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Elodie Saouter von Dach

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Par

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Methicillin-Resistant *Staphylococcus aureus*
Prevention and Fight Against this Pathogen

Thèse présentée et soutenue à Villejuif le 6 juillet 2017

Composition du Jury:

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SUISSE

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- **Comparative Genomics of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Shows the Emergence of Clone ST8-USA300 in Geneva, Switzerland.** von Dach E, Diene SM, Fankhauser C, Schrenzel J, Harbarth S, François P. *J Infect Dis.* 2016 May 1;213(9):1370-9.
- **Determinants of Successful Methicillin-Resistant *Staphylococcus aureus* Decolonization.** von Dach E, Landelle C, Agostinho A, Hausteiner T, François P, Renzi G, Schrenzel J, Harbarth S. *Infect Control Hosp Epidemiol.* 2016 Jun;37(6):732-6.
- Reply to Planet et al. François P, von Dach E, Diene SM, Harbarth S, Schrenzel J. *J Infect Dis.* 2016 Nov 15;214(10):1610-11.
- **Comparing The Cost-Effectiveness Of Linezolid To Trimethoprim/Sulfamethoxazole Plus Rifampicin From The Treatment Of MRSA Infection: A Health-Care System Perspective.** von Dach E, Morel C, Murthy A, Pagani L, Macedo-Vinas M, Olearo F, Harbarth S. *Clin Microbiol Infect.* 2017 Sep;23(9):659-666.

PRESENTATIONS

- **Direction Générale de la Santé - Groupe antibiorésistance (invited)**
Comparative genomics to investigate the emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) USA300 clone in Geneva, Switzerland.
- **Colloque Service Prévention et Contrôle de l'Infection - Hôpitaux Universitaires de Genève (invited)**
Comparative genomics to investigate the emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) USA300 clone in Geneva, Switzerland.
- **Colloque des maladies infectieuses - Hôpitaux Universitaires de Genève (invited)**
Comparing the cost-effectiveness of linezolid (LZD) to trimethoprim/sulfamethoxazole (TMP-SMX) plus rifampicin (RMP) for the treatment of MRSA infection: a health-care system perspective.
- **Colloque Service Prévention et Contrôle de l'Infection - Hôpitaux Universitaires de Genève (invited)**
Comparing the cost-effectiveness of linezolid (LZD) to trimethoprim/sulfamethoxazole (TMP-SMX) plus rifampicin (RMP) for the treatment of MRSA infection: a health-care system perspective.

International Consortium for Prevention & Infection Control (ICPIC), Geneva, 16-19 Juin 2015

- Determinants of successful MRSA decolonization among patients included in a clinical trial of polyhexanide. von Dach E, Landelle C, Haustein T, Agostinho A, Renzoni A, François P, Renzi G, Schrenzel J, Pittet D, Harbarth S. (Poster 192)
- Comparative genomics to investigate the emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) USA300 clone in Geneva, Switzerland. Diene SM, von Dach E, Fankhauser C, Bonetti EJ, Schrenzel J, Harbarth S, François P. (Poster 197)

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- Comparative genomics to investigate the emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) USA300 clone in Geneva, Switzerland. von Dach E, Diene SM, Fankhauser C, Bonetti EJ, Schrenzel J, Harbarth S, François P.

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- Comparative genomics to investigate the emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) USA300 clone in Geneva, Switzerland. von Dach E, Diene SM, Fankhauser C, Bonetti EJ, Schrenzel J, Harbarth S, François P.

ECCMID, Vienne, 22-25 Avril 2017

- Comparing the cost-effectiveness of linezolid (LZD) to trimethoprim/sulfamethoxazole (TMP-SMX) plus rifampicin (RMP) for the treatment of MRSA infection: a health-care system perspective. von Dach E, Morel CM, Murthy A, Pagani L, Macedo-Vinas M, Olearo F, Harbarth S. (ePoster 1381)

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- Comparing the cost-effectiveness of linezolid to trimethoprim/sulfamethoxazole plus rifampicin for the treatment of MRSA infection: a health-care system perspective. von Dach E, Morel CM, Murthy A, Pagani L, Macedo-Vinas M, Olearo F, Harbarth S.

Joint annual meeting of SSM, SSI, SSHH, SSTMP, Basel, 30 Août - 1 September 2017

- Comparing the cost-effectiveness of linezolid to trimethoprim/sulfamethoxazole plus rifampicin for the treatment of MRSA infection: a health-care system perspective. von Dach E, Morel CM, Murthy A, Pagani L, Macedo-Vinas M, Olearo F, Harbarth S.

RÉSUMÉ

Mots clés: *Staphylococcus doré* résistant à la méticilline (SARM), USA300, épidémiologie, décolonisation, facteurs de risque, infection, analyse coût-efficacité, analyse coût-utilité triméthoprime-sulfaméthoxazole, linézolide, rifampicine, QALYs

Le *Staphylococcus aureus* (*S. aureus*) est une bactérie pathogène pouvant causer des infections du sang, de la peau, des tissus mous et du tractus respiratoire, infections qui peuvent être d'origine communautaire ou hospitalière. En plus des personnes pouvant être infectées par *S. aureus*, de nombreuses personnes, environ 20-25% parmi les adultes en bonne santé, portent ce pathogène sur leurs corps, sans pour autant souffrir de quelque symptôme qu'il soit (ils sont dits "colonisés"). *S. aureus* est communément trouvé dans les narines, mais peut aussi coloniser la peau ou la gorge. Les lésions chroniques de la peau, tels que blessures, ulcères, ou eczéma, ainsi que les points d'insertion de dispositifs médicaux, sont d'importantes niches écologiques pour le développement d'infections dues à ce microorganisme. Bien qu'asymptomatique et souvent bénigne, la colonisation à *S. aureus* peut rarement induire un risque d'infection secondaire grave et représente le risque d'être transmise à une tierce personne. À l'hôpital, les risques d'infection et de transmission apparaissent comme étant particulièrement hauts pour la souche de *S. aureus* résistante à la méticilline (SARM), dont les complications peuvent s'avérer très graves. La prévention et la lutte contre le SARM sont des problèmes de santé publique importants.

L'objectif de cette thèse est d'investiguer via différentes études de nouveaux moyens pour contrôler et traiter le SARM. En particulier, l'emploi du séquençage du génome entier comme moyen d'investigation d'une épidémie communautaire de SARM ou encore l'utilisation d'anciens antibiotiques pour traiter les infections à SARM.

La première partie est une étude épidémiologique et moléculaire portant sur la souche de SARM communautaire nord-américaine de type USA300. Les enquêtes portant sur le SARM communautaire aux Hôpitaux Universitaires de Genève ont révélé une grande diversité de souches présentes, avec une identification seulement sporadique de la souche USA300 (ST8 PVL-positive) pendant la période 1994-2012. Cependant, en 2013 cette lignée représentait presque 50% des SARM communautaires identifiés, une soudaine augmentation comparativement aux années précédentes. Afin de mieux comprendre l'augmentation de cette prévalence et de décrire les profils cliniques, les facteurs de risques, ainsi que de retracer l'origine, la propagation et l'évolution de cette souche, une étude épidémiologique et moléculaire mixte a été réalisée. Grâce à la surveillance des SARM communautaires à

Genève, les informations cliniques, démographiques et d'expositions ont été recueillies pour chaque cas colonisé ou infecté par une souche USA300 et comparées aux autres souches de SARM communautaires. Le séquenceur Solexa-Illumina a été utilisé pour le séquençage du génome entier (WGS) de toutes les souches USA300 isolées en 2013. Toutes les caractéristiques moléculaires dont les polymorphismes d'un seul nucléotide (SNP), la présence du locus ACME, la structure SCCmec et le contenu des éléments mobiles ont été documentés et liés aux données du patient. Les génomes publiés de souches USA300 ont été utilisés à des fins de comparaison et pour étudier la relation entre les isolats.

En 2013, parmi les 46 souches isolées chez des patients, 22 étaient de type USA300 (14 cas d'infection clinique et 8 cas de portage asymptomatique). Les arbres phylogéniques obtenus par WGS ont montré deux groupes distincts de souches, porteurs ou non du locus ACME ; un locus reposant sur un élément génétique mobile, impliqué dans le catabolisme de l'arginine et contribuant à la résistance bactérienne contre les défenses de notre peau et de nos muqueuses. Presque toutes les souches ACME positives étaient résistantes à la ciprofloxacine et à l'érythromycine et sont regroupées dans un cluster plus homogène que les souches ACME négatives. Trois paires de 2 souches ont été clairement identifiées comme des événements de transmission familiale. La grande majorité des souches ACME négatives a été isolée à partir de patients qui ont voyagés ou étaient en Amérique du Sud dans les 12 derniers mois. La position des SNP a permis de retracer l'origine géographique des souches et de révéler un élément SCCmec IVc commun dans presque tous les isolats ACME négatifs.

Le WGS fournit une identification précise des événements de transmission et a révélé que l'augmentation soudaine de la prévalence du clone USA300 résulte de plusieurs événements individuels d'importations, et n'est pas attribuable à un événement unique d'importation qui ce serait par la suite propagé dans notre région.

La deuxième étude est une étude épidémiologique portant sur les déterminants de la décolonisation spontanée à SARM. Entre janvier 2011 et juillet 2014, un essai randomisé contrôlé (RCT) a été réalisé chez des porteurs de SARM pour évaluer l'efficacité de la polyhexanide comparée à une solution placebo pour la décolonisation à SARM. Les déterminants liés à une réussite de décolonisation de SARM chez les patients inclus dans cette RCT ont été évalués. Une étude rétrospective cas-témoins a été réalisée chez les patients de cette même cohorte. Les patients porteurs de SARM et traités par polyhexanide ou placebo avec un frottis de contrôle à 28 jours après la fin du traitement ont été inclus. Les cas ont été définis comme les patients non-porteurs de SARM à la fin de l'étude. Le groupe témoin était constitué de patients encore porteurs de SARM au même moment. Nous avons testé les facteurs potentiels favorisant la décolonisation en utilisant une analyse de régression logistique univariée. Les données ont été enregistrées au cours de l'étude prospective initiale après le consentement des patients. Un modèle de régression logistique multivariée a ensuite été construit. Nous avons gardé comme candidats potentiels pour l'analyse multivariée les déterminants avec une P value <0.2. Sur un total de 135 patients

identifiés: 46 étaient des cas et 89 des contrôles. Comparés aux témoins, les cas ont tendance à vivre sans assistance, possèdent un seul site colonisé et ont une durée de séjour hospitalier inférieur à 3 semaines. En analyse multivariée, deux déterminants sont restés associés avec la décolonisation du SARM: statut indépendant et un unique site corporel colonisé au départ; nos résultats ont été validés par le test de Hosmer-Lemeshow.

Les patients indépendants et coloniés en un seul site semblent donc être plus favorables à une décolonisation spontanée à du portage à SARM.

Pour clore ce projet de thèse, la dernière étude est une étude coût-efficacité comparant la combinaison de triméthoprime-sulfaméthoxazole (TMP-SMX) plus rifampicine (RMP) versus linézolide (LZD) seul dans le traitement des infections à SARM. Un essai clinique de non-infériorité, randomisé a été réalisé aux Hôpitaux Universitaires de Genève comparant ces deux traitements. Celui-ci montre que comparée au LZD l'efficacité d'un traitement de TMP-SMX et RMP est non-inférieure dans le traitement des infections à SARM. En raison de l'utilisation d'un versus deux médicaments et compte tenu de la différence de coût initial des médicaments de ces deux alternatives, nous avons décidé d'effectuer une analyse coût-efficacité pour évaluer l'impact économique ainsi que la toxicité potentielle de ces alternatives de traitement. Nous avons effectué une analyse coût-utilité en utilisant les données de cet essai ainsi que des données simulées provenant de la littérature.

Nous avons construit un modèle de décision par ordinateur à l'aide de ces données et d'un point de vue des fournisseurs de soins de santé. Notre mesure de l'efficacité repose sur les années de vie ajustées sur la qualité (QALYs). Plusieurs analyses de sensibilités ont été réalisées à partir des données de l'étude ou des données simulées, pour vérifier la fiabilité du modèle, telles que des simulations de Monte Carlo pour construire des courbes d'acceptabilité coût-efficacité et des analyses de sensibilités à une, deux et trois dimensions. Un traitement de TMP-SMX plus RMP et de LZD a un coût par QALY gagné de "174CHF/160€" et "3130CHF/2877€", respectivement. Le traitement de TMP-SMX plus RMP reste dominant avec tous les types d'analyses de sensibilité effectués même dans les scénarios extrêmes, comparé au LZD. Un rabais de 95% sur le prix d'achat actuel du LZD serait nécessaire pour qu'il devienne rentable par rapport à un traitement de TMP-SMX plus RMP. De plus, la mise sur le marché récente du LZD en version générique, nous a appris que son prix était diminué de 10% par rapport au prix du médicament original. Cependant, même avec cette baisse tarifaire, le LZD reste moins coût-efficace que le traitement de TMP-SMX plus RMP, dans tous les types d'analyses de sensibilité.

En conclusion, par rapport au LZD, un traitement combiné de TMP-SMX plus RMP est économiquement plus intéressant pour le traitement des infections à MRSA.

Ce travail de thèse offre de nouvelles perspectives en termes de santé publique et de prise en charge préventive et curative de la multi-résistance antibiotique du *S. aureus*. Les nouvelles techniques telles que le WGS, la meilleure compréhension des déterminants

de la décolonisation spontanée ou l'utilisation d'anciens antibiotiques aussi performants et moins chers sont des alternatives intéressantes permettant de mieux comprendre la dynamique du MRSA et d'offrir de nouvelles perspectives concernant la lutte contre ce pathogène important. Selon l'ONU, la lutte contre la résistance aux antibiotiques est une priorité mondiale. C'est pourquoi il est toujours pertinent d'étudier de nouvelles techniques de contrôle et de traitement contre les bactéries multi-résistantes, qui pourront servir d'exemples pour de nouveaux germes émergents.

ABSTRACT

Keywords methicillin-resistant *Staphylococcus aureus* (MRSA), USA300, epidemiology, decolonization, risk factors, infection, cost-effectiveness analysis, cost-utility analysis trimethoprim-sulfamethoxazole, linezolid, rifampicin, QALYs

Staphylococcus aureus (*S. aureus*) is a bacterium that can cause infections that can have a community or hospital origin. Among people infected with *S. aureus*, about 20-25% are healthy adults that carry the pathogen on their bodies (they are said "colonized"). Although asymptomatic and often benign, colonization with *S. aureus* can involve a low risk of secondary infection and represents a serious risk of transmission to a third party. In hospitals, the risk of infection and transmission appears to be unusually high for methicillin-resistant *S. aureus* (MRSA) and complications can be very severe while only very few treatments exists and only few available treatment to treat them. Prevention and the fight against MRSA is a major public health concern.

