1373	124B1 M+	Monocryl+ 2-0	water	0.01	0.35	12	360	219
1374	127B2 M+	Monocryl+ 2-0	water	0.01	0.35	12	480	255
1375	127B1 M+	Monocryl+ 2-0	water	0.01	0.35	12	480	212
1376	130B2 M+	Monocryl+ 2-0	water	0.01	0.35	12	600	163
1377	130B1 M+	Monocryl+ 2-0	water	0.01	0.35	12	600	184
1378	133B2 M+	Monocryl+ 2-0	water	0.01	0.35	12	720	209
1379	133B1 M+	Monocryl+ 2-0	water	0.01	0.35	12	720	214
1310	V-0-C1.1-B	Vicryl+ 0	subcutaneous	0.0063	0.22	12	168	4.11
1311	V-0-C1.2-B	Vicryl+ 0	subcutaneous	0.0063	0.22	12	168	3.97
1312	V-0-C2.1-B	Vicryl+ 0	subcutaneous	0.0063	0.22	12	168	6.76
1313	V-0-C2.2-B	Vicryl+ 0	subcutaneous	0.0063	0.22	12	168	10.4
1318	V-0-C3.1-B	Vicryl+ 0	subcutaneous	0.00495	0.1725	12	168	3.82
1319	V-0-C3.2-B	Vicryl+ 0	subcutaneous	0.00495	0.1725	12	168	8.28
1314	V-1-M1.1-B	Vicryl+ 1	intramuscular	0.01	0.35	12	168	1.45
1315	V-1-M1.2-B	Vicryl+ 1	intramuscular	0.01	0.35	12	168	2.57
1316	V-1-M2.1-B	Vicryl+ 1	intramuscular	0.01	0.35	12	168	2.04
1317	V-1-M2.2-B	Vicryl+ 1	intramuscular	0.01	0.35	12	168	2.13
1320	V-1-M3.1-B	Vicryl+ 1	intramuscular	0.01	0.35	12	168	1.62
1321	V-1-M3.2-B	Vicryl+ 1	intramuscular	0.01	0.35	12	168	2.12
1323	V-0-C1-B	Vicryl+ 0	water	0.01	0.35	12	168	82.5
1324	V-0-C2-B	Vicryl+ 0	water	0.01	0.35	12	168	75.1

1325	V-0-C3-B	Vicryl+ 0	water	0.01	0.35	12	168	87.1
1326	V-0-C4-B	Vicryl+ 0	water	0.01	0.35	12	168	85.7
1327	V-0-C5-B	Vicryl+ 0	water	0.01	0.35	12	168	88
1328	V-0-C6-B	Vicryl+ 0	water	0.01	0.35	12	168	92.2
1329	V-1-C1-B	Vicryl+ 1	water	0.01	0.35	12	168	145
1330	V-1-C2-B	Vicryl+ 1	water	0.01	0.35	12	168	141
1331	V-1-C3-B	Vicryl+ 1	water	0.01	0.35	12	168	127
1332	V-1-C4-B	Vicryl+ 1	water	0.01	0.35	12	168	122
1333	V-1-C5-B	Vicryl+ 1	water	0.01	0.35	12	168	108
1334	V-1-C6-B	Vicryl+ 1	water	0.01	0.35	12	168	123
8925	V0.p.total.1	Vicryl+ 0	EtHO/H2O 100% w/w	0.01	0.35		144	2100
8926	V1.p.total.1	Vicryl+ 1	EtHO/H2O 100% w/w	0.01	0.35		144	2250
8927	V2-0.p.total.1	Vicryl+ 2-0	EtHO/H2O 100% w/w	0.01	0.35		144	1860
8928	PDS2- 0.p.total.1	PDS+ 2-0	EtHO/H2O 100% w/w	0.01	0.35		144	16500
8929	V0.p.total.2	Vicryl+ 0	EtHO/H2O 100% w/w	0.01	0.35		144	1270
8930	V1.p.total.2	Vicryl+ 1	EtHO/H2O 100% w/w	0.01	0.35		144	1410
8931	V2-0.p.total.2	Vicryl+ 2-0	EtHO/H2O 100% w/w	0.01	0.35		144	1470
8932	PDS2- 0.p.total.2	PDS+ 2-0	EtHO/H2O 100% w/w	0.01	0.35		144	22100
8933	M2-0.p.total.1	Monocryl+ 2-0	EtHO/H2O 100% w/w	0.01	0.35		144	41000
8934	M2-0.p.total.2	Monocryl+ 2-0	EtHO/H2O 100% w/w	0.01	0.35		144	34000

