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Epidémiologie de la colonisation digestive à Staphylococcus aureus

Julie Gagnaire

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Julie Gagnaire

Epidémiologie de la colonisation digestive
à *Staphylococcus aureus*

Devant le jury composé de :

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Tables des matières

Résumé	7
Abstract	8
Introduction	9
Revue systématique de la littérature : Epidémiologie et impact clinique de la colonisation digestive à <i>Staphylococcus aureus</i>	11
Article 1 – Caractérisation du réservoir nasal et digestif à <i>Staphylococcus aureus</i> chez des patients de réanimation	33
Article 2– Colonisation intestinale à <i>Staphylococcus aureus</i> avant et après préparation colique chez des patients bénéficiant d’une colonoscopie	45
Discussion	66
Perspectives	77
Références	79
Annexes : autres publications et communications en lien avec la thèse	88

Résumé

La colonisation digestive à *Staphylococcus aureus* est mal connue avec des travaux portant majoritairement sur la colonisation à *S. aureus* résistant à la méticilline. Elle est majoritairement associée à une colonisation nasale mais peut aussi être retrouvée de manière exclusive chez les porteurs. La détection de ce portage n'est pas standardisée et les données concernant sa physiopathologie sont rares. L'origine de cette colonisation reste mal définie et l'interrelation avec la colonisation nasale est peu étudiée notamment chez les patients colonisés avec des souches de *S. aureus* sensible à la méticilline (SASM). Contrairement à la colonisation nasale dont l'impact clinique est bien décrit, l'impact clinique de ce réservoir en association ou non avec le réservoir nasal est peu étudié. Nous avons montré, chez des patients de réanimation, qu'il existe une diversité génétique entre les souches de SASM isolées des muqueuses nasales et rectales chez un même individu et nous avons également décrit un cas d'infection endogène chez un porteur au niveau digestif exclusif suggérant un rôle de ce réservoir dans les infections à *S. aureus*. Nous avons identifié, chez des patients communautaires bénéficiant d'une colonoscopie, la localisation de *S. aureus* au niveau de biopsies coliques suggérant sa présence au niveau de la muqueuse intestinale. De plus nous avons mis en évidence un rôle de la préparation colique dans la décolonisation de *S. aureus* au niveau de plusieurs sites de portage. Enfin nos travaux ont également montré que, comparé au dépistage nasal seul, le dépistage du portage digestif de *S. aureus* permettait de dépister jusqu'à 17% de porteurs en plus.

Abstract

Despite first description in the literature in the 1960s, *Staphylococcus aureus* intestinal colonization is not well known. Previous works mainly investigated the carriage of methicillin-resistant *S. aureus*. Overall, *S. aureus* intestinal colonization is associated with *S. aureus* nasal one but exclusive intestinal carriers are also described. The screening of this carriage is not standardized and different hypotheses about its physiopathology are debated. However, the origin of this colonization remains unclear and the interrelationship with nasal colonization has been poorly studied, particularly in patients colonized with methicillin-sensitive strains of *S. aureus* (MSSA). Unlike *S. aureus* nasal colonization, whose clinical impact is well described, the clinical impact of this reservoir in association or not with nose carriage is not well studied. We showed in intensive care unit patients, that there was in a same patient a significant genetic diversity between MSSA strains isolated from nasal and rectal mucosa. We also recorded a case of an endogenous infection in an exclusive rectal carrier suggesting a role of this reservoir during *S. aureus* infections. During the follow-up of community patients undergoing a colonoscopy, we identified, *S. aureus* strains in colonic biopsies suggesting the presence of this bacterium in the intestine mucosa. Additionally, we highlighted the potential role of bowel cleansing preparation to decolonize *S. aureus* carriers at several sites. Finally, compared to nose sampling only, our works showed the adding value of the intestinal screening that would detect up to 17% more *S. aureus* carriers.

Introduction

Staphylococcus aureus est une bactérie qui se distingue des autres staphylocoques par son pouvoir pathogène responsable de 1 à 5% des infections communautaires et jusqu'à 30% des infections nosocomiales. Avant d'être un pathogène redoutable, *S. aureus* est une bactérie commensale qui colonise la peau et les muqueuses de l'homme. Le nez et plus précisément, le vestibule nasal antérieur, est le principal réservoir de *S. aureus* (Wertheim et al., 2005). Les données de prévalence de portage nasal ces dernières années font état d'une tendance mondiale à la baisse et de disparités géographiques notamment en ce qui concerne la diffusion de clone résistants et/ou virulents (i.e. clone USA 300) (Verhoeven et al., 2014). Ce portage concerne en moyenne 24% de la population générale avec des prévalences plus élevées chez les enfants comparativement aux adultes (environ 50% dans les premiers mois de vie). Plusieurs déterminants du portage nasal de *S. aureus* ont été décrits dans la littérature avec les facteurs liés à l'hôte et les facteurs extrinsèques. Des facteurs liés à la bactérie ont également été rapportés notamment le rôle des adhésines et des interférences bactériennes (Sakr et al., 2019; Verhoeven et al., 2014). Le portage nasal de *S. aureus* est un facteur de risque majeur d'infection à cette bactérie avec notamment un risque multiplié par 9 pour les infections du site opératoire (Kalmeijer et al., 2000; Kluytmans et al., 1995). Dans 80% des cas, les souches nasales et d'infections sont reliées génétiquement (Wertheim et al., 2004).

Les infections à *S. aureus* ne surviennent cependant pas exclusivement chez les personnes avec un portage nasal et dans environ 20 à 50% des cas (Berthelot et al., 2010), les souches d'infection à *S. aureus* ne sont pas similaires aux souches isolées au niveau du nez. Outre une origine exogène possible notamment associée aux soins (Price et al., 2017), l'existence d'un autre réservoir endogène de *S. aureus* également source d'infection est plausible. Le pharynx, les voies respiratoires, le vagin, le tube digestif et la peau ont principalement été décrits comme sites de colonisation à *S. aureus*. Dans une cohorte de volontaires sains, par exemple notre équipe a rapporté que la colonisation pharyngée était associée au portage nasal dans 60% des cas et que le dépistage systématique de la gorge ne permettait pas d'augmenter significativement la détection de nouveaux

porteurs de *S. aureus*. Mais de façon intéressante, 40% des souches identifiées au niveau pharyngé étaient différentes des souches nasales (Verhoeven et al., 2015).

La colonisation digestive à *S. aureus* a été décrite dans les années 50 et 60 (Williams, 1963). D'après une revue de la littérature publiée il y a 10 ans, la prévalence du portage intestinal à *S. aureus* était estimée à 20% dont 9 % de *S. aureus* résistant à la méticilline (SARM) (Acton et al., 2009) avec environ 1/3 de porteurs exclusifs au niveau digestif. Cette colonisation a été associée à la survenue d'infections, principalement à SARM, quand les patients sont colonisés à la fois au niveau du nez et du rectum (Squier et al., 2002; Srinivasan et al., 2010; Szumowski et al., 2009) et plus récemment à des épidémies de colonisation (Senn et al., 2016; van Belkum, 2016). Dans la littérature, il y a souvent peu de données pour évaluer le portage digestif par rapport à la colonisation nasale dans une même population, avec notamment des études s'étant intéressées principalement au portage digestif de SARM.

En introduction de ce travail de thèse sur l'épidémiologie du portage digestif de *S. aureus*, nous avons souhaité réaliser une actualisation des connaissances sur la colonisation digestive à *S. aureus* à travers la rédaction d'une revue systématique de la littérature. Les lacunes identifiées dans la littérature nous ont permis de définir les objectifs de ce travail de thèse. Ces objectifs sont présentés dans le chapitre suivant.

Revue systématique de la littérature : Epidémiologie et impact clinique de la colonisation digestive à *Staphylococcus aureus*

Epidemiology and clinical relevance of *Staphylococcus aureus* intestinal carriage: a systematic review and meta-analysis.

Gagnaire J, Verhoeven PO, Grattard F, Rigaille J, Lucht F, Pozzetto B, Berthelot P, Botelho-Nevers E.

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Alors que les premières revues de la littérature sur la colonisation nasale à *S. aureus* ont été publiées dans les années 90, il a fallu attendre 2009 pour que la première revue systématique sur la colonisation intestinale à *S. aureus* soit réalisée par l'équipe d'Alex Van Belkum (Acton et al., 2009). Une dizaine d'année après, nous avons souhaité réaliser une nouvelle revue systématique de la littérature afin de faire le point sur les études ayant investigué ce réservoir de colonisation à *S. aureus* quel que soit sa méticillino-résistance. Pour cela nous avons suivi la méthodologie PRISMA avec plus de 95 références sélectionnées publiées entre 2002 et 2017. Afin de pouvoir mesurer la prévalence de ce portage digestif, nous avons compilé dans une méta-analyse les fréquences de portage intestinal, combiné au portage nasal ou digestif exclusif par sous-groupe de population et en fonction de la méticillino-résistance.

Brièvement en population générale, nous avons rapporté que la prévalence globale de *S. aureus* au niveau digestif était de 25% et qu'elle pouvait varier en fonction des catégories d'individus. Par exemple si on considère le portage de *S. aureus* incluant les souches de *S. aureus* sensible à la méticilline (SASM) et de SARM, nous rapportons des prévalences de l'ordre de 14% chez les patients communautaires, de l'ordre de 21% chez les patients hospitalisés et de l'ordre de 38 % chez les nouveaux nés. De plus, certaines études qui ont comparé le dépistage de *S. aureus*, surtout pour le SARM, à partir d'un ou plusieurs sites ont montré que le dépistage du site digestif permettait d'augmenter jusqu'à 50% le nombre de porteurs de *S. aureus*. Dans la lignée de la revue d'Acton *et al*, nous n'avons pas retrouvé d'études permettant de statuer sur un site de dépistage préférentiel (périnéal, pli de l'aîne, rectal, selles...) pour étudier ce portage digestif. Nous avons rapporté les études qui ont décrites l'impact clinique de la colonisation digestive et notamment des travaux récents qui se sont intéressés au risque de dissémination environnemental des souches de *S. aureus* à partir du site digestif.

Les études sur la colonisation digestive qui ont estimé la relation entre les souches isolées du nez et au niveau digestif ont surtout été réalisé chez des porteurs de SARM. En ce qui concerne l'évaluation de son impact clinique, les études sont également peu nombreuses. Parmi celles qui suggèrent un sur risque d'infection à *S. aureus* chez les porteurs digestif, là encore les porteurs sont

majoritairement colonisés ou infectés avec des souches de SARM. Ainsi, l'étude de la relation entre le portage nasal et digestif à *S. aureus* dans une cohorte de patients de réanimation adulte ayant des prélèvements en soins courants de nez et de rectum pour la recherche de bactéries multi-résistantes (BMR) aux antibiotiques nous a semblé pertinente pour évaluer la prévalence de *S. aureus* au niveau digestif quel que soit le profil de résistance à la méticilline. Nous avons également recensé de manière prospective les infections à *S. aureus* dans cette même cohorte dans le but de décrire leur lien avec le réservoir digestif de *S. aureus*.

Comme rapporté dans notre revue, la définition du réservoir digestif de *S. aureus* est souvent imprécise. Les articles de la littérature ayant comparé les prévalences de portage au niveau de ces différents sites ont montré qu'elles étaient similaires dans un même groupe de patients. Par contre aucune étude ne conclut sur la localisation précise du réservoir de *S. aureus* au niveau du tube digestif. Egalement peu de travaux ont été réalisés sur des stratégies de décolonisation ciblée de *S. aureus* au niveau digestif. Ainsi, il nous a paru intéressant d'évaluer la présence de *S. aureus* dans le tube digestif de patients communautaires, et plus particulièrement par la réalisation de prélèvements étagés (aine, rectum et colon) dans le cadre d'une colonoscopie. La dynamique de la colonisation à *S. aureus* avant et après examen chez ces patients bénéficiant d'une préparation colique a également été étudiée dans l'optique d'évaluer l'effet de ce traitement sur le portage de cette bactérie.



Epidemiology and clinical relevance of *Staphylococcus aureus* intestinal carriage: a systematic review and meta-analysis

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ABSTRACT

Introduction: Recent data highlight the importance of screening more than one site for improving the detection of *S. aureus* colonization. Intestinal carriage is frequently under-investigated and its clinical impact ought to be defined a better way.

Areas covered: This review and meta-analysis provide an updated overview of prevalence, characteristics and clinical significance of *S. aureus* intestinal carriage in different populations, both for methicillin-susceptible and -resistant *S. aureus* strains.

Expert commentary: Intestinal *S. aureus* carriage is documented with higher prevalence in children and in patients with *S. aureus* skin and soft tissue infections. This site of colonization was shown to be associated with a high risk of dissemination in the environment and with *S. aureus* infection. Intestinal carriage is frequently retrieved in nasal carriers, reflecting probably an association with a high bacterial load. Exclusive intestinal carriage present in one third of intestinal carriers can be associated with infection. Comparative genotyping analysis of different strains from nasal and extra-nasal sites of carriage, including the intestinal ones, in the same individuals, would allow a better comprehension of the pathophysiology of *S. aureus* endogenous infection. It could also permit to improve the prevention of these infections by decolonization of sites implicated in infection genesis.

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Staphylococcus aureus; carriage; intestinal colonization; digestive colonization; infection; prevention


1. Introduction

The nose is the most common site of carriage described in *Staphylococcus aureus* carriers [1]. Nasal carriage is a well-known risk factor for *S. aureus* infections that are related to the strain of carriage in more than 80% of cases [1]. However, several extra-nasal body sites have been more recently described as concomitant reservoirs for *S. aureus*; they include mainly the skin [2]; the pharynx [3,4]; the vagina [2]; the rectal, anal, and perineal areas [5]; plus stools [6]. The contribution of these extra-nasal sites, including the intestinal ones in the development of endogenous *S. aureus* infections is however poorly understood. Histologic similarities of epithelial surfaces (squamous epithelial cells) between anal canal and nasal mucosa suggest common mechanisms of *S. aureus* colonization [7]. The threat of methicillin-resistant *S. aureus* (MRSA) infection and the need for their prevention contributed to investigate the intestinal reservoir and its potential clinical implications. Most studies studying the potential role of *S. aureus* intestinal carriage in the development of endogenous infection focused on MRSA. Yet the epidemiology of MRSA changed and globally decreased [8]. MRSA remains a common bacterium involved in healthcare-associated (HA) infections worldwide and is also responsible for community-acquired

(CA) infections notably in North America [8]. However methicillin-sensitive *S. aureus* (MSSA) carriage is more widely reported and frequent in European countries [8]. *S. aureus* intestinal carriage was reviewed by Acton et al., more than 10 years ago [5] and has recently been by Claassen-Weitz et al., but focusing only on differences between community and hospital settings [6]. The aim of the present work was to assess the prevalence of *S. aureus* intestinal carriage among different types of populations by conducting a systematic review and meta-analysis but also to discuss its clinical impact.

2. Methods

This review followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [9]. The search was performed in three database including Medline via Pubmed, Science direct via EBSCOhost, and Web of Science via Web of Knowledge using the following keywords: (*Staphylococcus aureus*) AND (carriage OR carrier* OR colonization OR epidemiology OR incidence OR prevalence) AND (gut OR intestinal OR gastrointestinal OR rectal OR rectum OR anal OR anus OR perineal OR perineum OR stool OR feces OR faeces OR fecal OR faecal OR extranasal). The search

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was limited to studies published between January 2002 and May 2017, written in English or French language, and involving human beings. The reference list of the included papers was scanned to identify potentially relevant studies that could be considered. Inclusion and exclusion criteria are depicted in Table 1. For each selected study, the extracted data included the following items: name of first author; year of study; number of subjects; type of population; frequency of *S. aureus*, MSSA, and MRSA intestinal carriage; frequency of exclusive intestinal carriage of *S. aureus*, MSSA, and MRSA; type(s) of samples taken for screening; the use of broth enrichment; and the rate of *S. aureus* infection associated to this carriage. Meta-analyses of the proportions of *S. aureus* intestinal carriage in different subgroups were done with MedCalc Statistical Software version 17.6 (MedCalc Software bvba, Ostend, Belgium). Cochrane's Q test was used to assess the presence of heterogeneity. A *p* value of <0.2 was considered to indicate significant heterogeneity. The degree of heterogeneity was assessed by the I^2 statistic [10].

3. Results and discussion

3.1. Results of literature search: intestinal carriage according to methicillin susceptibility profile, population characteristics, and sampling methods

The literature search yielded 712 non-duplicate studies (Figure 1). A total of 95 articles were included in the systematic review and the meta-analysis (see Table 2). Forty-two studies reported only MRSA prevalence [14,17,28,32,48,49,52–54,56–58,61–71,74–80,83–85,90,92,93,95,97,99–101,104]. Twenty-three studies reported prevalence of intestinal carriage but without details on the susceptibility profile of *S. aureus* isolates [12,15,20,24,30,31,34,35,37,39–41,44,47,59,60,81,88,89,98,103] and 30 studies reported both MSSA and MRSA prevalences [2,11,13,16,18,19,21–23,25–27,29,36,38,43,45,46,50,51,55,72,73,82,86,87,90,91,94,96].

Data on *S. aureus* intestinal carriage were reported in different populations including healthy adults (20 studies) [2,11–29], healthy newborns (14 studies) [12,22,31,33,34,37,40–47], healthy children (14 studies) [12,25,30–41], hospitalized adults

Table 1. Eligibility criteria for systematic review and meta-analysis.

Inclusion criteria for systematic review	Exclusion criteria for systematic review
<ul style="list-style-type: none"> • Studies reporting <i>Staphylococcus aureus</i> carriage from fecal, anal, rectal, perineal, or rectovaginal specimens from humans whether or not reporting <i>S. aureus</i> carriage from other sites. • Studies providing data of prevalence of <i>S. aureus</i> carriage whether or not data about <i>S. aureus</i> infections associated. 	<ul style="list-style-type: none"> • Studies reporting no data about <i>S. aureus</i> carriage or infections. • Studies reporting samples from groin area only. • Studies reporting outbreak caused by <i>S. aureus</i> (i.e. foodborne illness) • Studies reporting acquisition rate of <i>S. aureus</i> only. • Studies including only a subset of patients (i.e. carriers of vancomycin-resistant enterococci) • Studies including parasites or bacteria other than <i>S. aureus</i>. • Articles not providing information on the number of participants screened for <i>S. aureus</i> fecal, anal, rectal, perineal, or rectovaginal carriage. • Full text not available from the electronics database of the University of Saint-Etienne, INSERM, and Web of Science.
<p>Inclusion criteria for meta-analysis of proportions</p> <ul style="list-style-type: none"> • Studies reporting <i>S. aureus</i> carriage rate from fecal, anal, rectal, perineal, or rectovaginal specimens; • Studies providing information for classifying patient populations (healthy adults, children and newborns, hospitalized adults, children and newborns, and community volunteers at risk of MRSA carriage). 	<p>Exclusion criteria for meta-analysis of proportions</p> <ul style="list-style-type: none"> • Studies not providing enough information for classifying patient populations.

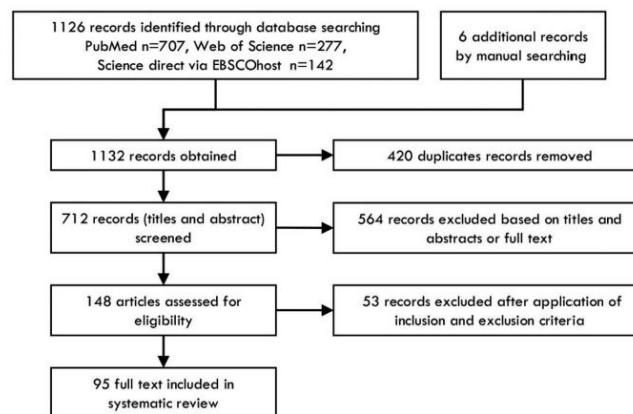


Figure 1. Prisma flow diagram for literature search about intestinal *S. aureus* carriage.

Table 2. Characteristics of studies included in the meta-analysis.

Studies	Ref.	Population types	Age range (mean) ^a	End of study	Country	Sample type	Broth enrichment
Healthy volunteers adults							
Aubry-Damon et al., 2004	[11]	Healthy volunteers (not pig farmer)	21–72	2002	France	Stools	No
Aubry-Damon et al., 2004	[11]	Healthy volunteers (pig farmers)	21–72	2002	France	Stools	No
Lindberg et al., 2004	[12]	Healthy volunteers (women with newborns)	-	2002	Sweden	Stools	No
Wertheim et al., 2005	[2]	Healthy volunteers	-	-	Netherlands	Perineal swab	No
Chen et al., 2006	[13]	Pregnant women	-	2005	USA	Rectovaginal swab	Yes
Szakacs et al., 2007	[14]	Healthy volunteers (from staff)	22–62	2006	Canada	Rectal swab	No
Harrison et al., 2008	[15]	Pregnant women	18–42	-	United Kingdom	Stools	No
Andrews et al., 2009	[16]	Pregnant women	(29)	2009	USA	Rectal swab	Yes
Thyagarajan et al., 2009	[17]	Outpatients with hip fracture (admitted from home)	-	-	Belgium	Perineal swab	-
Creech et al., 2010	[18]	Pregnant women	Median of 28	2006	USA	Rectovaginal swab	No
Top et al., 2010	[19]	Pregnant women	(27)	2009	USA	Rectovaginal swab	No
Onanuga et al., 2011	[20]	Healthy volunteers (villagers and students)	15–35	2009	Nigeria	Stools	No
Tomlinson et al., 2011	[21]	Pregnant women	(30)	2009	USA	Rectovaginal swab	Yes
Cursino et al., 2012	[22]	Pregnant women	15–48	2010	Brazil	Perineal swab (perianal)	Yes
Benito et al., 2013	[23]	Healthy volunteers	2–89	2011	Spain	Stools	Yes
Chan et al., 2013	[24]	Pregnant women	-	2011	Bangladesh	Rectovaginal swab	No
Gurnee et al., 2014	[25]	Pregnant women (with twin)	-	2014	USA	Stools	Yes
Albrecht et al., 2015	[26]	Healthy volunteers	18–82	2012	USA	Rectal swab	Yes
Kumar et al., 2015	[27]	Healthy volunteers	7 months–84 years	2012	USA	Perineal swab (perianal)	Yes
Frickmann et al., 2016	[28]	Healthy volunteers (soldiers returning from deployment)	-	2015	Germany	Stools	Yes
Singh J et al., 2016	[29]	Healthy volunteers (military trainees)	18–30	2014	USA	Perineal swab (perianal)	No
Healthy children							
Lindberg et al., 2004	[12]	Healthy children	8 weeks	2002	Sweden	Stools	No
Soysal et al., 2006	[30]	Healthy children (outpatients of pediatric clinic)	1–16	2003	Turkey	Perineal swab	No
Adlerberth et al., 2007	[31]	Healthy children	2 months	-	Sweden, UK, and Italy	Stools	No
Adlerberth et al., 2007	[31]	Healthy children	6 months	-	Sweden, UK, and Italy	Stools	No
Gustafsson et al., 2007	[32]	Healthy children	3–16 months	2005	Sweden	Perineal swab	No
Harrison et al., 2009	[33]	Healthy children	10 weeks	-	England	Stools	No
Harrison et al., 2009	[33]	Healthy children	7 months	-	England	Stools	No
Skov et al., 2009	[34]	Healthy children	47–64 weeks	2001	Denmark	Perineal swab	No
Faden et al., 2010	[35]	Healthy children	1–12	2008	USA	Rectal swab	-
Hewlett et al., 2010	[36]	Healthy children	6 weeks–5 years	-	USA	Rectal swab	No
Lindberg et al., 2011	[37]	Healthy children	6 months	2003	Sweden and Italy	Stools	-
Akinkunmi et al., 2012	[38]	Healthy children	<3	2006	Nigeria	Stools	No
Sánchez et al., 2012	[39]	Healthy children	(54 months)	-	Spain	Stools	Yes
González et al., 2013	[40]	Healthy children	15–90 days	2006	Mozambique	Stools	No
González et al., 2013	[40]	Healthy children	>181 days	2006	Mozambique	Stools	No
González et al., 2013	[40]	Healthy children	91–180 days	2006	Mozambique	Stools	No
Gurnee et al., 2014	[25]	Healthy children	-	2013	USA	Stools	Yes
Stokholm et al., 2016	[41]	Healthy children	1 year	2010	Denmark	Stools	No
Stokholm et al., 2016	[41]	Healthy children	1 month	2010	Denmark	Stools	No
Healthy newborns							
Lindberg et al., 2004	[12]	Healthy newborns	3 days	2002	Sweden	Rectal swab	No
Lindberg et al., 2004	[12]	Healthy newborns	1 week	2002	Sweden	Stools	No
Lindberg et al., 2004	[12]	Healthy newborns	2 weeks	2002	Sweden	Stools	No
Lindberg et al., 2004	[12]	Healthy newborns	4 weeks	2002	Sweden	Stools	No
Adlerberth et al., 2007	[31]	Healthy newborns	3 days	-	Sweden, UK, and Italy	Rectal swab	No
Adlerberth et al., 2007	[31]	Healthy newborns	7 days	-	Sweden, UK, and Italy	Stools	No
Adlerberth et al., 2007	[31]	Healthy newborns	14 days	-	Sweden, UK, and Italy	Stools	No
Adlerberth et al., 2007	[31]	Healthy newborns	28 days	-	Sweden, UK, and Italy	Stools	No
Lundell et al., 2007	[42]	Healthy newborns	2 weeks	2003	Sweden	Rectal swab	No
Lundell et al., 2007	[42]	Healthy newborns	4 weeks	2003	Sweden	Rectal swab	No
Gries et al., 2009	[43]	Healthy newborns	2 weeks	2007	USA	Stools	No
Harrison et al., 2009	[33]	Healthy newborns	2 weeks	-	England	Stools	No

(Continued)

Table 2. (Continued).

Studies	Ref.	Population types	Age range (mean) ^a	End of study	Country	Sample type	Broth enrichment
Skov et al., 2009	[34]	Healthy newborns	2 to 11 weeks	2001	Denmark	Perineal swab	No
Lindberg et al., 2011	[37]	Healthy newborns	2 weeks	2003	Sweden and Italy	Stools	-
Lindberg et al., 2011	[37]	Healthy newborns	4 weeks	2003	Sweden and Italy	Stools	-
Lindberg et al., 2011	[37]	Healthy newborns	8 weeks	2003	Sweden and Italy	Stools	-
Cursino et al., 2012	[22]	Healthy newborns	3 days–4 weeks	2010	Brazil	Perineal swab	Yes
González et al., 2013	[40]	Healthy newborns	<14 days	2006	Mozambique	Stools	No
Highet AR et al., 2014	[44]	Healthy newborns	3–52 weeks	2006	Australia	Stools	No
Highet AR et al., 2014	[44]	Newborns (sudden infant death syndrome)	3–52 weeks	1994	Australia	Stools	No
Benito et al., 2015	[45]	Healthy newborns (breastfeeding only)	7–35 days	-	Spain	Stools	No
El-Kersh et al., 2016	[46]	Healthy newborns	1–7 days	2013	Saudi Arabia	Stools	No
Kigbu et al., 2016	[47]	Healthy newborns	1 day	2013	Nigeria	Rectal swab	No
Kigbu et al., 2016	[47]	Healthy newborns	3 days	2013	Nigeria	Rectal swab	No
Kigbu et al., 2016	[47]	Healthy newborns	9 days	2013	Nigeria	Rectal swab	No
Kigbu et al., 2016	[47]	Healthy newborns	14 days	2013	Nigeria	Rectal swab	No
Stokholm et al., 2016	[41]	Healthy newborns	1 week	2010	Denmark	Stools	No
Hospitalized patients (adults)							
Dupeyron et al., 2002	[48]	Hospitalized patients (gastroenterology ward)	(57)	2001	France	Stools	No
Manian et al., 2002	[49]	Hospitalized patients (acute rehabilitation ward)	15–91 (53)	2000	USA	Perineal swab	No
Squier et al., 2002	[50]	ICU patients (surgical and transplant unit)	42–91 (65)	2001	USA	Rectal swab	Yes
Eposito et al., 2003	[51]	Hospitalized patients	-	2001	Italy	Rectal swab	No
Gnanalingham et al., 2003	[52]	Hospitalized patients (Neurosurgical unit)	16–64 (57.7)	1999	United Kingdom	Perineal swab	No
Ho et al., 2003	[53]	ICU patients	(61.3)	1999	Hong-kong	Rectal swab	No
Boyce et al., 2005	[54]	Hospitalized patients	33–107 (74.7)	2003	France	Stools	No
Cavalcanti et al., 2005	[55]	ICU patients	(48)	2003	Brazil	Perineal swab	No
Grmek-Kosnik et al., 2005	[56]	Hospitalized patients	-	-	Slovenia	Perineal swab	No
Marchaim et al., 2005	[57]	Hemodialysis patients	(68)	2001	Israel	Rectal swab	No
Marchaim et al., 2005	[57]	Hospitalized patients	(70)	2001	Israel	Rectal swab	No
Eveillard et al., 2006	[58]	Hospitalized patients (incl. ICU)	(76)	2003	France	Rectal swab	No
Singh et al., 2006	[59]	ICU patients (liver transplantation unit)	(54)	2004	USA	Rectal swab	No
Bhalla et al., 2007	[60]	Inpatients at least 3 days	(65)	-	USA	Stools	No
Zhang et al., 2007	[61]	Hospitalized patients	-	2007	Canada	Rectal swab	Yes
Batra et al., 2008	[62]	ICU patients	-	2006	United Kingdom	Rectal swab	Yes
Currie et al., 2008	[63]	Hospitalized patients	-	2007	Canada	Rectal swab	No
Furuno et al., 2008	[64]	Hospitalized patients	(52)	2005	USA	Perineal swab (perianal)	No
Reyes et al., 2008	[65]	Hospitalized patients (incl. ICU)	-	-	Canada	Perineal swab (perianal)	Yes
Ide et al., 2009	[66]	Hospitalized patients (general hospital)	-	-	Belgium	Perineal swab	Yes
Nishikawa et al., 2009	[67]	Patients admitted to a geriatric hospital	>65	2003	Japan	Perineal swab (perianal)	Yes
Baker et al., 2010	[68]	Veterans hospitalized for acute care	22–95	2009	USA	Perineal swab (perianal)	No
Bitterman et al., 2010	[69]	Hospitalized patients (general hospital)	-	2006	Israel	Perineal swab	No
Lauderdale et al., 2010	[70]	ICU patients	-	2006	Taiwan	Perineal swab	Yes
Collins et al., 2011	[71]	Hospitalized patients	-	2009	United Kingdom	Perineal swab	No
Maillet et al., 2011	[72]	Hospitalized patients (cardiac surgery ward)	-	2008	France	Rectal swab	-
Schechter-Perkins et al., 2011	[73]	Patients presenting to the Emergency Department	22–85	2010	USA	Perineal swab (perianal)	No
Chow et al., 2012	[74]	Hospitalized patients (dermatology and infectious disease wards)	44–77	2010	Singapore	Perineal swab (perianal)	No
Fiolic et al., 2012	[75]	In-patients admitted to vascular surgery ward	24–87	2010	Croatia	Perineal swab	Yes
Matheson et al., 2012	[76]	Patients admitted to acute-care hospital	>16	2010	United Kingdom	Perineal swab	Yes
Ng et al., 2012	[77]	Hospitalized patients with MRSA pos roommates	-	2010	Canada	Rectal swab	Yes
Datta et al., 2013	[78]	ICU patients	-	2010	India	Perineal swab	No
El-Bouri et al., 2013	[79]	Hospitalized patients	-	2012	Wales	Perineal swab	No
Popovich et al., 2013	[80]	HIV-negative patients	(51.7)	2012	USA	Perineal swab (perianal)	Yes
Dokter et al., 2016	[81]	Hospitalized patients with burn injury	0–98 (28.1)	2010	Sweden	Perineal swab	No
Dahlman D et al., 2017	[82]	Hospitalized patients	19–77	2015	Sweden	Perineal swab	No
Hospitalized children and newborns							
Thorburn et al., 2006	[83]	Children who required ventilation for at least 4 days	(5.4 months)	2003	United Kingdom	Rectal swab	Yes
Ohno et al., 2008	[84]	Pediatric surgical patients	0–24	2007	Japan	Stools	No
Srinivasan et al., 2010	[85]	Hospitalized children suffering from cancer	(8)	2007	USA	Rectal swab	No
Shehabi et al., 2013	[86]	Newborns hospitalized in neonatal ICU	-	2008	Jordan	Stools	No

(Continued)

Table 2. (Continued).