The objective of this thesis is to investigate new methods to better understand and control MRSA via different studies.

First, an epidemiological and molecular study of community-associated MRSA strain USA300 type from North America was conducted. Through monitoring of CA-MRSA in Geneva, clinical, demographic and exposure characteristics were collected for each patient colonized or infected with a USA300 strain compared to other CA-MRSA strains. The Solexa-Illumina instrument was used for the whole genome sequencing (WGS) of all USA300 strains isolated. The WGS provided accurate identification of transmission events and showed that the sudden increase in the prevalence of USA300 clone resulted from several individual events of imports, and is not attributable to a single event of importation subsequently spread in the region.

The second part is an epidemiological study on the determinants of spontaneous MRSA decolonization. A clinical trial was conducted among MRSA carriers for evaluating the effectiveness of polyhexanide compared with a placebo solution for MRSA decolonization. A case-control study among the patients of the same cohort was performed. Of a total of 135 patients included: 46 were cases, and 89 were controls. In the multivariate analysis, independent patients and carriers of a single MRSA carriage site tend to be more favorable to spontaneous MRSA decolonization.

To close this project, a clinical trial was performed at the Geneva University Hospitals comparing the combination of trimethoprim-sulfamethoxazole (TMP-SMX) plus rifampicin (RMP) versus linezolid (LZD) alone for the treatment of MRSA infections. We decided to

conduct a cost-utility analysis to assess the economic impact and potential toxicity of these treatment alternatives. The treatment of TMP-SMX plus RMP and LZD had a cost per QALY gained of "160€" and "2877€". A treatment of TMP-SMX plus RMP remained dominant with all types of sensitivity analysis performed. In conclusion, compared to LZD, combined treatment of TMP-SMX plus RMP is economically more advantageous for the treatment of MRSA infections.

The work of this thesis offers new perspectives in terms of public health and preventive and curative management of the antibiotic multidrug resistance of *S. aureus*. New techniques such as WGS, a better understanding of the determinants of spontaneous decolonization, or the use of older, effective and cheaper antibiotics are interesting alternatives to better understand the dynamics of MRSA and to provide new perspectives on the control of this important pathogen. According to the United Nations, the fight against antibiotic resistance is a global priority. This is why it is still relevant to study new techniques of control and fight against multi-resistant bacteria, which can further be used against new emerging germs.

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Abbreviations

ACER:	average cost-effectiveness ratio
ACME:	arginine catabolic mobile element
ADR:	adverse drug reaction
AE:	adverse event
AMR:	antimicrobial resistance
aOR:	adjusted odd ratio
aRR:	adjusted risk relatif
BMI:	body mass index
bp:	base-pairs
BSI:	bloodstream infection
β -lactam(ase):	beta-lactam(ase)
CA-MRSA:	community-associated methicillin-resistant <i>Staphylococcus aureus</i>
CC:	clonal complex
CDC:	The Center for disease control and prevention
CEA:	cost-effectiveness analysis
CEAC:	cost-effectiveness acceptability curve
CHF:	Swiss franc
CI:	confidence interval
COMER:	copper and mercury resistance mobile element
CRE:	carbapenem-resistant Enterobacteriaceae
dn:	synonymous
ds:	nonsynonymous
DNA:	deoxyribonucleic acid
DRG:	diagnosis related group
ESBL:	Extended spectrum beta-lactamase
HAI:	healthcare-associated infection
HA-MRSA:	healthcare-associated methicillin-resistant <i>Staphylococcus aureus</i>
HQORL:	health-related-quality-of-life
HUG:	Geneva university hospitals
ICER:	incremental cost-effectiveness ratio
ICU:	intensive care unit
IV:	intravenous
LA-MRSA:	livestock-associated methicillin-resistant <i>Staphylococcus aureus</i>
LZD:	linezolid
MC:	Monte Carlo
MIC:	minimum inhibitory concentration
MLST:	multilocus sequence typing

MGE:	mobile genetic element
mMRSA:	multidrug resistant MRSA
mRNA:	messenger ribonucleic acid
MRO:	multi-resistant organism
MRSA:	methicillin-resistant <i>Staphylococcus aureus</i>
MSSA:	methicillin-sensitive <i>Staphylococcus aureus</i>
MuH:	high-level resistance)
MuL:	low-level resistance
NmMRSA:	non-multidrug resistant MRSA
OR:	odds ratio
PBP:	penicillin-binding protein
PCR:	polymerase chain reaction
PFGE:	pulsed-field gel electrophoresis
PO:	per os
PVL:	panton-valentine leukocidin
QALY:	quality-adjusted life year
RMP:	rifampicin
RNA:	ribonucleic acid
RR:	relative risk
SAE:	serious adverse event
<i>S. aureus</i> :	<i>Staphylococcus aureus</i>
SD:	standard deviation
<i>S. epidermidis</i> :	<i>Staphylococcus epidermidis</i>
SCCmec:	staphylococcal cassette chromosome mec
SNP:	single-nucleotide polymorphism
SPA:	<i>Staphylococcus aureus</i> protein A typing
SSI:	surgical site infection
SSTI:	skinand soft tissue infection
ST:	sequence type
TEN:	toxic epidermal necrolysis
TMP:	trimethoprim
TMP-SMX:	trimethoprim-sulfamethoxazole
tRNA:	transfer ribonucleic acid
TSS:	toxic shock syndrome
USA300-LV:	USA300 Latin-America Variant
US\$:	US dollars
UTI:	urinary tract infection
VRE:	vancomycin-resistant enterococci
WHO:	The World Health Organisation
WGS:	whole-genome sequencing

Chapter 1

Introduction

"Whatever you do will be insignificant, but it is very important that you do it."

Mahatma Gandhi

In 1668, Antony van Leeuwenhoek observed for the first time a bacteria using a microscope of its manufacture. He called them "animalcules." His observations were published in a series of letters sent to the Royal Society [1]. Two century later, the German microbiologist Christian Gottfried Ehrenberg used the word "bacteria" for the first time, deriving from the Greek *βακτηριον*, meaning "stick." In the 19th century, bacteriology was revolutionized with the work of Louis Pasteur. In 1859, he evidenced that micro-organisms are responsible for the fermentation process [2]. It also showed the role of bacteria as potential infectious agents [3]. Later, the etiologic role of bacteria in disease was confirmed by Robert Koch. He was a pioneer in medical microbiology, and he performed seminal research on anthrax, tuberculosis, and cholera. He clearly demonstrated that bacteria could play a role as infectious disease agent and wrote a series of a couple of postulates (Koch's postulates), still used today [4]. In 1905, he was awarded the Nobel Prize in medicine. When bacteria were discovered in the 19th, no antibacterial treatment existed. In the early 20th century, Paul Ehrlich created a treatment to treat syphilis before the use of penicillin as bacterial treatment proposed by Ernest Duchesne in 1897 and studied by Alexander Fleming in 1929 [5]. During the 20th century, several antibiotics were developed. In 1940 the first antibiotic-resistant bacteria have been identified [6].

1.1 Bacteria

Bacteria are unicellular and prokaryotic (without a nucleus) organisms living in all environments. Most bacteria have a carbohydrate cell wall named the peptidoglycan. The larger bacteria are between $0.1\mu\text{m}$ and $2\mu\text{m}$ [7]. Bacteria have many forms: spherical (hulls), or elongate rods (bacilli) and spiral. Currently, there are about 7300 known species [8]. The estimated number of species varies between 5 and 10 million [9, 10]. Bacteria are ubiquitous and present in all types of habitats and can be isolated from many places (e.g.:

fresh, brackish, soil, or marine water, air, ocean depths, radioactive wastes [11], earth's crust, skin and in the intestines of animals and humans). For example, 1ml of water and 1g of soil contains respectively 1 and 40 millions of bacteria cells. In the world, it has been estimated that there are 4 to 6 thousand billion of a billion of a billion (4×10^{30} to 6×10^{30}) bacteria, representing a huge proportion of the entire biomass [12]. However, a significant portion of these bacteria still needs to be characterized. Unfortunately, many of them are non-cultivable in laboratories [13].

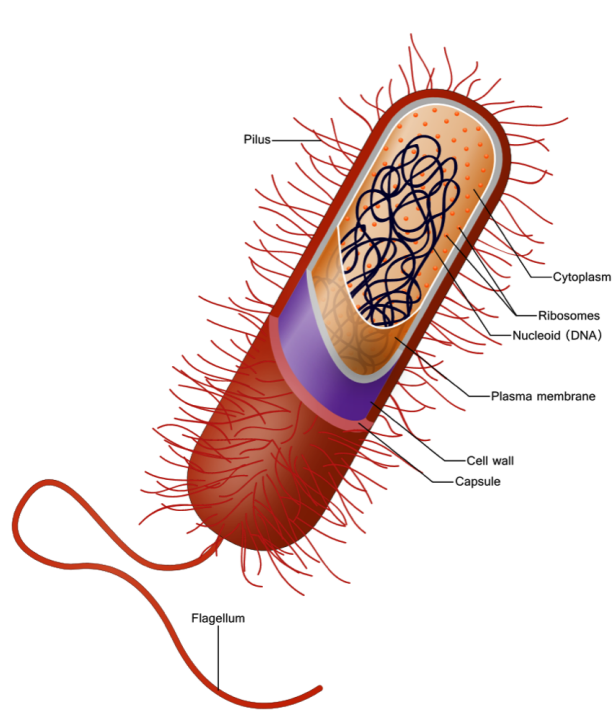
The human skin, mouth, and intestine are approximately colonized by 10^{12} , 10^{10} , 10^{14} bacteria, respectively. There are as much human cells as bacteria in the human body [14]. The majority of these bacteria are harmless and even beneficial to the body. However, there are several pathogenic species responsible for causing infectious disease (e.g., tuberculosis, syphilis, anthrax). Each year, tuberculosis kills about 2 million people mostly in sub-Saharan Africa: respiratory infection is the primary fatal bacterial disease. Some bacteria are responsible for intestinal disorders while others can cause wounds or respiratory infections. Antibiotics can be used to treat bacterial infections, inhibiting their vital functions.

Bacteria are characterized by an absence of a nucleus and other organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus and chloroplasts (Figure 1.1) [15]. They are relatively simple prokaryotic (coreless body) cells. The cell wall is a major component of the bacteria. The bacteria can be distinguished by the nature of their cell walls; Gram-positive or Gram-negative. This difference is based on the chemical composition and structure of the cell wall by highlighting the Gram stain (Figure 1.2). Walls of the Gram-positive bacteria have a peptidoglycan containing teichoic acids and thick. Gram-negative bacteria walls have a thin peptidoglycan localized in the periplasm between the cytoplasmic membrane and an outer cell membrane. The composition of the cell wall determines the bacterial form and rigidity. However, it exists bacteria without a wall: the mycoplasma.

1.2 *Staphylococcus aureus*

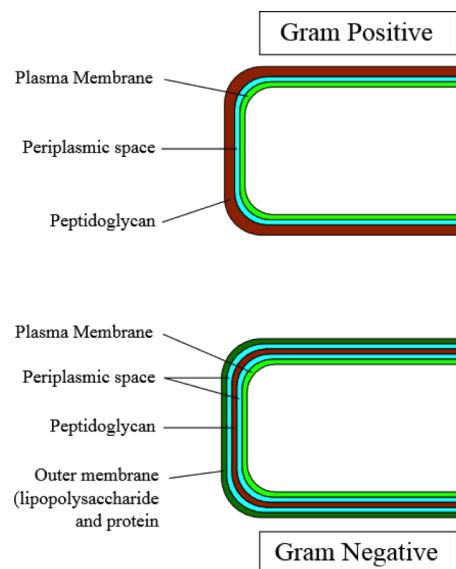
The surgeon Sir Alexander Ogston made the first identification of the bacteria "*Staphylococcus*" in 1880 in Aberdeen, United Kingdom. He discovered the microbe during a surgery in the pus of a knee joint abscess. In 1884, the name "*Staphylococcus aureus*" (*S. aureus*) was given by Rosenbach according to the official system of nomenclature at the time. *S. aureus* belongs to the phylum of Firmicutes, the class of bacilli, the order of Bacilliales and the family of Staphylococcaceae (Figure 1.3). *S. aureus* observed by microscope appears in the form of a cluster, like grapes. The colonies are large, round, golden-yellow (Figure 1.4) and when they are grown on blood agar plate, there is hemolysis (Figure 1.5) [16]. The reproduction of *S. aureus* is asexual and occurs by binary fission. After fission, the two daughter cells remain attached, explaining why the bacteria are in clusters. *S. aureus* is a Gram-positive coccal bacterium, facultative anaerobic. The particularity of a facultative

Figure 1.1: A diagram of a typical prokaryotic bacteria cell.



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Figure 1.2: Gram-positive and -negative cell wall structure.

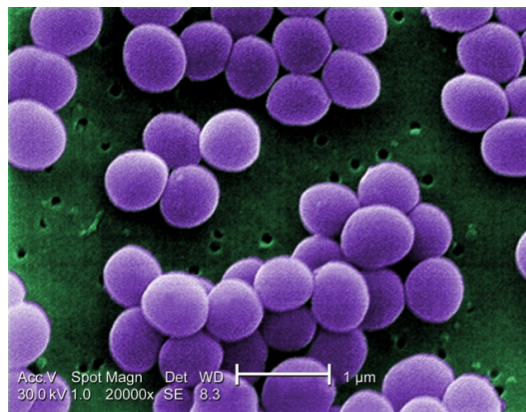


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Figure 1.3: Scientific classification of *Staphylococcus aureus*.

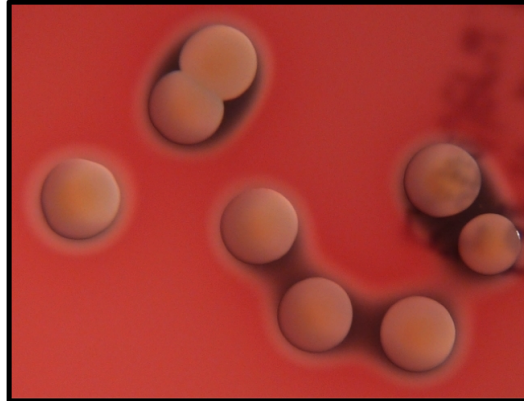
<u>Staphylococcus aureus</u>
Domain: Bacteria
Kingdom: Eubacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Staphylococcaceae
Genus: Staphylococcus
Species: <i>S. aureus</i>

Figure 1.4: Scanning electron micrograph of *S. aureus* (false color added).



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Figure 1.5: Yellow colonies of *S. aureus* on a blood agar plate (note regions of clearing around colonies caused by lysis of red cells in the agar; beta hemolysis).