Raw data-Modeling phase: Ethanol/Water 13.3% w/w and 3.3% w/w solutions

Sample Id	Tube	Suture Type & Caliber (USP)	Solvent/Medium	Solvent Volume (L)	Segment Length (m)	Immersion Time to Determination (Hours)	Triclosan Concentration Measured (µg/L)
8833	V0p.1.33_0_4	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	4	103
8834	V0p.2.33_0_4	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	4	94
8837	V0p.1.33_4_12	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	8	82.5
8838	V0p.2.33_4_12	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	8	78.5
8841	V0p.1.33_12_24	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	12	51.5
8842	V0p.2.33_12_24	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	12	66
8845	V0p.1.33_24_36	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	12	58
8846	V0p.2.33_24_36	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	12	53.5
8849	V0p.1.33_36_48	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	12	29.6
8850	V0p.2.33_36_48	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	12	50
8853	V0p.1.33_48_72	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	23.7
8854	V0p.2.33_48_72	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	26.9
8857	V0p.1.33_72_96	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	19.3
8858	V0p.2.33_72_96	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	18.2
8861	V0p.1.33_96_120	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	16.1
8862	V0p.2.33_96_120	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	12.7
8865	V0p.1.33_120_144	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	17.1
8866	V0p.2.33_120_144	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	9.57
8869	V0p.1.33_144_168	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	16.9
8870	V0p.2.33_144_168	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	7.71
8873	V0p.1.33_168_192	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	10.7
8874	V0p.2.33_168_192	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	7.24
8877	V0p.1.33_192_216	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	8.24
8878	V0p.2.33_192_216	Vicryl+ 0	EtHO/H2O 3.3% w/w	0.01	0.35	24	5.22
8835	V1p.1.33_0_4	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	4	133

8836	V1p.2.33_0_4	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	4	126
8839	V1p.1.33_4_12	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	8	125
8840	V1p.2.33_4_12	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	8	106
8843	V1p.1.33_12_24	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	12	87.5
8844	V1p.2.33_12_24	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	12	83
8847	V1p.1.33_24_36	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	12	65
8848	V1p.2.33_24_36	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	12	66.5
8851	V1p.1.33_36_48	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	12	51
8852	V1p.2.33_36_48	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	12	61
8855	V1p.1.33_48_72	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	28.7
8856	V1p.2.33_48_72	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	30.4
8859	V1p.1.33_72_96	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	16.7
8860	V1p.2.33_72_96	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	21.3
8863	V1p.1.33_96_120	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	13.9
8864	V1p.2.33_96_120	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	14.3
8867	V1p.1.33_120_144	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	10.9
8868	V1p.2.33_120_144	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	18.5
8871	V1p.1.33_144_168	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	7.97
8872	V1p.2.33_144_168	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	10.1
8875	V1p.1.33_168_192	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	15.2
8876	V1p.2.33_168_192	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	10.5
8879	V1p.1.33_192_216	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	8.32
8880	V1p.2.33_192_216	Vicryl+ 1	EtHO/H2O 3.3% w/w	0.01	0.35	24	9.32
8881	V0p.1.133_0_4	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	4	203
8882	V0p.2.133_0_4	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	4	175
8885	V0p.1.133_4_12	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	8	102
8886	V0p.2.133_4_12	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	8	120
8889	V0p.1.133_12_24	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	12	73.5
8890	V0p.2.133_12_24	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	12	73.5
8893	V0p.1.133_24_36	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	12	65
8894	V0p.2.133_24_36	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	12	65
8897	V0p.1.133_36_48	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	12	77.7
8898	V0p.2.133_36_48	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	12	42.9
8901	V0p.1.133_48_72	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	20.1
8902	V0p.2.133_48_72	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	30.4
8905	V0p.1.133_72_96	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	23.4
8906	V0p.2.133_72_96	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	28.6
8909	V0p.1.133_96_120	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	17.2
8910	V0p.2.133_96_120	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	23
8913	V0p.1.133_120_144	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	17.2
8914	V0p.2.133_120_144	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	22.1
8917	V0p.1.133_144_168	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	11.6
8918	V0p.2.133_144_168	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	13.7
8921	V0p.1.133_168_192	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	7.4
8922	V0p.2.133_168_192	Vicryl+ 0	EtHO/H2O 13.3% w/w	0.01	0.35	24	8.67
8883	V1p.1.133_0_4	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	4	243
8884	V1p.2.133_0_4	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	4	238
8887	V1p.1.133_4_12	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	8	227
8888	V1p.2.133_4_12	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	8	206
8891	V1p.1.133_12_24	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	12	114
8892	V1p.2.133_12_24	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	12	122
8895	V1p.1.133_24_36	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	12	58
8896	V1p.2.133_24_36	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	12	71
8899	V1p.1.133_36_48	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	12	36.8
8900	V1p.2.133_36_48	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	12	84.9
8903	V1p.1.133_48_72	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	22.4
8904	V1p.2.133_48_72	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	27.3
8907	V1p.1.133_72_96	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	19.5
8908	V1p.2.133_72_96	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	20.9
8911	V1p.1.133_96_120	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	11.7
8912	V1p.2.133_96_120	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	17.4
8915	V1p.1.133_120_144	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	11.7
8916	V1p.2.133_120_144	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	14.1