Studies	Ref.	Population types	Age range (mean) ^a	End of study	Country	Sample type	Broth enrichment
Nitsch-Osuch et al., 2015	[87]	Newborns hospitalized in neonatal ICU	-	2010	Poland	Stools	No
Gomez et al., 2016	[88]	Preterm neonates with enteral feeding tubes	0–49 days	2010	Spain	Stools	No
Non hospitalized participants at risk of MRSA carriage							
Waites et al., 2004	[89]	Patients with spinal cord injury	19–78 (33)	2002	Spain	Perineal swab	No
Roghmann et al., 2007	[90]	Patients with spinal cord dysfunction	(50)	2004	USA	Perineal swab	Yes
Mody et al., 2008	[91]	Nursing home residents	-	2004	USA	Perineal swab (perianal)	No
Mody et al., 2008	[91]	Nursing home residents with indwelling devices	-	2004	USA	Perineal swab (perianal)	No
Antoniou et al., 2009	[92]	MSM	(43)	2007	Canada	Rectal swab	No
O'Fallon et al., 2009	[93]	Resident of a senior institution	57–103 (88.5)	2003	USA	Rectal swab	No
Szumowski et al., 2009	[94]	MSM (HIV-infected)	Median of 41	2007	USA	Perineal swab (perianal)	Yes
Thyagarajan et al., 2009	[17]	Patients with hip fracture (admitted from residential home)	-	-	Belgium	Perineal swab	-
March et al., 2010	[95]	Residents and staff of a long-term care facility	22–96	2008	Italy	Rectal swab	No
Fouéré et al., 2012	[96]	MSM	17–71	-	France	Perineal swab	No
Miko et al., 2012	[97]	Outpatients from sexually transmitted disease clinic	(30.6)	2011	USA	Rectal swab	Yes
Dodiuk-Gad et al., 2013	[98]	Patients with drier disease	(44.6)	2010	Israel	Perineal swab	Yes
Joore et al., 2013	[99]	MSM (without SSTI)	31–48 (39)	2010	Netherlands	Perineal swab	Yes
Popovich et al., 2013	[80]	HIV-infected inpatients	(44.1)	2012	USA	Perineal swab (perianal)	Yes
Gibson et al., 2014	[100]	Nursing home residents with indwelling devices who acquired MRSA	-	-	USA	Perineal swab (perianal)	No
Lemma et al., 2015	[101]	HIV infected pediatrics patients	6–14 days	2014	Ethiopia	Perineal swab	No
Roghmann et al., 2015	[102]	Nursing home residents	(78)	-	USA	Perineal swab (perianal)	Yes
Alsterholm M et al., 2017	[103]	Outpatients with atopic dermatitis	26–66	2012	Sweden	Perineal swab	No
Alsterholm M et al., 2017	[103]	Outpatients with atopic dermatitis	26–66	2013	Sweden	Perineal swab	No
Alsterholm M et al., 2017	[103]	Outpatients with atopic dermatitis	26–66	2014	Sweden	Perineal swab	No
Dahlman D et al., 2017	[82]	Injecting drug users	23–68	2015	Sweden	Perineal swab	No
Lee CM et al., 2017	[104]	Residents of long-term care facilities	(80.3)	2015	Taiwan	Rectal swab	No
Lee CM et al., 2017	[104]	Residents of long-term care facilities	(80.3)	2016	Taiwan	Rectal swab	No
Lee CM et al., 2017	[104]	Residents of long-term care facilities	(80.3)	2017	Taiwan	Rectal swab	No

^aIf not precise age is in years. Mean is given in parenthesis if available.
- Data not available.

(inpatients) (33 studies) [48–74,76–80,82], hospitalized newborns (3 studies) [86–88], and hospitalized children (3 studies) [83–85]. Prevalence was also studied in patients at risk of MRSA colonization (19 studies) [17,80,82,89–104].

One of the major difficulties with this topic is that the definition of intestinal carriage is not homogeneous in the literature. In fact, the term 'intestinal carriage' has been used to designate a positive screening for *S. aureus* strains in various samples (see Table 2). Samples used to screen intestinal carriage included rectal swabs (27 studies) [12,14,16,31,35,36,42,47,50,51,53,57–59,61–63,72,77,83,85,92,93,95,97,104,105], samples from the perianal skin area (perineum, perianal or inguinal region) (39 studies) [2,17,22,27,29,30,32,34,35,49,52,55,56,64–71,73,74,76,78–80,82,89–91,94,96,98–103], recto-vaginal swabs (5 studies) [13,18,19,21,24], and stool specimens (25 studies) [1,11,12,15,20,23,25,28,31,33,37–41,43–46,48,54,60,84,86–88]. The prevalence of intestinal carriage varied widely according to the population tested and the methicillin susceptibility of the bacterium [5,6]. Prevalence determined in stool specimens was higher than that recovered from other anatomical sites. However this may have been biased by the fact these studies were performed in newborns, a population that exhibits high rates of carriage (see Table 2). In studies that compared multiple intestinal sites of sampling (rectum, stools, and perianal [91,106–110] or rectal and perineal [22]) in a same population, similar prevalence of *S. aureus* carriage was found among the different anatomical sites.

These similarities seem then to legitimate the equivalence of these body sites for determining intestinal carriage [5]. Globally, perianal skin area is used more frequently than rectum for studying *S. aureus* intestinal carriage, probably for practical reasons (see Table 2).

3.2. What is the prevalence of intestinal carriage according to the type of population screened?

Pooled analysis of eligible studies showed a proportion of intestinal carriage of 24.8% [95% confidence interval (CI), 20.8–29.1%] for *S. aureus* and 4.7% [95% CI, 3.7–5.7%] for MRSA only. This general frequency is similar to the one reported in previous reviews, as those of Acton et al., including older studies [5] or those of Claassen-Weitz et al., that differed however since they found a surprising high rate of MSSA carriage [6]. Figures 2–7 summarized the subgroup analysis done in different populations of individuals. In fact, these results allow comparison of prevalence of *S. aureus* intestinal carriage among newborns, children and adults, either healthy (Figures 2, 3 and 4), hospitalized (Figure 5, 6), or at risk of MRSA carriage (Figure 7).

In healthy participants, the subgroup analysis showed that intestinal carriage rates in adults were 13.8% [95% CI, 10.7–17.4%] for *S. aureus* and 1.4% [95% CI, 0.7–2.4%] for MRSA (Figure 2). Healthy newborns displayed a prevalence

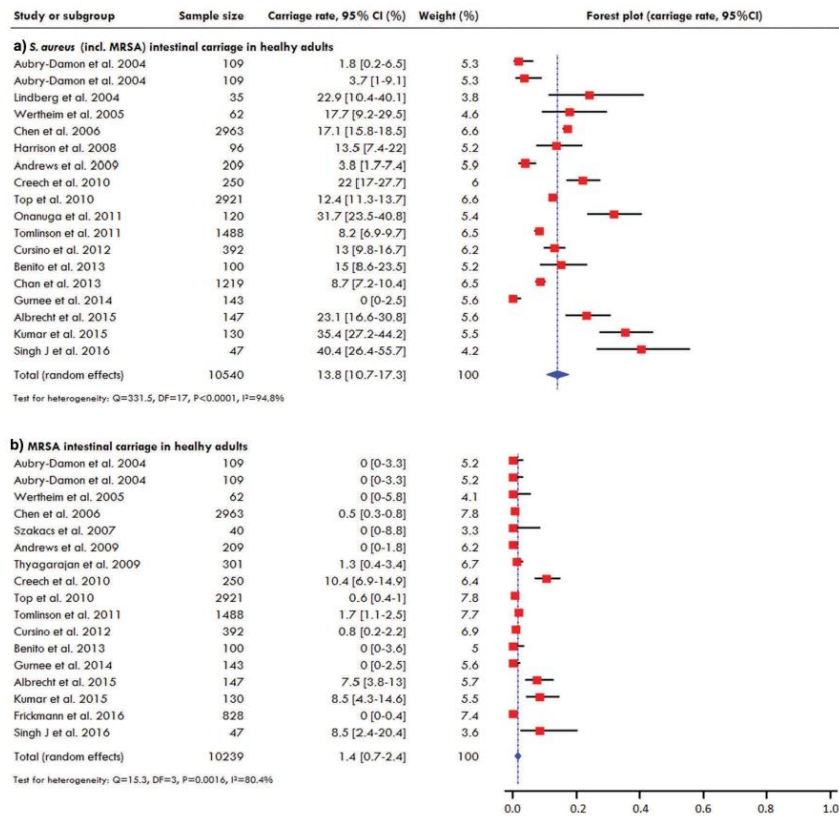


Figure 2. Subgroup forest plots for intestinal carriage rate of *Staphylococcus aureus* (A) and Methicillin resistant *S. aureus* (MRSA) only (B) in healthy adults. Size of the data markers is relative to study weight using random-effects models. Horizontal lines represent 95% confidence intervals (95% CI). Diamonds and vertical dotted line correspond to the pooled effects using random-effects models.

of 38.5% [95% CI, 30.7–46.5%] for *S. aureus* and 7.3% [95% CI, 2.3–14.8%] for MRSA (Figure 3), whereas it was 23.4% [95% CI, 11–38.7%] for *S. aureus* and 3.1% [95% CI, 1–11.2%] for MRSA in children (Figure 4). Higher prevalence for intestinal carriage was reported in newborns and neonates (Figures 3 and 6), probably because *S. aureus* strains can easily colonize the infant gut due to poor competition with other bacteria. This population harbored also very high rates of *S. aureus* nasal carriage [111,112]. In newborns, the prevalence of intestinal *S. aureus* carriage was then up to 73% [40] and those of nasal one up to 50% [112]. Breast-feeding was suggested to be involved in the development of *S. aureus* intestinal colonization in newborns [45]. However, the implications of this mother-to-infant microbial transmission in healthy neonates on the immune system development and on the establishment of intestinal microbiome remain poorly understood [45].

In studies that included adults hospitalized patients, intestinal carriage rates were 21.4% [95% CI, 9.1–37.3%] and 5.3% [95% CI, 3.9–7%] for *S. aureus* and MRSA, respectively. Most of

these studies investigated only the prevalence of MRSA carriage as depicted in Figure 5. Only few studies reported the prevalence of intestinal carriage in hospitalized children and newborns (Figure 6).

Among intestinal carriers, approximately one-third was exclusive *S. aureus* or MRSA intestinal carriers as summarized in Table 3 [2,15,17,27,30,33–35,43,50,55,56,58–63,65–70,75,77,80,82,83,85,86,90–92,96,102]. This notion of exclusive carriage depends however on the number of anatomical sites sampled in the studies (i.e. two or more sites) as shown in Table 3. In general *S. aureus* exclusive intestinal carriage was found in 0–26% of the whole populations included in studies (Table 3).

3.3. Relationship between intestinal and nasal carriage strains

Genetic comparison of *S. aureus* strains isolated from nasal and intestinal carriage revealed discordant results. Roghmann et al. described that all but one patient carried strains with the

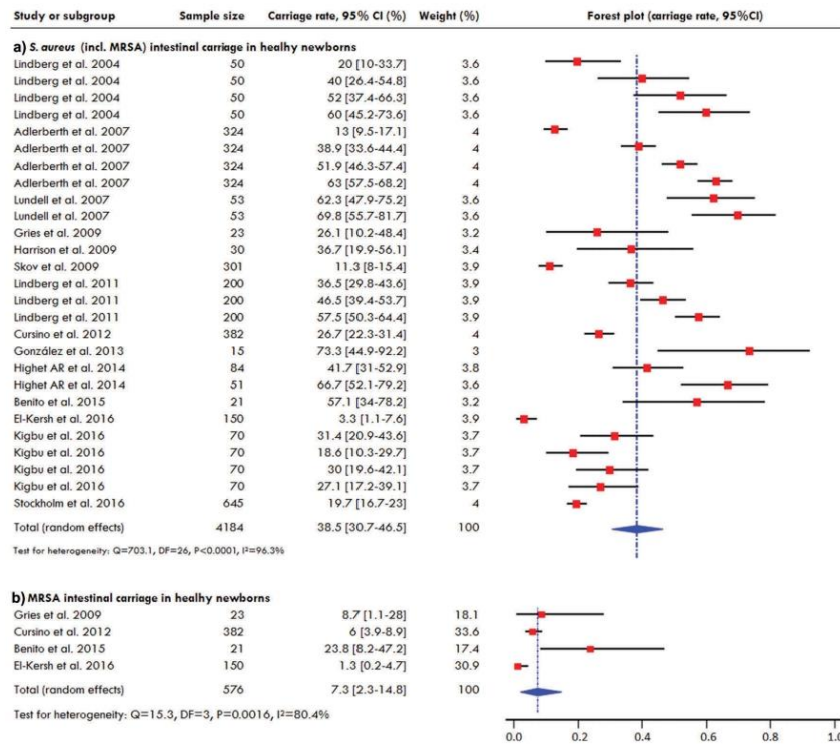


Figure 3. Subgroup forest plots for intestinal carriage rate of *Staphylococcus aureus* (A) and Methicillin resistant *S. aureus* (MRSA) only (B) in healthy newborns. Size of the data marker is relative to study weight using random-effects models. Horizontal lines represent 95% confidence intervals. Diamonds and vertical dotted line correspond to the pooled effects using random-effects models.

same spa-type in nose and perineum among 11 *S. aureus* carriers (MSSA and MRSA) in a population of community-dwelling people with spinal cord dysfunction [90]. Using MLST and spa-typing, Nakao et al. found, in a study conducted in Japan in 2013, that MRSA strains isolated in rectal samples were all identical to nasal samples in 21 nasal carriers among newborns and infants admitted to a neonatal intensive care unit [113]. Among children with USA300 *S. aureus* skin and soft tissue infections (SSTI), by using microarray analysis on 19 strains from nose, rectum, and abscesses, all strains were found to have similar genotypes [7]. By contrast to these three studies, a work conducted on 76 children scheduled for elective surgery in the USA showed that, among 8 *S. aureus* carriers colonized in both nasal and perianal sites, only 3 had the same strain in both sites [114]; this study included a majority (73%) of MSSA strains.

3.4. *S. aureus* genetic background is associated with rectal carriage

As shown in Table 2, a great proportion of studies included in this review focused on MRSA intestinal carriage. Since these studies took place in areas where MRSA is preponderant as shown in Table 2, it is difficult to conclude whether MRSA is really more

prevalent than MSSA in intestinal carriage. In a study involving hospitalized patients, Squier et al. described a higher persistence for MRSA rectal carriage compared to MSSA carriage at 1, 3, and 6 weeks after the initial positive culture ($p < 0.001$) [50].

Lowy and colleagues reported a rectal colonization rate of 64.3% (9/14) in the USA300 carriers compared to 17.3% (13/75) in non-USA300 carriers ($p < 0.001$) in patients seen in a sexually transmitted disease clinic [97]. Similarly, in a population of HIV-infected patients in which 50% were men having sex with men, colonized by CA-MRSA, perirectal colonization rate were 65.8% (52/79) and 41.7% (15/36) in USA300 and non-USA300 carriers, respectively (OR 2.7 [1.2–6.06], $p = 0.015$) [115]. Further studies are needed to understand the association between intestinal colonization and USA300 clone.

Recently Senn et al. reported an outbreak of *S. aureus* colonization with a ST228 MRSA strain involving 1600 patients in a tertiary care hospital [116]. Using whole-genome sequencing technology, they described that 70% (113/162) of ST228 *S. aureus* carriers had positive rectal swabs compared to 49% (19/39) for carriers colonized with the other clones ($p < 0.001$). They suggested that this strain belonging to the Clonal Complex (CC) 5 plays the role of a stealthy superbug able to maintain a long-term hospital outbreak by cross transmission. In a newborn population, Benito et al.,

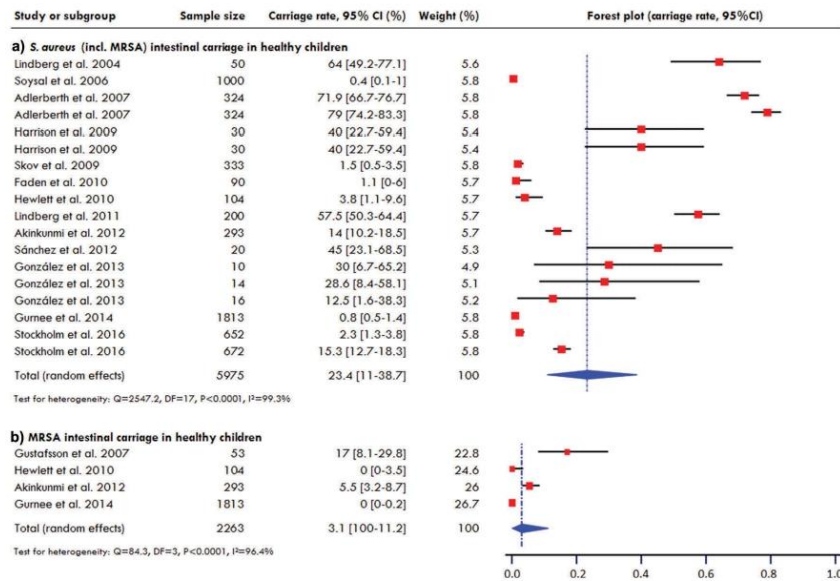


Figure 4. Subgroup forest plots for intestinal carriage rate of *Staphylococcus aureus* (A) and Methicillin resistant *S. aureus* (MRSA) only (B) in healthy children. Size of the data marker is relative to study weight using random-effects models. Horizontal lines represent 95% confidence intervals. Diamonds and vertical dotted line correspond to the pooled effects using random-effects models.

reported that 5/5 MRSA strains from stool carriage belonged to CC5 and 5/7 MSSA strain belonged to CC30 [45]. Nakao et al. reported that CC8 was the most important clone (11/21) recovered from rectal samples [113]. As a whole, despite the small-sized effectives and the heterogeneity of the populations studied, these results suggest that some clones are more frequently associated to intestinal carriage.

3.5. Why screening intestinal carriage?

3.5.1. Screening for intestinal carriage to increase detection sensitivity in *S. aureus* carriers

Recently, two systematic reviews clearly emphasized that the addition of screening for intestinal carriage improves the sensitivity of detection of MRSA carriage [117,118]. Compared to nasal swabbing only, screening at perineum or rectal site increased the detection of carriage up to 20% [117] and even up to 50% [118]. This added value is even increased in studies using broth enrichment [70] (for details see Table 2). In nursing home residents, a screening strategy of *S. aureus* carriage using swabbing at nose, groin, perianal area, and device sites detected 100% of the persistent *S. aureus* carriers and 97.4% of the transient carriers [100]. In a similar population, nasal cultures only missed 35% of *S. aureus* carriers [91]. In newborns, the combination of nasal and perineal screening allowed the detection of 85% and 83% of MRSA and MSSA strains, respectively, at birth [22]. In dermatology patients at high risk for MRSA carriage, an additional sampling of intestinal MRSA carriage increased the detection of MRSA by 11.7%,

whereas the detection was increased, respectively, by only 4.5% and 3.2% when screening the throat or the skin [74]. On the other hand, in patients not identified at risk for MRSA carriage, the screening for intestinal carriage did not increase the sensitivity of detection of new MRSA carriers [74]. These studies need to be confirmed in the general population since these data were obtained among patients at high risk of MRSA carriage. Moreover, screening for intestinal carriage could also allow detecting additional strains of *S. aureus* compared to the nose alone as observed in our prospective cohort of 400 ICU patients (Gagnaire J et al., article submitted).

3.5.2. Screening for intestinal carriage because of its clinical impact

3.5.2.1. Intestinal carriage is a risk factor for the development of *S. aureus* infection. It is well known that *S. aureus* nasal carriage, notably in persistent carriers, increases the risk of *S. aureus* infection. The role of *S. aureus* extra-nasal carriage as a risk factor for *S. aureus* infection remains however uncertain [1]. Squier et al. in a surgical intensive care unit and liver transplant unit reported that (i) patients with both rectal and nasal MRSA colonization developed, during the stay significantly, higher rates of *S. aureus* infections than did patients with nasal carriage alone (40% vs. 18%, $p < 0.001$) (ii) bacteremia occurred significantly more frequently in patients with *S. aureus* rectal carriage compared with nasal carriage only or no *S. aureus* carriage ($p = 0.002$) [50]. In children with cancer, MRSA carriage both in the nose and rectum was associated with a

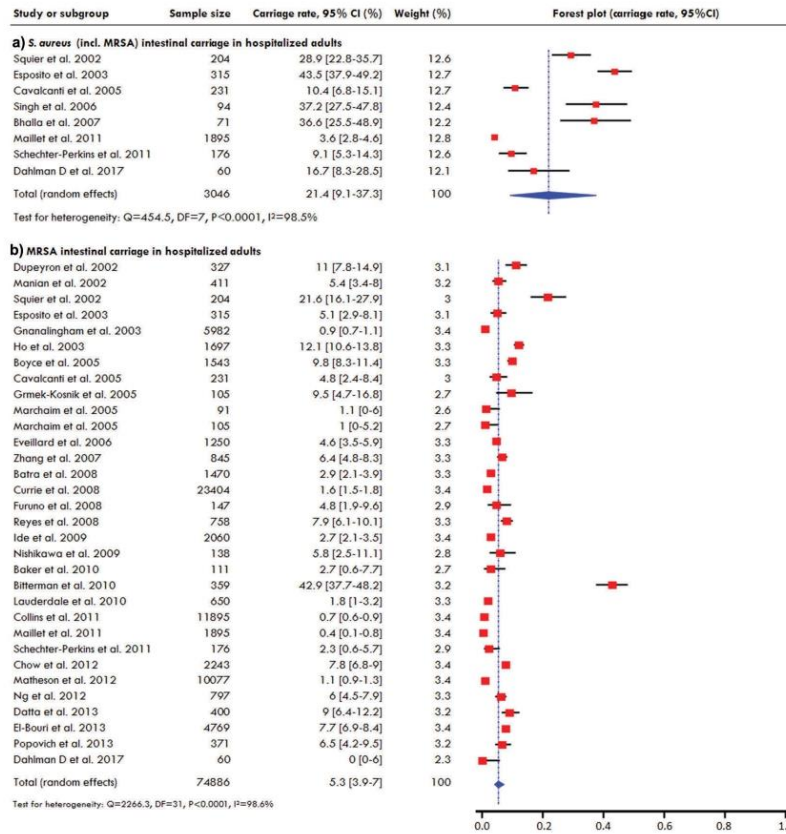


Figure 5. Subgroup forest plots for intestinal carriage rate of *Staphylococcus aureus* (A) and Methicillin resistant *S. aureus* (MRSA) only (B) in hospitalized adults. Size of the data markers is relative to study weight using random-effects models. Horizontal lines represent 95% confidence intervals. Diamonds and vertical dotted line correspond to the pooled effects using random-effects models.

significantly higher risk of MRSA infection compared to colonization at only one site ($p = 0.04$) [85]. In a prospective study conducted in an outpatient clinic in the USA, patients with MRSA perianal colonization (not nasal or wound colonization) had an independent 10-fold risk of presenting SSTI [94]. Additionally, in a study performed on 71 hospitalized patients (with a majority of MRSA carriers), subjects who had *S. aureus* positive nasal and stool culture more frequently had positive skin cultures (groin and axilla) than those with nasal colonization alone ($p = 0.001$) [60]. The authors suggested that this combination of colonization sites could potentially facilitate staphylococcal infections by increasing the risk of device contamination and nosocomial transmission. These data also support the ‘Trojan horse hypothesis’ proposed by Thwaites et al., reflecting the ability of *S. aureus* to be hidden inside immune cells and to circulate via the bloodstream without causing bacteremia and home to distant tissues [119]. This hypothesis is also supported by a recent

study using a murine gut-derived MRSA surgical site infection (SSI) model and suggesting the capability of MRSA strains originating from the gut to migrate to the surgical wound resulting in SSI without bacteremia [120]. However, in this model, contamination of surgical wound by external MRSA due to the animals’ habits cannot be excluded.

3.5.2.2. ‘Exclusive’ intestinal carriage is also at risk of infection. To the best of our knowledge, only three cases of *S. aureus* infections have been described among exclusive intestinal carriers. In a study including children with cancer, two out of nine patients with MRSA exclusive rectal carriage developed *S. aureus* infections [85]. Among 71 hospitalized patients, 1 out of 3 patients with exclusive stool colonization developed *S. aureus* infection [60]. Data related to the type of infection, the delay between colonization and infection, and the results of molecular typing to compare the respective strains were not specified in these two studies. An endogenous origin to these infections is however likely. In an ICU patient known to have *S. aureus*

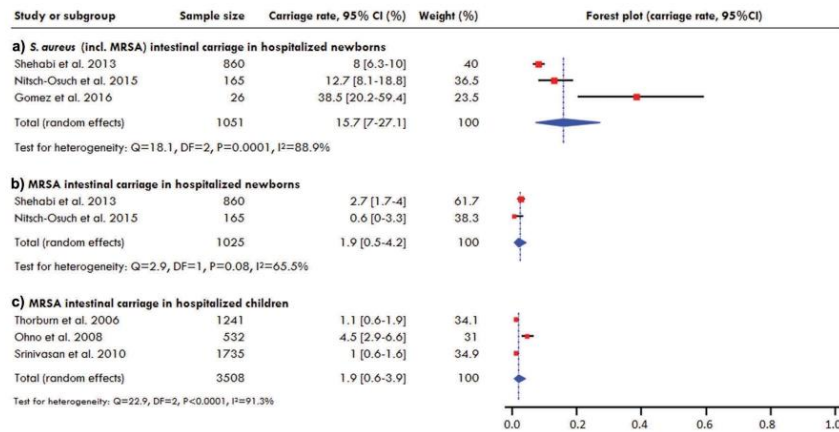


Figure 6. Subgroup forest plots for intestinal carriage rate of *Staphylococcus aureus* in hospitalized newborns (A), methicillin resistant *S. aureus* (MRSA) only in hospitalized newborns (B) and MRSA in hospitalized children. Size of the data markers is relative to study weight using random-effects models. Horizontal lines represent 95% confidence intervals. Diamonds and vertical dotted line correspond to the pooled effects using random-effects models.

intestinal carriage, we recently observed a bacteremia due to the same strain as those recovered at the site of exclusive intestinal colonization (Gagnaire J et al., article submitted). Despite the limited number of data available, it is probable that some *S. aureus* infections are linked to an intestinal *S. aureus* reservoir. In fact, since exclusive carriage is a rare condition, one way to determine in what proportion *S. aureus* infection originate from the gut is to compare by molecular typing both nasal and intestinal *S. aureus* colonizing strains together with the strain responsible for the infection.

3.5.2.3. Intestinal carriage is more frequently found in *S. aureus*-infected patients. In patients hospitalized with *S. aureus* infections (mainly SSTI) (not included in the meta-analysis), the subgroup analysis showed that intestinal carriage rates in adults were 39.4% [95% CI, 23.3–56.8] for *S. aureus* [26,27,29,35,105] and 23.4% [95% CI, 13.4–35.1%] for MRSA [26,27,29,69,99,121]. Prevalence in this population seems then higher than in noninfected people, despite a smaller effective. In fact, patients presenting SSTI were found to be frequently colonized at different anatomical sites and notably to be intestinal carriers [26,27,121] compared to usual rates of multiple site colonization [122]. In the USA, in 2007, Mc Cullough et al. described a higher incidence of CA-MRSA (USA 300 strains) perineal infections in toddlers (aged between 0 and 3) compared to older subjects (aged between 4 and 18) (respectively 76% vs. 6%), probably correlated to a higher rate of intestinal carriage in the first group [123]. In a comparative study including 147 subjects with SSTI and 147 uninfected controls, CA-MRSA was responsible for 48 infections and MSSA infection occurred in 36 cases [26]. In this study, using *spa* typing, the strain recovered from intestinal carriage was the same as the infectious one in 63.3% of cases for MRSA and 52.8% of cases for MSSA [26]. In a study that compared 60 children with *S. aureus* SSTI and 90 control children, the strains isolated from abscess were similar

to those of rectal carriage in 88% of cases (23/26) compared to nasal carriage similar in 75% of cases (12/16) (not statistically significant). Rectal isolates were significantly more likely to be MRSA compared to nasal isolates (31%) ($p = 0.022$) [35]. In a study that compared 74 MSM with SSTI and 137 MSM without symptoms, anogenital *S. aureus* colonization was significantly associated with the symptomatic group [99].