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anaerobe is the capacity to convert ATP by aerobic respiration in the presence of oxygen and to switch for anaerobic respiration (fermentation) without oxygen [16]. *S. aureus* is productive of catalase enzyme. The catalase enzyme transforms hydrogen peroxide (H_2O_2) to oxygen and water. Catalase-activity is used to distinguish staphylococci from streptococci and enterococci. Previously, the coagulase test was used to do this distinction, but it appears that all *S. aureus* strains are not coagulase-positive [16]. *S. aureus* is at the same time a frequent colonizer of human beings without causing any harm, but also a pathogenic bacterium that can cause infection in humans.

In humans, it is frequently found on the skin and respiratory tract. About 30% of the humans are healthy carriers of *S. aureus* [17], which can be found as a commensal bacteria of the skin and in anterior nares [18, 19].

1.3 Penicillins

Penicillins are beta-lactam (β -lactam) antibiotics. It is a toxin synthesized by certain species of fungi of the genus *Penicillium* family and is harmless to humans. They were not introduced to therapies until 1943, thirteen years after the discovery of penicillin G by Alexander Fleming. In 1940, a British research team, which included the physician and pharmacologist Australian Howard Florey, the German-born chemist Ernst Chain and Norman Heatley British biologist discovered how to produce enough penicillin to kill bacteria that infect a vivand body [18]. Penicillins and other β -lactam antibiotics act by inhibiting the formation of cross-links peptidoglycan in the bacterial cell wall. The β -lactam penicillin binds to (and thereby inactive) a transpeptidase enzyme which should bind together the

Figure 1.6: Penicillin core structure.

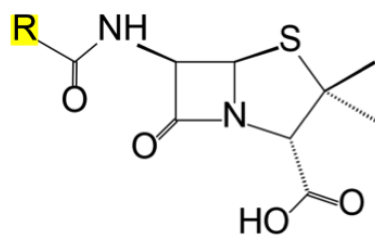
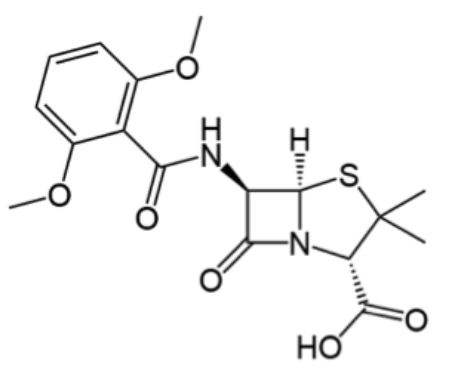


Figure 1.7: Methicillin core structure.



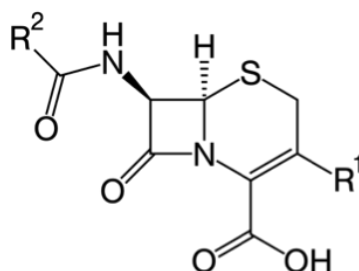
peptidoglycan molecules of the bacterial wall, creating penicillin inhibited wall synthesis which prevents the multiplication of bacteria. Penicillins are used in the treatment of bacterial infections, mainly against Gram-positive bacteria. Penicillins are active on bacteria multiplication and not in the resting phase.

However, certain bacterial strains or species develop resistance to penicillin by hydrolyzing the β -lactam ring (at the amide bond) (Figure 1.6) with a β -lactamase, which causes the inactivation of the antibiotic. To counter this, pharmaceutical industry developed an inhibitor of this enzyme, clavulanic acid. Thus, penicillin combined with clavulanic acid can see its spectrum of antibacterial activity restored or expanded (see co-amoxiclav, coticarclav). These new penicillins are methicillin, oxacillin, and cloxacillin; they all have the same spectrum of action.

1.4 Penicillinase-resistant penicillins

Discovered at Beecham Research laboratories, methicillin is an antibiotic. It was introduced in 1959 and is the first penicillinase-resistant semi-synthetic penicillin derived from penicillin nucleus, 6-aminopenicillanic acid (6-APA) (Figure 1.7) [20]. The methicillin prevents peptidoglycan synthesis by inhibiting the transpeptidase enzymes (PBP1a, 1b,

Figure 1.8: Cephalosporin core structure.



and 2). This process is similar to penicillin G against Gram-positive bacteria and some Gram-negative cocci (meningococci and gonococci). The activity is stable and active when the staphylococcal β -lactamase is present. It is then active against penicillin G-resistant (penicillinase-producing) and penicillin-susceptible staphylococci, but not against Gram-negative cocci (meningococci and gonococci). It has also very limited activity against Streptococci. Therefore, it cannot be used for treatment of severe streptococcal infections like [21].

The use of methicillin was wide but it appears that the drug was toxic, so it was replaced by other penicillinase-resistant penicillins such as cloxacillin, oxacillin, nafcillin, flucloxacillin and dicloxacillin. Currently, methicillin is no longer used for humans.

1.5 Cephalosporins

Cephalosporins are β -lactam antibiotics; they derive from fungi named *Acremonium* known as *Cephalosporium*. They constitute with cephamycins the subgroup of β -lactam antibiotics named cepheems. There are different generations of cephalosporins with different properties. The first one (1st) has activity against Gram-positive bacteria. The second and following generations (2nd, 3rd, 4th, 5th, 6th generation) have increased activity against Gram-negative bacteria. In most cases, the new generations with significantly greater Gram-negative antimicrobial activity, but they have a decreased activity against Gram-positive organisms. The different properties are due to a modification of the cephalosporin nucleus (Figure 1.8). However, the 4th generation of cephalosporins has right broad-spectrum activity. *Listeria*, atypical (including *Mycoplasma* and *Chlamydia*), MRSA, and enterococci are called "LAME" because these organisms are not sensible to cephalosporins, although, the 5th generation of cephalosporin has activity against MRSA. The resistance to cephalosporin antibiotics is due to the acquisition of a supplementary β -lactam-insensitive PBP or a reduced affinity of existing PBP components.

1.6 Methicillin-resistant *Staphylococcus aureus*

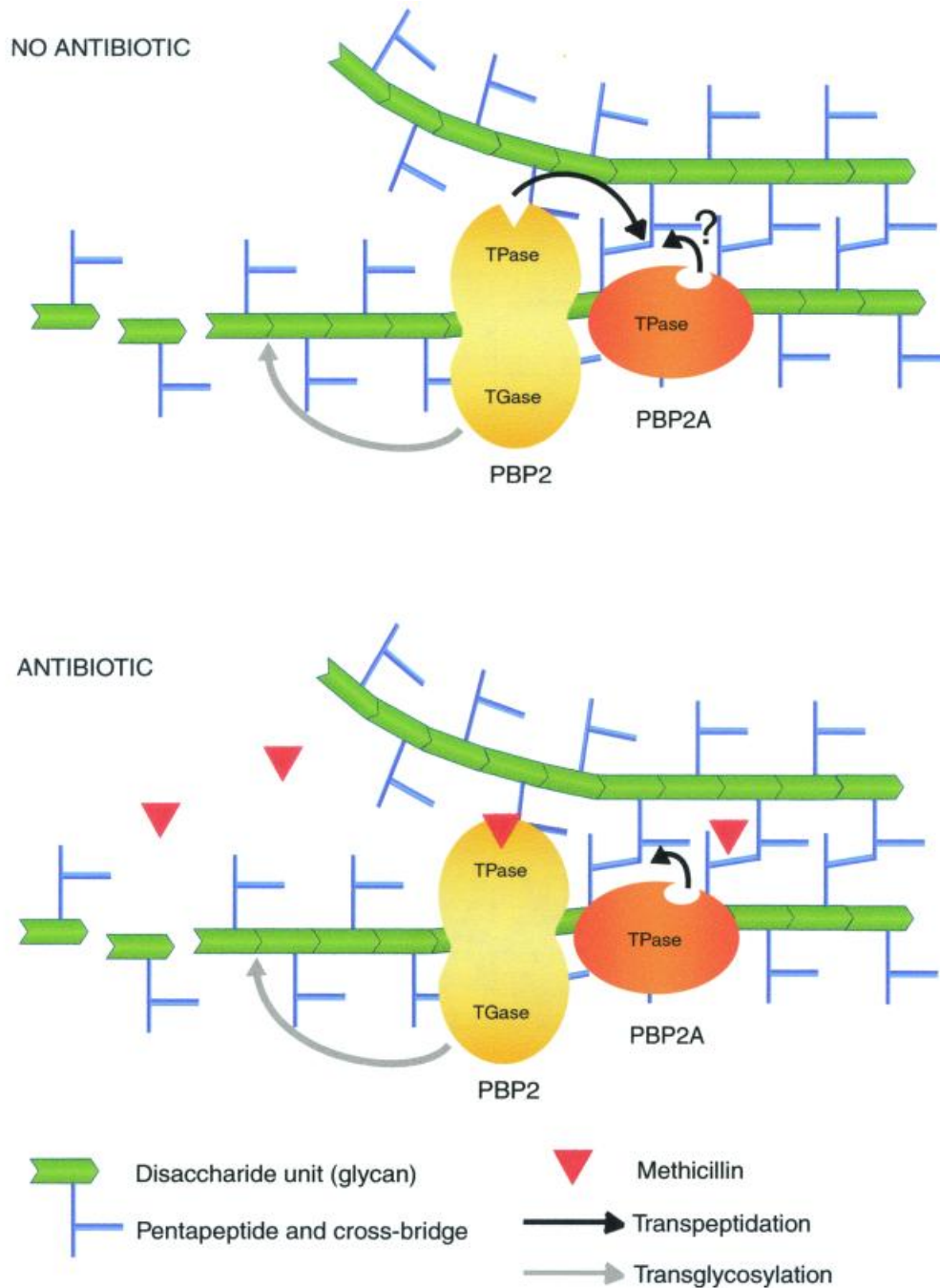
Methicillin-resistant *Staphylococcus aureus* (MRSA) is any strain of *S. aureus* that has developed resistance against the β -lactam antibiotics (including the penicillins), the penicillinase-resistant penicillins (methicillin, oxacillin, dicloxacillin) and the cephalosporins. This resistance emerged by natural selection. All the strains of *S. aureus* unable to resist against these antibiotics are called methicillin-sensitive *S. aureus* (MSSA). Although MRSA has developed such resistance only recently, they are not intrinsically more virulent than MSSA. The major consequence of this resistance is hard to treat infections caused by MRSA using standard antibiotics. Consequently, this type of infection causes therapeutic challenges. The first description of the MRSA was made in 1961 in the UK [22].

MRSA is commonly found in hospitals, long-term care and nursing homes. In these places, the patients presenting invasive devices and immunosuppression have a high risk to develop nosocomial infection due to MRSA. Although MRSA is mostly a hospital bacterium, they appear now more commonly in the community. Both the terms HA-MRSA (healthcare-associated MRSA) and CA-MRSA (community-associated MRSA) characterize this distinction. The emergence of MRSA is a worldwide problem in clinical medicine.

1.6.1 Resistance factors

S. aureus resistance to penicillin is due to a bacterial enzyme; penicillinase. The penicillinase inactivates the antibiotics, cleaving the β -lactam ring of the penicillin molecule. Penicillinase-resistant β -lactam antibiotics (methicillin, cloxacillin, oxacillin, dicloxacillin and flucloxacillin) can resist to the degradation by staphylococcal penicillinase. The resistance to penicillinase-resistant penicillins is mediated by the *mec* operon, a part of the staphylococcal cassette chromosome *mec* (SCC*mec*). SCC*mec* is a mobile element, with mainly five different types designated SCC*mec* I-V. Each SCC*mec* encodes specific *ccr* genes for recombinase enzymes. These enzymes allow the excision and integration of SCC*mec* [23]. SCC*mec* I, IV, and V code for resistance methicillin genes only, whereas SCC*mec* II and III code for plasmids and transposons for resistance to multiple antibiotics [24]. In *S. aureus*, there are four penicillin-binding proteins (PBP), named PBP1, PBP2, PBP3, and PBP4. *MecA* gene codes for an altered PBP called PBP2a or PBP2'. It performs the functions of PBP2 and has a lower affinity for binding β -lactams such as penicillinase-resistant penicillins, cephalosporin, and carbapenem, leading to resistance for all β -lactams and the impossibility to use them against MRSA infection [24]. It has been demonstrated that PBP2 is a cell wall and bifunctional enzyme. It implied both transpeptidation and transglycosylation which are important for linking and elongation of the glycan backbone in peptidoglycan [25]. PBP2 is the main enzyme implied in the peptidoglycan synthesis including peptidoglycan cross-linking, while the role of PBP1 is only during cell division (Figure 1.9) [26]. A couple of other factors are involved in methicillin resistance in the presence of PBP2a [27, 28]. Two of these factors are *mecI* (repressor) and *mecRI* (transmembrane β -lactam signal transducer) which switches off *mecI* in the

Figure 1.9: Model for the cooperative fonctionnement of the TGase domain of PBP2 and the TPase activity.



In the absence of antibiotic, it is assumed that both the TPase and TGase domains of PBP2 participate in the biosynthesis of staphylococcal peptidoglycan. Whether or not the TPase activity of PBP2A (present in methicillin-resistant strains) also functions in the cross-linking of the peptidoglycan in the absence of antibiotic in the medium is not clear at present. (Lower) When antibiotic is added to the medium, the TPase domain of PBP2 is acylated and is no longer capable of performing its peptide cross linking activity. However, the observations described in this paper demonstrate that the penicillin-insensitive TGase domain of PBP2 remains functional and cooperates with the TPase activity of the acquired PBP2A and is essential for cell-wall synthesis and bacterial growth in the presence of β -lactam antibiotics in the surrounding medium.

"Pinho MG, de Lencastre H, Tomasz A. An acquired and a native PBP cooperate in building the cell wall of drug-resistant staphylococci. *Proc Natl Acad Sci U S A*. 2001 Sep 11;98(19):10886-91."

also resistant to all penicillins, carbapenems and currently marketed cephalosporins. These observations have been confirmed by extensive clinical experiments in some serious MRSA infections, where MRSA do not respond to any of the cephalosporins or penicillinase-resistant penicillins [34–38]. However, two new cephalosporins, ceftaroline, and ceftobiprole, are active in vitro and in vivo against MRSA.

1.6.2 Toxins and mobile elements

Depending on the strain, *S. aureus* can secrete several exotoxins. Many of these toxins are associated with specific diseases. By producing potent toxins and expressing cell-surface proteins, disease-associated strains bind and inactivate antibodies, promoting infections.

1.6.2.1 Panton-Valentine Leukocidin (Exotoxins)

Panton-Valentine leukocidin (PVL) cytotoxin is part of the β -pore-forming toxins family. PVL was discovered in 1894 by Van deVelde and has the capacity to lyse leukocytes. Later, in 1932, Sir Philip Noel Panton and Francis Valentine named the toxin after they made the link with skin and soft tissue infection (SSTI) [39].