8919	V1p.1.133_144_168	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	6.2
8920	V1p.2.133_144_168	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	15.6
8923	V1p.1.133_168_192	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	11.7
8924	V1p.2.133_168_192	Vicryl+ 1	EtHO/H2O 13.3% w/w	0.01	0.35	24	6.82
8927	V2-0p. Total1	Vicryl+ 2-0	EtHO/H2O 100% w/w	0.01	0.35	144	1860
8931	V2-0p. Total2	Vicryl+ 2-0	EtHO/H2O 100% w/w	0.01	0.35	144	1470
8928	PDS2-0p. Total1	PDS+ 2-0	EtHO/H2O 100% w/w	0.01	0.35	144	16500
8932	PDS2-0p. Total2	PDS+ 2-0	EtHO/H2O 100% w/w	0.01	0.35	144	22100
8933	M2-0p. Total1	Monocryl+ 2-0	EtHO/H2O 100% w/w	0.01	0.35	144	41000
8934	M2-0p. Total2	Monocryl+ 2-0	EtHO/H2O 100% w/w	0.01	0.35	144	34000
8925	V0p.Total1	Vicryl+ 0	EtHO/H2O 100% w/w	0.01	0.35	144	2100
8929	V0p.Total2	Vicryl+ 0	EtHO/H2O 100% w/w	0.01	0.35	144	1270
8926	V1p.Total1	Vicryl+ 1	EtHO/H2O 100% w/w	0.01	0.35	144	2250
8930	V1p.Total2	Vicryl+ 1	EtHO/H2O 100% w/w	0.01	0.35	144	1410

Raw data–24-h Time-kill analysis *S. aureus* ATCC 29213

Solvent	Immersion Hours	Hour of Count	Count
1	1	0	1,640,000
1	4	0	1,640,000
1	12	0	1,810,000
1	24	0	1,760,000
1	48	0	2,400,000
1	1	24	0
1	4	24	0
1	12	24	25,100,000
1	24	24	40,400,000
1	48	24	57,000,000
2	1	0	1,520,000
2	4	0	1,390,000
2	12	0	1,810,000
2	24	0	1,810,000
2	48	0	1,810,000
2	1	24	0
2	4	24	0
2	12	24	23,300,000
2	24	24	31,100,000
2	48	24	38,900,000

Solvent codes: 1: 13.3% w/w 2: 3.3% w/w.