3.5.3. Screening intestinal carriage because it is a risk factor for environment dissemination

Intestinal *S. aureus* carriage might have a propensity to facilitate *S. aureus* environment dissemination as recently emphasized in the outbreak described above involving a single clonal ST228 *S. aureus* strain [116]. Whole genome sequencing showed that unknown long-term asymptomatic MRSA intestinal carriers were probably constantly admitted to the ward and contributed to maintain the outbreak by a cross-transmission via the environment. In a neonatal ICU, it was shown that feces of MRSA-carrier children contained significant amounts of *S. aureus*, contributing to the contamination of surrounding surfaces in contact with the healthcare workers' hands [113]. Acton et al., reviewed two outbreak investigations of CA-MRSA infection in football players documenting the role of extra-nasal MRSA carriers in the contamination of fomites [124,125]. The different body sites of *S. aureus* colonization were not investigated, but the role of this extra-nasal source was evoked as a possible reservoir for *S. aureus* transmission or reinfection [126]. Additionally, Senn et al. suggested that low-level resistance to mupirocin and the presence of the *qac* gene in ST228 *S. aureus* strain may also have played a role in its hospital environment dissemination because of possible non-efficacy of mupirocin and broad-spectrum antiseptics with cationic compounds like chlorhexidine or quaternary ammoniums on the *S. aureus* intestinal reservoir. This propensity to dissemination may be related to genetic characteristics of *S. aureus* strains and intestinal

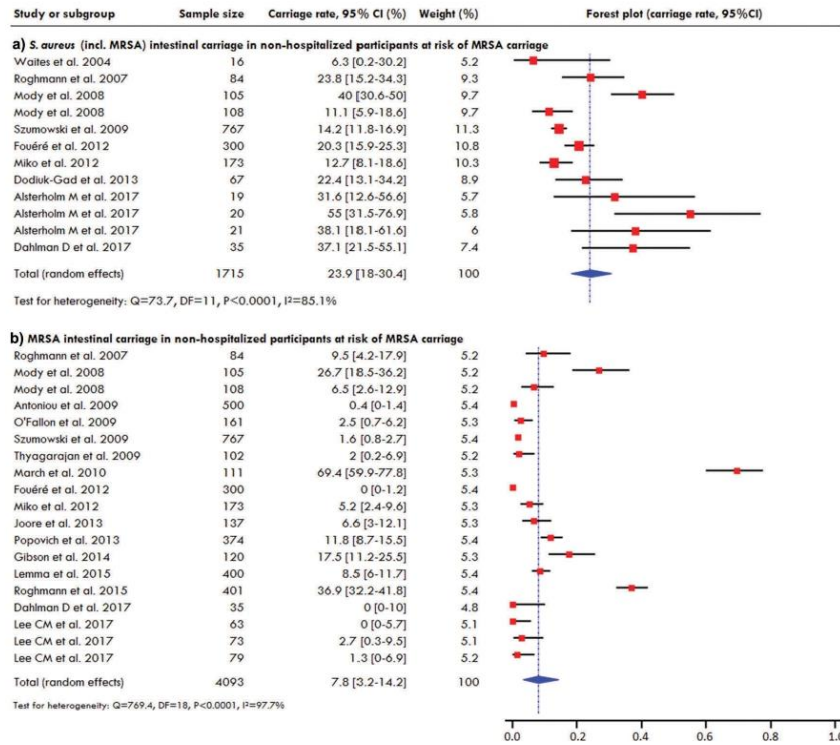


Figure 7. Subgroup forest plots for intestinal carriage rate of *Staphylococcus aureus* (A) and Methicillin resistant *S. aureus* (MRSA) only (B) in non-hospitalized participants at risk of MRSA carriage. Size of the data markers is relative to study weight using random-effects models. Horizontal lines represent 95% confidence intervals. Diamonds and vertical dotted line correspond to the pooled effects using random-effects models.

localization of *S. aureus* strains located at the intestinal level and not routinely screened [116].

3.6. Decolonization of *S. aureus* intestinal carriage

S. aureus nasal decolonization with topical mupirocin and chlorhexidine has shown to significantly reduce SSI in cardiac surgery [127] and is now recommended by the WHO to prevent these infections in orthopedic patients [128]. Presently, no prospective controlled study has been conducted to evaluate the effect of *S. aureus* intestinal decolonization on the reduction of *S. aureus* infection, and few *S. aureus* decolonization strategies have investigated this site of carriage.

Mupirocin is the preferred topical antibacterial agent used for nasal decolonization [129]. However mupirocin has a lower impact on the decolonization of MRSA extra-nasal sites compared to the nasal site [130]. Therefore in CA-MRSA carriers who are frequently colonized at nasal and intestinal site (see above), this strategy is possibly ineffective especially since they are exposed to contaminated fomites in their surrounding environment [131]. This strategy was also found non-effective in decolonizing extra-nasal sites of carriage (including perineum) in persistent nasal carriers [2].

Chlorhexidine gluconate (CHG) efficacy on the reduction of HA infections has been described in many studies [129]. CHG daily bathing at 2% in association with other bundle measures (e.g. contact precautions, geographic separation of patients) proved effective by reducing rectal colonization with carbapenemase-producing *Klebsiella pneumoniae* (CKP) [132] and with vancomycin-resistant enterococci (VRE) [133]. This efficacy may be due to CHG ability to reduce bacteria skin colonization and therefore to reduce the risk of recolonization and cross-transmission as shown for CKP [132] and VRE [134]. The direct effect of CHG on *S. aureus* intestinal colonization was not clearly evaluated. Although a recent meta-analysis has shown the reduction of MRSA colonization in ICU patients by the use of CHG, we cannot be sure that CHG specifically reduced intestinal carriage since the studies performing perineal or rectal MRSA screening did not provide details about it [135].

Few studies have been performed to measure the impact of oral antimicrobial agents on *S. aureus* intestinal carriage. Rifampicin used alone or in combination with novobiocin or trimethoprim-sulfamethoxazole, displayed a small decolonizing effect on MRSA carriers, including those colonized at multiple sites [136,137]. Furthermore, in combination with doxycycline and intranasal mupirocin, administration of

Table 3. Studies providing data on exclusive *S. aureus* intestinal carriage.

Studies	Ref.	SA/ MRSA	Total number of people screened	Exclusive intestinal carriage		All participants (%)	Site of screening	Other site (s) sampled	Population studied
				Intestinal carriage N (%)	Intestinal carriers N (%)				
Squier et al., 2002	[50]	SA	204	59 (28.9)	7 (11.9)	3.4	Rectal swab	ICU patients (surgical and transplant unit)	
Cavalcanti et al., 2005	[55]	SA	231	24 (10.4)	11 (45.8)	4.8	Perineal swab	ICU patients	
Wertheim et al., 2005	[2]	SA	62	11 (17.7)	1 (9.1)	1.6	Perineal swab	Healthy volunteers	
Singh et al., 2006	[59]	SA	94	35 (37.2)	9 (25.7)	9.6	Rectal swab	ICU patients (liver transplantation unit)	
Bhalla et al., 2007	[60]	SA	71	26 (36.6)	3 (11.5)	4.2	Stools	Infants at least 3 days	
Harrison et al., 2008	[15]	SA	96	13 (13.5)	9 (69.2)	9.4	Stools	Pregnant women	
Skov et al., 2009	[34]	SA	301	34 (11.3)	6 (17.6)	2.0	Perineal swab	Healthy newborns	
Gries et al., 2009	[43]	SA	23	6 (26.1)	0	0.0	Stools	Healthy newborns	
Harrison et al., 2009	[33]	SA	30	11 (36.7)	1 (9.1)	3.3	Stools	Healthy children	
Harrison et al., 2009	[33]	SA	30	12 (40)	7 (58.3)	23.3	Stools	Healthy children	
Harrison et al., 2009	[33]	SA	30	12 (40)	8 (66.7)	26.7	Stools	Healthy newborns	
Faden et al., 2010	[35]	SA	90	1 (1.1)	0	0.0	Rectal swab	Healthy children	
Fouéré et al., 2012	[96]	SA	300	61 (20.3)	30 (49.2)	10.0	Perineal swab	MSM	
Shehabi et al., 2013	[86]	SA	860	69 (8)	42 (60.9)	4.9	Stools	Newborns hospitalized in neonatal ICU	
Kumar et al., 2015	[27]	SA	130	46 (35.4)	9 (19.6)	6.9	Perineal swab	Healthy volunteers	
Dahlman et al., 2017	[82]	SA	35	13 (37.1)	3 (23.1)	8.6	Perineal swab	Injecting drug users	
Dahlman et al., 2017	[82]	SA	60	10 (16.7)	2 (20)	3.3	Perineal swab	Hospitalized patients	
Total SA			2647	443 (16.7)	148 (33.4)	5.6			
Grmek-Kosnik et al., 2005	[56]	MRSA	105	10 (9.5)	2 (20)	1.9	Perineal swab	Hospitalized patients	
Soysal et al., 2006	[30]	MRSA	1000	4 (0.4)	2 (50)	0.2	Perineal swab	Healthy children (outpatients of pediatric clinic)	
Eveillard et al., 2006	[58]	MRSA	1250	57 (4.6)	23 (40.3)	1.8	Rectal swab	Hospitalized patients (incl. ICU)	
Thorburn et al., 2006	[83]	MRSA	1241	14 (1.1)	3 (21.4)	0.2	Rectal swab	Children who required ventilation for at least 4 days	
Roghmam et al., 2007	[90]	MRSA	84	8 (9.5)	5 (59.5)	6.0	Perineal swab	Patients with spinal cord dysfunction	
Zhang et al., 2007	[61]	MRSA	845	54 (6.4)	14 (25.9)	1.7	Rectal swab	Hospitalized patients	
Batra et al., 2008	[62]	MRSA	1470	43 (2.9)	11 (25.6)	0.7	Rectal swab	ICU patients	
Reyes et al., 2008	[65]	MRSA	758	60 (7.9)	21 (35)	2.8	Perineal swab	Hospitalized patients (incl. ICU)	
Mody et al., 2008	[91]	MRSA	105	28 (26.7)	5 (17.8)	4.8	Perineal swab	Nursing home residents	
Mody et al., 2008	[91]	MRSA	108	7 (6.5)	5 (71.4)	4.6	Perineal swab	Nursing home residents with indwelling devices	
Currie et al., 2008	[63]	MRSA	23,404	382 (1.6)	160 (41.9)	0.7	Rectal swab	Hospitalized patients	
Thyagarajan et al., 2009	[17]	MRSA	301	4 (1.3)	2 (50)	0.7	Perineal swab	Outpatients with hip fracture (admitted from home)	
Thyagarajan et al., 2009	[17]	MRSA	102	2 (2)	0	0.0	Perineal swab	Patients with hip fracture (admitted from residential home)	
Nishikawa et al., 2009	[67]	MRSA	138	8 (5.8)	5 (62.5)	3.6	Perineal swab	Patients admitted to a geriatric hospital	
Ide et al., 2009	[66]	MRSA	2060	56 (2.7)	14 (25)	0.7	Perineal swab	Hospitalized patients (general hospital)	

(Continued)

Table 3. (Continued).

Studies	Ref.	SA/ MRSA	Total number of people screened	Intestinal carriage		Exclusive intestinal carriage		Site of screening	Other site (s) sampled	Population studied
				N (%)	N (%)	Intestinal carriers N (%)	All participants (%)			
Antoniou et al., 2009	[92]	MRSA	500	5 (1)	2 (40)	0.4	Rectal swab	Nares	MSM	
Srinivasan et al., 2010	[85]	MRSA	1735	17 (9.8)	9 (52.9)	0.5	Rectal swab	Nares	Hospitalized children suffering from cancer	
Bitterman et al., 2010	[69]	MRSA	359	154 (42.9)	57 (37)	15.9	Perineal swab	Nares, throat	Hospitalized patients (general hospital)	
Lauderdale et al., 2010	[70]	MRSA	650	12 (1.8)	4 (33.3)	0.6	Perineal swab	Nares, throat, skin (axilla)	ICU patients	
Baker et al., 2010	[68]	MRSA	111	3 (2.7)	0	0.0	Perineal swab	Oropharynx, skin (axilla, hand, wound), and catheter insertion site	Veterans hospitalized for acute care	
Fiolic et al., 2012	[75]	MRSA	58	7 (12.1)	0	0.0	Perineal swab	Nares, throat	In-patients admitted to vascular surgery ward	
Ng et al., 2012	[77]	MRSA	797	48 (6)	0	0.0	Rectal swab	Nares	Hospitalized patients with MRSA positive roommates	
Popovich et al., 2013	[80]	MRSA	374	44 (11.8)	17 (38.6)	4.5	Perineal swab	Throat, skin (axilla, inguinal, chronic wound)	HIV-negative patients	
Popovich et al., 2013	[80]	MRSA	371	24 (6.5)	5 (20.8)	1.3	Perineal swab	Throat, skin (axilla, inguinal, chronic wound)	HIV-infected inpatients	
Roghmann et al., 2015	[102]	MRSA	401	148 (37)	36 (24.3)	9.0	Perineal swab	Nares	Nursing home residents	
Total MRSA			38,327	1199 (3.1)	402 (33.5)	1.0				

SA: *S. aureus* including methicillin-resistant *S. aureus*; MRSA: methicillin-resistant *S. aureus* only.

rifampicin led to digestive adverse events [138]. Oral vancomycin was tested for eradicating MRSA intestinal carriage in ICU adult patients [139]. Although MRSA carrier rates were significantly reduced after administration of enteral vancomycin, 24% of patients remained colonized with MRSA strains in the rectum [139]. The effects of enteral vancomycin associated with throat and rectal MRSA colonization surveillance were assessed on MRSA gut overgrowth, transmission, infection, and mortality in a pediatric intensive care unit [83]. Interestingly, a significant MRSA gut overgrowth and a reduction of secondary endogenous infection was observed in children who received enteral vancomycin [83]. However, the risk of emergence of vancomycin-resistant *S. aureus* (VRSA) has been documented in patients carrying VRE and treated with vancomycin [140]. In their study, Thorburn et al. did not report any positive VRE or VRSA cultures from the 1611 screened samples of the above study [83].

Selective decontamination of the digestive tract (SDD), which consists in topical application of tobramycin, colistin, and amphotericin B in the oropharynx and stomach together with intravenous cefotaxime, was tested in ICU patients with the aim of having an impact on mortality and ICU-acquired *S. aureus* bacteremia in comparison to standard care patients [141,142]. In the most famous cluster-randomized crossover study on the subject, the reduction of intestinal *S. aureus* colonization was not evaluated but a reduction was observed as for the number of rectal swabs positive to Gram-negative bacteria [141]. The same investigators showed in another trial that SDD decreased the rates of ICU-acquired bloodstream infections and of rectal colonization by multidrug-resistant Gram-negative organisms compared to selective oropharyngeal decontamination (SOD) [143]. SDD and SOD do not seem to increase resistance in Gram-negative bacteria [144]. The impact of oral antibiotics or SDD on intestinal *S. aureus* carriage was not specifically tested. Due to the risk of antimicrobial resistance, guidelines on the treatment of MRSA infections do not recommend their use [145].

It was recently shown in a double-blind randomized controlled study conducted in a Veterans Affairs hospital that the daily use of the probiotic *Lactobacillus rhamnosus* HN001 could reduce the odds of *S. aureus* carriage in stool samples of patients [146,147]. More studies using different strains of probiotic are needed to determine their usefulness in *S. aureus* decolonization carriage.

4. Expert commentary

In this work, we evaluate *S. aureus* intestinal carriage prevalence to 25% of people, which is close to *S. aureus* nasal one [1] and exclusive intestinal carriage is present in one-third. This reservoir could then not be neglected. However, despite a large number of studies investigating *S. aureus* intestinal carriage, few studies focused on its role and its clinical implication. In SSTI linked in great part to CA-MRSA, the clinical importance of *S. aureus* intestinal carriage cannot be denied as reviewed above. For other endogenous infections, it would be necessary to better address the role of intestinal carriage as a possible risk factor. The recent paper of Krezalek et al. reinforces the interest in considering intestinal carriage of

MRSA as an underestimated MRSA reservoir. In fact, by using a murine model of gut-derived MRSA SSI, the authors highly suggested the silent migration of MRSA from the gut to the wound [120]. We showed in a multicenter study that strains implicated in orthopedic SSI were the same as the nasal strain in only 60% of cases [148]. This strongly suggests the presence of extra-nasal reservoir of *S. aureus*. In fact, it is known that some people can carry different strains at different body sites [3,4]. It also seems that colonization with MSSA could be more diverse than with MRSA. Indeed, MRSA strains are included into a small number of widely disseminated clonal lineages [149], which explains that different strains of MSSA could colonize a same individual at different sites. To determine the site reservoir that is at the origin of the infection, genotyping studies are warranted in order to better prevent *S. aureus* endogenous infections.

Moreover, we can hypothesize that patients with carriage at multiple sites have higher bacterial loads of *S. aureus*. Patients with a high load of *S. aureus* in the nose, defined as persistent carriers, are at higher risk of infections because of this bacterium [150,151]. Therefore, a patient with nasal and intestinal carriage might be at higher risk of *S. aureus* infection due to a higher load of *S. aureus* carriage.

As we have seen, different sites of screening are used to detect intestinal carriage, leading to confusion about where the actual reservoir of intestinal carriage is: the perineum skin or the digestive tract? Studies that compare strains from the different sites by molecular typing would be useful to verify whether all these sites harbor the same strain. For this purpose, whole genome sequencing is now the best tool for comparing strains [152]. Explorative studies to investigate the presence of *S. aureus* colonization in the gut are needed to better determine the origin of intestinal reservoir. They would allow to select the best screening site for intestinal carriage and subsequently lead to recommendations for homogenized screening practices.

As mentioned along this review, the prevalence of intestinal carriage varies largely according to each studied population. In newborns, where intestinal carriage prevalence is very high, we suggest, if hospitalized in ICU, that intestinal carriage should be systematically screened. This could be proposed as a preventive measure to avoid cross transmission and control outbreaks. We also suggest not only nasal screening but extra-nasal screening (notably intestinal) performed at the same time in populations where *S. aureus* carriage screening is recommended.

5. Five-year view

More and more data suggest the value of screening not only the nose but other sites to detect *S. aureus* carriage. In fact the screening of extra-nasal sites increases the detection of *S. aureus* carriers by up to 50% [118], mainly shown for MRSA. Rectal or perineal sites frequently represented the extra-nasal sites with the highest value for a better detection [58,69,70,91]. However, the optimal combination of sampled sites needs to be defined. Should intestinal carriage be considered for all patients or only in some well-identified situations? *S. aureus* infections associated to intestinal carriage

(exclusive or combined with other sites) have been reported in literature as reviewed herein. Further studies are needed to determine the ratio of infection linked to intestinal colonization, especially in population at high risk of *S. aureus* infection like ICU patients.

Before suggesting decolonization for intestinal carriage, it is necessary to identify the target population(s) who will benefit from this strategy. Patients with CA-MRSA SSSI with high frequency of *S. aureus* intestinal carriage could be identified as a target population. Alternative strategies for intestinal decolonization of *S. aureus* such as probiotics, fecal microbiota transplant, vaccines, or topical molecules will probably play a role in the future and should be preferred to SDD or oral antibiotics since the latter could select antimicrobial resistance in microbiota. Further studies must be performed to find the decolonization strategy associated to the best risk-benefit ratio.

Key issues

- Overall, *S. aureus* intestinal carriage prevalence is approximately of 25%. Among these carriers, one third have exclusive intestinal carriage.
- Prevalence of *S. aureus* intestinal carriage are very heterogeneous according to the studied population (age, healthy or hospitalized, risk factors for *S. aureus* colonization, SSSI patients...).
- Intestinal carriage, including exclusive one, may play a significant role in the occurrence of endogenous *S. aureus* infection.
- Screening for intestinal carriage increases detection sensitivity in *S. aureus* carriers.
- At individual level, the sites sampled to investigate *S. aureus* intestinal carriage (rectal, stools, perianal) seem to display similar sensitivity.
- Further studies are warranted to definitely homogenize screening practices
- Genotyping studies are needed to analyse the diversity of strains harboured by a single individual at different sites.
- Intestinal *S. aureus* carriage may have a propensity to facilitate *S. aureus* environment dissemination.
- Decolonization strategies using mupirocine and chlorhexidine probably have a marginal effect on intestinal *S. aureus* carriage.

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Declaration of interest

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Article 1 – Caractérisation du réservoir nasal et digestif à *Staphylococcus aureus* chez des patients de réanimation

Interplay of nasal and rectal carriage of *Staphylococcus aureus* in intensive care unit patients.

Gagnaire J, Botelho-Nevers E, Martin-Simoes P, Morel J, Zéni F, Maillard N, Mariat C, Haddar CH, Carricajo A, Fonsale N, Grattard F, Pozzetto B, Laurent F, Berthelot P, Verhoeven PO.

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Comme nous l'avons constaté dans la revue de la littérature, assez peu d'études ont été menées pour décrire l'épidémiologie de la colonisation digestive comparativement avec la colonisation nasale à SASM chez les patients hospitalisés. De la même façon, assez peu de données existent concernant les infections chez les patients hospitalisés porteurs de *S. aureus* au niveau digestif. La présente étude dénommée DIGESTAPH était une étude épidémiologique descriptive de la colonisation à *S. aureus* au niveau nasal et rectal chez des patients hospitalisés en réanimation adulte. Cette étude a été réalisée dans le cadre d'un Appel d'Offres Local (AOL) financé par le CHU de Saint-Etienne. L'objectif principal était de décrire la prévalence de la colonisation rectale à *S. aureus* dans cette population. Les objectifs secondaires étaient de réaliser chez un même patient la comparaison par typage moléculaire (i) des souches de *S. aureus* isolées au niveau nasal et rectal et (ii) des souches de *S. aureus* isolées de colonisation et d'infections. La population étudiée correspondait à une cohorte prospective de patients recrutés au cours de l'année 2013 dans 3 services de réanimation adulte du CHU de Saint Etienne (réanimation polyvalente et chirurgicale, médicale et néphrologique). Les patients inclus étaient des adultes ayant bénéficié d'un dépistage à la recherche de BMR, à l'admission en réanimation, au niveau nasal et rectal dans le cadre des soins courants.

Sur les 400 patients attendus, 363 ont été inclus dans l'étude. La prévalence du portage de *S. aureus* était de 30,9 % pour le SASM et de 2,2 % pour le SARM. La prévalence du portage rectal était de 14%, assez proche de la prévalence digestive moyenne de 21 % estimée chez les patients hospitalisés incluant des patients de réanimation adulte (Gagnaire et al., 2017). Aucune caractéristique comme le sexe, l'âge, l'IGS2 n'était associée statistiquement au portage digestif (données non publiées). Ce travail a permis de montrer que le dépistage du portage digestif, en plus du nez, permettait de détecter 10 % de porteurs de *S. aureus* supplémentaires et d'identifier 40 % de souches génétiquement non reliées aux souches nasales. Ce pourcentage de diversité entre souches nasales et rectales de colonisation était identique à celui qui avait été rapporté entre souches nasales et pharyngées dans une étude conduite par notre équipe dans une cohorte de patients hémodialysés chroniques (Verhoeven et al., 2015). Nous avons également identifié qu'un patient porteur d'une

souche de *S. aureus* appartenant au complexe clonal 5 au niveau du nez avait 1,8 fois plus de risque d'être porteur de cette même souche au niveau rectal ($p < 0.05$). De façon intéressante, ce clone a été identifié récemment dans la littérature comme associé à la colonisation intestinale à *S. aureus* dans un contexte épidémique (Senn et al., 2016).

Au total, 14 infections dont 8 d'origine endogène chez des porteurs de *S. aureus* ont été rapportées. Le risque de développer une infection à *S. aureus* était multiplié par 4 chez les patients colonisés. Dans cette étude nous n'avons pas trouvé d'association entre le risque d'infection à *S. aureus* et le nombre de sites colonisés comme décrit dans une étude publiée par l'équipe de Squier *et al* (Squier et al., 2002). Néanmoins, 2 infections ont été rapportées chez des porteurs à la fois au niveau nasal et rectal avec des spa-types et des complexes clonaux identiques. L'analyse par NGS qui aurait pu statuer sur le site de colonisation à l'origine de l'infection n'a pas identifié de différence entre les souches de colonisation. Enfin dans ce travail, pour la première fois à notre connaissance une infection d'origine endogène a été rapportée chez un porteur rectal exclusif avec une charge bactérienne élevée. En termes d'impact clinique de la colonisation digestive exclusive dans notre cohorte de patients de réanimation, il est intéressant de noter que l'incidence des infections endogènes chez les porteurs exclusifs au niveau rectal était identique à celle des porteurs exclusifs au niveau nasal. En parallèle, 1 seule infection à SARM sur les 9 diagnostiquées a été rapportée chez les porteurs ce qui suggère l'importance de la recherche en routine des colonisations à SASM. Comme récemment rapporté dans une étude conduite chez 9156 patients hospitalisés en réanimation le portage de *S. aureus* méticilline résistant mais aussi méticilline sensible, est un facteur de risque important d'infection avec cette bactérie avec un risque 10 à 15 fois plus important de développer une pneumopathie nosocomiale (Paling et al., 2017).

Ce travail a apporté des éléments de réponse sur la colonisation digestive à SASM, sur son lien avec la colonisation nasale et son impact clinique. Cependant du fait d'une exposition répétée aux antibiotiques et à un risque de pression de colonisation important en réanimation (Price et al., 2017), la généralisation de ces données ne peut se faire dans d'autres populations. C'est pourquoi le travail suivant a été conduit en communautaire.



Interplay of nasal and rectal carriage of *Staphylococcus aureus* in intensive care unit patients

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Abstract

The aim of this study was to investigate the relationship between nasal and rectal *Staphylococcus aureus* carriage in intensive care unit (ICU) patients and the occurrence of ICU-acquired infections related to *S. aureus* carriage. Three hundred and ninety-five patients admitted in ICU were screened for *S. aureus* nasal and rectal carriage and followed to record *S. aureus* infections during their stay. *S. aureus* strains were genotyped by arbitrarily primed PCR, spotyping, microarray and whole genome sequencing. At ICU admission, 112 of 363 (30.9%) patients carried *S. aureus* including 61 (16.8%) exclusive nasal carriers, 40 (11.0%) combined nasal and rectal carriers and 11 (3.0%) exclusive rectal carriers. The 152 *S. aureus* isolates from nasal and rectal swabs belonged to 19 clonal complexes (CCs). Patients colonized in both nose and rectum harboured different strains in at least 40% of cases according to arbitrarily primed PCR data. Nasal carriers of CC5 *S. aureus* had an increased risk of rectal carriage (RR = 1.85, $P < .05$). *S. aureus* nasal and rectal carriage was a risk factor of *S. aureus* ICU-acquired infection (RR = 4.04; 95%CI [1.38–11.76]). Incidence rates of endogenous ICU-acquired infections in exclusive nasal carriers, exclusive rectal carriers and in both nasal and rectal carriers were 0.08 (5/61), 0.09 (1/11) and 0.03 (1/40), respectively ($p = 0.47$). Rectal swabbing increased the detection of *S. aureus* carriage and revealed an important diversity of *S. aureus* strains in ICU patients. Further studies are needed to understand how *S. aureus* rectal carriage increases the risk of endogenous ICU-acquired infections.

Keywords *Staphylococcus aureus* · Colonization · Nose · Rectum · Staphylococcal infections · Intensive care unit

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Introduction

Staphylococcus aureus colonizes the skin and mucosal membranes of approximately one third of human beings [1–3]. Nasal colonization is a well-established risk factor of different types of *S. aureus* infections in all populations that have been studied [2]. In most cases, the *S. aureus* strains isolated from the nose and the infection site(s) are indistinguishable but the mechanisms of colonization-related infection (i.e. endogenous infection with the strain of carriage) are poorly understood [2]. Still, there is a lack of evidence to define whether *S. aureus* reaches preferentially the site of infection by contamination from the cutaneous site or by translocation through mucosal membrane [4]. The latest data indicate that the mean prevalence of *S. aureus* colonization is 24% and 14% in the nose and intestinal tract, respectively [1, 2]. Compared with the *vestibulum nasi*, which is the main site of carriage in humans, the gastro-intestinal tract also needs to be considered as an important reservoir of *S. aureus* in humans. However, most studies about *S. aureus* intestinal colonization conducted in the past decades have focussed on methicillin-resistant *S. aureus* (MRSA), and the role of methicillin-susceptible *S. aureus* (MSSA) intestinal carriage in colonization-related infection has been largely neglected. To the best of our knowledge, only a few epidemiological studies comparing nasal and intestinal carriage showed that *S. aureus* intestinal colonization could be an additional risk factor for *S. aureus* infections [5–7]. More recent data showed that a particular clone of *S. aureus* (i.e. ST228 MRSA) could be linked with lasting colonization of the intestinal tract [8]. Together, these results suggest that the *S. aureus* intestinal reservoir could play a significant role in colonization-related infections and outbreaks. Besides, *S. aureus* decolonization based on topical application of mupirocine and chlorhexidine for preventing *S. aureus* colonization-related infection did not reduce the intestinal colonization rate [9] and antibiotics-based decolonisation could not be recommended due to the risk of emergence of multidrug-resistant bacteria. Thus, prevention strategies taking into account intestinal *S. aureus* reservoir could help reduce colonization-related *S. aureus* infections, especially in surgical or critically-ill patients.

In order to better understand the relationship between *S. aureus* nasal and intestinal colonization whatever the susceptibility to methicillin, we conducted a prospective study in a cohort of ICU patients that aimed to investigate (i) the prevalence of *S. aureus* nasal and intestinal colonization, (ii) the clonality of *S. aureus* strains isolated in both sites and (iii) the risk of colonization-related infections during hospitalization in ICU.

Material and methods

Patient and sample collection The study took place at the University Hospital of St-Etienne, France, between February and July 2013 in the three adults ICUs including a nephrological unit of 6 beds, a polyvalent medical and surgical unit of 12 beds and one emergency medical unit of 15 beds. Patients were enrolled prospectively and sampled at admission in ICU using nasal and rectal swabs (eSwab 480 CE, Copan, Brescia, Italy). *S. aureus* infections were collected prospectively by the intensive care physicians during the patients' ICU stays. ICU-acquired infections were defined according to the surveillance definition of health care-associated infection in acute care settings elaborated by Centres for disease control and the National Healthcare Safety Network standards [10]. All medical records of infected patients were reviewed independently by both an infectious disease (EBN) and an infection control (JG) specialists. *S. aureus* infection was defined as ICU-acquired if it occurred more than 48 h after patient's admission in the ICU. Acquired-infections were considered endogenous if the strain responsible for infection shared both the same *spa*-type and the same microarray profile as the one(s) found in nasal and/or rectal swabs sampled before the occurrence of infection.

Microbiological methods Nasal and rectal swabs were plated on *S. aureus* chromogenic agar (BBL CHROMagar Staph aureus, Becton Dickinson, France) by using the EasySpiral Dilute instrument (Interscience, St-Nom-la-Bretèche, France) and incubated at 36 °C for 48 h. The Scan1200 plate reader (Interscience) was used to quantify *S. aureus* loads [11]. Clinical specimens were plated on blood agar (COS ref.: 43049, bioMérieux, France) and incubated at 36 °C for at least 48 h. All presumptive colonies were identified by MALDI-TOF MS according manufacturer recommendations (Microflex LT, Bruker, Bremen, Germany).

Genotyping of *S. aureus* strains *S. aureus* strains were analysed by arbitrarily-primed PCR [12], *spa*-typing [13] and DNA microarray (*S. aureus* Genotyping Kit v2.0, Alere, France) [14]. Clonal complexes (CC) of *S. aureus* strains were assigned by using the results of DNA microarray [14]. Microarray data and *spa*-types were analysed with BioNumerics software v7.6 (Applied Maths, Sint-Martens-Latem, Belgium). To analyse more closely the relationship between strains isolated from two patients colonized at both nasal and rectal sites and who presented *S. aureus* infection, Illumina 300 bp paired-end whole genome shotgun sequencing was performed as previously described [15]. Core genome single-nucleotide polymorphism (SNP) analysis and pairwise distance matrices were produced with Snippy v4.2.1 (<https://github.com/tseemann/snippy>), by using the rectal strain sequence as reference. *S. aureus* strains isolated from within

a single individual were considered as unrelated when pairwise SNPs were greater than 40 [16].

Statistical methods Statistical analysis was performed using SPSS software (IBM SPSS v20.0, Chicago, IL, USA) and MedCalc statistical software v18.11 (MedCalc Software bvba, Ostend, Belgium). Chi-squared and Fisher's exact tests were used to compare categorical variables. Mann–Whitney *U* test was used to compare continuous variables. *p* values below 0.05 were considered as statistically significant. Minimum spanning tree was computed with BioNumerics v7.6 (Applied Maths, Sint-Martens-Latem, Belgium).