In infected cells, PVL, a member of the synergohymenotropic family, creates pores in the membranes [40]. It is a bicomponent toxin encoded by a gene from a material of a bacteriophage [41]. This gene codes for two proteins named LukF-PV and LukS-PV (respectively 33 and 34 KDa). They are both subunits, which are assembled in the membrane of host defense cells like monocytes, white blood cells and macrophages [42]. A ring with a central pore is formed by both subunits which act as super-antigen allowing leakage of the cell contents [40, 43]

An increased virulence of certain strains of MRSA is associated with the presence of PVL. The toxin has been found in a majority of CA-MRSA studied and is often the cause of some skin or mucosa necrotic lesions and necrotic hemorrhagic pneumonia [41, 44]. According to epidemiological data, there is evidence for a link between strains harboring PVL and necrotizing pneumonia, deep vein thrombosis, large multiple subcutaneous abscesses and necrotizing fasciitis [39, 45, 46].

In community-acquired staphylococcal necrotizing pneumonia, 85% were PVL-positive whereas none of the hospital-acquired pneumonia was. Necrotizing pneumonia is an aggressive condition that affects younger and healthier patients often leading to the death of patients (>40%) within 72 hours [40].

1.6.2.2 Arginine catabolic mobile element

The arginine catabolic mobile element (ACME) is a mobile genetic element, identified in coagulase negative staphylococci and MRSA with a relatively high prevalence in *Staphylococcus haemolyticus* and *Staphylococcus epidermidis* (*S. epidermidis*) [47, 48]. Recently identified in MRSA (but not common in MSSA) [49], ACME has been found in the USA300 clone [50, 51], the predominant CA-MRSA clone in the United States (ST8). It was also retrieved with a low frequency in some non-ST8 clones [49]. The ACME locus contributing to arginine catabolism is involved in the mechanisms allowing skin infection; some enzymatic activity (arginine deiminase) promotes the survival of MRSA in the skin environment showing acidic pH. In addition the resistance to polyamines also conditioned by the ACME locus is prompted by genes composing the locus.

ACME is a staphylococcal genetic element which increases fitness and capacity of *Staphylococcus* to colonize the mucous and skin membrane [49], and also provides immune modulating functions, for example, resistance to polyamines. The polyamines have a role in non-specific immune response on intact skin and during the inflammatory response of a wound healing [52, 53]. ACME is composed of an ARC (arginine deiminase gene cluster) allowing the survival of bacteria in the acidic skin environment [52]. Moreover, speG, a polyamine-tolerance gene combined with ACME, enhances the resistance to polyamines produced by the human skin [54].

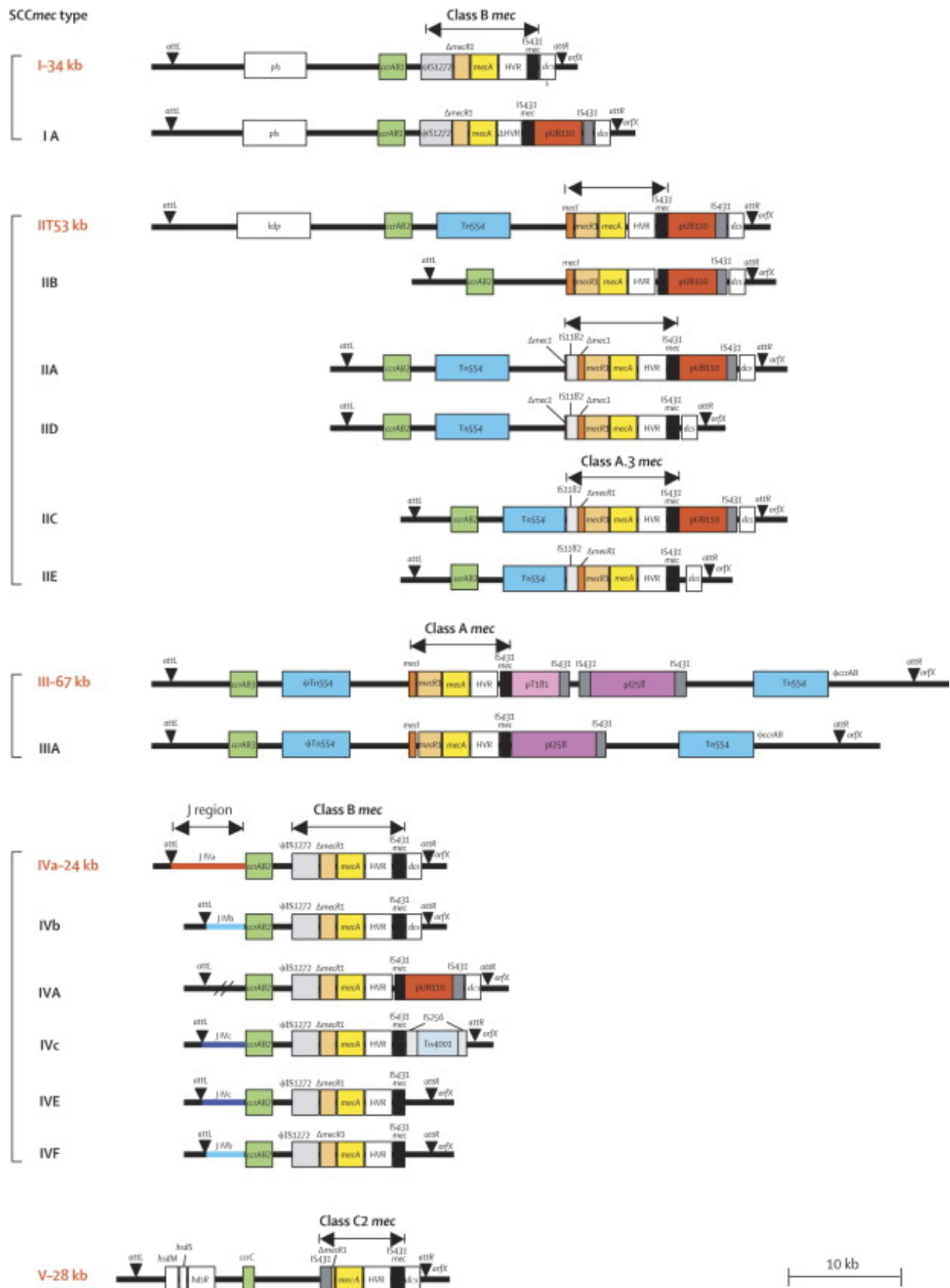
ACME has been located downstream of the SCC*mec* gene [51]. It seems to be assembled in *S. epidermidis* into the spe-G positive, ACME has been transferred in virulent USA300 clone, during the evolution [50, 54]. This transfer seems to happen because of the ccr gene (recombinase) complex which excises and inserted ACME in SCC*mec* among Staphylococci. ACME has enabled a substantial augmentation of MRSA's adaptability to spread and host rapidly person-to-person. It is particularly visible in the CA-MRSA USA300 clone [50, 51].

1.6.3 MRSA strains

Acquisition of SCC*mec* in MSSA gave rise to genetically different MRSA lineages. These variations may explain the variability in associated infections and the virulence of MRSA [55]. ST250-MRSA is the first strain of MRSA from ST250-MSSA and SCC integration *mec* [55]. There are 11 SCC*mec* structural types identified, with a size of 20 to 60kb, which are defined according to their combination of the *mecA* gene and the genetic proximity with ccr allotypes: ccr is a locus encoding for a recombinase involve in the mobility of this chromosomal island.

Five types of SCC*mec* have been described with a couple of variants [56–60]. They are identified according to the combination of *mec* and ccr gene complexes that they contain (Figure 1.11). Besides the differences between SCC*mec* and the ccr gene, there is also a difference for the various SCC*mec* elements. They differ from each other in their

Figure 1.11: Genomic organization of SCCmec types I-V and variants.



"Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. *Lancet*. 2006 Sep 2;368(9538):874-85."

antibiograms. They present different antibiotic resistance genes against none β -lactam antibiotics they carry. In regards to the SCC $_{mec}$ type I, IV and V, they only have the antibiotic resistance gene *mecA* (except for the variant Ia, IVa, and IVc). The cassette SCC $_{mec}$ types II and III contain other antibiotics and heavy metal resistance genes integrated into transposons or plasmids [60].

1.6.3.1 MRSA typing methods

There are several methods to characterize MRSA strains. For example PFGE (Pulsed-field gel electrophoresis), the *S. aureus* protein A (SPA) typing and multilocus sequence typing (MLST), in decreasing order of discriminative power. It means that many SPA types can belong to the same sequence type (ST). Furthermore, closely related ST can be organized into so-called clonal complexes (CC). Each CC has an ST as founder and many STs as members.

PFGE is a genetic typing method used to study the genetic diversity of MRSA. The genome of MRSA (~ 2.8 Mb) is digested with an enzyme and then separated by an agarose gel electrophoresis. A comparison of the banding pattern is made with software to assign the specific strain of the bacteria analyzed.

Another technique for identifying MRSA is SPA typing. It is a technique in which the genetic sequence of the *S. aureus*-specific SPA is used. The polymorphic repeated of the SPA gene is identified to attribute it to a unique code of the strain. The succession of a specific strain determines its SPA type. This technique has been widely used in routine for sub-typing of MRSA in the hospital setting. Due to its labour-intensity and introduction of novel, more rapid genotyping methods, it has been abandoned in many references and research laboratories

The characterization of bacterial isolates can be made by the sequences of 450 base-pairs (bp) internal fragments of seven house-keeping genes (Table 1.1). It is the MLST technique, which is useful in studying the relatedness and secular trends of MRSA strains [61].

Primers are designed using the sequences of the highly conserved regions that flank the most variable regions. Each primer pair amplifies an internal fragment of the housekeeping gene and allows an accurate sequencing of fragments of each gene on both strands [62]. Each gene fragment is defined according to their distinct alleles (different sequences of the gene), each bacterial isolate is specified by the alleles of the seven housekeeping loci, normally the ST. The seven housekeeping genes have many alleles; strains are highly unlikely to have identical allelic profiles by chance. Some isolates with the same allelic profile for these genes can be defined as members of the same clone [61, 63].

Table 1.1: Sequence variation at the seven loci.

Gene	Sequence length (bp)	No. of alleles	No. of polymorphic sites
arcC : carbamate kinase	456	17	19
aroE : shikimate dehydrogenase	456	17	23
glpF : glycerol kinase	465	11	14
gmk : guanylate kinase	429	11	13
pta : phosphate acetyltransferase	474	15	18
tpi : triosephosphate isomerase	402	14	18
yqiL : acetyl coenzyme A acetyltransferase	516	16	19

"Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. *J Clin Microbiol.* 2000 Mar;38(3):1008-15."

Usually, information on SCC mec type and MLST are combined to describe a MRSA clone (e.g. ST8-MRSA IV means that the ST of MRSA is 8 and the clone contains the SCC mec type IV element). Although a specific ST can be identified as CC, because they are closely related the clone differs amongst each other by only a couple of alleles in house-keeping genes (1 or 2 alleles). Typing information can lead to a better understanding of the spread of a clone across the world, its evolution and its emergence [64].

1.6.3.2 HA-MRSA

For some time the emergence of MRSA has been considered as originally from the spread of a single clone between hospitals. Recent epidemiological and molecular data have shown us that since the first MRSA detected in 1961 in the UK many clones have emerged. Typically, HA-MRSA strains are multi-resistant and are the consequence of selective pressure due to a high usage of antibiotics to which they are resistant. HA-MRSA can be a real cause of infection in patients who have medical interventions such as mechanical intervention, major surgery or invasive devices. In some cases, patients have an asymptomatic carriage of MRSA (see 1.8 MRSA colonization).

Currently, only five CC and 11 primary clones have been identified as responsible for HA-MRSA and spread worldwide: in CC5, ST5-MRSA-II, and ST228-MRSA-I; CC8, ST8-MRSA-II, ST239-MRSA-III, ST247-MRSA-I, and ST250-MRSA-I; in CC30, ST36-MRSA-II; in CC22, ST22-MRSA-IV; and in CC45, ST45-MRSA-IV [64]. Different clones can be attributed to a different place in the world. For example, ST239-MRSA-III or the closely related ST241-MRSA-III are predominant in Western Pacific Australia and Asia, except in Korea and Japan, where the dominant HA-MRSA clone is ST5-MRSA-II [65]. HA-MRSA strains are now present in most countries in the world to a low or high prevalence [66].

1.6.3.3 CA-MRSA

In the 1990s, MRSA strains completely unassociated with hospital acquisition were observed in different places of the world. CA-MRSA appear to have emerged independently

of HA-MRSA and each other [67]. The first observation of CA-MRSA has been made in Western Australia in remote communities in the 1990s, a couple of distinct clones appeared in several parts of the world like in the USA, Australia or Europe [68]. Molecular typing of CA-MRSA and genome comparison between HA-MRSA and CA-MRSA strains showed that new MRSA strains integrated the *SCC_{mec}* cassette into an MSSA strain separately on its own [69]. A majority of CA-MRSA carry the *SCC_{mec}* IV or V and are PVL positive. Over time, genetics analysis revealed that for CA-MRSA, PVL-negative strains have emerged several times on different continents [45]. In the 2000s, CA-MRSA strains have been introduced into the health care system with epidemiological and clinical features distinguished from the HA-MRSA. CA-MRSA is known to be more virulent and easier to treat than HA-MRSA [67]. CA-MRSA has known to be non-multiresistant and susceptible to several classes of antibiotics other than β -lactams. They are responsible for many skin and SSTI, including furunculosis in young people [46, 70–72]. CA-MRSA could also be responsible for severe infections like necrotizing pneumonia, bacteremia and rare cases of infectious endocarditis [73]. The carriers of CA-MRSA are relatively young compared to HA-MRSA carriers. Many outbreaks of CA-MRSA were observed in isolated populations, prisoners, military recruits, sports teams and among sexually active homosexual men [74].