APPENDIX 2: TRICLOSAN MIC AND TS ACTIVITY- STUDY 1 & STUDY 3 COMBINED

Minimum inhibitory concentration and raw time-kill with P+ suture results of all 117 collected species in Study 1 and Study 3

Microbial species	Id strain	center	surgery	days	MIC	CFU 0h	CFU 4h	CFU 8h	CFU 24h
Achromobacter	80501	Pellegrin	orthopedic		30				
Bacillus pumilus	1	PBNA	spinal SSI	0	30				
Bacillus pumilus	6	PBNA	spinal SSI	35	30				
Bacillus cereus	80581	Pellegrin	orthopedic		30				
Citrobacter braaki	83899	Pellegrin	orthopedic		30				
Enterobacter bugandensis	83850	Pellegrin	orthopedic		30				
Enterobacter cloacae	82334	Pellegrin	orthopedic		30				
Enterobacter cloacae	ATCC5383	reference	reference		30				
Enterococcus avium	78191	Pellegrin	spinal non-SSI		30				
Enterococcus faecalis	5	PBNA	spinal SSI	35	30				
Enterococcus faecalis	8	PBNA	spinal SSI	98	30				
Enterococcus faecalis	11	PBNA	spinal SSI	26	30				
Enterococcus faecalis	23	PBNA	spinal SSI	42	30				
Enterococcus faecalis	38	StMarin	spinal SSI	21	30				
Enterococcus faecalis	82333	Pellegrin	spinal SSI	38	30	3100000	5550	66	1
Enterococcus faecalis	ATCC29212	reference	reference		30				
Enterococcus faecium	2	PBNA	spinal SSI	0	30				
Enterococcus faecium	14	PBNA	spinal SSI	18	30				
Enterococcus faecium	19	PBNA	spinal SSI	177	30				

Microbial species	Id strain	center	surgery	days	MIC	CFU 0h	CFU 4h	CFU 8h	CFU 24h
Enterococcus faecium	24	PBNA	spinal SSI	40	30				
Enterococcus faecium	33	StMarin	spinal SSI	10	30				
Enterococcus faecium	40	Pellegrin			30				
Enterococcus faecium	ATCC51299	reference	reference		30				
Enterococcus hirae	ATCC10541	reference	reference		30				
Escherichia coli	32	StMarin	spinal SSI	0	30				
Escherichia coli	37	StMarin	spinal SSI	21	30				
Escherichia coli	42	Pellegrin			30				
Escherichia coli	80272	Pellegrin	spinal SSI	18	30				
Escherichia coli	82376	Pellegrin	spinal non-SSI		30				
Escherichia coli	84052	Pellegrin	spinal SSI	10	30	1960000	51200	140	1
Escherichia coli	ATCC25922	reference	reference		30	721000	0.0407	0.00219	0.0127
Escherichia coli ESBL	CIP BAA-2326	reference	reference		30				
Klebsiella pneumoniae	CIP8291T	reference	reference		30				
Lactobacillus spp	4	PBNA	spinal SSI	35	30				
MRSA	ATCC33592	reference	reference		30	1311667	27105	17697	38672
MRSA	clinical strain	reference	reference		30				
Propionibacterium acnes	14833	Pellegrin	spinal non-SSI		30	1720000	833000	71500	200
Propionibacterium acnes	14842	Pellegrin	orthopedic		30	1390000	733000	53200	185
Propionibacterium acnes	15602	Pellegrin	spinal SSI	23	30				
Propionibacterium acnes	15656	Pellegrin	orthopedic		30				
Staphylococcus aureus	7	PBNA	spinal SSI	35	30				
Staphylococcus aureus	9	PBNA	spinal SSI	98	30				
Staphylococcus aureus	10	PBNA	spinal SSI	26	30				
Staphylococcus aureus	15	PBNA	spinal SSI	18	30				