Ethical statement This study was approved by an Institutional Review Board (“Comité de Protection des Personnes Sud-Est I” No 2012-28) and by the National Security Agency of Medicines and Health Products.

Results

Patient population From the 400 patients enrolled, 363 were analysed in this study (Fig. 1). The sex ratio (M/F) was 1.6; the mean (\pm SD) age was 61 ± 16 years and the median (range) of Simplified Acute Physiologic Score II was 38 (6–115).

***S. aureus* nasal and rectal carriage** At ICU admission, 112 of 363 (30.9%) patients carried *S. aureus*: 61 (54.5%) were exclusive nasal carriers including 6 MRSA carriers, 40 (35.7%) were co-carriers (i.e. carriers of *S. aureus* in both nose and rectum) including 2 MRSA carriers, and 11 (9.8%) were exclusive rectal carriers. Thus, rectal swab improved the

detection of *S. aureus* carriage by 10% (11/112). The characteristics of the studied population, according to *S. aureus* carriage status, are described in Table 1. Except for the risk of acquired infection (see below), characteristics of patients were similar between *S. aureus* carriers and non-carriers. Notably, exposure to antimicrobials with activity against *S. aureus* was similar between *S. aureus* carriers and non-carriers (Table 1).

At inclusion, the mean (\pm SD) *S. aureus* load was significantly higher in nasal specimens (5.1 ± 2.2 log CFU/swab) than in rectal specimens (3.3 ± 1.3 log CFU/swab) ($p < 0.0001$) (Fig. 2a). The mean (\pm SD) *S. aureus* nasal loads were 5.6 ± 2.1 and 4.7 ± 2.2 log CFU/swab in co-carriers and in sole nasal carriers, respectively ($p = 0.053$) (Fig. 2b). The mean (\pm SD) *S. aureus* rectal loads were 3.4 ± 1.3 and 3.1 ± 1.4 CFU/swab in co-carriers and in sole rectal carriers, respectively ($p = 0.6$) (Fig. 2c).

Genetic diversity of *S. aureus* strains The 152 *S. aureus* isolates recovered from nasal and rectal swabs belonged to 20 clonal complexes (CCs). Sixteen (10.5%) isolates were methicillin-resistant including 14 isolates belonging to CC8 (Lyon clone, EMRSA-2) and two isolates belonging to CC22 (EMRSA-15) (Fig. 3).

Arbitrarily primed PCR showed that strains recovered from patients with combined nasal and rectal *S. aureus* carriage are different between the two sites in 16 patients out of 40 (40%). In other words, the rectal sampling improved the recovery of *S. aureus* strains colonizing humans up to 24% (27/112).

Interestingly, nasal carriers of *S. aureus* strains belonging to CC5 have been found to have an increased risk of *S. aureus* rectal carriage (RR = 1.85, 95%CI [1.14–3.02]). No other

Fig. 1 Flowchart: study design and distribution of the studied population in the three adult ICUs of the University hospital of St-Etienne, France

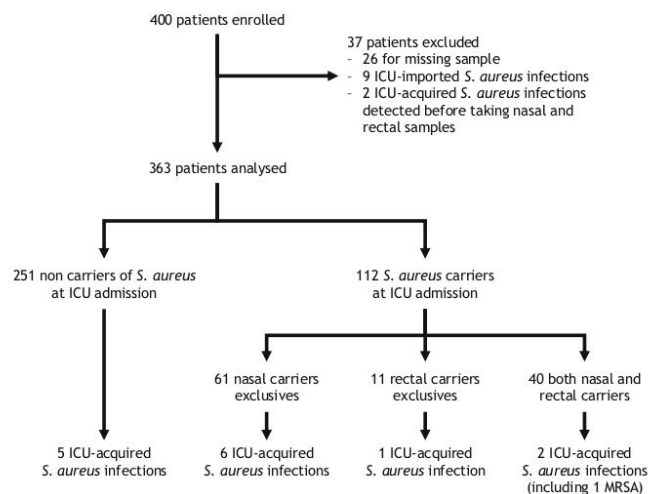


Table 1 Characteristics of patients admitted in ICUs according to their *S. aureus* carrier state

	<i>S. aureus</i> carriers at ICU admission n = 112 (%)	Non <i>S. aureus</i> carriers at ICU admission n = 251 (%)	P value
Type of ICU			
Medical and surgical	55 (49.1)	111 (44.2)	ns
Emergency medical	38 (33.9)	81 (32.3)	ns
Nephrological	19 (17.0)	59 (23.5)	ns
Sex ratio (M/F)	1.8	1.5	ns
Age (mean ± SD)	60 ± 17	62 ± 15	ns
SAPSII ^a (mean ± SD)	42 ± 22	41 ± 20	ns
<i>S. aureus</i> acquired infections	9 (8.0)	5 (2.0)	<0.05
Bacteremia ^b	6 (5.4)	1 (0.4)	<0.01
Pneumonia (incl. VAP)	3 (2.7)	4 (1.6)	ns
Antimicrobial treatment with activity against <i>S. aureus</i> before nasal and rectal samplings	45 (40.2)	112 (44.6)	ns

^a Simplified acute physiologic score II^b Including four cases of catheter-related bacteremia in carrier group and one case non-carrier group
VAP ventilator-associated pneumonia

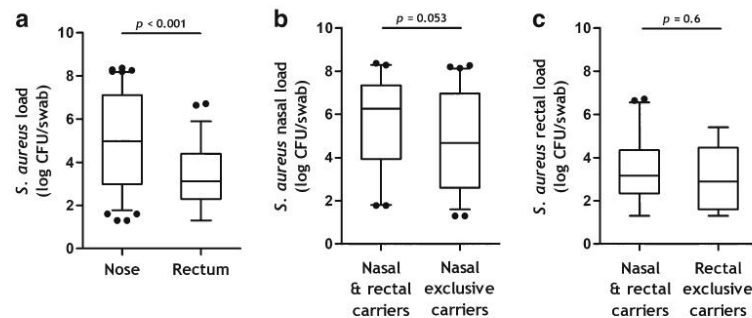
clonal complex was found to influence the risk of associated rectal carriage (Table 2).

***S. aureus* acquired infections** Fourteen ICU-acquired *S. aureus* infections, including one due to MRSA, were diagnosed during the study period and are summarized in Table 3. Nine *S. aureus* infections occurred in carriers and 5 in non-carriers. *S. aureus* carriage was significantly associated with the occurrence of *S. aureus* ICU-acquired infections (RR = 4.30; 95% CI [1.41–13.14]). Overall, the density incidence of ICU-acquired *S. aureus* infections was 2.2 per 1000 ICU-days, respectively, 4.66 and 1.1 per 1000 ICU-days in *S. aureus* carriers and non-carriers ($p < 0.01$). The median (interquartile range) of infection onset after ICU admission was 16 (11 to 19) and 12 days (3.75 to 27.5) in carriers and non-carriers, respectively ($p = 0.39$).

S. aureus infections occurring in carriers were due to their endogenous strain in 8 out of 9 cases (Table 3). Incidence rates

of endogenous infections were, respectively, 0.08 (5/61), 0.09 (1/11) and 0.03 (1/40) in exclusive nasal carriers, exclusive rectal carriers and in both nasal and rectal carriers ($p = 0.47$). One patient (case no. 6) acquired a *S. aureus* bloodstream infection related to a femoral arterial-catheter and had a previous rectal colonization with high bacterial load (10^5 CFU/swab) and no *S. aureus* nasal colonization (Table 3). Two patients (cases number 9 and 10) were found to carry *S. aureus* in the nose and in the rectum and acquired *S. aureus* after ICU admission (Table 3). Briefly, in case no. 8, *S. aureus* isolates recovered from the nose and the rectum harboured similar DNA microarray profiles and as little as 6 SNPs (>97% reference genome mapped). On the one hand, the strain isolated in blood culture showed a deletion of 2 spa repeats compared to the nasal and rectal isolates using spa-typing method. On the other hand, whole genome sequencing showed 894 and 896 pairwise SNPs compared to the nasal and rectal isolates respectively. In case no. 9, *S. aureus* isolates

Fig. 2 *S. aureus* loads recovered from nasal and rectal specimens of the 363 patients included in the study (a), from nasal specimens of co-carriers and nasal carriers sole (b) and from rectal specimens of co-carriers and rectal carrier sole (c)



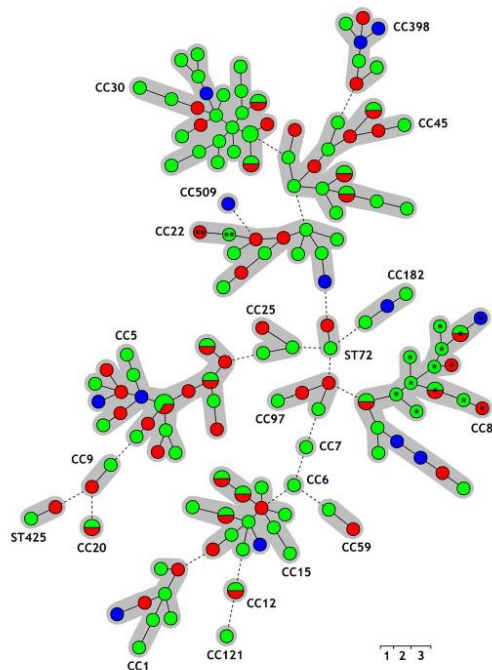


Fig. 3 Minimum spanning tree based on DNA microarray analysis of 165 *S. aureus* isolates recovered from nose (green), rectum (red) and infection site (blue) in intensive care unit patients. Clonal complexes (CC) or sequence type (ST) assigned by microarray analysis are highlighted in light grey. The node size represents the number of isolates according to the scale given. *CC8-MRSA-IV [sea+], Lyon Clone/UK-EMRSA-2. **CC22-MRSA-IV [fnbB-sec/1-], UK-EMRSA-15/Barnim EMRSA

grown from the nasal swabs, rectal swabs and blood culture shared the same spa-type and similar DNA microarray profiles, and harboured no more than 9 pairwise SNPs (Table 3).

Table 2 Risk of *S. aureus* rectal carriage in ICU patients according to the clonal complex of the *S. aureus* nasal carriage strain

Clonal complex of <i>S. aureus</i> nasal isolate (no. of patients)	Relative risk of rectal carriage	95% confidence interval	<i>P</i> value
CC30 (21)	1.11	0.63–1.95	ns
CC8 (15)	0.82	0.38–1.75	ns
CC5 (12)	1.85	1.14–3.02	< 0.05
CC45 (12)	1.31	0.70–2.44	ns
CC15 (11)	0.87	0.38–1.96	ns
CC22 (8)	0.94	0.37–2.38	ns
CC398 (3)	0.31	0.02–4.13	ns
CC1 (5)	0.49	0.08–2.89	ns
CC97 (2)	1.27	0.31–5.18	ns
Others CCs (12)	0.60	0.22–1.65	ns

Discussion

In ICU patients, we found a prevalence of nasal (28%) and rectal (14%) carriage very similar to other settings [1, 2] despite frequent exposure to antimicrobial drugs with activity against *S. aureus* in this population. Exclusive rectal carriage occurred rarely in our cohort (3%), which confirms the results found in other populations [1, 17]. Although nasal carriage was more frequent than rectal carriage, we found that the incidence of endogenous infection in exclusive rectal carriers is similar to those observed in exclusive nasal carriers. To date, few is known about the risk of infection related to colonization at other sites than the nose. In this prospective cohort, we reported a well-documented case of endogenous infection in a patient with exclusive rectal carriage (see case 6; Table 3) and 2 other cases of endogenous *S. aureus* infections occurring in patients colonized at both sites (cases 8 and 9; see Table 3). Previously, two studies reported three cases of *S. aureus* infections in *S. aureus* exclusive rectal carriers. However, molecular typing of colonization and infection strains was not performed and the time between colonization and the occurrence of infection was not specified [6, 18]. Additionally, Szumowski et al. showed that MRSA perianal colonization was associated with skin and soft tissue infection (SSTI) but they did not compare perianal and SSTI isolates [7]. Squier et al. reported that patients with both rectal and nasal carriage were significantly more likely to develop *S. aureus* infection than those with nasal carriage only in ICU but they failed to find infection in exclusive rectal carriers [5]. Here, in the case of bacteremia occurring in an exclusive rectal carrier, it can be hypothesized that the catheter proximity to the colonized site and the high bacterial load in the rectum led to the occurrence of a bloodstream infection. For the two other cases, since strains of colonization were similar, it is impossible to know which site of colonization was at the origin of the infection. In case 8, the strain isolated from the blood culture was considered as different from those

Table 3 Characteristics of patients with *S. aureus* infections acquired in ICU (among carriers and non carriers)

Case no. (ID)	Age/ gender	Comorbidities	Admission diagnosis	SAPS II	Type of infection	Outcome	Delay between admission and infection (days)	<i>S. aureus</i> positive specimen(s) (CC) ^c	Clonal complex (CC) ^c	Spa-type	Repeat succession
1 (008)	71/F	Arterial hypertension Abdominal aortic aneurysm	Meningeal haemorrhage	44	Bloodstream infection	Discharge	28	Nasal swab ^a Blood culture	CC398 CC398	t1250 t1250	08-16-02-25-02-25 08-16-02-25-02-25
2 (014)	62/M	Chronic obstructive pulmonary disease	Necrotizing Myositis Respiratory failure	41	Ventilator-associated pneumonia	Death	12	Nasal swab ^a Tracheal aspirates	CC22 CC22	1852 1852	11-19-12-21-17-34-24-34-22-25 11-19-12-21-17-34-24-34-22-25
3 (120)	63/F	Kidney transplantation Systemic lupus Arterial hypertension Atrial fibrillation	Haemorrhagic stroke Respiratory failure	25	Ventilator-associated pneumonia	Discharge	16	Nasal swab ^a Tracheal aspirates	CC7 CC398	t091 t571	07-23-21-17-34-12-23-02-12-23 08-16-02-25-02-25-34-25
4 (150)	71/M	Arterial hypertension Atrial fibrillation	Respiratory failure	50	Catheter related bloodstream infection	Discharge	44	Nasal swab ^a Blood culture	CC8 CC8	t008 t008	11-19-12-21-17-34-24-34-22-25 11-19-12-21-17-34-24-34-22-25
5 (340)	62/F	Mesenteric artery aneurysm Anxio-depressive syndrome	Heart attack	44	Bloodstream infection	Death	4	Nasal swab ^a Blood culture	CC8 CC8	t190 t190	11-17-34-24-34-22-25 11-17-34-24-34-22-25
6 (343)	50/M	-	Subarachnoid haemorrhage	15	Femoral arterial-catheter related bloodstream infection	Discharge	11	Rectal swab ^a Blood culture	CC1 CC1	t127 t127	07-23-21-16-34-33-13 07-23-21-16-34-33-13
7 (366)	53/M	-	Multiple injuries due to a road accident	39	Ventilator-associated pneumonia	Death	16	Nasal swab ^a Tracheal aspirates	CC182 CC182	t493 t493	04-34-17-66-32-17-23-24 04-34-17-66-32-17-23-24
8 (399)	58/M	Pulmonary embolism	Pneumectomy due to sarcoma Lower limb ischaemia	39	Bloodstream infection	Discharge	29	Nasal swab ^a Rectal swab ^a Blood culture	CC5 CC5 CC5	t539 t539 t062	26-23-17-34-17-12-17-16 26-23-17-34-17-12-17-16 26-23-17-12-17-16
9 (400)	50/M	Cirrhosis Arterial hypertension	Status epilepticus	70	Catheter related bloodstream infection	Discharge	16	Nasal swab ^a Rectal swab ^a Blood culture ^b	CC8 CC8 CC8	t008 t008 t008	11-19-12-21-17-34-24-34-22-25 11-19-12-21-17-34-24-34-22-25 11-19-12-21-17-34-24-34-22-25
10 (113)	19/M	-	Suicide attempt Facial injury	11	Pneumonia	Discharge	14	Tracheal aspirates	CC30	t4322	04-44-33-31-12-16-34-12-25-22-34
11 (256)	67/M	Chronic obstructive pulmonary disease Aortic aneurysm Cerebral vascular accident Chronic alcoholism	Acute pancreatitis	22	Catheter related bloodstream infection	Discharge	68	Blood culture	CC5	t045	26-17-20-17-12-17-16

Table 3 (continued)

Case no. (ID)	Age/ gender	Comorbidities	Admission diagnosis	SAPS II	Type of infection	Outcome	Delay between admission and infection (days)	<i>S. aureus</i> positive specimen(s)	Clonal complex (CC) ^c	Spa-type	Repeat succession
12 (260)	73/M	–	Aortic dissection	48	Pneumonia	Discharge	12	Tracheal aspirates	nd	nd	
13 (322)	47/M	Arterial hypertension Hypothyroidism	Head injury Occupational accident	37	Ventilator-associated pneumonia	Discharge	3	Tracheal aspirates	CC509	t489	49-13-23-05-34-34-33-34
14 (383)	52/M	Cervical myelopathy Mensiscectomy	Spinal cord injury tetraplegia Occupational accident	33	Ventilator-associated pneumonia	Discharge	4	Tracheal aspirates	CC15	t084	07-23-12-34-34-12-12-23-02-12-23

^a Carriage isolate sites

^b MRSA strain responsible for infection

^c Clonal complex was assigned by microarray genotyping (*S. aureus* genotyping kit V2.0, Alere) *nd* not done because missing strain

recovered from the nose and the rectum of this patient given the large number of pairwise SNPs observed. In this case, the delay of infection occurred 29 days after ICU admission, which could be enough to let the bacteria adapt to the host [19]. In case 9, WGS sequencing showed that the strain isolated in blood culture and those isolated from nasal and rectal sites were closely related, and despite the use of high-throughput method for investigation [20], it was not possible to determine whether nasal or rectal colonization originated the infection. Both cases of bacteremia might be related to rectal carriage. As we observed here, the combination of colonization sites is also associated to a high *S. aureus* bacterial load and then could potentially facilitate staphylococcal infections, increasing the risk of device contamination [18]. These observations evoke that the rectal reservoir could play a role in autoinfection [21]. The combination of colonization sites is probably associated to a dispersion of *S. aureus* colonization on the body (skin and mucosa) and in the environment of the patient [18]. Additionally, Senn et al., who reported a large outbreak of *S. aureus* colonization, suggested that the *S. aureus* rectal strain plays the role of a stealthy superbug able to maintain a long-term hospital outbreak by cross-transmission [8]. In our work, the genotyping of the 165 *S. aureus* isolates revealed a great diversity within patients indicating that no or few cross-transmissions occurred in the ICUs. Interestingly, we showed that strains belonging to CC5 seem to be more implicated in rectal carriage, notably when the patient was co-colonized with this clone in nose and rectum compared to other clones. The ST228 was already related to rectal carriage in an outbreak of *S. aureus* colonization [8].

Staphylococcus aureus is one of the most frequent causes of infections in the ICU [22–24] and we confirmed that *S. aureus* carriage is a risk factor for *S. aureus* ICU-acquired infections. This risk factor had been largely described previously for MRSA carriage [22, 25–29] but not so extensively for MSSA carriage [30, 31]. In recent studies [22, 25–30, 32], the relative risk of acquisition of *S. aureus* infections in ICU associated with *S. aureus* nasal or throat carriage ranged from 2.5 to 17.8 and our data are in concordance to previous findings. In fact recently, studies showed that screening patients for MRSA carriage at ICU admission is a valuable tool for guiding decision to treat ICU-acquired infections [27, 28]. We highlighted in the present work that not only MRSA carriage but also MSSA carriage could have an impact on the risk of infection in ICU as also showed by Paling et al. in a post hoc analysis of two cohort studies in critically ill patients [32]. Interestingly, molecular typing of *S. aureus* strains in ICU-patients harbouring combined nasal and rectal carriage showed that approximately half of them were colonized with two different strains of *S. aureus*. Similarly, we reported that *S. aureus* naso-pharyngeal carriers harboured two different strains in approximately half of cases in a cohort of haemodialysis patients [33]. These results suggest that

S. aureus carriers could harbour two or three different strains in the nose, the throat and the rectum in hospitalized patients exposed to colonization pressure and antimicrobial drugs [34]. Therefore, screening *S. aureus* carriage at ICU admission, for whatever methicillin resistance profile, would allow physicians, especially among patients with pneumonia, to adapt a probabilistic antibiotic treatment for patients according to the antimicrobial drug susceptibility of their *S. aureus* carriage strain with potential antimicrobial stewardship implications [35]. The prevention of *S. aureus* (MSSA and MRSA) endogenous infections may also be questioned in further studies since a recent meta-analysis showed that mupirocin decolonization was protective against *S. aureus* infections among adult ICU patients [36].

There are some limitations to our study. First, patients have been enrolled in 2013 and the study data may not represent the present situation. In the present work, we did not investigate other reservoirs as the study was observational without additional sampling of the patients. Additionally, because of the high rate of patients exposed to antimicrobial drugs, the prevalence of *S. aureus* carriage was probably underestimated and not reliable to determine the *S. aureus* nasal carrier status, as described by our team [37, 38] when we investigated the relationship between persistent and non-persistent carriers and the risk of developing a ICU-acquired *S. aureus* infection. However, this study is relevant in a population of ICU patients because of the frequent prescription of antibiotics in all ICUs.

Conclusion

This study confirms that nasal colonization by *S. aureus* is an important risk factor for *S. aureus* ICU-acquired infections and suggests that *S. aureus* rectal carriage could be an additional risk factor for infection. Rectal swabbing could improve the detection of an unrecognized *S. aureus* strain. Screening of *S. aureus* carriage in ICU patients, for whatever methicillin resistance profile, may help the prescription of antibiotics. Further studies are needed to better understand the role of the different reservoirs of *S. aureus* strains and their clinical impact on the risk of *S. aureus* infection, notably in ICUs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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Article 2– Colonisation intestinale à *Staphylococcus aureus* avant et après préparation colique chez des patients bénéficiant d’une colonoscopie

Staphylococcus aureus intestinal colonization in patients undergoing bowel preparation for colonoscopy

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Submitted in Alimentary Pharmacology and Therapeutics

Comme nous l'avons vu dans la revue, le choix d'un site plutôt qu'un autre pour dépister une colonisation digestive à *S. aureus* varie selon les populations étudiées et selon les auteurs. Bien que les données de la littérature montrent une équivalence entre ces différents sites, la notion de réservoir digestif reste équivoque. La présente étude dénommée COLOSTAPH était une étude épidémiologique descriptive de la colonisation à *S. aureus* dans une cohorte de patients ambulatoires devant bénéficier d'une colonoscopie de dépistage de cancer colorectal en service de Gastro-entérologie. Cette étude pilote descriptive a été réalisée dans le cadre d'un AOL financé par le CHU de Saint-Etienne. L'objectif principal était d'évaluer la prévalence du portage digestif (au niveau du pli de l'aîne, rectal et colique) de *S. aureus* dans cette population. L'objectif secondaire était d'évaluer l'effet de la préparation colique sur la colonisation à *S. aureus*. Dans cette cohorte prospective, les patients inclus étaient des adultes de moins de 80 ans qui ne devaient entre autres ne pas avoir d'antécédent de pathologie chronique intestinale, pas de corticothérapie au long cours ni de traitement antibiotique à visée anti staphylococcique dans le but d'exclure le plus de facteurs confondants possibles qui auraient pu sous ou sur estimer la prévalence de la colonisation digestive à *S. aureus*. Trois types de préparation colique ont été prescrits: 2 solutions à base de polyéthylène glycol (PEG) dont une additionnée d'acide ascorbique et 1 solution à base de picosulfate de sodium additionnée de citrate de magnésium. La qualité de la préparation colique a été évaluée à partir du score de Boston (Lai et al., 2009). Lors de la consultation pré-colonoscopie, 4 prélèvements ont été réalisés au niveau du nez, gorge, pli de l'aîne et du rectum. Le jour de l'examen, ces 4 prélèvements ont été renouvelés avant l'examen endoscopique et des biopsies du colon droit et/ou gauche ont été réalisées si des biopsies étaient nécessaires dans le cadre de l'investigation gastro-entérologique.

Sur les 143 patients attendus, 113 ont été inclus dans l'étude. Au moment de l'inclusion, il y avait 41,6 % des patients colonisés par *S. aureus* tout site de dépistage confondu. La prévalence du portage digestif de *S. aureus* était de l'ordre 10 %, au niveau du pli de l'aîne comme au niveau rectal, assez proche de la prévalence digestive moyenne de 14 % estimée en population communautaire (Gagnaire et al., 2017). Bien que la prévalence soit similaire entre ces 2 sites de dépistage, nous avons identifié seulement 3 patients colonisés à la fois au niveau du pli de l'aîne et

au niveau du rectum. Aucune des caractéristiques patients comme le sexe, l'âge, l'indice de masse corporelle, la notion d'immunodépression, ou encore le tabagisme n'étaient associées statistiquement au portage digestif (données non publiées). Egalement, aucune souche de SARM n'a été identifiée chez les porteurs au niveau digestif.

Le jour de la colonoscopie, des souches de *S. aureus* ont été isolées à partir de 6 biopsies coliques chez 3 patients. A notre connaissance, c'est la première fois que l'identification de *S. aureus* au niveau colique a été réalisée chez l'homme. Malgré quelques limites que nous discuterons, ces données vont dans le sens de l'existence d'un réservoir digestif de *S. aureus*. Après préparation colique, on observait une tendance significative à la baisse du nombre de porteurs de *S. aureus* ainsi que des charges bactériennes quel que soit le site de colonisation prélevé. Il n'y avait pas d'association statistique entre le type de produit utilisé, le score de Boston et la négativation des porteurs après lavement colique. Il a pu être montré que la préparation colique pouvait avoir un impact sur le microbiote intestinal (Harrell et al., 2012) mais à notre connaissance aucune étude ne décrit son effet pour la décolonisation de *S. aureus*.

Ce travail apporte quelques éléments de réponse sur le réservoir digestif de *S. aureus* et sur l'effet de la préparation colique avec une décolonisation de *S. aureus* au niveau de plusieurs sites de portage dont le nez.

***Staphylococcus aureus* intestinal colonization in patients undergoing bowel preparation for colonoscopy**

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Running title: *S. aureus* carriage during colonoscopy

Key words: *Staphylococcus aureus*, intestinal colonization, nose colonization, colonoscopy, bowel preparation

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ABSTRACT

Background

The existence and the role of intestinal *Staphylococcus aureus* reservoir in the occurrence of endogenous infection due to this bacterium are debated.

Aims

To estimate the prevalence of intestinal *S. aureus* carriage and to investigate the dynamics of *S. aureus* colonization before and after bowel cleansing preparation.

Methods

We conducted a prospective observational study in community patients undergoing a colonoscopy for cancer screening. Nasal, throat, groin and rectal swabs were performed before and at the day of colonoscopy. Colonic biopsies from the right and the left colon were performed at the day of colonoscopy. The bowel preparation was based on polyethylene glycol or sodium picosulfate.

Results

Among the 113 enrolled patients, the overall prevalence of *S. aureus* carriage, according to the screening of 4 colonization sites was estimated at 41.6 % (IC 95% 32.5 – 50.7] whose 2.1% of MRSA carriers. At the inclusion, 11 (9.7 %) and 12 (10.6%) patients were identified as groin and rectal carriers. At the day of colonoscopy, 3 (2.6%) patients were identified as *S. aureus* intestinal carriers with 6 positive colonic biopsies. After the bowel preparation, there was a trend to a decrease of the number of *S. aureus* carriers and of the *S. aureus* loads in the studied population (OR = 0.60, CI [0.35 – 1.05]).

Conclusions

Despite some limitations we reported for the first time, in community patients, the identification of *S. aureus* in colon and we documented the decolonizing effect of bowel preparation on 4 *S. aureus* colonization sites including the nose.

INTRODUCTION

Staphylococcus aureus is both a major pathogen and a commensal bacterium that colonizes the skin and the mucosae in humans. The prevalence of *S. aureus* intestinal carriage ranges from 13.8% to 21.4% in healthy adults and inpatients, respectively. It was shown that the screening for intestinal carriage increases the sensitivity of detection in *S. aureus* carriers ¹. Intestinal carriage, including exclusive one, may play a significant role in the occurrence of endogenous *S. aureus* infection and was shown to be associated with a high risk of *S. aureus* dissemination in the environment ¹.

The intestinal carriage of *S. aureus* is still far from being well described because various samples (including rectal, recto-vaginal, perianal or inguinal swabbing or stools) are used to screen this reservoir ¹. Studies aimed at identifying *S. aureus* colonization of the digestive mucosa in humans are scarce ^{2,3}. Additionally, the relationship between *S. aureus* nasal and intestinal sites remains unclear. The few data available were conducted in patients hospitalized, probably not reflecting what occurred in community patients. Moreover, the way of colonization of the intestinal tract is also debated ⁴. It could be the consequence of perianal skin area colonization or of the nasopharyngeal colonization.

The bowel preparation was found to afflict, at least temporarily, the intestinal microbiota ⁵ but the impact of this colon cleansing regimen on *S. aureus* intestinal carriage was not studied. Here, we conducted a prospective study in a cohort of community patients undergoing a colonoscopy in a Gastroenterology department aimed at investigating (i) the prevalence of *S. aureus* intestinal colonization, (ii) the effect of bowel preparation on *S. aureus* colonization.

MATERIAL AND METHODS

Study population. Patients were enrolled in the Gastroenterology department of the University Hospital of St-Etienne, France, between February 2015 and October 2017. The inclusion criteria were adults who scheduled to undergo a colonoscopy for cancer screening due to positive fecal blood test, family history of colorectal cancer, polyps monitoring or digestive disorders. The exclusion criteria were pregnant women, adults other 80 years, patients with anticoagulant or antiplatelet therapy, with a known hemostasis disorder, with known inflammatory bowel diseases (IBD), receiving long-term corticosteroid therapy and under anti-staphylococcal antibiotic treatment.

Sample collection and bowel preparation. Patients were enrolled prospectively and sampled during the gastro-enterology's consultation using nasal, throat, groin and rectal swabs (eSwab 480CE, Copan, Brescia, Italy). Patients received instructions for bowel preparation before colonoscopy based on polyethylene glycol (PEG) containing or not ascorbic acid or a preparation with sodium picosulfate containing magnesium citrate according to clinician preferences or any prior experiences ⁶. No preoperative shower was required before the exam. On the day of colonoscopy, patients were sampled before colonoscopy at the same sites than in the inclusion to investigate *S. aureus* patient's colonisation. The efficacy of mechanical bowel preparation was evaluated with the Boston bowel preparation scale (BBPS) score ⁷. If colon biopsies were necessary, according to the clinical context, two additional biopsies from right and left colon were taken for *S. aureus* screening (Captura Biopsy Forceps, Cook medical, USA). The endoscopy specialists were blinded to any results of *S. aureus* colonization.

Microbiological methods. A 10 µL of Amies liquid was plated on *S. aureus* chromogenic agar (BBL CHROMAgar Staph aureus, Becton Dickinson, France) for a semi quantification of *S. aureus* bacterial loads and incubated at 36°C for 48h. All presumptive colonies were identified by MALDI-TOF MS according manufacturer recommendations (Microflex LT, Bruker, Bremen, Germany). Biopsy specimens were dissected and ground using a manual disposable homogenizer in a tube containing 1 mL of brain heart infusion broth. *S. aureus* cultures on chromogenic agar were performed with and without a 48h broth enrichment culture at 36°C. The relationships between the strains isolated from the different sites of colonisation was established by using spa-typing as previously described ⁸.