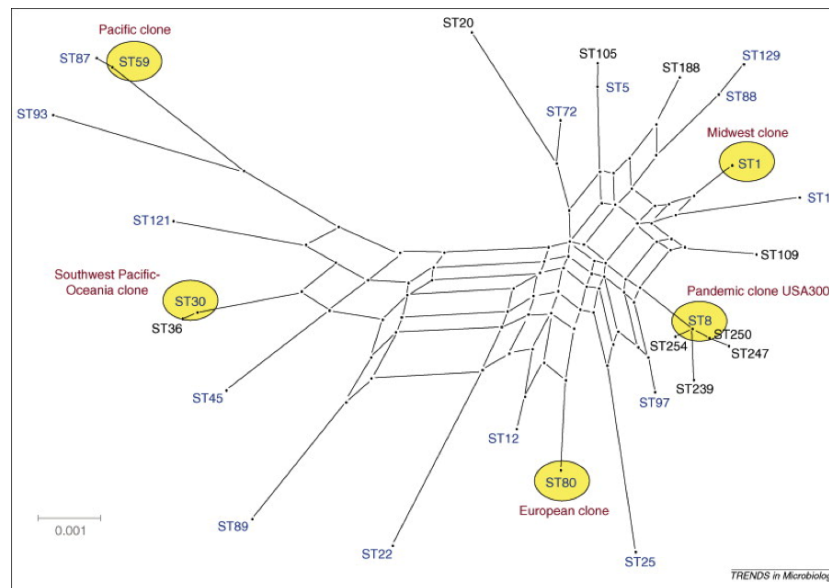
All around the world, there are five main CA-MRSA strains identified all. Being *SCC_{mec}* IV and PVL positive (Figure 1.12) The first CA-MRSA highlighted is the strain commonly named *the Midwest clone*. It was named like this due to the region in the USA where it emerged [75,76]. However, this clonal lineage was prevalent earlier in some communities in Western Australia [68]. MLST analysis has identified this strain as an ST1 clonal lineage [68]. The second CA-MRSA strain is the ST30 clonal lineage. It was implicated in an outbreak in Mexico, Australia, Greece and the USA and known as the Southwest Pacific/Oceania clone [77–81]. The third CA-MRSA clone is the ST80 clonal lineage, it has been identified in many European communities and is known as the European clone [45]. The fourth CA-MRSA strain is the Pacific clone, which is resistant to many non- β -lactam antibiotics. It is called ST59 and is present in places like Taiwan, Vietnam and in the USA [80, 82, 83]. The last CA-MRSA clone identified to be spread worldwide is the ST8 clonal lineage. It is better known as USA300 and USA400 strains due to its unique PFGE profile [83].

1.6.4 MRSA epidemiology

1.6.4.1 In the world

MRSA are present around the world. However, the prevalence is heterogeneous between countries. In some places of the world, there was a very high prevalence of MRSA among *S. aureus* in clinical cultures with up to greater than 50% in Colombia, Hong Kong, Singapore, Malta, Romania and in Irak in 1998 (Figure 1.13). A high prevalence was present all around the world, in America (USA, Brazil, and Argentina), Europe (France, UK, Spain, Portugal, Italia, Greece, Slovenia, and Croatia), Asia (Turkey), Africa (Algeria, Tunisia,

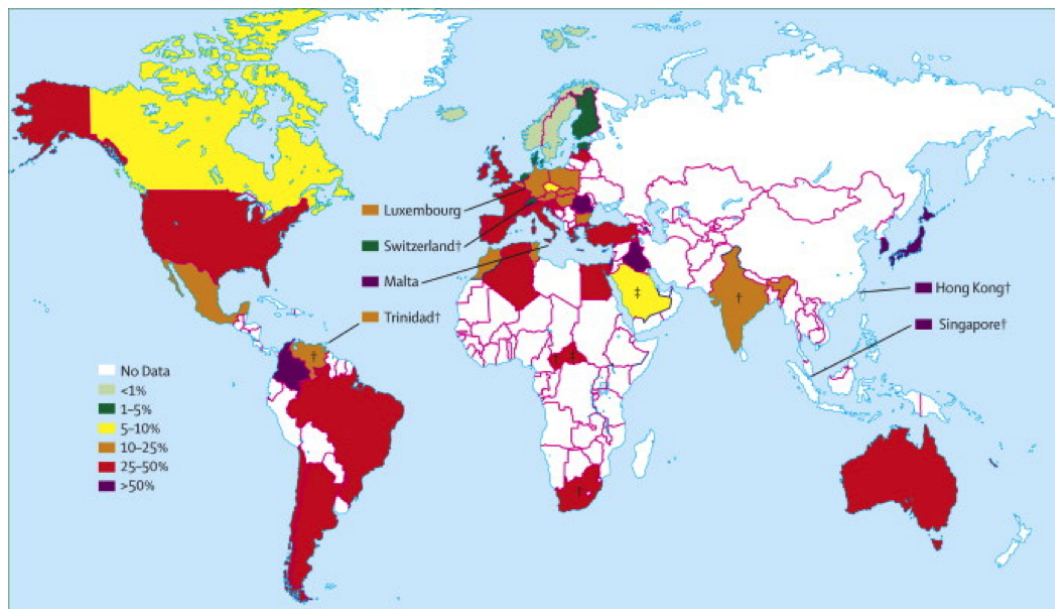
Figure 1.12: Worldwide CA-MRSA clonal lineage.



Genetic diversity among *S. aureus* strains that have acquired type IV SCC_{mec} independently (blue). The five predominant CA-MRSA clones (red) all have the *lukPV* gene operon encoding PVL. The graph was constructed with Splitstree (version 4.8) using concatenated sequences of seven housekeeping gene fragments used in MLST for *S. aureus*. The scale bar represents the evolutionary distance (number of substitutions per nucleotide position).

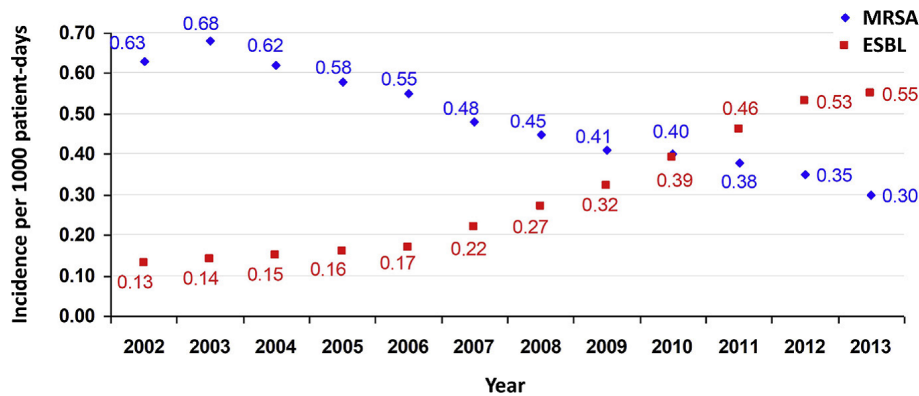
"The role of virulence determinants in CA-fMRSA pathogenesis. Diep BA, Otto M. *Trends Microbiol.* 2008 Aug;16(8):361-9."

Figure 1.13: Worldwide prevalence of MRSA by countries.



"Grundmann H, Aires-de-Sousa M, Boyce J et al. Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. *Lancet* 2006 Sep 2; 368(9538): 874-85."

Figure 1.14: French National Surveillance data showing changes in the incidence density of MRSA and ESBL (2002-2013).



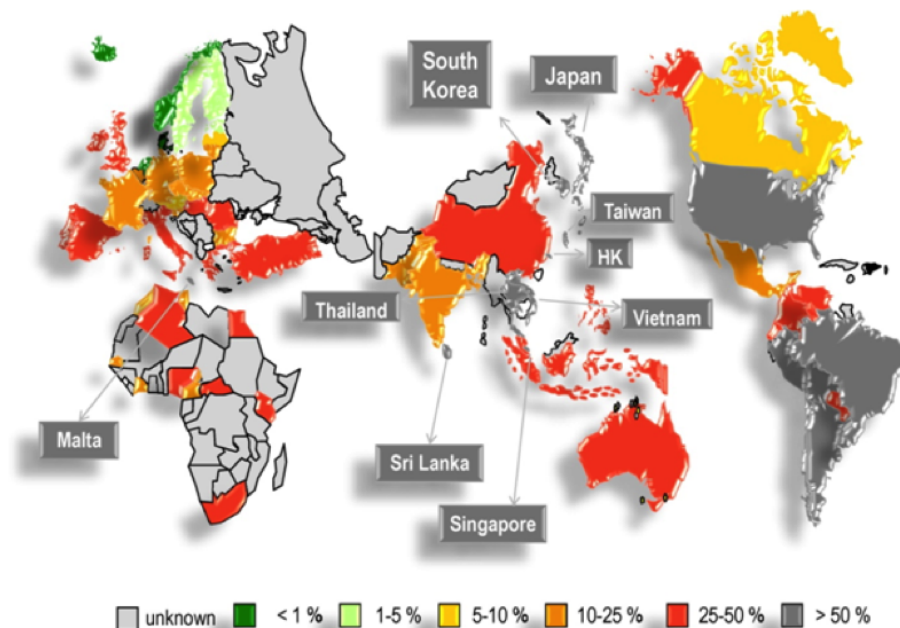
"<http://www.invs.sante.fr/Publications-et-outils/Rapports-et-syntheses/Maladies-infectieuses/2015/Surveillance-desbacteries-multiresistantes-dans-les-etablissements-de-sante-en-France.>" - 05/30/2016

South Africa, and the Central African Republic) and Oceania (Australia). However, there are also some places in the world with low prevalence rates (<5%) like in Finland, Denmark, the Netherlands, and German-speaking Switzerland (except for Geneva city). The dissemination of HA-MRSA took place in the 1960s, CA-MRSA in the 1990s and livestock-associated MRSA (LA-MRSA) since the 2000s [67, 84, 85]. However, since the beginning of the 2000s, the prevalence of MRSA in the world was begun his decrease until now. For example, in France, this decline seemed to follow the introduction gradually between 1993 and 2004 of a national control program (Figure 1.14).

In the United States, no data are recently available. MRSA increased from 1998, with in 2005 about 53% of *S. aureus* clinical isolates [86]. Since 2005, adjusted national estimated healthcare-associated infection (HAI) incidence rates have decreased, with healthcare-associated hospital-onset infections decreasing by 54% [87]. Again, a series of infection control interventions during this period may have contributed to declining MRSA. A large observational study describes an example of such interventions [88]. In 2007, Veterans Affairs Hospitals throughout the United States introduced a multimodal strategy involving MRSA screening, contact precautions, hand hygiene promotion, and institutional culture change [88, 89]. This program was associated with a reduction in MRSA by 62% (P-value <0.001 for trend) in intensive care units (ICU) and 45% (P-value <0.001 for trend) in non-ICU area.

In 2005, two separate teams in France and the Netherlands announced the discovery of a strain associated with pig farming. The strain belongs to the ST398: appearing to be responsible for LA-MRSA infections, and known to colonize calves and pigs persistently [90, 91]. There exist some cases of human LA-MRSA carriers with few described cases of endocarditis, pneumonia and necrotizing fasciitis [90, 91].

Figure 1.15: Worldwide prevalence of HA-MRSA in 2000.

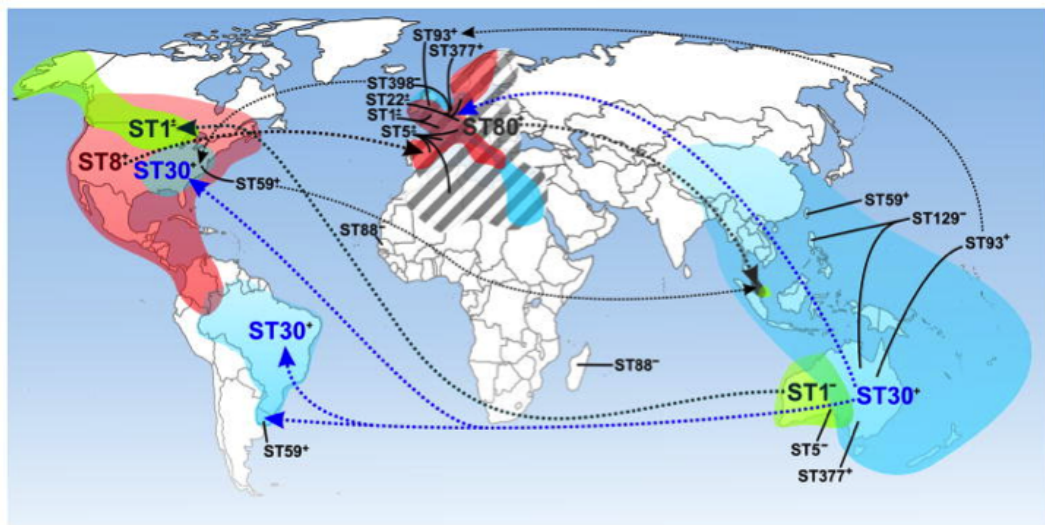


"<http://www.handinscan.com/global-epidemiology-of-mrsa/>" - 05/31/2016

HA-MRSA In the world, there is a high prevalence of HA-MRSA (Figure 1.15). It is tough to have real epidemiological data from different studies due to the difference in the population sample and due to the fact the studies conducted are often not comparable. However, the available data clearly show a high prevalence (>50%) of HA-MRSA in North and South America, Malta and Asia (especially in Sri Lanka (86.5%), South Korea (77.6%), Vietnam (74.1%), Taiwan (65.0%), Thailand (57.0%) and Hong Kong (56.8%), whereas the prevalence is lower in the Philippines (38.1%) and India (22.6%); intermediate rates (25-50%) in Australia, Africa, and some European countries [e.g. Portugal (49%), Greece (40%), Italy (37%) and Romania (34%)]. Except for few places, other European countries have low MRSA prevalence rates (e.g. Scandinavia and The Netherland) (Figure 1.14) [92–95].

CA-MRSA The Figure 1.16 represents the distribution of the CA-MRSA clones around the world. We focus on the USA300 strain. It appears in the 2000s and is currently pandemic in some places in the USA (38 US states), Canada, in several European countries and South America [80]. This epidemic clone is usually responsible for human diseases including pneumonia, endocarditis, sepsis, skin infections, toxic shock syndrome (TSS) and necrotizing fasciitis [96–98]. The introduction of USA300 strain in new geographic areas was associated with a displacement of local CA-MRSA strains like ST1, ST59 and

Figure 1.16: Global distribution of CA-MRSA as indicated by multilocus sequence type.



Dotted lines indicated the possible route of dissemination for the indicated clones. Major CA-MRSA clones are indicated by larger font and color. Colored regions are an estimate of the area in which infections have been reported for the indicated clone (not all are shown). ST1, green; ST8, red; ST30, blue; ST80, gray hatched. +, PVL-positive; -, PVL-negative; ±, the combination of PVL-positive and PVL-negative strains isolated from the region.

"DeLeo FR, Otto M, Kreiswirth BN et al. Community-associated methicillin-resistant *Staphylococcus aureus*. *Lancet* 2010; 375: 1557-68."