Microbial species	Id strain	center	surgery	days	MIC	CFU 0h	CFU 4h	CFU 8h	CFU 24h
Staphylococcus aureus	18	PBNA	spinal SSI		30				
Staphylococcus aureus	25	PBNA	spinal SSI	40	30				
Staphylococcus aureus	28	StMarin	spinal SSI	84	30				
Staphylococcus aureus	29	StMarin	spinal SSI	1365	30				
Staphylococcus aureus	31	StMarin	spinal SSI	0	30				
Staphylococcus aureus	35	StMarin	spinal SSI	10	30				
Staphylococcus aureus	36	StMarin	spinal SSI	11	30				
Staphylococcus aureus	41	Pellegrin			30				
Staphylococcus aureus	76996	Pellegrin	orthopedic		30				
Staphylococcus aureus	77070	Pellegrin	spinal SSI	0	30				
Staphylococcus aureus	82247	Pellegrin	orthopedic		30				
Staphylococcus aureus	84054	Pellegrin	spinal SSI	17	30	2520000	6220	44	1
Staphylococcus aureus	ATCC29213	reference	reference		30	566667	16583	6638	11832
Staphylococcus capitis	77083	Pellegrin	orthopedic		30				
Staphylococcus epidermidis	76969	Pellegrin	orthopedic		30				
Staphylococcus epidermidis	77029	Pellegrin	orthopedic	29	30				
Staphylococcus epidermidis	77078	Pellegrin	orthopedic		30	2230000	81100	8410	1
Staphylococcus epidermidis	83892	Pellegrin	orthopedic		30				
Staphylococcus epidermidis	CIP8155T	reference	reference		30	650000	9397	2680	3487
Staphylococcus haemolyticus	39	Pellegrin			30				
Staphylococcus hominis	30	StMarin	spinal SSI	1365	30				
Staphylococcus saprophyticus	17	PBNA	spinal SSI		30				
Actinomyces radingae	78219	Pellegrin	orthopedic		60				
Bacillus pumilus	26	PBNA	spinal SSI	40	60				
Enterobacter aerogenes	81198	Pellegrin	spinal SSI	11	60				

Microbial species	Id strain	center	surgery	days	MIC	CFU 0h	CFU 4h	CFU 8h	CFU 24h
Escherichia coli	16	PBNA	spinal SSI		60				
Escherichia coli	21	PBNA	spinal SSI	42	60				
Escherichia coli	34	StMarin	spinal SSI	10	60				
Escherichia coli ESBL	83655	Pellegrin			60				
Klebsiella pneumoniae	78738	Pellegrin	orthopedic		60				
Proteus mirabilis	20	PBNA	spinal SSI	177	60				
Staphylococcus aureus	22	PBNA	spinal SSI	42	60				
Staphylococcus aureus	27	StMarin	spinal SSI	21	60				
Staphylococcus aureus	77282	Pellegrin	orthopedic		60				
Staphylococcus aureus	82075	Pellegrin	spinal SSI	59	60				
Staphylococcus epidermidis	82122	Pellegrin	orthopedic		60				
Staphylococcus hominis	81115	Pellegrin	orthopedic		60				
Staphylococcus warneri	83655	Pellegrin			60				
Streptococcus canis	80641	Pellegrin	orthopedic		60				
Citrobacter koseri	81038	Pellegrin	spinal SSI	14	125				
Clostridium sporogenes	81166	Pellegrin	orthopedic		125				
Corynebacterium striatum	77279	Pellegrin	orthopedic		125	1620000	655000	72200	20
Proteus mirabilis	82123	Pellegrin	orthopedic		125				
Staphylococcus lugdunensis	84000	Pellegrin	orthopedic		125				
Corynebacterium striatum	82160	Pellegrin	orthopedic		250				
Enterobacter cloacae	80144	Pellegrin	orthopedic		250				
Hafnia alvei	80495	Pellegrin	orthopedic		250				
Staphylococcus aureus	77117	Pellegrin	orthopedic		250				
Streptococcus	81484	Pellegrin	orthopedic		250				
Streptococcus constellatus	77813	Pellegrin	spinal non-SSI		250				