Statistical methods. Statistical analysis was performed using SPSS software (IBM SPSS v20.0, Chicago, IL, USA). It was calculated that for a prevalence of digestive carriage expected at 14%⁹, by choosing a lower bound of the confidence interval of not less than 10% compatible with the data in the literature, the number of subjects required was 143 patients. The *S. aureus* quantity was categorized in 5 semi-quantitative classes (classe 1: 10^2 - 10^3 UFC/swabs; classe 2: $>10^3$ - 10^4 UFC/swabs; classe 3: $>10^4$ - 10^5 UFC/swabs; classe 4: $>10^5$ - 10^6 UFC/swabs; classe 5 $> 10^6$ UFC/swabs). Univariate analysis was performed for several factors like BBPS score and type of bowel preparation to analyse their impact of the presence or lack of *S. aureus* colonization after bowel preparation in *S. aureus* carriers at inclusion. Chi squared, chi squared for trend and Fisher's exact tests were used to compare categorical variables. Mann–Whitney *U* test was used to compare continuous variables. *P*-values below 5% were considered as statistically significant. Dendogram was computed with BioNumerics v7.6 (Applied Maths, Sint-Martens-Latem, Belgium).

Ethical statement. This study was approved by an Institutional Review Board (“Comité de Protection des Personnes Sud-Est I 2014-28) and by the French Security Agency of Medicines and Health Products.

RESULTS

Study population. Among the 122 patients enrolled, 113 were included in the study (see **Figure S1 for the flowchart**). All were outpatients. The mean delay between the inclusion and the colonoscopy was 53 days (range 1-159). Ninety two (81%) patients had at least one colon biopsy for *S. aureus* research because other biopsies were necessary according to the clinical context. A total of 1085 microbiological samples were analyzed. Among the 113 included patients, the sex ratio (M/F) was 1.3. The mean (\pm SD) age of participants was 60.5 ± 10 years and the mean (\pm SD) body mass index was 26.2 ± 5 kg/m². Sixteen (14%) patients reported smoking habits, 6 (5%) reported suffering from diabetes mellitus, 4 women were using contraception. None patient with immunodeficiency was identified in the included population. Twelve patients (11%) were healthcare workers.

Dynamics of *S. aureus* colonization.

At inclusion, 47 of 113 (41.6 %) patients carried *S. aureus* including 2.1% of methicillin-resistant *S. aureus* (MRSA) carriers. Among them 34 (30.1 %) were nasal carriers, 22 (19.5 %) throat carriers, 11 (9.7%) groin

carriers and 12 (10.6 %) rectal carriers. None of intestinal *S. aureus* carriers were found as MRSA carriers. A total of 3 patients were colonized simultaneously at groin and rectum. Among the previous characteristics of the study population, none were statistically associated with the intestinal carriage of *S. aureus*.

At the time of the colonoscopy, 34 of 113 (30.1%) patients carried *S. aureus*. Among them 22 (19.5%) were nasal carriers, 17 (15.0%) throat carriers, 3 (2.6%) groin carriers and 8 (7.1%) rectal carriers. The dynamics of *S. aureus* load in all carriers before and at colonoscopy was illustrated in **Figure 1**. After the bowel preparation, there was a trend to a decrease of *S. aureus* carriage in the studied population (OR = 0.60, CI [0.35 – 1.05]), a trend to decrease of nasal *S. aureus* carriage (OR = 0.56, CI [0.30 – 1.03]) and a significant decrease for groin *S. aureus* carriage (OR = 0.25, CI [0.06 – 0.93]). As a whole, the *S. aureus* load decreased for the nose, throat and the rectum sites as illustrated by the **Figure 1E** (respectively $p=0.1$, $p=0.2$, $p<0.05$; chi squared for trend). Results of a univariate analysis that was performed on 43 patients identified as *S. aureus* carriers at inclusion according to their *S. aureus* colonization status after the bowel preparation were synthesised in **Table 1**. Briefly, the type of preparation, the BBPS scores and the number of colonization sites were not associated with the negativation of *S. aureus* carriage after bowel preparation. Seven patients were found to acquire *S. aureus* by comparison to the carriage status at the inclusion including 5 throat carriers, 1 nasal carrier and 1 rectal carrier. A great diversity of *S. aureus* strains was observed in the studied population (see **Figure S2**) but all *S. aureus* strains identified from 27 *S. aureus* carriers before and on the day of colonoscopy were genetically similar.

Identification of *S. aureus* carriers in colon biopsies. *S. aureus* was recovered from both right and left colon biopsies in 3 (2.6%) patients. These 3 patients were *S. aureus* carriers at other sites at the time of colon biopsy and at inclusion (**Table 2**). Their *S. aureus* strains harbored the same spa type whatever the site of carriage. Among them, one patient had a BBPS score under 5, one had a BBPS between 7-8 and one had a BBPS between 8-9.

DISCUSSION

In this study that included community patients, the prevalence of *S. aureus* carriage, according to the screening of 4 colonization sites, was estimated at 41.6 % (IC 95% 32.5 – 50.7). Many studies that focused on MRSA carriage have shown that the prevalence increased with the number of colonization sites screened^{10,11}. In this study, 98 % of carriers were colonized with methicillin-susceptible *S. aureus* (MSSA) strains. The prevalence of *S. aureus* rectal carriage was estimated at 10.6 % [IC 95 % 4.9 – 16.3] in accordance with the results of the meta analyse that we performed in healthy patients¹. As described in other studies^{12,13}, the frequencies of *S. aureus* carriage in rectal and groin sites were similar. Remarkably, only 3 patients were colonized simultaneously at groin and rectum. In this context, the hypothesis that colonization of the intestinal tract could be the consequence of perianal skin area colonization⁴ seems debatable.

To the best of our knowledge, in our cohort of 92 healthy patients, this is the first time that *S. aureus* was recovered from colon biopsies. Lyra *et al*, in a study aimed to compare the bacterial quantities in left and right colon biopsies didn't detect, by using quantitative polymerase chain reaction, *S. aureus* in these samples¹⁴. The presence of *S. aureus* in colon samples suggests a specific intestinal reservoir of these bacteria. One study demonstrated the adhesion of *S. aureus* to human intestine mucus². Gries *et al* reported, using an *in vivo* model in mice, the possibility of persistent intestinal *S. aureus* colonization after oral administration of MRSA strains³. They also found significant amounts of *S. aureus* in the mucus of the caecal mucosa of infected mice. Further fundamental works need to be done to study the extra or intracellular localization of the bacteria on the colon mucosa as already described for *S. aureus* colonization in the *vestibulum nasi*. Recently, Krezalek *et al*, using also an intestinal *S. aureus* colonization model in mice, showed that the translocation of *S. aureus* strain from the digestive mucosa to a wound causing secondary surgical site infections (SSI) was possible, particularly via neutrophils¹⁵. The consequences and the risk associated with this carriage are not known. Interestingly, dysbiosis have been reported in IBD patients¹⁶ and *S. aureus* intestinal colonization have been recently negatively associated with disease or condition of the gastrointestinal tract including IBD¹⁷. It would be interesting to study *S.*

aureus intestinal carriage in IBD patients to compare the prevalence of *S. aureus* carriage to our healthy population and to look for the localization of *S. aureus* carriage in the digestive tract as many colonic biopsies are regularly performed in the follow-up of this disease.

This is the first time, to our knowledge, that the impact of bowel preparation on *S. aureus* carriage was described investigating 4 screening sites, including the nose. After the bowel preparation, there was a trend to a decrease in the total number of *S. aureus* carriers ($p = 0.07$), a trend to decrease of nasal *S. aureus* carriage ($p=0.06$) and a significant decrease for groin *S. aureus* carriage ($p=0.03$). Interestingly, *S. aureus* load tend to decrease significantly for the 4 screening sites in *S. aureus* carriers detected at inclusion. It was already described that the bowel preparation, notably with the use of PEG, is associated with a loss of bacteria viability¹⁸, with an impact on the composition and on the diversity of mucosal-adherent microbiota¹⁹. The use of bowel preparation to prevent infection was slightly studied. Cheng *et al*, reported that a combination of environmental disinfection, patient isolation, bowel preparation using PEG, a five-day course of linezolid and daptomycin and oral *Lactobacillus rhamnosus*, had an effect on gastrointestinal vancomycin-resistant *Enterococcus faecium* clearance²⁰. In 2017, the WHO guidelines for the prevention of SSI concluded that mechanical bowel preparation without administration of oral antibiotics should not be used for the purpose of reducing SSI in adult patients undergoing elective colorectal surgery²¹. The use of bowel lavage in association to fecal transplantation has also been recommended in the treatment of recurrent *Clostridium difficile* infection²². But adverse events have been also reported including patient discomfort, electrolyte abnormalities and dehydration at the time of surgery²¹. The effect of bowel preparation on *S. aureus* carriage at distance of the colonoscopy was not evaluated in our study. Recently, Nagata *et al* described that bowel preparation has a profound impact on the gut microbiome and metabolome but the overall composition recovers to baseline within 14 days⁵. Our study showed that bowel preparation, at least temporarily, could be a tool to decrease *S. aureus* carriage and loads whatever the site of carriage.

This study has several limitations. Colon cleansing regimen probably underestimated the *S. aureus* identification present in the intestine. Fecal contamination on colon mucosa, estimated by the BBPS

score, could not be excluded for the 3 patients found colonized in colon biopsies even if one patient was not recovered colonized in the rectum at the day of the colonoscopy. Additionally, we did not sample the stools to look for *S. aureus* colonization just before colonoscopy. However, using molecular typing we were able to show that patients persistently colonized harbored the same *S. aureus* strains excluding potential cross transmission during the procedure.

Because of the pragmatic design of the study we were not able to describe the specific activity of each regimen used for bowel preparation of patients.

However the major strength of this study, including 113 healthy and community patients without chronic diseases or healthcare expositions, was to well describe the *S. aureus* colonization from nose to rectum, with to the best of our knowledge, the first report of *S. aureus* identification on colonic biopsies. We were able to document the effect of bowel preparation on *S. aureus* colonization with a decrease in the number of colonized sites but also of the *S. aureus* loads. This observation could be a path for further studies in *S. aureus* colonized patients, particularly those multi-colonized or harboring a high bacterial load, in order to better prevent the risk of infection due to this bacterium during invasive procedures.

CONCLUSION

In the present study, we well described the *S. aureus*, particularly the intestinal one, colonization of community patients and brought arguments for the presence of *S. aureus* in colon. We were able to measure at the day of the colonoscopy the effect of the mechanical bowel preparation on the global *S. aureus* patients' colonization suggesting a possible use of this regimen to decolonize them, at least temporarily

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TABLES

Table1. Univariate analysis of factors associated with the presence or lack of *S. aureus* colonization after bowel preparation in positive *S. aureus* carriers at inclusion

	Positive <i>S. aureus</i> carriers at inclusion and negative after bowel preparation (n=19)*	Positive <i>S. aureus</i> carriers at inclusion and positive after bowel preparation (n=24)*	<i>P</i> value
Gender (Male)	9	17	0.2
Age (mean \pm SD)	62.8 \pm 9.1	61.8 \pm 11.7	0.1
Body mass index (mean \pm SD)	26.8 \pm 4.4	26.1 \pm 4.3	0.6
BBPS score between 8-9 vs BBPS score under 8-9	16	19	1
Bowel preparation with polyethylene glycol vs sodium picosulfate preparation	7	15	0.1
<i>S. aureus</i> carriers at multiple sites at inclusion vs exclusive carriers	8	14	0.3

*43 patients for which the type of bowel preparation has been recorded / 4 patients with missing data

Table 2. *S. aureus* load according to the sampled sites in patients with *S. aureus* strains found in colon biopsies

Patient number	inclusion 039	045	056
Load of <i>S. aureus</i> before colonoscopy (UFC/swab)			
Nose	10 ⁶	-	10 ⁷
Throat	10 ²	10 ⁴	10 ²
Groin	-	-	10 ⁷
Rectum	-	10 ³	10 ²
Load of <i>S. aureus</i> at the day colonoscopy (UFC/swab)			
Nose	10 ⁵	-	10 ⁵
Throat	10 ³	-	-
Groin	10 ²	-	-
Rectum	10 ³	10 ²	-
Right colon biopsy	+	+	+
Left colon biopsy	+	+	+
+ Broth enrichment			

LEGENDS OF FIGURES

Figure 1. Dynamics of *S. aureus* load carriers before and at the day of colonoscopy in the total population (A-D) and dynamics of *S. aureus* load before and at the day of colonoscopy in *S. aureus* carriers at inclusion (E). The arrows represent 5 groups of patients which are defined according to (from left to right) : loss of *S. aureus* carriage, decrease of *S. aureus* load, steady state of *S. aureus* carriage, increase of *S. aureus* load, acquisition of *S. aureus* carriage.

Figure 2. Flowchart of the studied population

Figure 3. Dendogram based on spa typing analysis of 92 *S. aureus* isolates recovered from nose, throat, groin and rectum at inclusion (green) and the day of the colonoscopy (red) in *S. aureus* carriers at inclusion

|

FIGURES

Figure 1

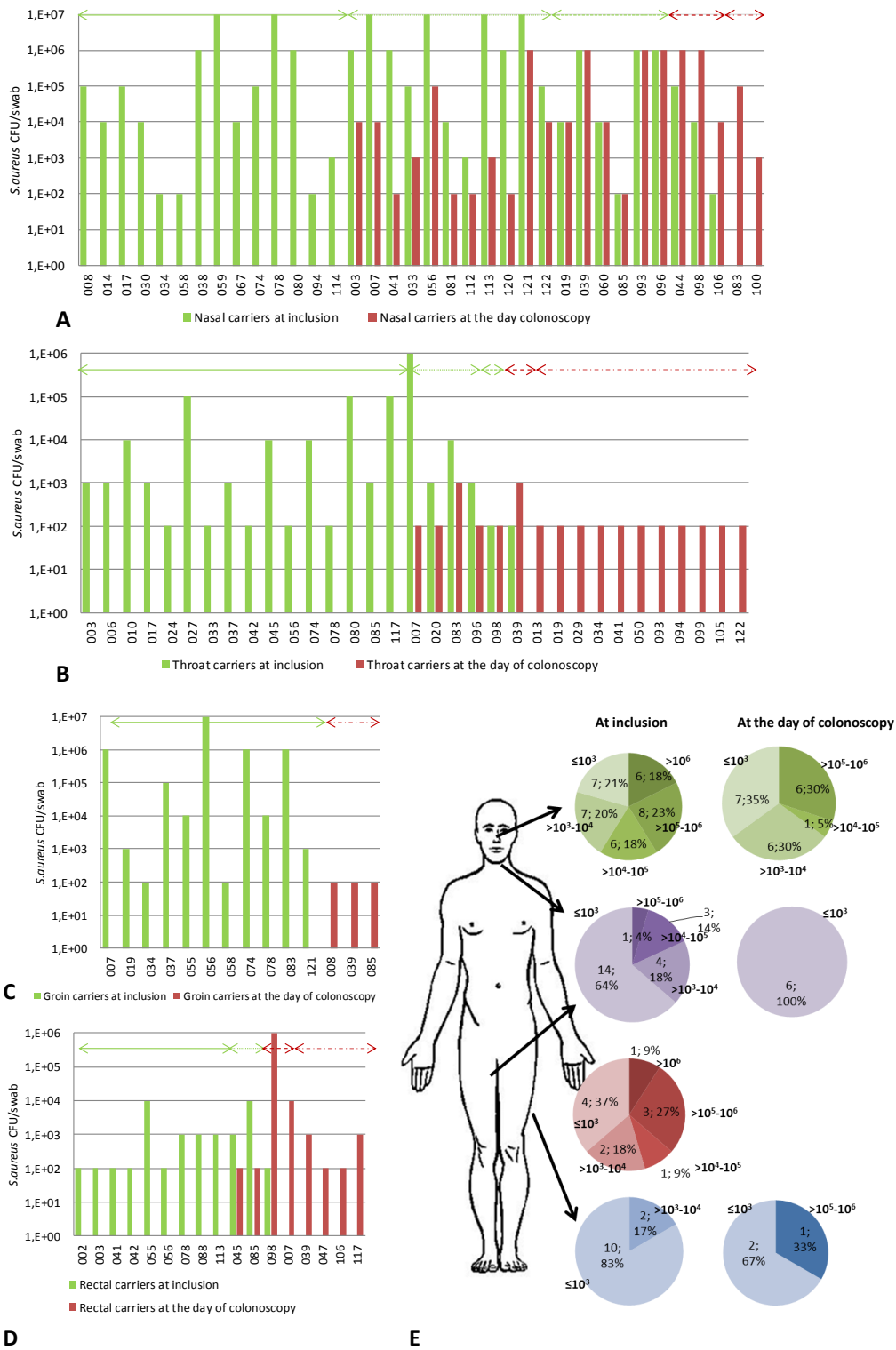


Figure 2

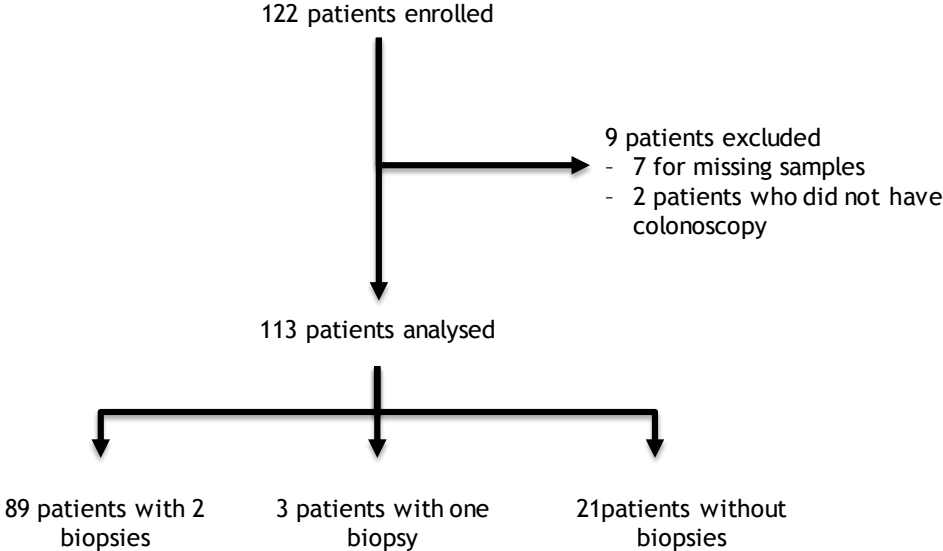
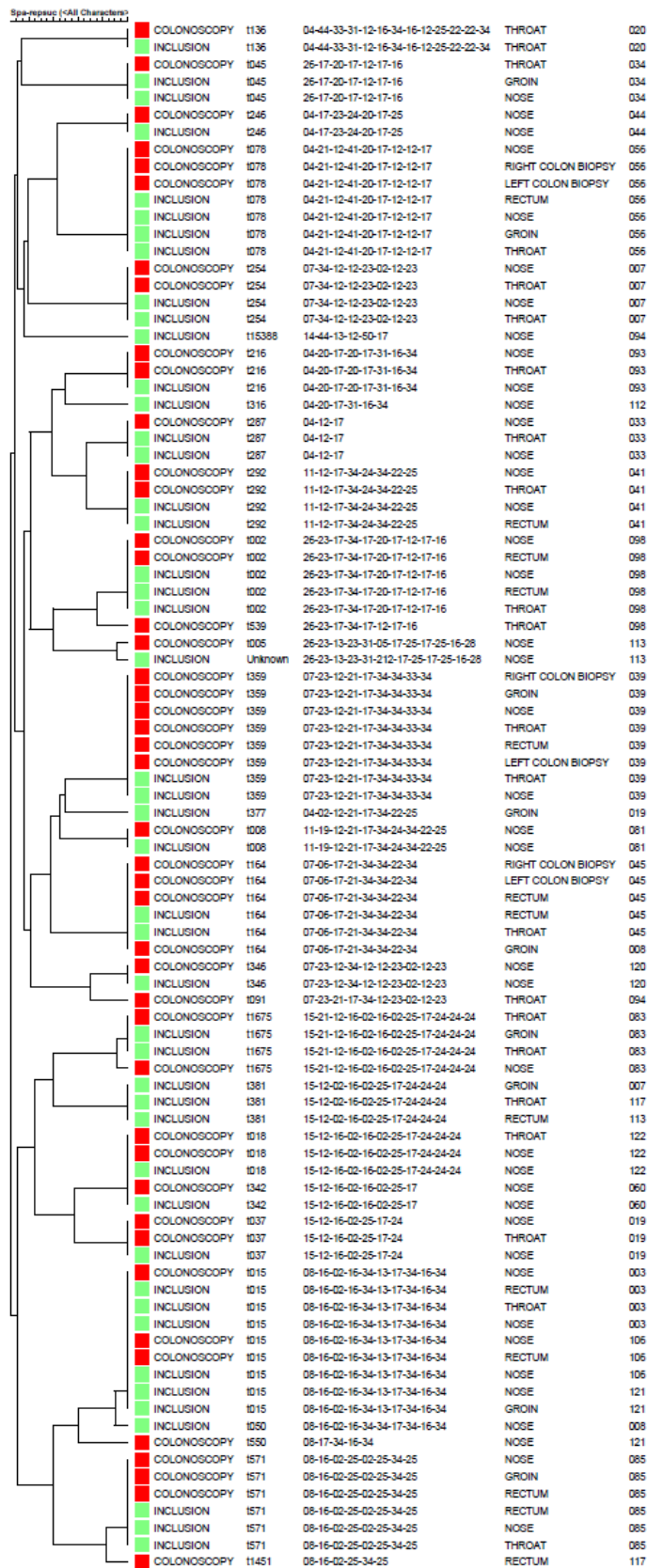


Figure 3



Discussion

Epidémiologie de la colonisation digestive

Les données de prévalence, et d'impact clinique des colonisations extra-nasales sont rares en comparaison à celles du portage nasal. Au cours de ce travail de thèse, nous avons apporté par des travaux d'épidémiologie clinique des données de prévalence du portage digestif en population hospitalière et communautaire. Nous allons discuter ces données au regard de la littérature.

En agrégeant l'ensemble des populations pour lesquelles la colonisation digestive à *S. aureus* a été étudiée dans notre méta-analyse, la prévalence globale de *S. aureus* au niveau digestif de 25 % est finalement assez similaire à celle de la colonisation nasale (Gagnaire et al., 2017). Néanmoins, on observe une grande hétérogénéité des prévalences de portage en fonction des populations étudiées : de 14 % pour les patients communautaires jusqu'à 70 % chez les nouveaux nés. Dans notre étude conduite en réanimation adulte, la prévalence du portage de *S. aureus* au niveau rectal était de 14 %, assez proche de celle décrite dans notre revue chez les patients hospitalisés de l'ordre de 21 % (IC 95% [9-37]) (Gagnaire, et al. 2019; Gagnaire et al. 2017). Cette prévalence de portage de *S. aureus* peu élevée peut probablement s'expliquer de par la fréquente utilisation d'antibiotiques chez les patients de réanimation (60 % des patients de réanimation à l'admission : rapport ReaRaisin2017). Dans notre étude conduite chez 113 patients ambulatoires bénéficiant d'une colonoscopie, la prévalence de la colonisation digestive à *S. aureus* était de l'ordre de 10 %, similaire à celle rapportée dans notre revue chez les volontaires sains de l'ordre de 14 % (IC 95% [11-17]) (Gagnaire, et al. 2019; Gagnaire et al. 2017).

Le portage digestif est souvent associé à un portage nasal mais peut être aussi exclusif et dans ce cas le dépister en plus du nez permet de mieux détecter les patients colonisés à *S. aureus*. En ce qui concerne les porteurs exclusifs, qui représentent généralement 30 % des patients porteurs digestifs de *S. aureus* (Acton et al., 2009; Gagnaire et al., 2017), des proportions un peu plus faibles ont été rapportées dans nos deux cohortes, soit 17 % en communautaire et 21 % en réanimation.

Il est décrit dans la littérature que le prélèvement d'autres sites de colonisation permet d'augmenter la détection de *S. aureus* chez les porteurs (McKinnell et al., 2013). En ce qui concerne le dépistage du site digestif, la recherche systématique de SARM au niveau digestif permettrait d'augmenter de 20 à 50 % l'identification de patients colonisés (Chipolombwe et al., 2016; McKinnell et al., 2013). Dans nos cohortes de patients hospitalisés en réanimation adulte et de patients ambulatoires avant la réalisation de leur colonoscopie, la prévalence de *S. aureus* globale (SARM compris) est augmentée respectivement de 10 % quand un prélèvement rectal est réalisé et de 17 % quand l'aine et le rectum sont prélevés en plus du nez (Gagnaire et al., 2019b, 2019a).

Jusqu'à ce travail, la majorité des études qui se sont intéressées à la colonisation digestive ont porté sur l'évaluation du portage de SARM (Gagnaire et al., 2017). Nos études en incluant le dépistage de SASM ont ainsi permis de mieux estimer le portage digestif et d'estimer la part des SASM au sein de ce réservoir. Dans nos études conduites en France, la part de SASM au sein du portage de *S. aureus* était de 91 % dans notre cohorte de réanimation et de 98 % dans notre étude conduite en communautaire (Gagnaire et al., 2019a, 2019b).

Ce travail de thèse a également apporté des données sur la diversité des souches de portage digestif et nasal chez un même individu. Les études antérieures qui ont comparé par typage moléculaire les souches de *S. aureus* isolées au niveau du nez et du tube digestif chez un même patient ont pour la plupart été réalisées chez des patients porteurs de SARM et rapportent que dans la majorité des cas, les souches sont similaires (Lemmens et al., 2011; Nakao et al., 2014). Ceci peut s'expliquer par une moindre diversité des souches de SARM de part une diffusion clonale (Harris et al., 2010). Etant donné la diversité génétique des souches de SASM, il paraît vraisemblable de penser que la probabilité est plus grande d'héberger des souches différentes chez ces porteurs. Ainsi une étude conduite chez 76 enfants hospitalisés pour une chirurgie, a retrouvé pour pour 73 % d'entre eux une colonisation avec des SASM, et a montré que les souches de *S. aureus* isolées au niveau nasal et péri-anal chez un même enfant étaient différentes dans 37,5 % (3/8) des cas (Mongkolrattanothai et al., 2011). Notre étude prospective conduite chez des patients de réanimation a permis d'identifier 40 % de clones supplémentaires quand le rectum était prélevé en plus du nez (Gagnaire et al.,

2019a). A l'inverse dans notre étude réalisée chez des patients communautaires, la diversité était beaucoup moins importante entre les souches de *S. aureus* isolées au niveau nasal, rectal et du pli de l'aîne. Le pourcentage de similarité des souches isolées au niveau de ces 3 sites dépistés était de 78 %. Ces résultats suggèrent que la diversité génétique des souches isolées au niveau nasal et digestif semble influencée par l'exposition aux soins, la pression de colonisation et la pression de sélection antibiotiques. L'analyse des souches de colonisation digestive et nasale nous a également permis de montrer que certaines souches de *S. aureus* semblent être plus à même de coloniser le site digestif. Dans la littérature, peu d'études se sont intéressées aux clones de *S. aureus* potentiellement impliqués dans la colonisation digestive. L'équipe de Van Belkum a rapporté assez récemment une épidémie hospitalière de colonisations avec une souche de SARM de type ST228 appartenant au complexe clonal 5 (Senn et al., 2016). Dans cette étude, cette souche épidémique a le plus souvent été isolée au niveau rectal par rapport au niveau nasal chez les porteurs (70% vs 49 %, $p < 0.01$). De façon intéressante, notre étude conduite en réanimation adulte a mis en évidence qu'un patient porteur d'une souche de *S. aureus* appartenant au CC5 au niveau nasal avait quasiment 2 fois plus de risque d'être porteur de cette même souche au niveau rectal.

En comparaison aux études publiées sur la colonisation nasale à *S. aureus*, peu de facteurs de risque de colonisation digestive liés à l'hôte ou à la bactérie ont été rapportés dans la littérature. En ce qui concerne les facteurs prédisposant de l'hôte, des fréquences élevées de portage au niveau digestif ont été rapportées chez des patients ayant développé des infections cutanées suppuratives récidivantes notamment avec le clone de *S. aureus* communautaire USA 300 producteur de leucocidine de panton valentine (PVL). Ces populations seraient possiblement plus colonisées au niveau de ce site plutôt qu'un autre (Gagnaire et al., 2017). Toutefois comme la recherche de portage digestif précède très rarement l'infection il est difficile de dire si ce portage est cause ou conséquence de ces infections cutanées et des parties molles. Récemment une étude conduite chez 625 patients, s'est intéressée aux facteurs de risque associés à la colonisation digestive (Kates et al., 2018). Les patients qui présentaient des pathologies digestives (reflux gastro œsophagien, rectorragies et pathologies inflammatoires) présentaient un sur risque d'être colonisé au niveau

digestif par *S. aureus* (OR: 1.96, 95% CI: 1.04-3.7; aOR: 13.9, 95% CI: 1.67-115.7). Les autres variables étudiées comme l'âge, le sexe, l'utilisation de laxatifs, d'antispasmodiques, les diarrhées, les douleurs abdominales n'ont statistiquement pas été associées à ce même risque. Au cours de ce travail de thèse, les caractéristiques des individus porteurs au niveau digestif ne différaient pas statistiquement de celles des non porteurs au niveau digestif.

A notre connaissance peu d'étude ont cherché à caractériser les déterminants bactériens associés à la colonisation digestive. En situation d'épidémie hospitalière, le fond génétique CC5 a été identifié (Senn et al., 2016). Les « wall teichoic acids » (WTA) qui ont été décrit comme indispensable à l'établissement de colonisation nasale *in vivo* dans un modèle de « cotton rat » (Weidenmaier et al., 2008) ont, de la même façon, été décrits comme essentiels à la colonisation intestinale chez la souris grâce à l'utilisation de *S. aureus* souches délétées pour le gène *tagO* (Misawa et al., 2015).

Impact clinique de la colonisation digestive

Si l'impact clinique de la colonisation nasale à *S. aureus* est bien connue avec le sur-risque d'infection endogène (Wertheim et al., 2004), l'impact clinique de la colonisation digestive, qu'elle soit associée à la colonisation nasale ou exclusive a été peu étudié dans la littérature. Deux études, dont une portant uniquement sur les SARM, ont rapporté qu'un portage combiné de *S. aureus* au niveau du nez et du rectum comparativement à un portage exclusif au niveau nasal était associé statistiquement à un sur-risque d'infections (bactériémies chez des patients hospitalisés et infections cutanées chez des enfants atteints de cancer) (Squier et al., 2002; Srinivasan et al., 2010). La colonisation simultanée des 2 sites (nasal et rectal) en comparaison au portage nasal exclusif a également été associée à une colonisation plus importante des sites cutanés (pli de l'aîne et aisselle) chez des patients d'un hôpital militaire majoritairement colonisés à SARM (41 % versus 77 %; $p = 0,001$) (Bhalla et al., 2007). Ceci pourrait suggérer que le risque d'infection pourrait être associé au fait d'avoir une charge bactérienne colonisante globale plus élevée. Dans notre cohorte de réanimation le nombre de sites colonisés n'était pas statistiquement associé au risque d'infection à *S. aureus* mais les charges bactériennes avaient tendance à être plus élevées chez les patients

colonisés au niveau nez et rectum en comparaison à celles des patients colonisés seulement au niveau nasal ($p=0,053$) (Gagnaire et al., 2019a). Dans notre étude en réanimation le risque d'infection endogène était similaire entre les porteurs exclusifs au niveau du nez et les porteurs au niveau des 2 sites. Dans ce dernier cas de figure d'infection endogène chez des porteurs à la fois au niveau du nez et du rectum, malgré l'utilisation d'une technique discriminante de typage, nous n'avons pas pu relier la souche d'infection à l'un des sites de portage, les souches étant génétiquement trop proches.