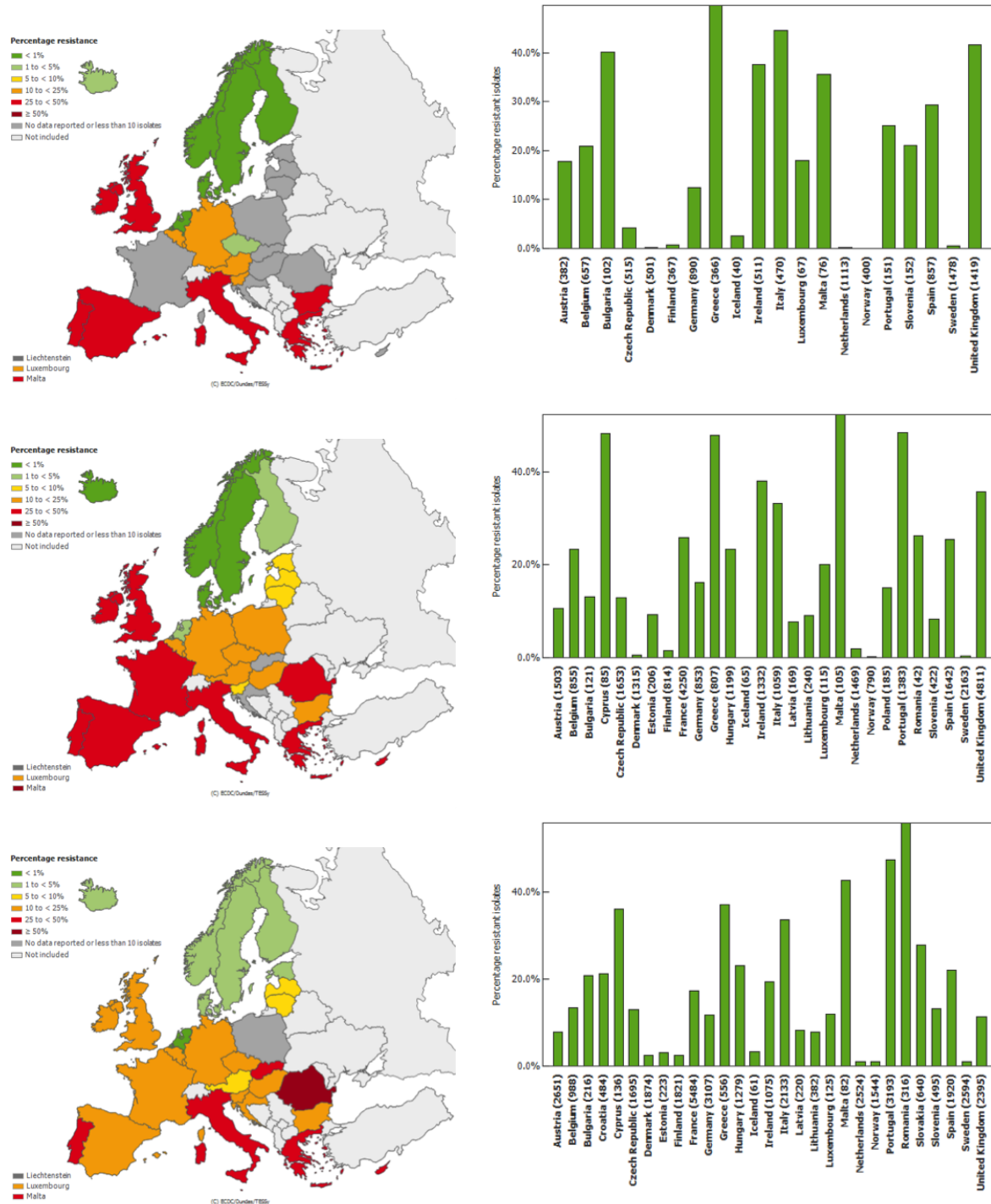
ST80 clones [81]. In many places, the USA300 clone accounts for >50% of all infections caused by *S. aureus* (MSSA and MRSA), which demonstrates the huge capacity of this clone to spread and infect sustainably among humans [50, 99].

1.6.4.2 Europe

The prevalence of MRSA in Europe is very heterogeneous with a north-south separation. The Southern European countries tend to have a high MRSA prevalence while North European countries seem to be less affected (Figure 1.17). From 2000 to 2009, the prevalence of MRSA remained relatively stable in European countries. However, since 2010 the prevalence of MRSA seems to decrease in some European countries like France, Spain, Portugal, Estonia, Croatia, Hungary and UK.

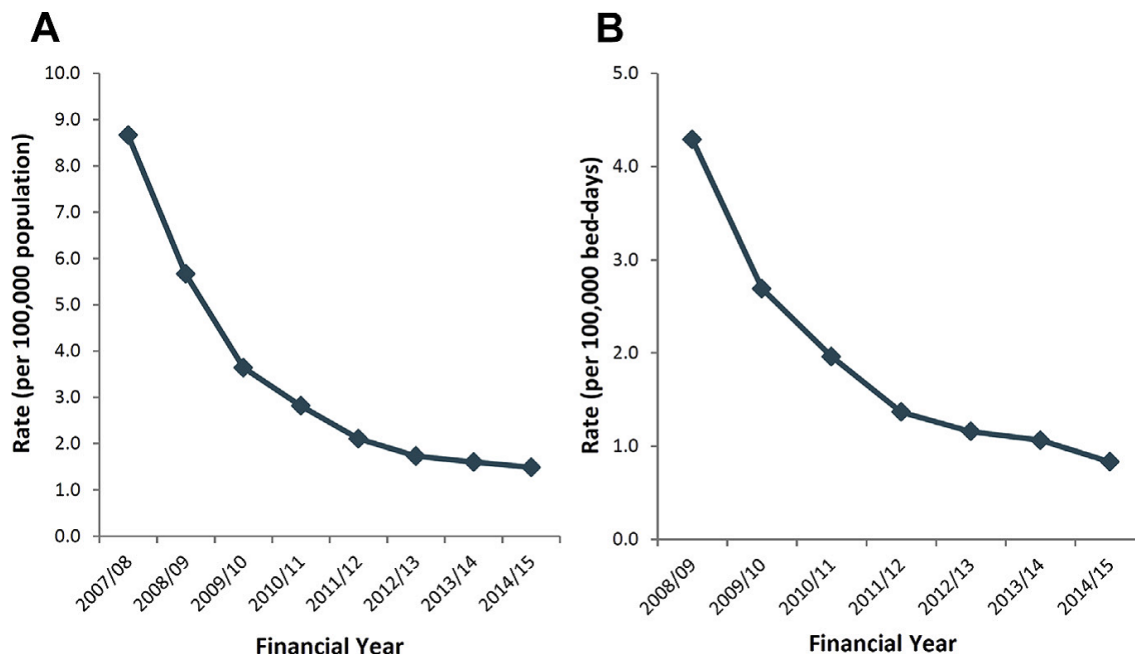
In the United Kingdom, MRSA bloodstream infections (BSI) increased during the 1990s, peaking in 2003 with a subsequent yearly reduction in rates (Figure 1.18). Like the French experience, this favorable trend followed the implementation of a national strategy [100]. Mandatory reporting of HAI began in 2001. After the 2003 peak in BSI, a target was set of 50% reduction in the national total to be achieved by 2008. In addition, a hand hygiene campaign was launched nationally in 2004, and in 2005, care bundles were implemented for catheter care, MRSA screening, and ventilated patients [101]. Despite initial skepticism, the target was surpassed, with a 62% decrease in MRSA BSI from 7700

Figure 1.17: Proportion of MRSA isolates by countries in 2000 (top), 2007 (center) and 2014 (bottom).



"European Center for Disease Prevention and Control"

Figure 1.18: Trends in rates of MRSA bacteremia in England per 100,000 population (A) and health care trust apportioned cases per 100,000 bed-days (B).



"[https://www.gov.uk/government/uploads/system/uploads/attachment](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/530000)" - 05/30/2016

in 2003 to 2004 to 2932 in 2008 to 2009 [100]. Although the hand hygiene campaign is considered a major contributor to the decline in MRSA, it is likely that the multifaceted approach, together with a clear target and support from the Department of Health, has resulted in this marked improvement [101].

In addition to France and the United Kingdom, there is now a diverse group of European countries, with varying baseline MRSA prevalence, that has been able to reverse previously increasing trends in MRSA. These countries, including Belgium, Germany, and Ireland, have seen a reduction in MRSA prevalence between 2011 and 2014 (European Antimicrobial Resistance Surveillance Network). Surveillance data from Australia also show significant reductions in the incidence of both healthcare-associated MRSA and MSSA BSI since 2002 [102]. These trends also coincided with the introduction of a range of infection control initiatives at national and local levels [102].

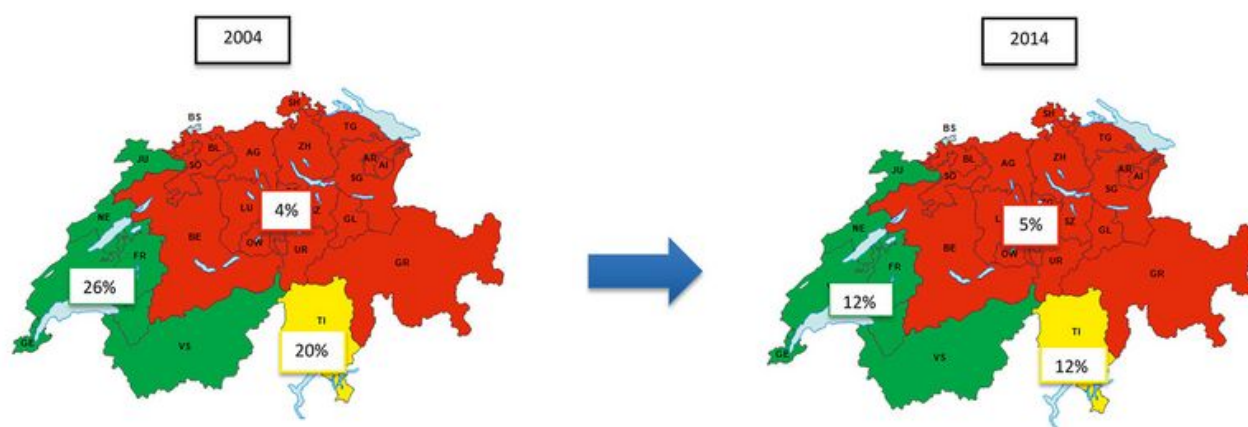
1.6.4.3 Switzerland

In Switzerland, the incidence of MRSA among *S. aureus* positive clinical culture decreased from 9.0% to 7.5% respectively between 2013 and 2015 (Table 1.2) according to data from ANRESIS (Swiss Centre for Antibiotic Resistance) [103]. In Switzerland, there are different linguistic regions. The ANRESIS data showed differences according to this regions

Table 1.2: MRSA incidence in Switzerland in 2013, 2014 and 2015.

Region	2013			2014			2015		
	Susceptible %	Resistant %	n	Susceptible %	Resistant %	n	Susceptible %	Resistant %	n
Switzerland East	94.6	5.4	4943	95.0	5.0	5444	94.6	5.4	5704
Switzerland West	93.6	6.4	6459	93.6	6.3	7400	93.0	7.0	7960
Switzerland Mid	85.2	14.8	6069	88.6	11.4	6722	90.1	9.9	6767
All	91.0	9.0	17471	92.3	7.7	19566	92.5	7.5	20431

"Olearo F, Albrich WC, Vernaz N et al. *Staphylococcus aureus* methicillin resistant in Switzerland: regional differences and trends from 2004 to 2014. *Swiss Med Wkly* 2016; 146:w14339."

Figure 1.19: Proportion of MRSA among *S. aureus* by region.

red = German-speaking; green = French-speaking; yellow = Italian-speaking.

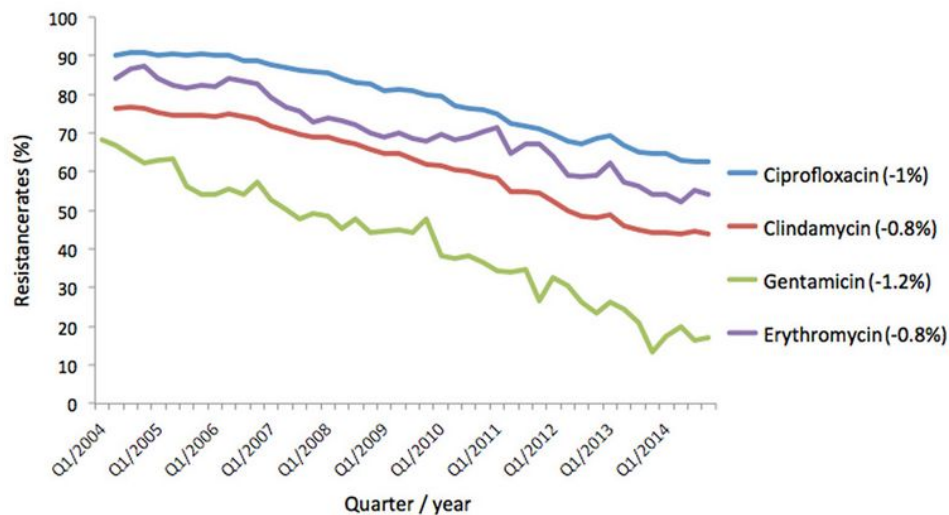
"Olearo F, Albrich WC, Vernaz N et al. *Staphylococcus aureus* methicillin resistant in Switzerland: regional differences and trends from 2004 to 2014. *Swiss Med Wkly* 2016; 146:w14339."

[103]. MRSA proportions were decreasing over time in Italian- and French-speaking regions (Figure 1.19), while MRSA trends were on a slightly upward trajectory in German-speaking region. However, MRSA proportions were still significantly higher in the Italian- and French-speaking regions.

Between 2004 and 2014, the resistances in MRSA to erythromycin, gentamicin, clindamycin and ciprofloxacin decreased (Figure 1.20). However, the resistances to rifampicin, fusidic acid, trimethoprim-sulfamethoxazole (TMP-SMX) and tetracycline remained lower and constant during the study period.

An extrapolation to the whole Swiss population, allowed to indicate an incidence of mMRSA (multidrug resistant MRSA) bacteraemia decreasing from 21 to 7 per 100'000 admissions. This incidence of other invasive mMRSA infections decreased from 11 to 10 per 100'000 admissions (Figure 1.21.a). According to Figure 1.21.b, we observed an increase in NmMRSA in invasive infections from 4 to 14 per 100'000 admissions. Concerning the

Figure 1.20: Time series analysis of antibiotic resistance among MRSA isolates.



Trends per quarter are given in parantheses.