Microbial species	Id strain	center	surgery	days	MIC	CFU 0h	CFU 4h	CFU 8h	CFU 24h
Streptococcus dysgalactiae	80642	Pellegrin	orthopedic		250				
Staphylococcus epidermidis	80099	Pellegrin	orthopedic		500				
Streptococcus anginosus	77848	Pellegrin	spinal non-SSI		500				
Candida albicans	ATCC10231	reference	reference		3000	662000	0	0	0.107
Candida albicans	clinical strain	reference	reference		3000				
Pseudomonas aeruginosa	79526	Pellegrin	orthopedic		128000	1550000	2110000	3890000	86300000
Pseudomonas aeruginosa	77076	Pellegrin	orthopedic		256000				
Pseudomonas aeruginosa	83895	Pellegrin	orthopedic		256000				
Pseudomonas aeruginosa	ATCC27853	reference	reference		256000				
Pseudomonas balearica	3	PBNA	spinal SSI	35	256000				
Pseudomonas aeruginosa	12	PBNA	spinal SSI	26	512000				
Brevibacillus spp	76655	Pellegrin	spinal SSI	20					
Candida albicans	clinical strain	reference	reference			401000	5	19	249
Escherichia coli	75730	Pellegrin	spinal SSI	43					
Escherichia coli ESBL	clinical strain	reference	reference			1220667	39567	8997	714187
Haemophilus parainfluenzae	75489	Pellegrin	spinal SSI	16					
MRSA	clinical strain	reference	reference			1090000	6987	3730	8195
Pseudomonas aeruginosa	clinical strain	reference	reference			879000	5840000	22700000	100000000
Staphylococcus aureus	74648	Pellegrin	spinal SSI	29					
Staphylococcus aureus	79414	Pellegrin	spinal SSI	11					
Staphylococcus aureus	81398	Pellegrin	spinal SSI	44					
Staphylococcus epidermidis	76639	Pellegrin	spinal SSI	20					
Staphylococcus epidermidis	75097	Pellegrin	spinal SSI	13					

APPENDIX 3: SUPPLEMENTAL MATERIAL - STUDY 4

Daoud, F.C.; Coppry, M.; Moore, N.; Rogues, A.-M. Do Triclosan Sutures Modify the Microbial Diversity of Surgical Site Infections? A Systematic Review and Meta-Analysis. *Microorganisms* 2022, 10, 927.

The published supplemental material file is copied below.

Do Triclosan Sutures Modify the Microbial Diversity of Surgical Site Infections? A Systematic Review and Meta-Analysis

SUPPLEMENTARY MATERIAL

Frederic C. Daoud, MD, MSc, Maïder Coppry, PharmD, PhD, Nicholas Moore, MD, PhD¹ Anne-Marie Rogues, MD, PhD

List of excluded randomised clinical trials

Baracs 2011

Baracs J, Huszar O, Sajjadi SG, Horvath OP. Surgical site infections after abdominal closure in colorectal surgery using triclosan-coated absorbable suture (PDS Plus) vs. uncoated sutures (PDS II): a randomized multicenter study. *Surg Infect (Larchmt)* 2011;12(6):483-9. [Other: NCT01123616; PubMed: 22142314]

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Chen S Y, Chen T M, Dai N T, Fu J P, Chang S C, Deng S C, et al. Do antibacterial-coated sutures reduce wound infection in head and neck cancer reconstruction? *Eur J Surg Oncol* 2011;37(4):300-4. [DOI: doi.10.1016/j.ejso.2011.01.015; PubMed: 21296544]

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Galal 2011

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Karip A B, Celik K, Aydin T, Yazicilar H, Iscan Y, Agalar C, et al. Effect of Triclosan-Coated Suture and Antibiotic Prophylaxis on Infection and Recurrence after Karydakis Flap Repair for Pilonidal Disease: A Randomized Parallel-Arm Double-Blinded Clinical Trial. *Surg Infect (Larchmt)* 2016;17(5):583-8. [DOI: doi.10.1089/sur.2015.207; PubMed: 27383814]

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Roy 2019

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Table S1. Source data - Microbial count suture treatment arm and per study