Concernant l'impact clinique du portage exclusif digestif avant ce travail de thèse, seules quelques données pouvaient suggérer qu'il existait. En effet dans le cadre d'un Projet Hospitalier de Recherche Clinique coordonné par notre équipe, il avait été observé que moins de 50% des infections sur matériel orthopédique prothétique pouvaient être reliées au portage nasal (Berthelot et al., 2010). En dehors de possibles infections d'origine exogène, la colonisation extra-nasale et notamment digestive à *S. aureus*, pourrait être une source alternative d'infections endogènes au décours d'une chirurgie orthopédique d'autant plus que le site opératoire est souvent à proximité du tube digestif. Dans la littérature, avant ce travail, seules 3 infections chez des porteurs digestifs exclusifs ont été rapportées mais aucune comparaison moléculaire n'avait été réalisée entre les souches d'infection et de colonisation. Dans notre étude conduite en réanimation, une infection d'origine endogène a pu être rapportée chez un porteur exclusif au niveau rectal. En comparaison aux infections rapportées chez les porteurs exclusifs au niveau nasal, le pourcentage d'infection endogène chez porteurs exclusifs au niveau rectal était identique. Cependant cette comparaison est à interpréter avec prudence au vu des faibles effectifs. Outre la proximité du site digestif avec différentes portes d'entrées possibles (point d'insertion de cathéter, site opératoires...), Krezalek et al ont étudié sur l'animal l'hypothèse d'une translocation possible de *S. aureus* présent au niveau digestif vers le site opératoire (Krezalek et al., 2017). A partir d'un modèle de colonisation intestinale chez la souris, la présence de *S. aureus* a été identifiée dans le cytoplasme de neutrophiles circulants qui pourraient servir de véhicules pour transporter le microorganisme vers la

plaie. De façon moins fréquente, la colonisation digestive à *S. aureus* a été également associée aux diarrhées post antibiothérapie dont la revue d'Acton 2009 fait la synthèse (Acton et al., 2009).

Le réservoir digestif de *S. aureus*

La physiopathologie de la colonisation digestive est peu connue avec plusieurs hypothèses formulées quant à l'origine de ce portage. Récemment, 3 hypothèses ont été proposées par l'équipe de M Otto : la première serait qu'il existe un continuum entre la colonisation nasale, pharyngée puis digestive ; la seconde serait plutôt de dire qu'elle est le résultat d'une contamination du périnée puis du tube digestif par une souche présente sur la peau ; la troisième et dernière hypothèse plus controversée est de dire que la colonisation digestive à *S. aureus* est à l'origine des autres sites de colonisation dont le nez (Piewngam and Otto, 2019). Ces hypothèses différentes quant à l'origine de la colonisation digestive à *S. aureus* proviennent probablement du fait que cette colonisation digestive peut être définie dans la littérature par l'isolement d'une souche *S. aureus* au niveau de plusieurs sites : périnée, prélèvement péri anal, anus, rectum, selles et pli de l'aîne. La plupart des études qui ont comparées les prévalences de portage de *S. aureus* selles *versus* rectum, rectum *vs* périnée, périnée *vs* pli de l'aîne rapportent des prévalences identiques quel que soit le type de site prélevé (Acton et al., 2009; Gagnaire et al., 2017). D'après ces résultats, toutes ces localisations pourraient s'apparenter a priori au réservoir digestif. Cependant ces comparaisons de prévalence n'ont pas été réalisées à l'échelle individuelle (Buehlmann et al., 2008; Cursino et al., 2012). Dans notre étude conduite en population communautaire, seulement 3 patients ont été retrouvés simultanément colonisés au niveau du pli de l'aîne et au niveau du rectum. Ces données ne sont pas suffisamment étoffées pour remettre en question l'équivalence du dépistage du site périnéal avec le site rectal pour la recherche d'une colonisation digestive à *S. aureus*.

Rares sont les études à ce jour qui se sont intéressées à l'identification de *S. aureus* au niveau du tube digestif lui-même. Lyra *et al* en 2012 ont conduit une étude chez 14 patients sans pathologie inflammatoire qui visait à comparer, par ARN 16S, la présence de bactéries pathogènes dans des biopsies coliques et dans les selles de ces mêmes patients (Lyra et al., 2012). *S. aureus* n'a

pas été retrouvé dans ces prélèvements. Aujourd'hui les analyses de microbiote intestinal réalisées par NGS sont plus des analyses de phylum que de bactéries spécifiques et ainsi apportent peu de réponse à la question de la présence de *S. aureus* au niveau digestif. Ainsi l'identification de Firmicutes dans des biopsies coliques ne nous apporte pas d'information quant à la présence ou l'absence de *S. aureus* qui appartient à ce phylum (Eckburg et al., 2005). D'autres travaux se sont intéressés à décrire dans le détail le microbiote jusqu'à l'identification d'espèces que ce soit par culturomique ou séquençage nouvelle génération par exemple (Gurwara et al., 2019; Mailhe et al., 2018). Ces études ne mentionnent pas la présence de *S. aureus* au niveau de plusieurs étages du tube digestif incluant le colon. Quand à la question de savoir si la présence de *S. aureus* au niveau rectal (écouvillonnage) est corrélée à la présence de *S. aureus* au niveau colique la plupart des publications sur le microbiote intestinal rapportent des différences entre les bactéries retrouvées au sein du tube gastro intestinal et celles retrouvées dans les selles de mêmes patients (Momozawa et al., 2011; Vasapolli et al., 2019). Notre étude en population communautaire a identifié 3 patients porteurs de *S. aureus* dans des biopsies coliques chez des patients tous porteurs au niveau rectal et au niveau du pli de l'aîne pour deux d'entre eux (Gagnaire et al., 2019b). Même si une contamination par des selles résiduelles suite à la préparation colique ou une contamination de la biopsie lors de la colonoscopie ne peut être exclue cette observation suggère l'existence d'un véritable réservoir digestif de *S. aureus*. Au vu de l'effet décolonisant du lavement colique utilisé en amont de la colonoscopie, il est également probable que l'identification de *S. aureus* au niveau des biopsies ait été sous-estimée. L'adhésion de *S. aureus* au niveau de la muqueuse colique a été démontrée pour la première fois en 2006 (Vesterlund, 2006). Les expérimentations *in vitro* ont été réalisées sur du mucus provenant de biopsies coliques d'un patient atteint d'un cancer colorectal. Gries *et al* ont, de leur côté, fait l'expérience *in vivo* chez la souris après administration orale de souches de SARM, d'une colonisation digestive persistante à *S. aureus*. Ils ont également retrouvé des quantités importantes de *S. aureus* dans le mucus de la muqueuse caecale des souris infectées (Gries et al., 2005). Ce modèle de colonisation digestive à *S. aureus* chez la souris a depuis été utilisé dans d'autres études pour notamment démontrer la translocation des souches de *S. aureus* de

la muqueuse intestinale vers des sites infectés (Krezalek et al. 2017) ou encore tester l'activité probiotique de *Bacillus subtilis* sur la colonisation digestive à *S. aureus* (Piewngam et al., 2018). Depuis, d'autres modèles de colonisation intestinale persistante à *S. aureus* ont été élaborés (Flaxman et al., 2017).

Risque de dissémination environnementale et colonisation digestive à *S. aureus*

Par ailleurs, la colonisation digestive à *S. aureus* a été associée à un risque de dissémination environnementale importante. C'est ce qu'illustre une épidémie de colonisations et d'infections avec un SARM du clone ST228 qui a concerné plus de 1600 patients dans un établissement de santé sur une période de 4 ans (Senn et al., 2016). La dissémination dite « silencieuse » de cette souche lui aurait potentiellement permis d'acquérir des mécanismes de résistances à la mupirocine et à la chlorhexidine. Si le dépistage de BMR en réanimation est souvent réalisé de manière systématique pour tout nouvel entrant et de manière hebdomadaire (Coppéré et al., 2018), la recherche de *S. aureus* est uniquement ciblée sur le SARM et uniquement au niveau nasal. En néonatalogie, où les nouveaux nés sont fréquemment colonisés au niveau digestif, Nakao *et al* identifient dans les selles des enfants hospitalisés des densités bactériennes qui pourraient être à l'origine d'une contamination de l'environnement de soins notamment par l'intermédiaire des changes (Nakao et al., 2014). En témoigne les nombreuses épidémies à *S. aureus* rapportées en néonatalogie (Bertin et al., 2006; Dowsett et al., 1984; Giuffrè et al., 2015; Howells and Jones, 1961) dont 2 rapportées par notre équipe concernant d'une part la diffusion d'un clone unique de type CC398 et d'autre part la diffusion de plusieurs clones reliés à ceux identifiés chez des professionnels de santé (Annexes pages 106-107). Les épidémies de *S. aureus* communautaires responsables de SSTI mais aussi d'infections gravissimes telles que des pneumopathies nécrosantes en lien avec la production de PVL sont connues pour être difficilement maitrisables. Comme discuté dans le chapitre précédent, les populations qui développent des SSTI sont fréquemment colonisées par *S. aureus* au niveau digestif (Gagnaire et al., 2017). Il est décrit que les récurrences cliniques de ces infections pourraient être dues à la non identification d'un réservoir environnemental ou à l'émergence de souches de *S.*

aureus résistantes à la mupirocine (Baud et al., 2014; Davis et al., 2012). En plus, la diffusion de souches de *S. aureus* virulentes d'origine digestive, non recherchées en systématique chez les cas et leur entourage, pourrait être une des hypothèses pour lesquelles le contrôle de ces épisodes récidivants est difficile. Les recommandations françaises sur la conduite à tenir lors d'épisodes de cas groupés d'infections cutanées suppuratives liées aux souches de SARM Co ne recommandent qu'après échec d'une première décolonisation de rechercher les réservoirs de *S. aureus* autre que le nez. En dehors du traitement par antibiothérapie systémique des cas, la décolonisation ne concerne que le portage nasal, cutané et pharyngé (HCSP, 2014).

Décolonisation du portage digestif à *S. aureus*

Si on considère que le portage digestif de *S. aureus* dans certaines situations entraîne un risque infectieux pour le patient lui-même ou la collectivité, qu'en serait-il des possibilités de décolonisation ? La mupirocine en pommade nasale et la chlorhexidine en douche et gargarismes sont actuellement proposés pour la décolonisation nasale de *S. aureus* mais ont-ils une efficacité sur le réservoir digestif ? Aucune étude n'a été réalisée dans cet objectif ciblé et pour le moment les données ne vont pas dans le sens de l'utilisation de ces 2 produits. En ce qui concerne l'utilisation d'antibiotiques oraux, les études ont plutôt décrits des effets limités sur la décolonisation de *S. aureus* (Gagnaire et al., 2017). Récemment une étude conduite chez des patients d'hématologie devant bénéficier d'une greffe allogénique de moelle a démontré l'efficacité de la décontamination digestive par vancomycine et gentamicine sur la décolonisation digestive de *S. aureus* (Wilk et al., 2017). Cependant, les recommandations américaines pour la prise en charge des infections à SARM ne sont pas favorables à leur utilisation au vu du risque d'émergences de résistances bactériennes (Liu C et al., 2011). La recherche sur les probiotiques et le microbiote intestinal a permis de montrer que la souche de *Lactobacillus rhamnosus* HN001 pouvait, dans une étude contrôlée, randomisée en double aveugle, réduire la quantité de *S. aureus* de 83 % dans les selles de patients (Eggers et al., 2017). Récemment un article publié dans Nature montre que la présence de *Bacillus subtilis* dans le tube digestif serait négativement corrélée à la colonisation digestive de *S. aureus* (Piewngam et al.,

2018). Des lipopeptides nommés les fengycines sécrétés par *Bacillus subtilis* auraient un effet inhibiteur sur le système « agr quorum sensing » de *S. aureus*. Dans cette étude, 200 personnes en bonne santé et vivant dans une zone rurale de Thaïlande ont été incluses. La fréquence de portage de *S. aureus* était de 12,5 % dans les selles et de 13 % dans le nez. La culture microbiologique des selles a permis de mettre en évidence que la présence de *Bacillus subtilis* n'était jamais associée à la présence de *S. aureus* et vice versa. Des expérimentations sur un modèle de colonisation intestinale chez la souris ont ensuite été conduites pour faire la preuve des interactions bactériennes entre cette bactérie et *S. aureus*. Brièvement du côté des thérapies vaccinales, bien qu'aucun vaccin efficace avec une activité anti staphylococcique n'ait pour le moment été identifié (Botelho-Nevers et al., 2013), il a été publié récemment que les réponses immunes adaptatives Th1 et Th17 pouvaient avoir un effet sur la clairance gastro-intestinale de *S. aureus* (Zhang et al., 2018). Dans notre étude conduite chez des patients bénéficiant d'une colonoscopie, la préparation colique semble avoir un effet sur la colonisation à *S. aureus* au niveau nasal, gorge, du pli de l'aîne et rectal. Une tendance significative à la réduction du nombre de porteurs et de la charge bactérienne chez les porteurs a été observée avant et après colonoscopie quel que soit le site de colonisation. Trois types de préparation colique ont été utilisés dont le point commun était d'avoir une activité dite « mécanique » sur le contenu digestif par le biais de laxatifs osmotiques. Les 2 principaux principes actifs contenus dans ces lavements étaient le polyéthylène glycol (PEG) encore appelé macrogol 3350 et le picosulfate de sodium associé à du citrate de magnésium. En 1966, Cox *et al* ont rapporté l'efficacité du PEG *in vitro*, notamment chez les entérobactéries, avec réduction de la viabilité bactérienne (Cox, 1966). *In vivo* chez l'homme, les purges à base de PEG ont d'ailleurs été associées à une altération du microbiote adhérent à la muqueuse colique (Harrell et al., 2012). Le lavement colique est présenté dans cette étude comme facteur confondant possible dans les analyses du microbiote digestif.

Dans la littérature, assez peu d'études se sont intéressées à l'effet de ces préparations dans la réduction des infections bactériennes et notamment dans la réduction des infections à *S. aureus*. Les quelques études qui ont testé l'activité, notamment du PEG, ont porté sur la clairance des entérocoques résistants à la vancomycine en combinaison à d'autres méthodes (Cheng et al., 2014)

ou encore dans le traitement des récurrences d'infection à *Clostridium difficile* en association avec la transplantation fécale (van Nood et al., 2013). L'utilisation de ces lavements coliques est controversée dans la réduction notamment des infections de site opératoire en chirurgie colorectale (World Health Organization 2016). Des effets indésirables sont également rapportés dans le résumé des caractéristiques des produits utilisés pour la préparation colique. Si on devait envisager d'utiliser ces préparations pour la décolonisation digestive à *S. aureus*, une étude récente réalisée par Nagata *et al*, a montré chez l'homme que le microbiote intestinal retrouvait sa composition initiale 14 jours après la réalisation du lavement colique (Nagata et al., 2019).

Perspectives

Ce travail a permis de mieux évaluer la colonisation digestive à *S. aureus* dans différentes populations mais de nombreuses données manquent encore et des points restent à approfondir. Comme nous l'avons vu, il paraît vraisemblable de considérer le tube digestif comme un réservoir muqueux de *S. aureus*. D'autres études seront nécessaires pour identifier précisément la localisation de ce réservoir au niveau des étages du tractus gastro-intestinal et sa situation extra ou intracellulaire dans la muqueuse digestive comme cela a été étudié dans le *vestibulum nasi* (Hanssen et al., 2017; Rigai et al., 2018). Ceci permettra sans doute d'avancer sur la physiopathologie du portage digestif qui suscite encore beaucoup d'interrogations et de comprendre comment sont intriqués les différents réservoirs de *S. aureus* les uns avec les autres. L'étude de la prévalence et de la localisation de *S. aureus* dans des populations connues pour avoir des dysbioses intestinales comme les patients atteints de maladie inflammatoire chronique de l'intestin permettrait de comparer ces données avec celle des populations communautaires.

Au vu de l'hétérogénéité des données de prévalence du portage digestif en fonction des populations étudiées et d'un l'impact clinique probablement différent, identifier les populations cibles éligibles au dépistage de ce réservoir est nécessaire. Deux populations ont été identifiées comme très fréquemment colonisées au niveau digestif avec *S. aureus* et pour lesquelles un risque de dissémination environnemental important décrit. Il s'agit des nouveaux nés et des patients atteints d'infections de la peau et des tissus mous à SARM ou SASM communautaires. La détection en amont de cette colonisation chez les nouveaux nés hospitalisés dans les unités de néonatalogie pourrait permettre de mieux appréhender le risque d'infection à cette bactérie dans cette population. La mise en place rapide des mesures de contrôle en cas d'épidémies de colonisations (cohorte, prélèvements d'environnements, bionettoyage, renforcement des précautions standard d'hygiène ...) pourrait permettre de limiter le risque de transmission croisée via le péril fécal. La poursuite d'études cliniques et épidémiologiques dans cette population est d'autant plus intéressante que les enfants naissent avec un tube digestif stérile et qu'ils ne sont exposés qu'à l'environnement hospitalier. Nous avons participé à plusieurs travaux (Annexes pages 106-107) conduits dans cette

dernière population en parallèle de ce travail de thèse. Les données sur la colonisation digestive des nouveaux-nés prématurés et des infections à *S. aureus* ont été collectées prospectivement et leur analyse est en cours de finalisation (données non présentées). Un article sera soumis prochainement sur la période d'étude 2015-2017 (3 ans de suivi).

Les patients atteints d'infections suppuratives de la peau et des tissus mous à *S. aureus* communautaires, pourraient également être des patients cibles pour la réalisation de dépistage de *S. aureus* au niveau digestif. Des études épidémiologiques basées notamment sur des analyses de diversité génétique entre les souches de portage au niveau nasal et digestif sont nécessaires pour confirmer l'intérêt de cette recherche supplémentaire dès les premiers épisodes infectieux, notamment en situation épidémique.

En ce qui concerne les stratégies de décolonisation de ce réservoir digestif d'autres études seront nécessaires pour (i) identifier les thérapeutiques les plus efficaces et les populations à qui proposer cette décolonisation ainsi que pour (ii) mesurer l'impact clinique ou environnemental d'une telle décolonisation. De façon inattendue, nous avons montré que les préparations coliques avaient un effet sur la réduction globale et sur les différents sites de portage chez les patients colonisés à *S. aureus* avant et après lavement. Des études interventionnelles seront nécessaires pour évaluer dans des populations plus larges les effets de ces préparations sur la réduction des infections à *S. aureus* dans des populations ciblées (ex : patients bénéficiant de chirurgies ou en cas de récurrence d'infection cutanée suppurative avec un *S. aureus* communautaire).

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Annexes : autres publications et communications en lien avec la thèse

Publication originale dans une revue internationale avec comité de lecture

-Verhoeven PO, Gagnaire J, Botelho-Nevers E, Grattard F, Carricajo A, Lucht F, Pozzetto B, Berthelot P. Detection and clinical relevance of *Staphylococcus aureus* nasal carriage: an update. *Expert Rev Anti Infect Ther.* 2014

Publication originale acceptée dans une revue nationale avec comité de lecture

-Botelho-Nevers E, Gagnaire J, Verhoeven PO, Cazorla C, Grattard F, Pozzetto B, Berthelot P, Lucht F. Decolonization of *Staphylococcus aureus* carriage. *Med Mal Infect.* 2017

Communications affichées acceptées dans des congrès internationaux

-J Gagnaire, F Grattard, A Cantais, Vuillard C, Barriere F, S Rapin, B Pozzetto, E Botelho-Nevers, H Patural, P Berthelot and P.O Verhoeven. Outbreak of MSSA CC 398 strains in a neonatal intensive care unit. Presented at: *28th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)*. Madrid, Spain, 21-24 April 2018.

-J Gagnaire, F Grattard, C.H Haddar, S Rapin, S Magnin, F Jaziri, C Fichtner, I Rayet, B Pozzetto, E Botelho-Nevers, H Patural, P Berthelot and P.O Verhoeven . Molecular dynamics of *Staphylococcus aureus* carriage after decolonization of NICU healthcare workers during an outbreak and one-year follow up. Presented at: *28th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)*. Madrid, Spain, 21-24 April 2018.

-J Gagnaire, F Grattard, C.H Haddar, S Rapin, S Magnin, F Jaziri, C Fichtner, I Rayet, B Pozzetto, E Botelho-Nevers, H Patural, P Berthelot and P.O Verhoeven . Molecular epidemiology of *Staphylococcus aureus* strains isolated from newborns and healthcare workers during an outbreak in a neonatal intensive care unit: effectiveness of control measures. Presented at: *26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)*. Amsterdam, Netherlands, 9–12 April 2016.

Detection and clinical relevance of *Staphylococcus aureus* nasal carriage: an update

Expert Rev. Anti Infect. Ther. Early online, 1–15 (2014)

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Staphylococcus aureus nasal carriage is a well-defined risk factor of infection with this bacterium. The increased risk of *S. aureus* infection in nasal carriers is supported by the fact that the strains isolated from both colonization and infection sites are indistinguishable in most of the cases. Persistent nasal carriage seems to be associated with an increased risk of infection and this status could be defined now in clinical routine by using one or two quantitative nasal samples. There is evidence for supporting the detection of nasal carriage of *S. aureus* in patients undergoing cardiac surgery and in those undergoing hemodialysis in order to implement decolonization measures. More studies are needed to determine which carriers have the highest risk of infection and why decolonization strategies failed to reduce *S. aureus* infection in some other groups of patients.

KEYWORDS: carrier state • nasal mucosa • persistent carriage • risk factor • screening test • *Staphylococcus aureus*

Staphylococcus aureus is an important cause of hospital-acquired infections [1] and a raising source of community infections, especially with the emergence and the spreading of community-associated methicillin-resistant *S. aureus* (CA-MRSA) in patients without previous health care contact (for recent review see [2]).

S. aureus is a commensal bacterium of the skin and the mucosal membranes in humans colonizing 15–36% of the whole population [3–5]. The *vestibulum nasi* is the main reservoir of *S. aureus* in humans. To our knowledge, the nasal colonization is a well-defined risk factor of *S. aureus* infection in all of the categories of patients that have been studied [6]. The relationship between colonization and infection sites is supported by the fact that *S. aureus* strains isolated from nasal carriage and infection are genetically undistinguishable in most of the cases [3]. However, the pathophysiology of endogenous infection with the strain of carriage remains unclear, and there is no evidence to define whether *S. aureus* reaches preferentially the site of infection by contamination from the cutaneous site or by translocation.

The decolonization strategies used for preventing *S. aureus* infection have been found effective in patients undergoing cardiac surgery or chronic dialysis [7]. In contrast, nasal decolonization showed no evidence of reduction of *S. aureus* infection in patients undergoing orthopedic surgery underlying the need to develop more effective strategies for prevention [8].

Nasal carriers of *S. aureus* have been classified into three groups. Unlike intermittent and non-carriers of *S. aureus*, persistent nasal carriers seem to harbor an increased risk of *S. aureus* infection [9]. However, the determinants involved in the development of infection from colonization are probably multifactorial and depend on both environmental, bacterial and host factors.

This review updates on the characterization of *S. aureus* nasal carriage in patients and its clinical relevance.

What is recently known about the *S. aureus* nasal carriage?

Epidemiology of *S. aureus* nasal carriage

Nasal carriage of *S. aureus* has been extensively studied in the past and continues to be a major topic in literature as illustrated by the

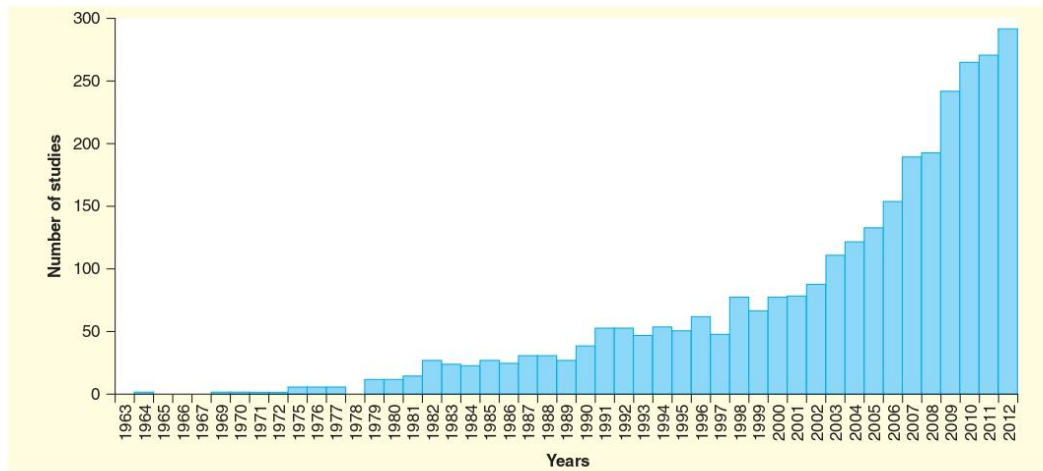


Figure 1. Increasing number of studies indexed in the PubMed database between 1963 and 2012 regarding the colonization with *Staphylococcus aureus* in humans.

increased number of papers published in this field notably in the past decade (FIGURE 1). Thousands of studies about *S. aureus* show that the nasal carriage rate continues to decrease in the general population to reach a mean prevalence of 24% from 2005 to 2012 (FIGURE 2), probably due to better individual hygiene and improvements in standard of living [3]. The

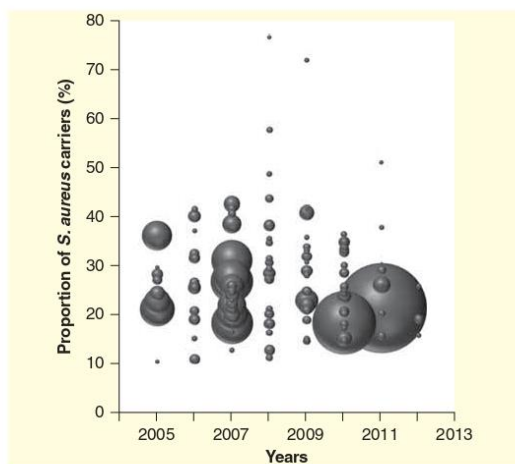


Figure 2. Prevalence of the *Staphylococcus aureus* nasal carriage from 2005 to 2012 according to the year of screening. The bubble sizes are proportional to the number of volunteers and patients enrolled in the studies. Studies reporting the prevalence of *S. aureus* nasal carriage were identified in PubMed by using 'Carrier State' [Mesh] AND '*Staphylococcus aureus*' [Mesh] with the following filters: English language, human studies and publication in the last 5 years.

prevalence of *S. aureus* nasal carriage in the community was estimated in 2011 at 21.6% in nine European countries in a cohort of more than 32,000 pediatric and adult patients without infectious disorder; MRSA was recovered from 1.3% of nasal samples and these strains exhibited a high genotypic heterogeneity with 53 spa types identified from 91 strains [10]. However, the global distribution of the prevalence of *S. aureus* nasal carriage and its antimicrobial drug resistance vary from one country to another (FIGURE 3) [4,5,10,11] especially regarding the spreading of CA-MRSA clone designated USA300 [2,12].

The prevalence of nasal carriage in children depends on the age. Newborns are typically more colonized than adults, but the prevalence of *S. aureus* nasal carriage decreases in the first year of life [13]. In Bogaert's study, the pediatric carriage of *S. aureus* was age related with a parabolic distribution and a peak incidence at 11 years [14].

Contributing factors of *S. aureus* nasal carriage

Host factors

Host factors associated with an increased risk of *S. aureus* nasal carriage are depicted in TABLE 1. Several patient-related factors influenced the nasal carriage of *S. aureus*: age, gender, ethnic origin, immune status, comorbidities, chronic illness or behavioral habits [15]. Recent studies have highlighted the role of contaminated environment hospital (for review see [16]) or household (for review see [17]) as a reservoir of *S. aureus* and therefore it can contribute to the transmission of this bacterium. The exposure to a colonized patient or a household member leads to a higher risk of colonization as demonstrated notably for hospital-acquired MRSA [18] or CA-MRSA [19]. There are conflicting results about the role of active, passive or previous smoking habits in the risk of *S. aureus* nasal carriage [20–22].

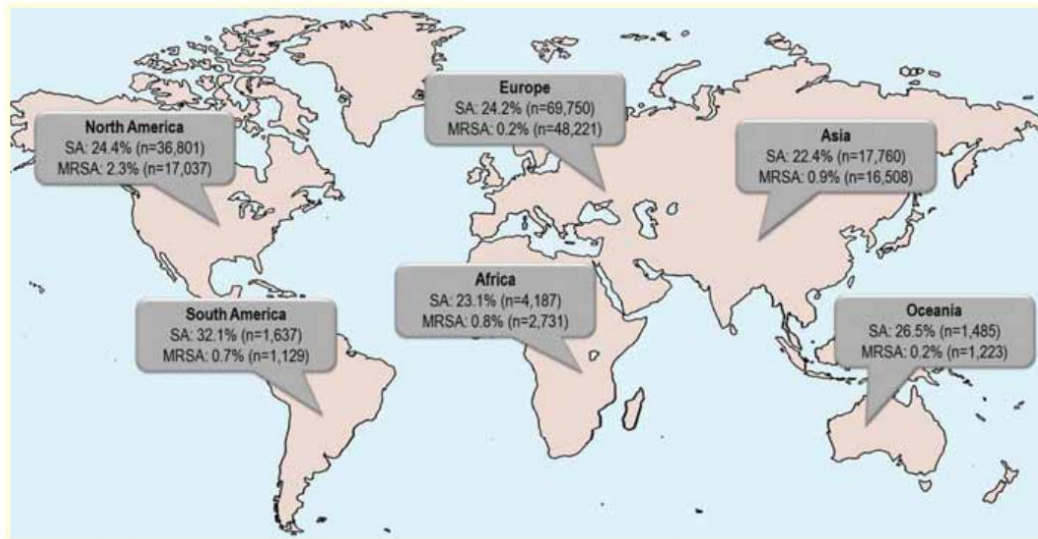


Figure 3. Global distribution of the prevalence of the *Staphylococcus aureus* nasal carriage between 2005 and 2012. Values in parenthesis are the total number of patients and volunteers screened. Studies reporting the prevalence of *S. aureus* nasal carriage were identified in PubMed by using 'Carrier State' [Mesh] AND '*Staphylococcus aureus*' [Mesh] with the following filters: English language, human studies and publication in the last 5 years. SA: Prevalence of *S. aureus* nasal carriage; MRSA: Prevalence of methicillin-resistant *S. aureus* nasal carriage.