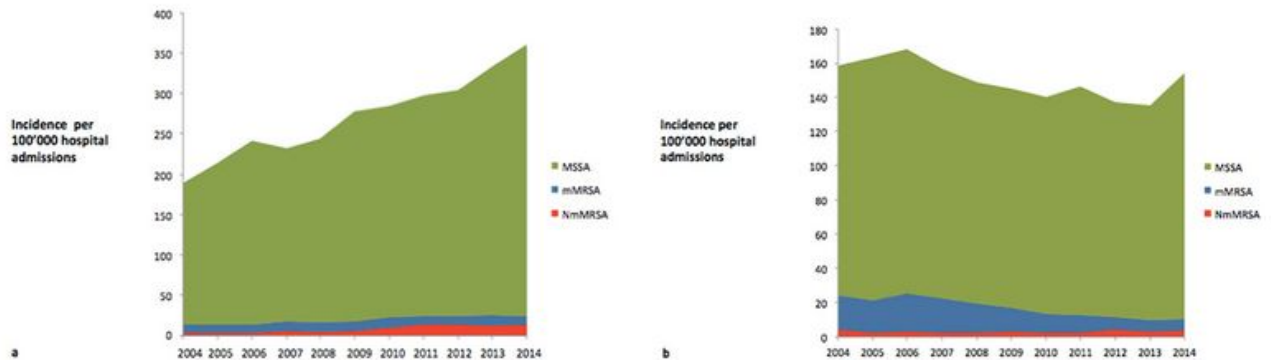
"Olearo F, Albrich WC, Vernaz N et al. Staphylococcus aureus methicillin resistant in Switzerland: regional differences and trends from 2004 to 2014. Swiss Med Wkly 2016; 146:w14339."

incidence NmMRSA (non-multidrug resistant MRSA) bloodstream infections, it remained stable over time.

1.6.4.4 Geneva University Hospitals (HUG)

The prevalence of MRSA at Geneva University Hospitals (HUG) was high compared to the rest of Switzerland until recently. According to the literature, the endemic threshold is estimated at 0.5 new cases for 100 admissions. Between 1992 and 1994, the incidence rate of new cases for MRSA was slightly above the endemic threshold, and since 1998 a significant increase in this incidence was observed at HUG, underpassing an incidence (colonization and infection) of about 2 cases per admissions during six years, between 2002-2007 (Figure 1.22). However, a significant reduction of this incidence started from 2007 until now, reacting the endemic threshold in 2014. Regarding the incidence of new nosocomial cases, defined as carrier of acquired in the hospital, a decrease was also observed an the HUG since 2007. This decrease did not hold in 2011 but resumed in 2012, and this until now, confirming a significant decline of the MRSA prevalence in Geneva. The monthly cumulative incidence of new MRSA carrier patients (nosocomial acquisition) at HUG confirms the decrease of MRSA with a homogenous diminution of this incidence since 2007 until now (Figure 1.23). At HUG, since the beginning of the outbreak, some chromosomal cassettes are more prevalent than others. Until now, the SCC mec I was the predominant cassette for HA-MRSA while SCC mec IV is the predominant cassette for CA-MRSA (Figure 1.24). However, for HA-MRSA, it was found that the proportion of SCC mec

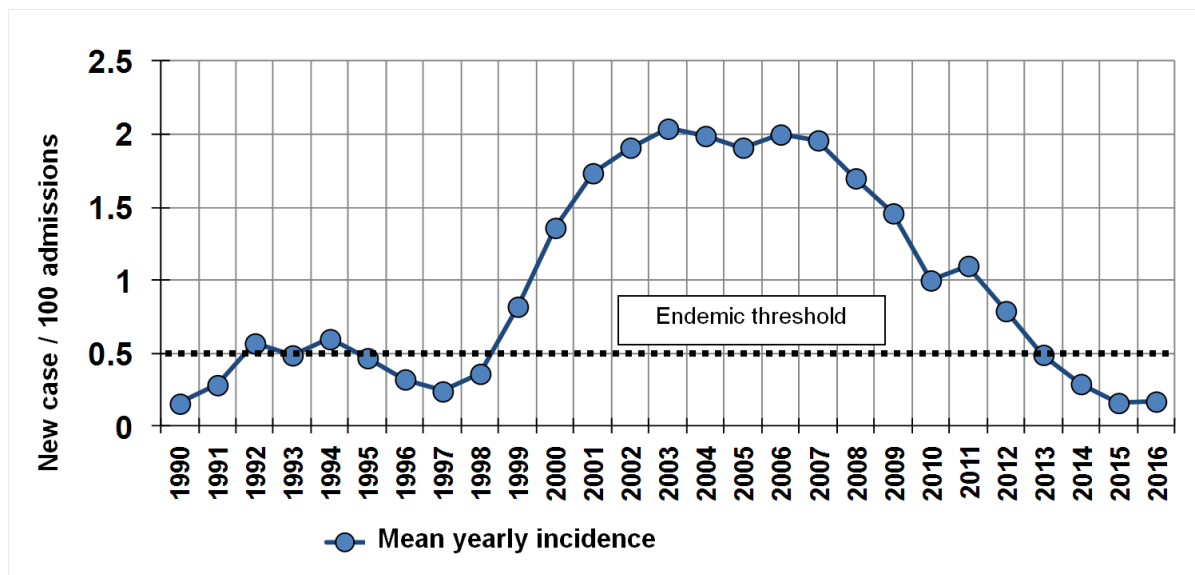
Figure 1.21: incidence of MSSA, non-multidrug resistant MRSA (NmMRSA) and multidrug resistant MRSA (mMRSA) per 100 000 hospitals admissions.



(a) in invasive isolates (excluding bacteraemia isolates) and (b) in bacteraemia.

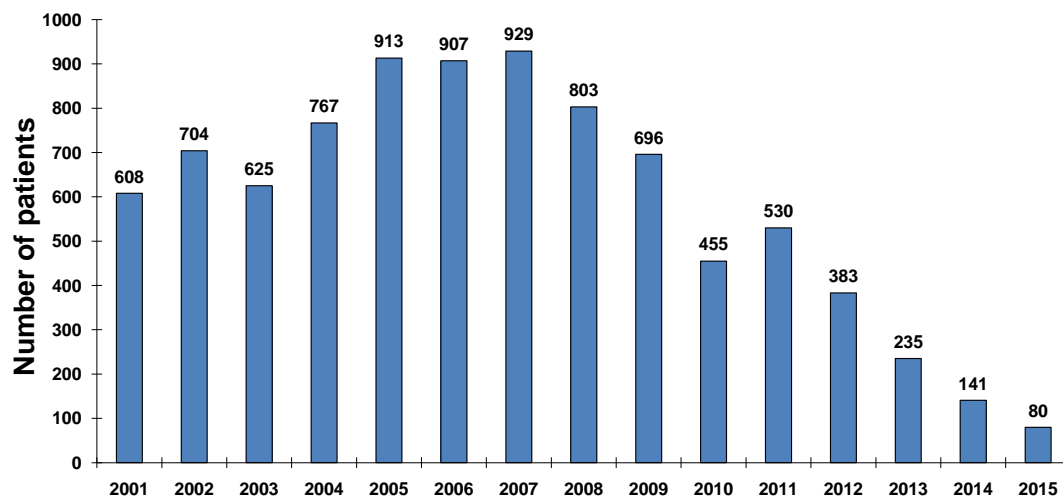
"Olearo F, Albrich WC, Vernaz N et al. *Staphylococcus aureus* methicillin resistant in Switzerland: regional differences and trends from 2004 to 2014. *Swiss Med Wkly* 2016; 146:w14339."

Figure 1.22: Annual MRSA incidence at HUG in hospitalized patients, 1990-2015.

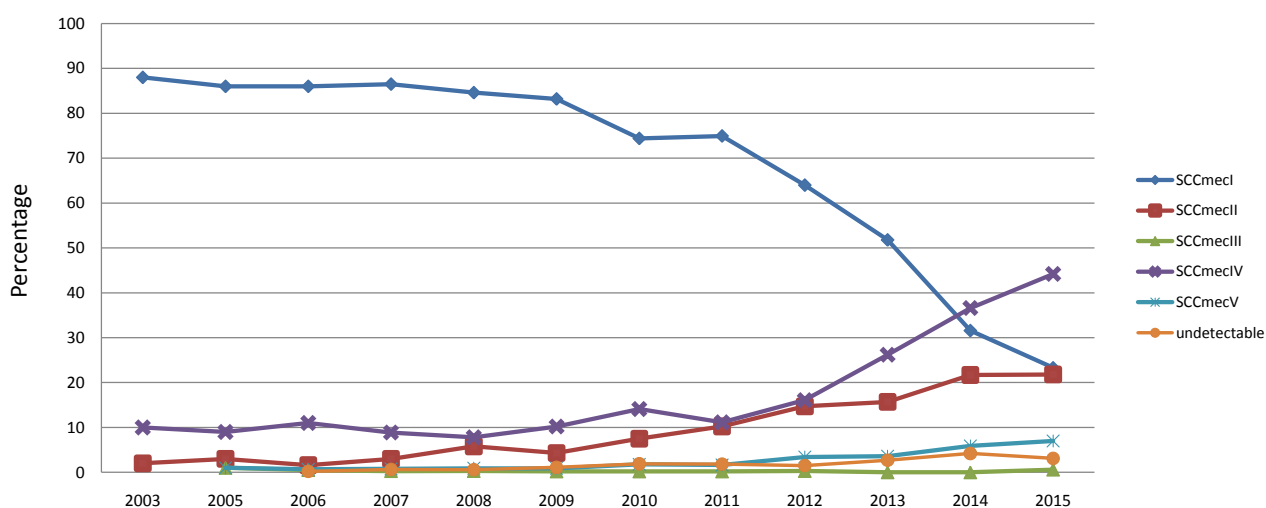


"Infection Control Program Geneva - HUG - C. Fankhauser."

Figure 1.23: New cases of nosocomial MRSA at HUG, 2001-2015.

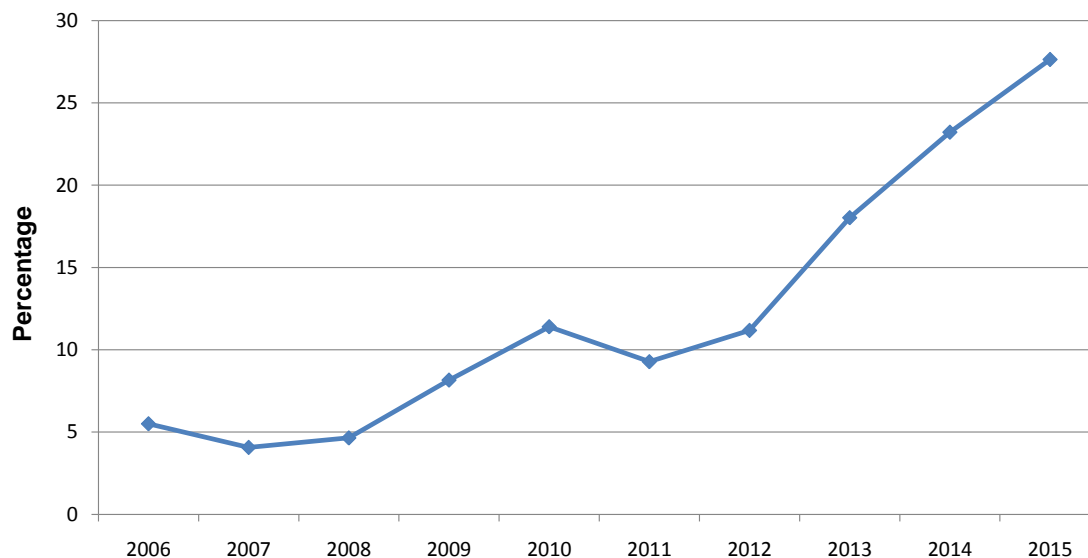


"Infection Control Program - HUG - C. Fankhauser."

Figure 1.24: MRSA: Distribution of chromosomal cassette SCC_{mec} predominant within HUG, 2003-2015.

"Infection Control Program - HUG - C. Fankhauser."

Figure 1.25: MRSA: Distribution of PVL toxin within HUG, 2003-2015.



"Infection Control Program - HUG - C. Fankhauser."

I tends to decrease while that of *SCCmec* II increases. We also observed an increase of detection of CA-MRSA compared to HA-MRSA in HUG with a more important proportion of *SCCmec* IV and *SCCmec* V now compared to 2005. These results are confirmed by the increase of PVL-positive MRSA strains identified in Geneva (Figure 1.25).

1.7 MRSA colonization

Colonization consists of the presence of MRSA on skin or in the noses without the presence of symptoms of clinical manifestations of infection or illness. We say that a carrier is colonized with MRSA. MRSA grows preferably in moist and warm place as groin, nostrils, armpits, belly button, etc. They are commonly found in the nose, but can also colonize the skin or the throat. Chronic skin lesions such as wounds, ulcers, or eczema, and the points of insertion of medical devices, are important ecological niches for the development of infections by this microorganism. Although asymptomatic and often benign, colonization with *S. aureus* involves a risk of secondary infection and represents a serious risk of transmission to a third party. The MRSA colonization can be transient or intermittent. According to a Sweden study, the average duration of MRSA colonization is about 5.9 months [104]. The same design performed in Paris identified a median duration of MRSA colonization of 282 days [105], with a chance of 50% to cleared the organism within 1 year. Some risk factors were identified to increase the duration of colonization like having household contact with MRSA carriers, young age, spa-type-t002, and colonization in two or more locations [104].

After discharge of carriers, a transmission of the MRSA strain to the households occurred in 20% of cases [105]. This transmission is associated with older age and participation in health care of the index patient [105].

According to some studies, it has been proved that the best way to perform an efficient MRSA detection is a multi-sites screening [106–109]. Indeed, the previous studies also conclude that the best body regions to test control MRSA colonization are first the nares [106], followed by the inguinal region [107](96% of patient are detected if the nares and the inguinal region are tested [110]), the third region is the throat (sometime more difficult to test) [109]. CA-MRSA tended to colonized the perianal and the inguinal region whereas HA-MRSA more often colonized the nares [111].

1.7.1 Decolonization treatments

The process of decolonization aims at reducing or eradicating the asymptomatic carriage of MRSA. Among the sites of colonization, nares are the most important, but there are other important sites like skin fold or perineum. When a patient is colonized with MRSA, there is a high risk to contaminate his environment and clothes. This contamination occurs touching surfaces with contaminated hands due to the shedding of skin scales. This environmental contamination can cause subsequent contamination of other patients.

1.7.1.1 Mupirocin

Mupirocin is a topical treatment and belongs to the monoxycarboic acid class of antibiotic. Originally, it was isolated from Gram-negative bacteria (*Pseudomonas fluorescens*) developed by the British pharmaceutical company Beecham [112]. The action of mupirocin is both bacteriostatic and bactericidal, respectively at low and high concentration [113–115]. It is active against Gram-positive bacteria and particularly MRSA [116]. Mupirocin is compounded at 90% by pseudomonic acid A and 10% by pseudomonic acid B, pseudomonic acid C and pseudomonic acid D (Figure 1.26). Mupirocin is used to treat bacterial SSTI like abscess or furuncle, and for MRSA decolonization. However, it is suggested not to use mupirocin for an extended period of time due to the rapid emergence of mupirocin resistance or the overgrowth of non-susceptible bacteria. In *Staphylococcus*, mupirocin inhibits the protein synthesis binding to the isoleucyl transfer ribonucleic acid (t-RNA) synthetase.