Group TS	Arslan	Ichida	Isik	Justinger	Mattavelli	Mingmalairak	Nakamura	Rozelle	Ruiz-Tovar2015	Thimour-Bergström	Lin	Ruiz-Tovar2020	total
total	11	72	5	28	18	1	12	2	5	22	0	4	180
<i>Proteus mirabilis</i>	0	0	0	0	0	0	0	0	0	2	0	0	2
<i>Proteus vulgaris</i>	0	0	0	0	2	0	0	0	0	0	0	0	2
<i>Morganella morganii</i>	0	0	0	0	1	0	0	0	0	0	0	0	1
<i>Citrobacter koseri</i>	0	0	0	0	1	0	0	0	0	0	0	0	1
<i>Citrobacter freundii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
Staph E coli K pneumoniae mix	0	11	0	0	0	0	0	0	0	0	0	0	11
<i>Coryne bacterium ssp</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Candida albicans</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
Gram+ Gram- mix	0	0	0	0	0	0	0	0	0	1	0	0	1
<i>Moraxella catarrhalis</i>	0	0	0	0	0	0	0	0	0	1	0	0	1
<i>Serratia marcescens</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
MRSA	0	0	0	0	0	0	0	1	0	0	0	0	1
Coag. neg staph	0	0	0	0	0	0	0	1	0	3	0	0	4
<i>S aureus</i>	0	0	0	0	1	1	1	0	0	7	0	0	10
<i>S epidermidis</i>	3	0	1	0	0	0	1	0	0	0	0	0	5
<i>Staphylococcus spp</i>	0	18	0	7	0	0	0	0	0	0	0	0	25
<i>P aeruginosa</i>	0	0	1	0	1	0	0	0	2	3	0	0	7
<i>Pseudomonas spp</i>	0	4	0	2	0	0	0	0	0	0	0	0	6
<i>E coli</i>	6	10	0	0	3	0	0	0	2	0	0	1	22
<i>Bacteroid fragilis</i>	2	0	0	0	1	0	1	0	0	0	0	0	4
<i>Bacteroid ovatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacteroid thetaiota</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacteroid spp</i>	0	0	0	0	0	0	2	0	0	0	0	0	2
<i>K oxytoca</i>	0	0	0	0	1	0	0	0	0	0	0	0	1
<i>Klebsiella pneumoniae</i>	0	10	3	0	0	0	0	0	0	0	0	0	13
<i>Klebsiella spp</i>	0	0	0	0	0	0	0	0	1	1	0	2	4
<i>Enterococcus faecalis</i>	0	0	0	0	2	0	4	0	0	2	0	0	8
<i>Enterococcus faecium</i>	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Enterococcus spp</i>	0	8	0	9	0	0	1	0	0	0	0	0	18
<i>E avium</i>	0	0	0	0	1	0	0	0	0	0	0	0	1
<i>Enterobacter cloacae</i>	0	0	0	0	3	0	1	0	0	0	0	0	4
<i>Enterobacter spp</i>	0	4	0	1	0	0	0	0	0	0	0	0	5
<i>Streptococcus anginosus</i>	0	0	0	0	1	0	0	0	0	0	0	0	1
<i>Streptococcus mutans</i>	0	0	0	0	0	0	0	0	0	2	0	0	2
<i>Streptococcus spp</i>	0	0	0	2	0	0	0	0	0	0	0	1	3
<i>Peptostreptococcus magnus</i>	0	0	0	0	0	0	1	0	0	0	0	0	1
Other bactereria	0	7	0	7	0	0	0	0	0	0	0	0	14