However, extrinsic factors (i.e., the exposure to *S. aureus*) do not explain the different nasal carriage patterns in humans. Twins and family studies failed to provide evidence for genetic determinants of the *S. aureus* nasal carriage [23–25]. Nonetheless, genetic host polymorphisms have been found associated with the nasal carriage of *S. aureus* (TABLE 1). Studies of the vitamin D receptor genes pointed out discrepant results; polymorphisms in the *Apal* and *TaqI* genes coding for the vitamin D receptor were found associated with an increased rate of *S. aureus* nasal carriage in patients with type 1 diabetes [26], whereas no association was found in elderly healthy persons from the Rotterdam cohort [27]. Polymorphisms of the Fc γ receptor gene were heritable risk factors for the development of disease relapses in Wegener's granulomatosis and might be associated with nasal carriage of *S. aureus* [28]. *S. aureus* nasal carriage was not affected by polymorphisms of genes coding for TNF- α (A863C), complement factor H (C402T), α -defensin-1/3 (G1623T and C1748T), α -defensin-4 (3'-UTR) and β -defensin-1 (5'-UTR) [29,30]. The host innate immune response (TABLE 1) might influence the *S. aureus* carriage as exemplified by antimicrobial peptides including human defensins (see the *S. aureus* nasal carriage patterns section). These results illustrate that host susceptibility represents only a part of determinants of *S. aureus* nasal carriage.

Bacterial factors

S. aureus factors involved in the nasal colonization process are mainly adhesion factors such as microbial surface components recognizing adhesive matrix molecules and cell wall

teichoic acids (TABLE 2). It has been shown that clumping factor B (ClfB) and iron-regulated surface determinant A bind to squamous epithelial cell envelope protein loricrin and cytokeratin 10 and promote nasal colonization in rodents (ClfB and iron-regulated surface determinant A) and humans (ClfB) [31–33]. Recent studies on nasal microbiome have reported an antagonism between several bacterial species of the nasal flora and *S. aureus*. *Staphylococcus epidermidis* was found more prevalent in noncarriers than in carriers of *S. aureus* [34]. Iwase and coworkers [35] demonstrated that purified *S. epidermidis* serine protease (Esp) was able to inhibit *S. aureus* biofilm formation and to destroy pre-existing *S. aureus* biofilms; the bactericidal activity of the Esp protein against *S. aureus* was enhanced by the presence of human β -defensin-2. *S. epidermidis* secreting the Esp protein artificially inoculated in volunteers was effective to decolonize *S. aureus* nasal carriers. Similar findings were observed with nasal application of a mixture of *Lactobacillus* that was found effective to decolonize a small number of MRSA nasal carriers [36]. Other resident bacteria can interfere with *S. aureus* nasal colonization as suggested with *Streptococcus pneumoniae* in epidemiological studies [14,37] and in an *in vivo* study with *Corynebacterium* sp. [38].

Overall, bacterial interferences, colonization and antibiotic pressures can modify overtime the patient nasal carrier status of *S. aureus* and therefore the risk of infection due to this bacterium.

Table 1. Human host factors increasing *Staphylococcus aureus* nasal carriage.

Categories	Human host factors	Ref.
General	Age: young people (<30 years), elderly people (>60 years) [†]	[14,37,44,110,112,117,123–125]
	Sex: male	[3,44,112,117,126]
	White people	[3,3,112,127]
	Country of residence	[10,128,129]
	Promiscuity and hospital crowding	[17,18]
	Colonized household member (peoples and pets) and contaminated environment	[3,17,117,130]
Behavior	Tobacco use [‡]	[20–22,44,117,123]
	Nose picking	[131]
	Contraception [§]	[49]
	Occupation: health care workers, livestock workers, pig farmers, sportsmen	[3,132,133]
	IV drugs use	[112,133,134]
	Male having sex with male [¶]	[133,135]
Diseases and medical history	Obesity	[3,117]
	Chronic wounds or skin lesions	[3,112]
	HIV patients	[3,44,112,136]
	Diabetes	[44,112]
	Chronic illness, severity of illness ^{†,¶}	[44,133]
Health care exposure	Previous antibiotic use	[112,133,137]
	History of hospitalization, exposure to health care facilities (surgery, intensive care units etc.)	[16,18,47,91,110,112,117,133,138]
	Medical devices (urinary or IV catheter, dialysis, tracheotomy, mechanical ventilation)	[3,47,112,133,138,139]
Genes	HLA-DR3 (no adjustment for underlying disease)	[140]
	Polymorphisms or haplotypes in genes coding for:	
	– Vitamin D receptor (in type 1 diabetics) [‡]	[15,26,27]
	– IL-4 (524 C/C and 524 C/T genotypes)	[30,141]
	– C-reactive protein (haplotype C-C-G)	[30,141]
	– Glucocorticoid receptor [§] (haplotype combination)	[54]
	– β -defensin-1/3 [§] (DEF1 5'-UTR)	[52]
	– Mannose-binding lectin (WT 54 and 57)	[29]
	– TLR2 (R753Q) ^{††}	[142]
	– Serine protease C1 inhibitor (V480M)	[15,143]
Antimicrobial peptides	High level of α -defensins (HNP 1–3) and β -defensin-2	[29,51]
	Low level of mRNA-encoding β -defensin-1/3 [§]	[52,53]
Others	Low level of vitamin D (in nonsmoker men) [§]	[22]
	Hemoglobin in nasal secretion	[144]

[†]MRSA carriage.

[‡]Controversial risk factor.

[§]Persistent carriage.

[¶]CA-MRSA carriage.

^{††}Children.

CA-MRSA: Community-associated methicillin-resistant *Staphylococcus aureus*.

Table 2. Bacterial factors involved in *Staphylococcus aureus* nasal carriage.

Factors	Mechanisms	Evidences	Ref.
WTA	Binds to human nasal epithelial cells	Essential for nasal colonization of cotton rat (during the early stages of the colonization)	[145]
ClfB [†]	Major adhesion factors associated with nasal carriage. Binds to fibrinogen, cytokeratin 10, cytokeratin 8 and loricrin	ΔClfB mutant was defective for nasal colonization in rodents and humans. Vaccination with ClfB prevented <i>S. aureus</i> colonization in rodents	[31,146,147]
IsdA [†]	Major adhesion factors associated with nasal carriage expressed under iron-limited conditions. Binds to fibronectin and fibrinogen, involucrin, loricrin, cytokeratin 10	ΔIsdA mutant was associated with decrease of <i>S. aureus</i> nasal carriage in rodents. Vaccination with IsdA prevented <i>S. aureus</i> colonization in rodents	[32,33,147]
SasG [†]	Binds to epithelial cells	Promote adhesion to hDNECs	[148]
SdrC/D [†]	Binds to epithelial cells	Promote adhesion to hDNECs	[148]
FnBPs [†]	Binds to fibronectin, fibrinogen and elastin	Essential for the internalization of <i>S. aureus</i> in non-phagocytic cells and expressed during nasal colonization in humans	[33,147,149]
Spa [†]	Binds Fc fragment of Ig	Not associated with persistent carriage	[150]
Coa	Binds prothrombin	Not associated with persistent carriage	[150]
IEC	Located on β-hemolysin-converting bacteriophages carrying <i>scn</i> , <i>chip</i> , <i>sea</i> and <i>sak</i> genes	Not required for early stage of colonization in humans but expressed during nasal colonization in humans	[33,151]
KatA	Increase the H ₂ O ₂ tolerance	Associated with nasal carriage in a cotton rat model	[143]
AhpC	Increase the H ₂ O ₂ tolerance	Associated with nasal carriage in a cotton rat model	[143]
Genetic lineages	No specific genetic lineage was associated with carriage isolates	Nasal carriage and clinical isolates belonged to the same genetic clusters	[44,56]

[†]Belongs to the family of microbial surface component recognizing adhesive matrix molecules.
AhpC: Alkyl hydroperoxide reductase, ClfB: Clumping factor B, Coa: Coagulase, FnBPs: Fibronectin-binding protein, hDNECs: Human desquamated nasal epithelial cells
IEC: Immune evasion cluster, IsdA: Iron-regulated surface determinant A, KatA: Catalase, SasG: *S. aureus* surface protein, SdrC/D: Serine-aspartic acid repeat proteins C and D, Spa: Protein A, WTA: Wall teichoic acids.

***S. aureus* nasal carriage patterns: persistent versus others (intermittent or noncarriers)**

Early in the 1960s, the three status of nasal carriage were defined including persistent carriers, intermittent carriers and noncarriers [39]. In the general population, the proportion of *S. aureus* nasal carriers varies from 10 to 35% for persistent carriers, 20–75% for intermittent carriers and 5–50% for noncarriers [3,39]. However, the probability to detect an intermittent carrier increases according to the number of samples taken and the duration of follow-up [3].

S. aureus-persistent nasal carriers

Persistent nasal carriers of *S. aureus* exhibit a higher nasal bacterial load than intermittent carriers or noncarriers [40,41]; a higher dispersion of *S. aureus* in the environment (which increase the risk of cross transmission to household, other patients and health care workers) [17]; and have a higher risk of infection [9,42] (see the ‘What is the clinical relevance of *S. aureus* nasal carriage?’ section). Moreover, persistent nasal carriers of *S. aureus* can be distinguished from intermittent ones by a lower exchange rate of *S. aureus* clones in repeated cultures [40,43–45]. This notion was, however, recently controverted by a 3-year

longitudinal study reporting that persistent carriers harbored the same high exchange rate than intermittent ones [46]. Persistent carriers, who had been decolonized and recolonized artificially with a mixture of *S. aureus* strains, reacquired their autologous strain in approximately 50% of cases, suggesting that host–bacterium interactions are highly specific [43]. In the latter study, it was shown that the duration of carriage was longer for persistent carriers (median >153 days) than for nonpersistent ones (median of 14 days). Since the study of van Belkum *et al.* [43], it is admitted that *S. aureus* nasal carriers are classified into two groups: persistent and others, suggesting that only persistent carriers should be targeted for preventive strategies during at-risk situations such as surgery or long-term venous catheterization.

Factors specifically related to *S. aureus*-persistent nasal carriage Although the proportion of *S. aureus* nasal carriers has been found higher in men than in women [47], this trend is even more pronounced regarding persistent nasal carriers [48]. Very recently, hormonal contraception has been found as a risk factor for persistent carriage in women [49], whereas active smoking was found to be a protective factor [22]. Persistent carriers

exhibited higher serum antibody levels for several *S. aureus* antigens (TSST-1, SasG, SEA, ClfA, ClfB, CHIP) than non-persistent ones [43,50], but the role of these circulating antibodies in nasal colonization remains unknown. Antimicrobial peptides levels including human neutrophil peptides 1–3 and β -defensin-2 were found higher in nasal fluids of nasal-persistent carriers compared with those of noncarriers [51]. Genetic host factors implicated in the innate immune response have been related to *S. aureus*-persistent nasal patterns. Persistent carriage was associated with polymorphism in the 5'-UTR of DEFB1 gene leading to a lack of expression of messenger RNA of human β -defensin-1 (hBDF1) and -3 (hBDF3) in the experimentally wounded skin [52,53]. In contrast, despite the nasal carriage, patterns have been found associated with a polymorphism of the glucocorticoid receptor [54], the long-term cortisol level was independent of the *S. aureus* nasal carriage status [55]. A study performed in a large Amish population failed to show a familial predisposition for persistent nasal carriage [25], suggesting that intrinsic host factors are not sufficient to increase the risk of persistent carriage. A longitudinal study of 32 twin pairs confirmed that host genetic background have a very limited effect on carrier status [23]. All these studies highlight that the genetic host factors determinants are highly complex and multifactorial. To date, no specific bacterial factor was associated with persistent nasal carriage of *S. aureus*. It has been shown that strains isolated in persistent carriers belong to the same genetic backgrounds from those isolated during infection [56].

What is new in the detection of *S. aureus* nasal carriage?

Sampling methods for the screening of *S. aureus* nasal carriage

The nasal sampling procedure for the screening of *S. aureus* nasal carriage should be performed following guidelines [201,202] in order to standardize the performance of the microbiologic procedures. The *vestibulum nasi*, corresponding to the first two centimeters of the anterior nostril, should be sampled by performing at least five rotations of the swab. The *S. aureus* load seems to be higher on the septum than on the nostril wings [51]. Different types of swab are commonly used but they could impact the sensitivity of the sampling procedure. Indeed, we have shown that nylon flocked swabs improved both the detection of *S. aureus* nasal carriers and the bacterial load recovered from the swab [57]. Despite *S. aureus* is able to persist on different surfaces, it is recommended to use a transport medium (i.e., liquid Amies) that meets the Clinical and Laboratory Standards Institute criteria for the recovery of most bacteria including *S. aureus* [58]. For qualitative studies, liquid Amies swab transport system kept at room temperature was shown to allow the recovery of *S. aureus* up to 3 weeks after sampling [59]. Moreover, Jones *et al.* conducted a large observational study from 1,367 patients attending a Medical Assessment Unit and requiring MRSA screening; the prevalence of *S. aureus* nasal carriage changed from 22% with dry swab to 31% with moistened swab ($p < 0.001$) [60]. These results

encourage the use of moistening nylon flocked swab especially when the nasal mucosa is dry.

Microbiological procedures used for detecting *S. aureus* nasal carriage

Selective media facilitate the reading and the identification of *S. aureus* especially from plurimicrobial mucosal samples. Selective chromogenic media were shown to exhibit a higher sensitivity and specificity for the detection of *S. aureus* by comparison to standard media (i.e., blood agar, mannitol salt agar) [61–66]. The sensitivity of chromogenic media could be improved by incubating agar plates for 48 h, whereas the specificity decreased significantly [62,63,65]. An overnight pre-enrichment step in salt broth followed by streaking on chromogenic medium was shown to improve the sensitivity of the screening of *S. aureus* colonization but delayed the report of results [67].

For the identification of *S. aureus* colonies, it is advisable to use the combination of culture onto chromogenic media and matrix-assisted laser desorption ionization–time of flight mass spectrometry that is highly sensitive and specific in case of plurimicrobial samples [68].

Finally, the nucleic acid amplification tests (NAATs) are able to detect genomic products of *S. aureus* directly from the sample without needing culture. Real-time PCR assays can be used to generate a result in <2 h using fully automated NAATs including the extraction step (i.e., Cepheid Xpert SA Nasal Complete, BD MAX StaphSR Assay) without specific knowledge in molecular biology [69,70] or in several hours with PCR assays including a separated extraction step (i.e., Roche Light-Cycler MRSA Advanced Test). Third-generation PCR assays targeting a species gene (*nuc* or *spa*), the *mecA* gene and the *orfX*–*SCCmec* junction are able to identify in most cases MRSA and a mix of MSSA and coagulase negative *Staphylococcus* carrying the *mecA* gene.

The conventional culture on chromogenic agar plate combined with matrix-assisted laser desorption ionization–time of flight mass spectrometry is an accurate and low-cost approach adapted for screening of *S. aureus* nasal carriage on a routine basis. The fully automated NAATs increase the cost of the screening strategy and could be reserved to specific clinical contexts for which a result should be available in emergency (patients undergoing surgery or admitted in emergency in intensive care unit). Currently, the clinical relevance of NAATs for screening *S. aureus* carriage (especially regarding MRSA) remains unclear [71,72].

How to characterize *S. aureus*-persistent carriage?

Definition of *S. aureus*-persistent nasal carriage

The microbiological diagnosis of persistent nasal carriers of *S. aureus* remains a challenge for clinicians and microbiologists. Despite the fact that persistent carriers are considered at high risk of infection, there is still no consensual definition of the persistent carriage of *S. aureus*. The characterization of persistent nasal carriers requires usually at least five nasals samples taken at an interval of 1 week (TABLE 3). In order to standardize

Table 3. Protocols used to define persistent nasal carriage of *Staphylococcus aureus* in the most relevant studies between 1995 and 2013.

Study (year)	Nasal samples (n)	Delay between two samples	Duration of follow-up	Ref.
Muthukrishnan <i>et al.</i> (2013)	2–18 (median of 4)	1 month to 1 year	Up to 3 years	[46]
Nurjadi <i>et al.</i> (2013)	4	Variable	75 days	[52]
Verhoeven <i>et al.</i> (2012)	6 to 7	Once a week	5 weeks	[41]
Alexander <i>et al.</i> (2011)	4	Every 3 months	12 months	[152]
Fritz <i>et al.</i> (2011)	3	3–6 months	12 months	[153]
Roghamann <i>et al.</i> (2011)	At least 9	Once a week	At least 9 weeks	[25]
Souly <i>et al.</i> (2011)	5	1–3 months	8 months	[154]
Panierakis <i>et al.</i> (2009)	2	At least 3 months	At least 3 months	[26]
Van Belkum <i>et al.</i> (2009)	5–10	Every 2–3 weeks in mean	6 months (approximately)	[43]
Cespedes <i>et al.</i> (2005)	1–3	6 months	18 months	[155]
Nouwen <i>et al.</i> (2004)	12	Once a week	12 weeks	[40]
Peacock <i>et al.</i> (2003)	10	Every 2 weeks then once a month	6 months	[124]
VandenBergh <i>et al.</i> (1999)	10 to 12	Once a week	12 weeks and 8 years	[73]
Eriksen <i>et al.</i> (1995)	6–21 (median of 14)	Every month (approximately)	19 months	[48]

the definition of the *S. aureus* nasal carriage state, Vandenberg *et al.* [73] proposed the use of an index of carriage defined by the number of samples yielding *S. aureus* divided by the total number of nasal samples taken in a patient. Subjects with an index of carriage ≥ 0.8 are defined as persistent carriers; subjects with an index of carriage equal to zero are defined as noncarriers; others are defined as intermittent carriers [25,41,43,48,73]. Seven successive nasal swab cultures were shown to reliably distinguish noncarriers from intermittent carriers [3,40].

How to predict persistent nasal carriers of *S. aureus* in clinical routine?

In different studies, this status was determined by using from 5 to 12 consecutive specimens taken over several weeks to months with a positive rate of at least 80% [9,25,41,43,48,57,73]. Nouwen *et al.* [40] proposed a strategy based on two nasal samples taken in 7 days apart; the combination of qualitative and quantitative results of two nasal swab cultures allowed predicting the persistent *S. aureus* carriage state with a positive predictive value of 0.79 and a negative predictive value of 0.99. The so called 'culture rule' was applied to epidemiological studies [49,54,74,75] but was not evaluated in clinical trials. A similar approach reported a strong correlation between the mean *S. aureus* load of two quantitative nasal samples and the persistent nasal carriage status [25].

Recently, an algorithm based on one or two quantitative nasal samples was proposed for predicting the persistent nasal carriage of *S. aureus* (FIGURE 4) [41]. From a clinical prospective cohort of hemodialysis patients and healthy volunteers, the algorithm was found able to distinguish persistent and

nonpersistent nasal carriers with a sensitivity of 95.2% (95% CI: 83.8–99.4%), a specificity of 91.0% (95% CI: 83.6–95.8%) a positive predictive value of 81.6% (95% CI: 68.0–91.2%) and a negative predictive value of 97.9% (95% CI: 92.5–99.7%) [76]. The *S. aureus* loads can be determined by using either culture on chromogenic media [41] or fully automated NAATs that provide a result on the day of collection [77].

What is the clinical relevance of *S. aureus* nasal carriage?

Relationship between nasal & infection strains of *S. aureus*

Few studies have analyzed the genetic relationship between nasal carriage of *S. aureus* strains and those isolated from clinical infection. Lamers *et al.* [56] showed that *S. aureus* strains responsible for nasal colonization belonged to the same genetic clusters than those responsible for invasive infection. Some differences in the presence of virulence genes were reported between *S. aureus* strains responsible for nasal colonization and invasive ones [78–80], but the lack of sampling representativeness and of the characterization of the nasal carriage status (intermittent or persistent) did not allow to conclude about virulence determinants responsible for colonization or infection. In general, no or very few differences were found between infecting and colonizing *S. aureus* strains, as shown by Young *et al.* that compared by whole-genome sequencing the colonizing strain and the strain leading to fatal bacteraemia in the same patient [81]. This emphasizes the role of extrinsic factors as invasive devices, skin lesion or surgery in the risk of developing an infection with this bacterium.

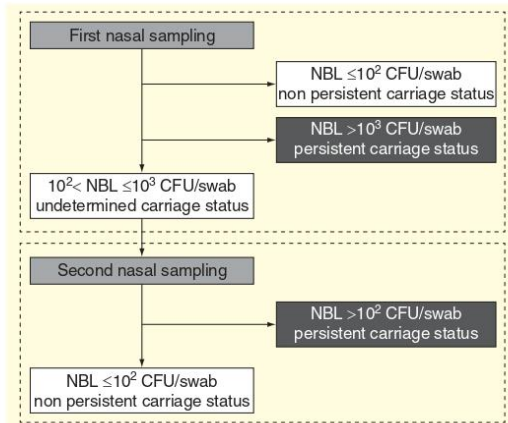


Figure 4. Definition of the *Staphylococcus aureus*-persistent nasal carriage state from the algorithm proposed in [41].
 NBL: Nasal bacterial load.
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The apparent association reported by several studies between MRSA nasal colonization and severe infection was shown to be related to a selection bias favoring the recruitment of patients with more severe illness and more frequent sampling to detect colonization [82]. Further research is needed to identify effective methods able to eradicate durably MRSA carriage and to reduce the high risk of subsequent infection.

Infections associated with *S. aureus* nasal carriage

Since 1930, numerous studies have confirmed the association between nasal carriage and staphylococcal infection in different populations [42,83–87]. In most cases, staphylococcal infections are endogenous, as shown for bacteraemia [85] and surgical site infection [88], with around 80% of strains genetically indistinguishable between those recovered from nose and infection site.

Extranasal sites of carriage, such as throat [5] and digestive tract [89], sometimes without associated nasal carriage may also play a role in the endogenous origin of infections [90]. Extranasal sites may also be colonized with strains that differ from the nasal one [74].

Exogenous acquisition of *S. aureus* at the site of infection has also been documented, particularly in health care settings by cross-transmission involving others patients, health care workers and medical devices [16,18,91].

Historically, *S. aureus* nasal carriage was associated in many studies as a risk factor for subsequent infection due to this bacterium, as synthesized by Williams *et al.* [39].

Surgical patients

In surgical patients, *S. aureus* nasal carriage has been identified as a risk factor for infection. Kluytmans *et al.* reported that nasal carriage was associated with *S. aureus* surgical site

infections (SSIs) in cardiac surgery patients, with a ninefold increased risk [84]. Then, others studies confirmed this finding [92–94]. Furthermore, a 60% decrease of SSI in cardiac surgery was observed by using a strategy of decolonization using mupirocin and chlorhexidine bathing [11].

In orthopedic surgical patients, *S. aureus* SSIs have been also associated with nasal carriage of the bacterium. The first evidence was reported by Kalmeijer *et al.* who found a ninefold increased risk of SSI and a 16-fold increased risk of *S. aureus* SSI in *S. aureus* nasal carriers [42]. In a large trial including more than 4,000 patients, *S. aureus* carriage was an independent risk factor for staphylococcal SSI in prosthetic orthopedic surgery [83]. Similar results were reported in patients with MRSA carriage [95,96]. For the majority of *S. aureus* orthopedic SSI reported in reference [83], either an endogenous origin could not be demonstrated or preoperative nasal colonization retrieved a strain that was different from the one recovered from the surgical site. To date, attempts to show that decolonization strategy is effective in orthopedic surgery failed [97] or did not reach the statistical significance [11]. *S. aureus* nasal carriage was also found to be a risk factor of SSI in other types of surgery [11,88,98]. Therefore, patients scheduled for cardiac surgery have been included in a clinical trial evaluating a staphylococcal vaccine targeting IsdB (V710) with an endpoint of reduction of *S. aureus* surgical infection: this vaccine failed to show an efficacy [99]. To date, no data about efficacy of vaccine on *S. aureus* carriage have been published.

Patients undergoing hemodialysis & continuous peritoneal dialysis

The most common microorganism responsible for infection in patients undergoing long-term hemodialysis or continuous peritoneal dialysis (CPD) is *S. aureus*, and these infections are typically associated with *S. aureus* nasal colonization. Indeed, hemodialysis and CPD patients are another group of patients for which *S. aureus* carriage has been found to be associated with infection [87,100–102]. Infections associated with nasal carriage in CPD patients are peritonitis and exit site infection [87,101], often leading to catheter loss [47]. Kluytmans *et al.* summarized relative risks of infection in carriers submitted to CPD ranging from 1.8 to 14, higher than those described in hemodialysis patients (range from 1.8 to 4.7) [47]. These infections are mostly endogenous [101]. In hemodialysis patients, catheter-related bacteremia are usually associated with carriage [102], with an endogenous origin [103]. Moreover, a meta-analysis showed the effectiveness of decolonization in preventing infection both in hemodialysis and CPD patients [104]. This population was enrolled in a *S. aureus* vaccine clinical trial performed by Nabi Biopharmaceuticals with a staphylococcal vaccine targeting the capsular polysaccharides (cap) 5 and 8 (StaphVAX). This study failed to show a decrease of bacteremia due to *S. aureus* harboring the cap5 and 8 among vaccinated patients compared with the placebo group [105]. An ancillary study investigating the impact of StaphVAX on nasal

carriage of *S. aureus* showed no reduction of nasal colonization rate among vaccinated hemodialysis patients [106].

Patients with recurrent skin & soft tissue infection

Patients with recurrent skin and soft tissue infection (SSTI) have an increased *S. aureus* carriage rate and are at increased risk of staphylococcal infection [86,107]. Nasal carriage was found to be more frequently associated with recurrent furunculosis compared with simple furuncle (88 vs 29%; $p < 0.007$) [86]. In patients with impetigo, the *S. aureus* nasal carriage rate was higher than that in the general population, but the prevalence was not different among patients with bullous and nonbullous impetigo [86]. Decolonization strategies have been recommended in patients with recurrent SSTI associated with nasal carriage of MRSA [108]. However, the quality of evidence for this recommendation is poor (evidence of grade C level). Although no recommendation exists for MSSA carriage, most physicians use the same strategy than for MSSA carriers in order to prevent recurrent SSTI.

Other groups of patients

Hospitalized nonsurgical patients carrying *S. aureus* in the nose have a threefold increased risk of nosocomial *S. aureus* bacteraemia but the mupirocin prophylaxis failed to reduce the infection rate versus placebo [109,110]. Several epidemiological studies showed that nasal carriers of *S. aureus* who harbored medical devices had an increased risk of infection due to this bacterium [111,112]. A higher risk of infection was also associated to nasal carriage of *S. aureus* in HIV-infected patients, patients with liver cirrhosis, transplanted patients and patients admitted in intensive care unit as reviewed previously [47].

Infections associated with nasal carriage according to the carriage patterns

If multiple evidences demonstrated that *S. aureus* nasal carriers are at high risk of staphylococcal infections, few clinical studies formerly concluded that *S. aureus*-persistent nasal carriers have a higher risk of infection compared with intermittent or noncarriers. Since 1944, some studies investigated the *S. aureus* nasal carriage over time and showed differences in the presence or persistence of this carriage, reflecting possible incorrect swabbing technique or variations in the numbers of cocci harbored in the nose [39]. Kalmeijer *et al.* showed that patients undergoing orthopedic surgery and harboring high nasal bacterial load had the highest risk of prosthetic infection [42]. Later, Nouwen *et al.* showed that patients undergoing CPD had a higher risk of *S. aureus* infection in case of persistent nasal carriage of *S. aureus*, whereas intermittent nasal carriers and noncarriers exhibited a low risk of *S. aureus* infection [9]. In this group of patients, *S. aureus* infection of dialysis catheter is predominant and could be due to external contamination with the strain of carriage colonizing the skin and especially the hands of the persistent carriers. MRSA-persistent carriers are also at higher risk of subsequent infection with increased morbidity and mortality [113].

To summarize, there is a clear evidence of association between *S. aureus* nasal carriage and increased risk of infection due to this bacterium. However, the relationship between *S. aureus* strain

colonizing the nose and the one responsible for infection may be influenced by the clinical context and notably by the site of infection. Eradicating the nasal carriage of *S. aureus* associated or not to cutaneous decontamination was proved to be effective in cardiac surgery and dialysis but not in orthopedic surgery. The dynamic of *S. aureus* carriage depending on host and bacterial factors, colonization, antibiotic pressure and environmental exposure need to be better understood to target the time period for setup of preventive measures. Studies must be done for evaluating whether universal decolonization is more effective to prevent *S. aureus* infection than targeted decolonization, with particular vigilance for emerging resistance due to widespread use of mupirocin and chlorhexidine. Finally, there is a need for clinical studies to better understand which carriers have the higher risk of *S. aureus* infections during an at-risk situation.

Expert commentary & five-year view

In the majority of cases, the strain responsible for *S. aureus* infection is carried in the nose of the patient, the most common site of colonization. Despite extranasal sites are often colonized by *S. aureus* regardless the nasal carriage state [89,114], the potential role of these other anatomical sites in the triggering of staphylococcal infection remains unknown. The relationship between strains colonizing mucosal sites and those recovered from infection site should be investigated to evaluate the clinical relevance of multisite sampling.

Many questions relative to the pathophysiology of infection in carriers are not still resolved. First, what are the mechanisms of infection: translocation or contamination by proximity? The variable efficacy of nasal decolonization to decrease SSI suggests that different ways and mechanisms are involved according to the site of infection. Second, what is the at-risk period for *S. aureus* infection in a carrier, and what is the dynamics of *S. aureus* carriage? Eradicating *S. aureus* nasal carriage was found effective to decrease the risk of infection in cardiac surgery: Bode *et al.* [11] demonstrated that patients' decolonization, although begun very shortly before cardiac surgery, decreased significantly the rate of surgical site infections due to *S. aureus*. But what is the best period to start preventive strategies in order to decrease the risk of *S. aureus* infection? Third, as persistent carriers of *S. aureus* seem to exhibit higher risk of infection, it could be useful to determine the patient nasal carriage status in clinical routine by using new methods [40,41]. The additional value of the characterization of *S. aureus* nasal carrier status and the impact of targeted decolonization of these patients need to be evaluated by clinical prospective trials.

As explicated in this review, persistent nasal carriage of *S. aureus* can be defined in different ways; for some authors, persistent carriers are patients exhibiting failure of nasal mupirocin decolonization [115,116]. Additionally, the presence in patients of wounds or of invasive devices, dependency for activities of daily living and exposure to antibiotics can all contribute to the chronic colonization of the patient, especially with MRSA strains [117]. Therefore, the definition of persistent nasal

carriers of *S. aureus* must be improved or at least specified according to the methicillin susceptibility of the *S. aureus* strain.

If a strategy of patients' decolonization is decided, which from targeted or universal decolonization is the best way to do it? Is the characterization of patient's nasal carriage cost effective. Does it permit to decrease the risk of mupirocin resistance? As persistent nasal carriers of *S. aureus* exhibit a high dispersion of *S. aureus* in the environment mainly by hand carriage, is there an interest to better characterize patients' nasal colonization for limiting cross-transmission in health care facilities and even in the household? Despite some conflicting results [118], several works addressing the problematic of MRSA transmission [118–121] documented a decrease of cross-transmission with the strategy 'search and isolate' or 'search and destroy'. To come back to the prevention of *S. aureus* infection at the individual level during at-risk procedures like invasive procedures (surgery, foreign material, dialysis, etc.), there are now clinical results for hypothesizing that preventive strategies like decolonization or even vaccine despite recent disappointing results (for review see [105]) can decrease the infectious risk due to *S. aureus* nasal carriage. To date, very few results have been provided about the effect of vaccines on carriage because vaccine clinical trials did not specifically targeted carriers [106]. A vaccine reducing carriage would be probably better than one with no effect on carriage. Recently, a review about this topic [105] pleaded for the future evaluation of vaccine efficacy not only on staphylococcal infections but also on mucosal carriage. Future vaccine strategies should include a large panel of antigens targeting factors involved in infection and colonization. In the hypothesis that these preventive strategies are found able to decrease infection linked to *S. aureus* nasal carriage,

will the global risk of health care-associated infection decrease? Very few studies investigated this essential issue.

Finally, despite many studies on the role of nasal *S. aureus* carriage in the risk of infection due to this bacterium, the dynamic of bacterial colonization and the balance between bacterial and host factors are important issues that are not yet solved. Although *S. aureus* nasal colonization is a well-recognized risk factor of infection with this bacterium, only a small proportion of patients develops an infection. Prospective studies must be done to investigate factors that promote infection in colonized patients, especially for MSSA. Indeed, people are not equally resistant to colonization or infection with *S. aureus* and host-*S. aureus* interactions are highly specific. The role of human microbiome in preventing colonization and infection is more and more studied, and strategies aimed to harness this compartment for preventing colonization are investigated [122]. The factors that contribute to establish *S. aureus* colonization and those that disrupt the balance between host and *S. aureus* must be better characterized for optimizing preventive strategies, especially during at-risk procedures in health care facilities.

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Key issues

- Nasal carriers of *Staphylococcus aureus* were recently reclassified into two types: persistent and others.
- Simplified methods are now available for predicting the patient nasal carriage status, persistent or not, on a routine basis.
- Unlike others, persistent nasal carriers of *S. aureus* exhibit a higher nasal bacterial load, a higher dispersion of *S. aureus* in the environment and are at higher risk of infection with this bacterium. These observations suggest that this population should be targeted for preventive strategies during at-risk situations.
- *S. aureus* nasal carriage is a well-defined risk factor of *S. aureus* infection in almost all of the categories of patients that have been studied.
- *S. aureus* infections are usually caused by the strain carried in the nose of infected patients.
- The role of extra nasal sites of *S. aureus* carriage in the development of infection due to this bacterium remains poorly investigated.
- Screening of *S. aureus* nasal carriage should be done in patients undergoing cardiac surgery or undergoing hemodialysis or continuous peritoneal dialysis in which nasal decolonization of carriers leads to reduce *S. aureus* infection rate.

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General review

Decolonization of *Staphylococcus aureus* carriage in 2016

Décolonisation de *Staphylococcus aureus* en 2016

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Abstract

Staphylococcus aureus nasal colonization is a well-known independent risk factor for infection caused by this bacterium. Screening and decolonization of carriers have been proven effective in reducing *S. aureus* infections in some populations. However, a gap remains between what has been proven effective and what is currently done. We aimed to summarize recommendations and current knowledge of *S. aureus* decolonization to answer the following questions: Why? For whom? How? When? And what are the perspectives?

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Keywords: Carriage; *Staphylococcus aureus*; Decolonization; Prevention of infections

Résumé

La colonisation nasale à *Staphylococcus aureus* est un facteur de risque bien connu d'infections à cette bactérie. Il a été démontré que le dépistage et la décolonisation de *S. aureus* permettent de réduire les infections à *S. aureus* dans certains groupes de patients. Il existe toutefois une différence entre ce qui a été démontré comme efficace et ce qui est fait dans la réalité. Nous avons synthétisé les connaissances et recommandations concernant la décolonisation de *S. aureus* en répondant à ces questions simples : pourquoi, pour qui, comment, quand et quelles sont les perspectives sur ce sujet ?

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Mots clés : Portage ; *Staphylococcus aureus* ; Décolonisation ; Prévention des infections

1. Introduction

Staphylococcus aureus is a leading cause of potentially life-threatening nosocomial and community-acquired infections [1]. It is also a commensal bacterium of the skin and mucosa, colonizing 15 to 36% of the whole population [1,2]. Various sites of carriage have been reported [1] such as the nose [2],

skin [1], pharynx [3,4], vagina [5], and rectum [6]. Although exclusive pharyngeal or digestive carriage has been reported [3,6], the main reservoir of *S. aureus* carriage in humans remains the nose [2].

S. aureus carriage is usually asymptomatic, at least before the staphylococcal infection onset. Nasal colonization is a well-defined risk factor for *S. aureus* infection in all types of patients who have so far been studied [2] with a 2- to 12-fold increased risk of infection [7]. The relation between colonization and infection is supported by the fact that *S. aureus* strains isolated from nasal carriage and infections cannot usually be genetically distinguished [1]. Among carriers, *S. aureus* persistent carriers

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seem to have the highest risk of infection [2,3]. This is probably due to a higher bacterial load [8,9] compared with intermittent or non-carriers [10,11]. Despite literature data supporting the effectiveness of decolonization strategies for reducing *S. aureus* infections in various populations such as dialysis patients and surgical patients [12,13], official recommendations are heterogeneous.

We aimed to summarize current recommendations and proven data on *S. aureus* decolonization.

2. Decolonization of *S. aureus* in 2016: why?

Carriers of *S. aureus* are at higher risk of *S. aureus* infections. Determinants of *S. aureus* carriage are mixed, with both host and bacterial factors, and have already been reviewed [2]. The association between *S. aureus* nasal carriage and staphylococcal disease was first reported in furunculosis patients in the early 1930s [1]. Since then carriers of *S. aureus* have been shown to be at higher risk of *S. aureus* infection in various settings. This increased risk has been extensively studied in surgical patients [9,14,15] and in patients undergoing hemodialysis and peritoneal dialysis [2,16,17]. *S. aureus* carriage has also been shown to increase the risk of recurrent soft and skin tissue infection [18], bacteremia [19,20], and *S. aureus* infection in preterm infants [21]. Methicillin-resistant *S. aureus* (MRSA) carriers may be at higher risk of *S. aureus* invasive infection than carriers of methicillin-susceptible *S. aureus* (MSSA), even though a recruitment bias with higher frailty of MRSA carriers could be hypothesized [22].

Staphylococcal infections in *S. aureus* carriers are mainly endogenous. *S. aureus* strains responsible for nasal colonization have been shown to belong to the same genetic clusters as those responsible for invasive infections [23]. Very few differences were observed between infecting and colonizing *S. aureus* strains in a single patient using whole genome sequencing in a comparison model of the colonizing strain and the strain responsible for fatal bacteremia [24]. An endogenous origin (from the carriage strain) of staphylococcal infections was shown in patients presenting with bacteremia [19,20], surgical site infections (SSI) [14,15], skin and soft tissue infections [25], and in patients undergoing dialysis [26,27]. Endogenous origin was shown in 60–93% of *S. aureus* infections among carriers of this bacterium [14,21].

Decolonization of carriers has proven effective in reducing staphylococcal infection occurrence in carriers. *S. aureus* is the leading cause of SSIs and the second cause of nosocomial infections in France. The use of mupirocin (nasal route) or mupirocin and chlorhexidine baths – as decolonization strategies in *S. aureus* carriers – has proven effective in reducing staphylococcal infections in various populations of carriers [7,12,13,28]. A recent meta-analysis highlighted the clinical effectiveness of mupirocin in preventing *S. aureus* infections in nonsurgical settings. It reduced the risk of *S. aureus* infections by more than 40% [29]. Active screening and decolonization of MRSA carriers in intensive care units was shown to be effective in reducing related infections but it also proved to be cost-effective [30].

Bode et al. performed a recent longitudinal follow-up of patients included in a randomized controlled study [12] and showed that detecting and decolonizing *S. aureus* carriage not only prevented *S. aureus* surgical site infections but also reduced the 1-year mortality in surgical patients undergoing clean surgery [31].

The reduction of endogenous infections in *S. aureus* carriers therefore supports decolonization in specific situations.

3. Decolonization of *S. aureus* in 2016: for whom?

Identification of *S. aureus* carriers is performed using nasal swabs. Screening results can even be available within 2 hours using real-time PCR [8,12]. Our team also recently showed that it was possible to characterize the carrier status and to identify persistent carriers using only one sample [11]. Indeed, clinical studies concluded that *S. aureus* persistent nasal carriers are at higher risk of infection compared with intermittent and non-carriers [9–11]. Persistent carriers are defined as having a higher bacterial load or, in longitudinal studies, recurrent *S. aureus* nasal swabs positive to the same or different strains [32,33]. To date, when suggested, decolonization targets all carriers and not just persistent ones. Several well-conducted studies highlighted that decolonization of carriers was associated with a risk reduction of staphylococcal infections in four distinct populations as follows.

3.1. Dialysis patients

Several studies have been conducted in hemodialysis or peritoneal dialysis patients. They showed the effectiveness of mupirocin with a reduction of the risk of *S. aureus* infection (bacteremia, exit-site infection, or peritonitis) of 60%, as summarized by Tacconelli et al. and Nair et al. [13,29]. However, very few randomized studies have been conducted [34] and mupirocin treatment modalities are usually heterogeneous (nasal or catheter exit-site application of mupirocin, as well as different times of application). To date, the French national guidelines have not been edited for this population of patients. We recently performed a survey among French nephrologists participating in a national congress and showed that less than 50% of them screened and decolonized their patients [35].

3.2. Patients undergoing surgery

The authors of several studies showed that *S. aureus* decolonization of carriers before scheduled surgery was associated with a reduction in staphylococcal SSIs [12] or in *S. aureus* nosocomial infections in post-surgery settings [28]. The occurrence of *S. aureus* SSIs has been shown to be reduced by decolonization in patients undergoing cardiothoracic surgery in a well-conducted randomized study [12]. However, despite a general trend, all other published studies did not reach statistical significance in all surgical specialties such as in orthopedics [36]. A recent study conducted in orthopedic surgery settings aimed to evaluate a bundle intervention including decolonization of carriers and adequate perioperative prophylaxis (for MRSA or MSSA). Despite the non-randomized design, the

authors further supported the evidence that decolonization of *S. aureus* carriers may help reduce the occurrence of *S. aureus* SSIs [37]. The effectiveness of *S. aureus* carriage decolonization prior to surgery remains unproven in other types of surgery (neurosurgery, digestive surgery, gynecological surgery).

3.3. Patients presenting with recurrent SSTIs

Patients presenting with recurrent SSTIs are the third population for whom a decolonization strategy of *S. aureus* (MSSA or MRSA) has proven effective in reducing the occurrence of SSTIs [38]. *S. aureus* strains of infection and carriage may secrete Panton-Valentine leukocidin, and decolonization of households and contacts may be recommended in an outbreak context [39]. Despite a good decolonization rate after treatment, recurrence of carriage in this population is frequently observed after the procedure. Carriage of *S. aureus* is also frequent among preterm infants hospitalized in neonatal intensive care units, and decolonization with topical mupirocin seems to be effective in reducing MRSA infections [21].

3.4. MRSA carriers

Decolonization of MRSA carriers in a hospitalization setting, notably in the ICU or before surgery, has proven effective in reducing MRSA infections [7,40]. It also seems to be a cost-effective measure [40].

Decolonization of *S. aureus* carriage is now officially recommended in France before cardiac surgery [41] and in the context of grouped cases of community-acquired SSTI due to MRSA [42]. There is no official recommendation to decolonize other patients (hemodialysis and peritoneal dialysis patients, non-cardiac surgical patients, etc.).

4. Decolonization of *S. aureus* in 2016: how? When?

Mupirocin (pseudomonic acid A) has been widely available for use as a topical antimicrobial agent for many years and is considered effective in reducing *S. aureus* infections in nasal carriers [43]. Mupirocin alone, used topically twice a day, is the most effective way to decolonize *S. aureus* and reduce *S. aureus* infections in dialysis patients [44]. In other populations such as surgical patients, mupirocin alone failed to reduce *S. aureus* SSI occurrence among carriers [28,45]. This might be due to the high rate of skin colonization among *S. aureus* nasal carriers [1]. However, Perl et al. [28] and Kalmeijer et al. [45] performed decolonization in all patients; not just carriers. Their studies were probably designed to show a reduction in *S. aureus* SSI in the whole population, not just carriers. This design could have impacted the power of the study. In contrast, in this same population, intranasal mupirocin 2% (2 to 3 times a day) associated with chlorhexidine bathing (2–4%) once a day for 5 days was associated with a reduction in *S. aureus* SSI [12,36]. This strategy is most frequently used in surgical patients and in patients presenting with recurrent SSTIs [41,42]. No study was however conducted to compare mupirocin alone versus mupirocin + chlorhexidine to decolonize *S. aureus* carriers

and to reduce staphylococcal infections. As pharyngeal carriage may also be present [46], mouth rinses with chlorhexidine 0.1 to 0.2% has also been proposed for nasal and skin decolonization [39,41] despite the lack of evidence of an increased effectiveness in decolonization.

The emergence of mupirocin and chlorhexidine resistance has already been reported, and has been associated with lower decolonization rates [47–49]. Resistance to mupirocin has mainly been reported among MRSA strains, possibly due to a more intensive use of mupirocin in countries with a high incidence of MRSA strains [50]. Resistance was indeed more common in MSSA strains when mupirocin was widely used in community settings and in countries with a low incidence of MRSA strains [51]. Mupirocin resistance was also reported among coagulase-negative staphylococcal (CoNS) isolates [52]. Moreover, recurrence rates of *S. aureus* colonization and failure of decolonization using mupirocin and chlorhexidine have been reported (20–60%) [53,54]. Alternatives to mupirocin ointment or mupirocin and chlorhexidine bathing have therefore been proposed such as nasal [55] or nasal and cutaneous povidone-iodine use [56], cutaneous octenidine-based wash solution [39], and hypochlorite sodium (bleach) bath [57]. Comparison of mupirocin alone, mupirocin and chlorhexidine baths, and mupirocin and bleach water baths in patients presenting with recurrent SSTIs revealed that the inexpensive latter strategy seemed to be equivalent and even better than the mupirocin and chlorhexidine strategy [57]. Topical, nasal, and skin tea tree oil has also proved equivalent to the administration of mupirocin and triclosan as a decolonization strategy for MRSA in a small uncontrolled trial [58]. The authors of a recent study did not manage to prove the effectiveness of polyhexanide in eradicating MRSA carriage [59]. Alternative ways to perform *S. aureus* decolonization than topical procedure have also been proposed, notably in case of topical decolonization failure. Systemic antibiotics have been tested: doxycycline and rifampicin associated with mupirocin and chlorhexidine were shown to be effective in reducing MRSA carriage for at least 3 months in susceptible isolates [60]. Other oral antibiotics used to decolonize patients were reviewed by Ammerlaan et al. [61]. However, systemic antibiotic regimens have not yet been compared to topical mupirocin-based regimen in *S. aureus* decolonization. The impact of such regimen associated with systemic antibiotics on resistance would certainly be higher than that of topical regimen and should not be used in first line.

Two strategies are currently being discussed in the literature as to which patients should be decolonized: should we perform universal or targeted decolonization?

The targeted strategy is the so-called “search and destroy” strategy. It relies on the detection of carriers who are at risk of endogenous infection (i.e., surgery, invasive procedure, admission in the intensive care unit, undergoing dialysis, etc.) to then be able to decolonize the identified *S. aureus* carriers. By contrast, universal decolonization strategy is used without prior screening of carriers. Findings obtained with a stochastic, individual-based model of MRSA transmission supported targeted screening and decolonization strategy instead of the universal strategy [62]. It was associated with a similar effectiveness

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despite a significant reduction in the number of individuals to screen and decolonize. The authors of a monocentric prospective interventional cohort study, conducted with a crossover design and including more than 20,000 surgical patients, did not observe that universal screening of MRSA carriers associated with decolonization of carriers before surgery was associated with a significant reduction in MRSA infections when compared with standard MRSA control measures [63]. This disappointing result may be partly explained by the fact that more than 50% of infected patients acquired MRSA carriage during hospitalization and were therefore MRSA-free at admission. This real-life trial rather suggested the use of targeted screening and decolonization strategy in surgical populations at high risk of MRSA infections. A recent universal decolonization performed in the ICU, i.e. without screening MRSA carriers, resulted in a significant reduction in MRSA-positive clinical cultures as well as in a significant reduction in bloodstream infections from any pathogen, MRSA bloodstream infections not being significantly reduced compared with targeted decolonization [64]. The threat of bacterial resistance emergence in MRSA [65] (observed in the study of Huang et al.) and CoNS [66] as well as adverse effects due to universal decolonization is, however, ongoing and this strategy has not been universally adopted. However, a recent study using a mathematical model suggested that the increased selection of high-level mupirocin resistance in CoNS does not constitute an important risk for high-level mupirocin resistance in *S. aureus* [67]. Another mathematical model study also suggested that universal decolonization might be less expensive than targeted decolonization [68]. These latter studies provided results that should be interpreted with caution as the emergence of mupirocin resistance in MSSA is less studied and understood than in MRSA. Another universal intervention aiming at preventing *S. aureus* infection could be to immunize patients against this pathogen before invasive procedures. To date *S. aureus* vaccine development failed to prevent *S. aureus* infections and *S. aureus* colonization [69].

Among *S. aureus* carriers belonging to populations where decolonization has proved to reduce *S. aureus* infections (hemodialysis and peritoneal dialysis patients, patients presenting with recurrent SSTIs, and surgical patients – notably cardiac surgery), the most studied protocol to date is mupirocin 2% nasal ointment twice a day associated with chlorhexidine bathing once a day for 5 days. It should be performed before scheduled surgical procedures or at least initiated before surgery [12]. With regard to patients presenting with recurrent SSTIs, decolonization is suggested concomitantly with the SSTI treatment [42]. Despite arguments to decolonize dialysis patients [13,44], additional evidence on the use and effectiveness of mupirocin in this population is needed. The right time to perform decolonization remains unclear in dialysis carriers, and notably persistent carriers as they repeatedly undergo invasive care (dialysis three times a week, insertion of a catheter, etc.) and are at high risk of infections and recurrent colonization. Patients undergoing multiple courses of decolonization are at risk of resistance emergence to both mupirocin and chlorhexidine, as definitive eradication of *S. aureus* carriage is rarely achieved, all the more in this population [70].

5. Decolonization of *S. aureus*: conclusion and perspectives

We aimed to summarize well-established data on decolonization of *S. aureus* carriers. Surgical patients, dialysis patients and patients presenting with recurrent SSTIs would benefit from decolonization. Decolonization of *S. aureus* carriers therefore seems to be the adequate solution to prevent staphylococcal infections. The use of mupirocin or chlorhexidine is effective in reducing carriage and *S. aureus* infections in several situations such as before cardiac surgery and in the context of grouped cases of community-acquired SSTI due to MRSA. Robust data from a well-designed study is needed in at-risk situations of *S. aureus* SSI such as orthopedic surgery, despite the potential efficacy of decolonization in preventing infections [36]. The choice between universal or targeted decolonization strategy also needs to be made. Mupirocin administered to dialysis patients seems to be effective in reducing endogenous *S. aureus* infections. However, decolonization protocols should be better defined.

The increased resistance to mupirocin and chlorhexidine associated with failures of decolonization stress the need for alternatives. New molecules have been or are currently being tested in humans such as the highly bactericidal XF-73. The agent is currently being evaluated in phase II clinical trials for nasal decolonization. The impact of *S. aureus* vaccines, which are currently being evaluated, on carriage is unknown. However, the role of *S. aureus* vaccines on the carriage of the bacterium would be an interesting additional marker of vaccine effectiveness [69].

Disclosure of interest

The authors declare that they have no competing interest.

Contributors

E.B.N. wrote the first draft of the article.

J.G., P.O.V., C.C., F.G., and B.P. contributed to searching the literature.

C.C., F.L., and P.B. reviewed the article.

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INTRODUCTION AND PURPOSE

We report an outbreak of methicillin susceptible *S. aureus* strains belonging to t571 spa type and clonal complex (CC) 398 in a neonatal intensive care unit (NICU). CC 398 MSSA is epidemiologically related to CC 398 livestock-associated MRSA that has been described as a major animal pathogen that can infect humans. CC 398 MSSA carriage and infections have been previously described in adults but never in an epidemic context in neonates.

MATERIALS and METHODS

A *S. aureus* outbreak occurred in the NICU between June and October 2017. During that period, 19 neonates were screened weekly in throat and rectal swabs. Screening of 57 health care workers (HCWs) was proposed in August including nasal and throat sampling. A total of 16 environment samples were also done during the outbreak. *S. aureus* isolates were genotyping by spa typing and *S. aureus* genotyping microarray (Aler).

Acknowledgement: the authors thank all the HCWs of the NICU for their participation to this study

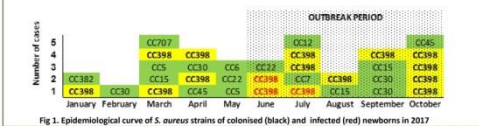
26th ECCMID ESCMID

RESULTS

Description of the outbreak
3 severe *S. aureus* infections were diagnosed in June and July 2017. They included 1 pneumonia and 2 bacteremias leading to a death of one extremely premature newborn. Interestingly, all 3 strains exhibited resistance to erythromycin, with inducible resistance to clindamycin. Molecular typing using spa typing and microarray confirmed that these isolates belonged to a single clone from Clonal Complex (CC) 398 with a t571 spa type. This CC398/t571 strain harboured none of the major staphylococcal virulence genes (such as PVL, TSST of enterotoxin genes) but *chp* and *scn* genes carried by the hly-converting phage that contribute to inhibition of *S. aureus* phagocytosis by human neutrophils.

Epidemiological survey of newborns
Following a previous outbreak in 2014, newborns from the NICU had been weekly screened for *S. aureus* infection in throat and rectum. From January to October 2017, 31 newborns other than the 3 infected cases were found colonised with *S. aureus*; 16 were found colonised with the epidemic clone and among them 16/16 (100%) had an erythromycin resistant strain. During the outbreak period and among the 19 carriers, 13 were colonized at both sites (throat and rectum).

Incidence of *S. aureus* infection and colonisation in the NICU.
From June to October, the overall incidence of *S. aureus* infection and colonization including all types of strains or only the CC398/t571 *S. aureus* strain was 8.92 and 5.16 per 1000 hospitalized days, respectively. From January to October 2017 no *S. aureus* infection occurred with any other type of *S. aureus* strain.



RESULTS

Environmental survey
S. aureus was cultured from 5 environment samples out of 16 (including incubator and monitoring devices) and 3 of them recovered the CC398/t571 *S. aureus* strain.

Screening of HCWs
Detection of *S. aureus* carriage was proposed to 57 HCWs. The prevalence of carriage in the NICU during the summer period was high, with respectively 22 and 34 HCWs carrying *S. aureus* in nose (38%) and in nose and throat (60%). Among the carriers, 16 (47%) carried the CC398/t571 clone (Fig. 2) and among them 13/16 (81%) had an erythromycin resistant *S. aureus* strain.

Epidemiology of CC398 strains during the 2017 year
Surprisingly, the CC398/t571 was shown to spread and persist in the NICU both in HCWs and newborns (Fig. 1 and Fig. 2). This suggests to survey and control the spread of this emergent CC398 MSSA in paediatric settings.

Fig. 2. Dendrogram by microarray of *S. aureus* strains isolated from HCWs (in blue) or newborns (in orange) during the outbreak period. One isolate depicted for each HCW or newborn.

CONCLUSION

Molecular typing of *S. aureus* strains highlighted the epidemiological link between strains isolated from newborns, HCWs and NICU environment. Interestingly, this is the first outbreak highlighting the spread of the CC398 MSSA strain in a NICU, suggesting a particular environmental or human fitness of this clone.

INTRODUCTION AND PURPOSE

S. aureus nasal decolonization with topical mupirocin and chlorhexidine has been shown to significantly reduce surgical site infection due to this bacterium in cardiac surgery and is now recommended by the WHO to prevent these infections in orthopaedic patients.

Moreover, *S. aureus* decolonization of healthcare workers (HCWs) can be set up in addition to other classic hygienic control measures during the course of a *S. aureus* outbreak.

The aim of this work was to describe the epidemiology of *S. aureus* strains harbored by HCWs during an outbreak and one year after their decolonization.

METHODS

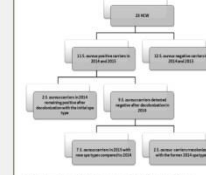
Nasal and throat samples were taken from HCWs during an outbreak occurring in November 2014 in a neonatal intensive care unit (NICU), one week after decolonization of carriers in December 2014 and one year later in November 2015.

HCWs carrying *S. aureus* were decolonized by using intra-nasal mupirocin, mouth wash and shower with chlorhexidine during 5 days.

S. aureus isolates were genotyped by spa typing.

26th ECCMID ESCMID

RESULTS



N° of HCW	New types identified among <i>S. aureus</i> carriers before (November 2014), one week after (December 2014) and one year (November 2015) after decolonization. Sites of carriage (see text and for details) indicated in parenthesis.		
	November 2014	December 2014	November 2015
F	1088 (N7)	-	1045 (N7)
G	1012 (N7)	-	1718 (N7)
H	1234 (N)	-	1718 (N)
I	1084 (N)	1084 (N)	1012 (N)
J	1539 (N7)	1539 (N)	1539 (N)
K	1023 (N1-N5/N7)	-	1539 (N)
L	1246 (N7)	-	1246 (N)
M	1289 (N7)	-	1289 (N)
N	1289 (N) (N1/N7)	-	1289 (N)
O	1482 (N) (N1/N7)	-	1482 (N)

Fig. 2. Results of *S. aureus* carriage in 11 HCWs (A to K) after decolonization

From a total of 67 HCWs screened during the initial outbreak in 2014, 23 HCWs could be sampled during the three periods (Fig. 1)

Among 11 HCWs (48%) identified as *S. aureus* carriers in 2014, 9 (82%) were found effectively decolonized one week after decolonization but all of them were found positive again for *S. aureus* carriage one year later.

S. aureus strains with a different spa type were identified in 7 (64%) of these 11 cases (Fig. 1 and 3)

Among the 4 HCWs who harbored a *S. aureus* strain epidemiologically related to the first isolate, 2 cases corresponded to an initial failure of the decolonization treatment (Fig. 2)

Twelve (52%) HCWs, found non *S. aureus* carriers on the first screening in 2014, were negative one year later.

The prevalence of *S. aureus* nasal carriage in the NICU HCWs was high, reaching up to 46% (31/67) in November 2014, and remaining at 38% (18/47) in 2015, one year after decolonization.

The genotypic diversity of *S. aureus* strains was 58% in 2014 during the outbreak and 44% in 2015, one year after. 6 spa types were found common between the 2 periods in the NICU (Fig. 4)

RESULTS

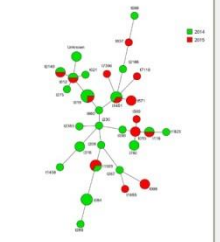
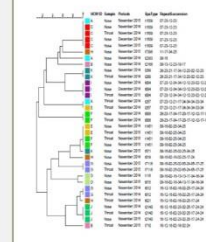


Fig. 4. Minimum spanning tree (spa typing) of *S. aureus* strains isolated from all nasal-colonized HCWs during the outbreak (green) and one year after (red)

CONCLUSION

The decolonization of HCWs during an outbreak in a NICU did not significantly reduce one year later the high prevalence of *S. aureus* carriage observed in the HCWs. However, the strategy had an impact on the diversity of the strains circulating in the unit, including those identified in HCWs still working during the one-year period.

Acknowledgement: the authors thank all the HCWs of the NICU for their participation to this study

26th ECCMID ESCMID

INTRODUCTION AND PURPOSE

S. aureus is a frequent cause of healthcare-associated infections in hospitalised infants contributing to increased morbidity and mortality in this population. Following a severe infection in a neonatal intensive care unit (NICU), a longitudinal evaluation of *S. aureus* infections was performed during a 8-month period.

METHODS

A *S. aureus* outbreak occurred in the NICU in September 2014. Control measures were implemented including:

- reinforcement of hand hygiene,
- use of gowns and mask,
- reinforcement of environmental cleaning

Nasal samples were taken in healthcare workers (HCWs) in November 2014, in December 2014 (one week after decolonization of nasal carriers) and 6 months later in March 2015.

- HCWs carrying *S. aureus* were decolonized by using intra-nasal application of mupirocin, mouth wash and chlorhexidine shower.
- The surveillance of infections was performed in all newborns until March 2015.

S. aureus strains isolated from newborns and HCWs were analyzed by microarray and spa-typing.



RESULTS

Five *S. aureus* infections including 3 pneumonia and 2 bacteremia were observed in the NICU between September and November 2014, leading to the death of one newborn. During this period, the overall incidence of *S. aureus* infections was 4.7 per 1000 days of hospitalization.

After implementation of control measures, *S. aureus* infections decreased significantly between December 2014 and March 2015 ($P=0.2$).

The prevalence of *S. aureus* nasal carriage in HCWs was **46% and 36% in November 2014 and April 2015**, respectively ($P>.05$) although nasal decolonization was achieved in 90% of cases in December 2014.

Molecular typing of *S. aureus* strains highlighted the epidemiological link between strains isolated from newborns (shown in green in Figure) and HCWs (in light/dark blue in Figure).

- 3 pneumonia were due to 3 different clones belonging to spa-types/clonal complexes (CC) t209/CC9, t780/CC45 and t056/CC101. Strains t209 and t780 were epidemiologically linked to respectively 1 and 2 HCW *S. aureus* nasal strains.
- 2 bacteremia were due to 2 different clones, t316/CC59 and t012/CC30.
- Strain t316 involved in a newborn death harbored the gene coding for enterotoxin B. Strains t316 and t012 were similar to those of 2 and 1 HCW *S. aureus* nasal strains, respectively.

HCWs were colonized by *S. aureus* strains belonging to spa-types t780 (n=2), t316 (n=2), t019 (n=3), t1451 (n=2), t084 (n=3) and others (n=19) (light blue circles in Figure).

After decolonization, *S. aureus* nasal carriage strains belonged to spa-types t1509 (n=3), t571 (n=2), t012 (n=3) and others (n=15) (shown in dark blue in Figure).

Interestingly, CC59/t316 strain disappeared from the NICU after HCW decolonization.

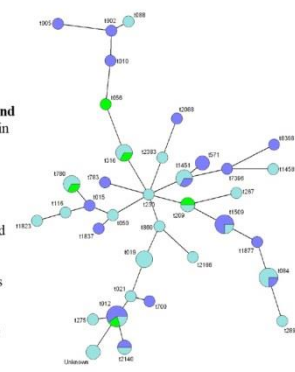


Figure. Molecular epidemiology (spa-typing) of *S. aureus* strains isolated from infected newborns (green) and from colonized HCW during an outbreak (light blue) and after implementation of control measures (dark blue)

CONCLUSIONS

This study highlights the effectiveness of control measures including reinforcement of standard precautions and targeted decolonization of HCWs to end of *S. aureus* outbreak in NICU. Molecular characterization of *S. aureus* strains shows that decolonization of HCWs contributes to modify the epidemiology of *S. aureus* nasal carriage among HCWs but not to decrease significantly the prevalence of nasal carriage.

Acknowledgement: the authors thank all the HCWs of the NICU for their participation to this study.