Group NTS	Arslan	Ichida	Isik	Justinger	Mattavelli	Mingmalairak	Nakamura	Rozelle	Ruiz-Tovar2015	Thimour-Bergström	Lin	Ruiz-Tovar2020	total
total	22	59	9	30	13	1	17	8	35	29	1	22	246
Proteus mirabilis	0	0	0	0	0	0	0	0	0	0	0	0	0
Proteus vulgaris	0	0	0	0	0	0	0	0	0	0	0	0	0
Morganella morganii	0	0	0	0	1	0	0	0	0	0	0	0	1
Citrobacter koseri	0	0	0	0	0	0	0	0	0	0	0	0	0
Citrobacter freundii	0	0	0	0	0	0	0	0	0	1	0	0	1
Staph E coli K pneumoniae mix	0	0	0	0	0	0	0	0	0	0	0	0	0
Coryne bacterium ssp	0	0	1	0	0	0	0	0	0	0	0	0	1
Candida albicans	0	0	1	0	1	0	0	0	0	0	0	0	2
Gram+ Gram- mix	0	0	0	0	0	0	0	0	0	0	0	0	0
Moraxella catarrhalis	0	0	0	0	0	0	0	0	0	0	0	0	0
Serratia marcescens	0	0	0	0	0	0	0	0	0	1	0	0	1
MRSA	0	0	0	0	0	0	1	1	0	0	0	0	2
Coag. neg staph	0	0	0	0	0	0	0	3	0	4	0	0	7
S aureus	2	0	5	0	1	0	0	3	0	15	0	0	26
S epidermidis	3	0	2	0	0	0	0	0	0	0	0	0	5
Staphylococcus spp	0	19	0	10	0	0	0	0	0	0	0	0	29
P aeruginosa	2	0	0	0	2	1	2	1	9	0	0	0	17
Pseudomonas spp	0	3	0	0	0	0	0	0	0	0	0	0	3
E coli	12	11	0	0	3	0	0	0	16	1	0	9	52
Bacteroid fragilis	3	0	0	0	1	0	2	0	0	0	0	0	6
Bacteroid ovatus	0	0	0	0	0	0	1	0	0	0	0	0	1
Bacteroid thetaiotao	0	0	0	0	0	0	1	0	0	0	0	0	1
Bacteroid spp	0	0	0	0	0	0	1	0	0	0	0	0	1
K oxytoca	0	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella pneumoniae	0	11	0	0	1	0	0	0	0	0	1	4	17
Klebsiella spp	0	0	0	0	0	0	0	0	5	2	0	4	11
Enterococcus faecalis	0	0	0	0	0	0	4	0	5	3	0	0	12
Enterococcus faecium	0	0	0	0	1	0	1	0	0	0	0	0	2
Enterococcus spp	0	5	0	10	0	0	1	0	0	0	0	0	16
E avium	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	0	0	0	0	2	0	3	0	0	0	0	0	5
Enterobacter spp	0	5	0	2	0	0	0	0	0	0	0	0	7
Streptococcus anginosus	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus mutans	0	0	0	0	0	0	0	0	0	2	0	5	7
Streptococcus spp	0	0	0	2	0	0	0	0	0	0	0	0	2
Peptostreptococcus magnus	0	0	0	0	0	0	0	0	0	0	0	0	0
Other bactereria	0	5	0	6	0	0	0	0	0	0	0	0	11

SENSITIVITY ANALYSES

Table S2. Sensitivity analysis of the relative risk of culture-confirmed SSIs

Study removed	Pooled RR
None	0.62 [0.47, 0.82]
Isik 2012	0.60 [0.44, 0.82]
Arslan 2018	0.63 [0.46, 0.86]
Ichida 2018	0.58 [0.44, 0.75]
Jüstinger 2013	0.62 [0.45, 0.87]
Mingmalairak 2009	0.62 [0.46, 0.82]
Rozzelle 2008	0.65 [0.49, 0.85]
Lin 2018	0.62 [0.47, 0.83]
Mattavelli 2015	0.58 [0.45, 0.75]
Nakamura 2013	0.64 [0.47, 0.86]
Ruiz-Tovar 2015	0.67 [0.51, 0.87]
Ruiz-Tovar 2020	0.64 [0.48, 0.86]
Thimour-Bergström 2013	0.67 [0.49, 0.91]

Table S3. Sensitivity analysis of the association between genera and suture types

Study removed	Total N	Fisher's exact test p	Pearson's Chi² p	Cramer's V
None	375	0.704	0.719	0.1097
Isik 2012	363	0.635	0.646	0.1187
Arslan 2018	342	0.710	0.726	0.1142
Ichida 2018	267	0.558	0.596	0.1438
Jüstinger 2013	330	0.756	0.770	0.1113
Mingmalairak 2009	373	0.700	0.717	0.1103
Rozzelle 2008	365	0.686	0.703	0.1129
Lin 2018	374	0.702	0.718	0.1100
Mattavelli 2015	350	0.732	0.746	0.1106
Nakamura 2013	347	0.657	0.683	0.1178
Ruiz-Tovar 2015	335	0.786	0.786	0.1085
Ruiz-Tovar 2020	349	0.787	0.795	0.1053
Thimour-Bergström 2013	330	0.657	0.664	0.1226

9.
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