Rapidly after the first introduction of mupirocin, some strains of MRSA appeared to become resistant. There are two distinct populations of mupirocin-resistant bacteria: MuL (low-level resistance) and MuH (high-level resistance) [117]. In MuL strains, the emergence probably comes from a mutation in the organism's wild-type isoleucyl-tRNA synthetase. However, for the MuH strain, the resistance is due to a gene acquisition of a separate Ile synthetase the *mupA* gene [118]. Cross-resistance between mupirocin and other antibiotics is unlikely because the mechanism of resistance for mupirocin differs from the mechanism for other antibiotics [117]. The *MupA* gene may however be transferred with some other

Figure 1.26: Pseudomonic acid A-D core structure labeled A-D respectively.

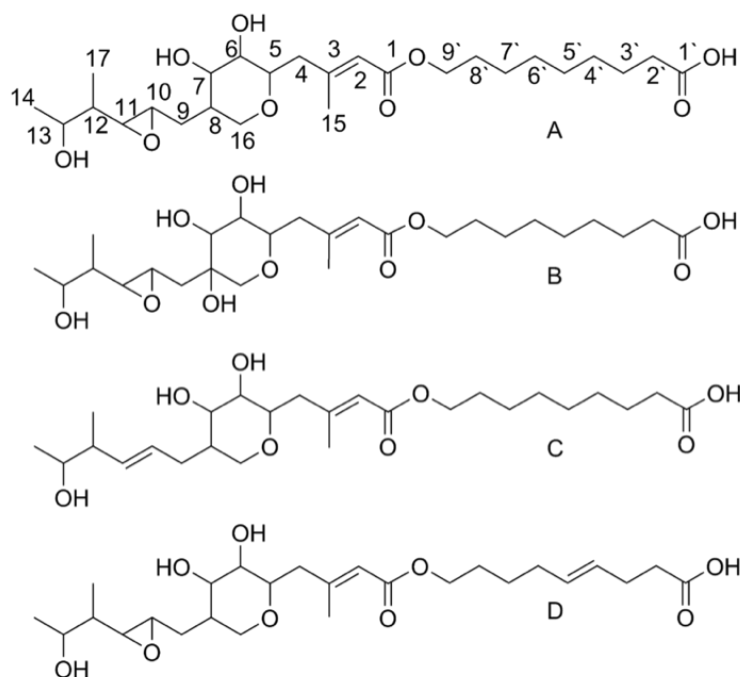
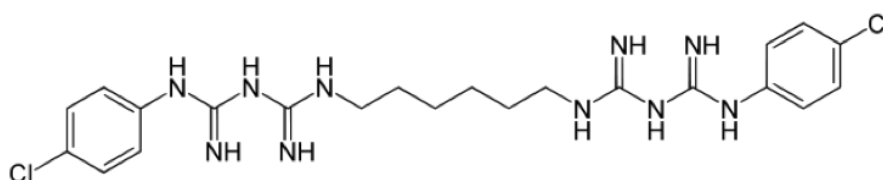


Figure 1.27: Chlorhexidine core structure.



antibacterial gene of resistance, like trimethoprim (TMP), tetracycline or triclosan-resistant genes [117].

1.7.1.2 Chlorhexidine

Chlorhexidine is an antibacterial which is used as an antiseptic but also for other applications. It is a cationic polybiguanide (Figure 1.27), primarily used for its salts. It is employed in several products like cosmetics (antiperspirants, deodorants, toothpaste and additive to creams), pharmaceutical products (antiseptic mouthwashes, the active substance in wound dressings and preservative in eye drops) and disinfectants [119].

Like mupirocin, chlorhexidine has a bacteriostatic effect at low concentrations and a bactericidal effect at high concentrations due to the fact that the membrane disruption

results in cell death [120]. Chlorhexidine has an action against Gram-negative and Gram-positive bacteria, aerobes, facultative anaerobes and yeasts [120]. The best efficacy is against Gram-positive bacteria (with a concentration $\geq 1\mu\text{g}/\text{l}$), whereas it needs higher concentration to have an action against Gram-negative bacteria and yeast (with a concentration 10 to more than $75\mu\text{g}/\text{ml}$). Chlorhexidine is used as a cleanser for skin wound and surgical scrubs, germinal hand rinses and preoperative skin preparation [120].

1.8 MRSA infection

Whereas MRSA colonization is only associated with the presence of MRSA on the skin, MRSA infection is declared when there are clinical signs of inflammation and illness (e.g., fever, pus, swelling, localized pain and tenderness, redness and warmth). Infection is due to tissue damage because of MRSA invasion and requires treatment.

We can distinguish two types of acquisition of infection classified as CA-MRSA or HA-MRSA.

1.8.1 HA-MRSA infection

In term of incidence, the most common MRSA infections are SSI, bacteremia, osteoarticular and pneumonia. It is one of the most common causes of post-surgical wound and nosocomial infection. HA-MRSA is associated to surgical procedures, intravenous catheter and contact with devices that can be found in a hospital. There are several risk factors that can be associated with HA-MRSA infection: hospitalization, weakened immune system, dialysis or nursing home residence. HA-MRSA causes severe illness and can spread in bones, blood or others tissues. Infection caused by HA-MRSA often causes severe problems such as sepsis, pneumonia, bone infection. The principal symptoms of this infection are fever, fatigue, headaches, muscle aches, malaise, chills, cough, chest pain, rash.

1.8.2 CA-MRSA infection

Regarding CA-MRSA infections, the risk factors are close personal contact, exposure to contaminated objects and poor hygiene, athletes who share equipment (razors, towels), contact sports, residence in prison, military training camps, homosexual relation (a higher incidence of MRSA in homosexual population), attendance of day care facilities and also touching surfaces contaminated with MRSA. There are risk factors mostly reported in the United States, for Europe, the picture is somewhat different. According to a study of Longtin et al. in Switzerland, risk factors [121] associated with CA-MRSA infection were nationality other than European or Swiss, absence of healthcare contact.

CA-MRSA infection is mainly restricted to the skin and causes skin infection. CA-MRSA infection can develop in an area that has been scratched, rubbed or with a cut. Parts of the body with more hair are more susceptible to develop an infection (such as

the armpits, buttocks, beard and back of the neck). During infection, the infected area is swollen (fluid-filled), red and painful to touch.

1.8.3 Types of infection

MRSA is responsible for the types of infections:

Minor infections:

- SSTI (Skin and Soft Tissue Infection): abscesses, boils, furuncles, cellulitis, folliculitis
- UTI (Urinary Tract Infection)

Severe infections:

- Pneumonia
- SSI (Surgical Site Infection)
- Bacteremia, Sepsis and toxic shock syndrome (TSS)
- Osteoarticular infection: osteomyelitis, infected medical device
- Endocarditis
- Meningitis

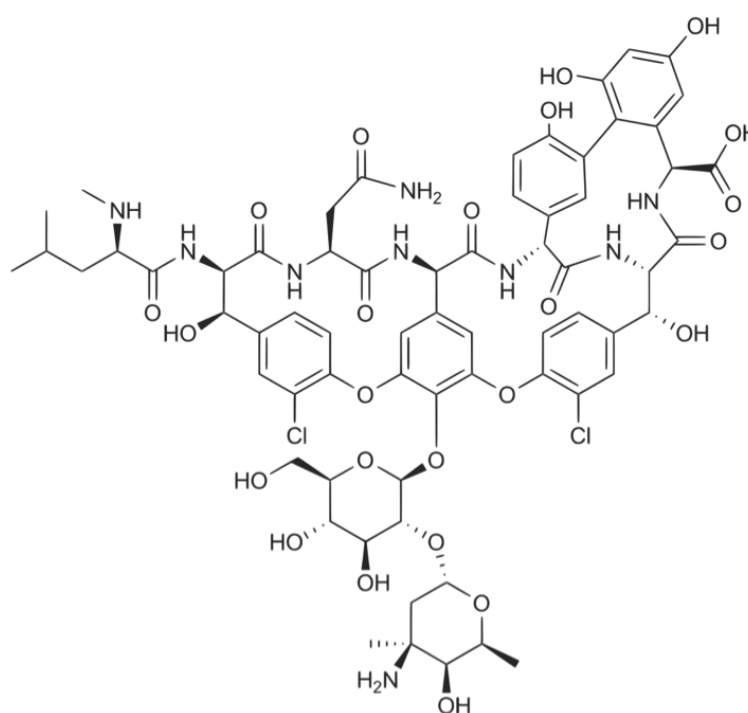
Infections associated with deep-seated foci:

- Bacteremia, Sepsis and toxic shock syndrome (TSS)
- Osteoarticular infection: osteomyelitis, infected medical device

1.8.4 Antibiotic treatment

Despite having developed numerous resistances to antibiotics, there are still several antibiotics that can treat MRSA infection. They are selected depending on the location, severity of the infection, the MRSA strain and the hospital or community acquisition (different resistance profile). The two principal antibiotics to treat MRSA are vancomycin and linezolid (LZD). However, several other antibiotic treatment options exist (e.g. daptomycin, teicoplanin, etc). In particular, a combination of trimethoprim-sulfamethoxazole plus rifampicin (RMP) is also effective [122].

Figure 1.28: Vancomycin core structure.



1.8.4.1 Vancomycin

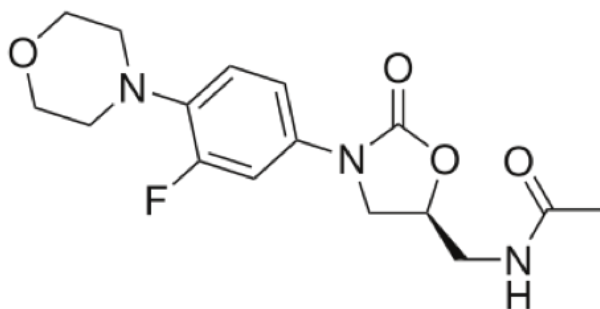
Vancomycin is a glycopeptide. Large (1 500 to 2 000 Daltons) and complex (Figure 1.28), it is, like β -lactam, an inhibitor of the peptidoglycan synthesis of the bacterial wall.

Vancomycin is bactericidal, but the bacterial killing is slow to appear; its oral bioavailability is negligible, the intravenous route is therefore required in all directions. The main side effects are kidney and hearing impairment; it is, therefore, justified to undertake regular monitoring of plasma levels of vancomycin. Despite its use in last resort only since 1956, vancomycin is not so far away from the development of a resistance. There are vancomycin-resistant enterococci (VRE), in which the structure D-Ala-D-Ala of peptidoglycan precursors is replaced by a structure D-Ala-D-lactate does not allow binding of vancomycin. Very few last lines of treatments are available against these bacteria (e.g. LZD). The risk of such resistance among other groups of bacteria, including *S. aureus*, further restricts its use.

1.8.4.2 Linezolid

LZD has been discovered in the 1990s and approved in 2000. It is a novel oxazolidinone agent that has demonstrated activity against Gram-positive bacteria and specifically MRSA. The structural core of LZD is composed by 1,3-oxazolidin-2-one moiety with an

Figure 1.29: Linezolid core structure.



S-methyl group and an aryl group (Figure 1.29) [123]. LZD is a synthetic drug unlike others antibiotics like penicillin; we do not find it in nature. It does not have a natural original skeleton (unlike most β -lactam, which is semi-synthetic).

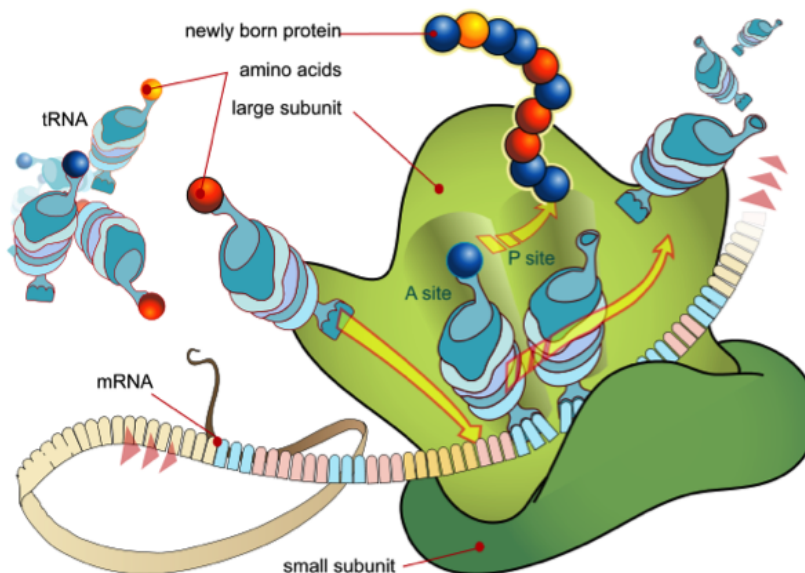
LZD can stop the production of protein in bacteria then inhibit their growth; it's a protein synthesis inhibitor. LZD does not have a bacteriocidal activity but only a bacteriostatic activity. The activity of LZD is not yet fully understood [124]. It seems to act against bacteria using a unique mechanism of action. It can block the early step of protein production, but it has no activity against the later stages (Figure 1.30) [125]. Specifically, it can stop the reproduction and growth of bacteria interrupting the translation of messenger ribonucleic acid (mRNA) into a protein in the core of the ribosome [125,126].

This mechanism of action is relatively unique. Therefore, cross-resistance with other protein synthesis inhibitors is infrequent or nonexistent [127,128]. Currently, the incidence of LZD resistance is low since his first approval, but could be increasing.

It is available intravenously and orally, which is a significant advantage for its clinical use and has been used successfully to treat HA- or CA-MRSA infections. It is now widely employed in many countries with endemic MRSA infections. Several studies have even suggested that LZD may be superior to parenteral vancomycin, although this claim has not been supported by the FDA [129–138].

LZD has the potential for interaction with adrenergic and serotonergic agents via a reversible enhancement of the pressor response to agents such as dopamine or epinephrine and a risk of serotonin syndrome (e.g., cognitive dysfunction and hyperpyrexia) in patients receiving concomitant serotonergic agents. Due to its weak activity as a monoamine oxidase inhibitor, LZD should not be used concomitantly with many psychotropic agents such as tramadol, pethidine, duloxetine, venlafaxine, milnacipran, sibutramine, chlorpheniramine, brompheniramine, cyproheptadine, citalopram, and paroxetine [139].

Figure 1.30: Simplified schematic of mRNA translation. Linezolid occupies the A site (at center) and prevents tRNA from binding.



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1.8.4.3 Trimethoprim-sulfamethoxazole

TMP-SMX, also known as Co-trimoxazole, was first sold in 1974. It is part of the WHO list of essential medicines needed in a primary health system. It is now an inexpensive drug available as a generic agent. TMP-SMX is an association of both antibiotics, TMP and SMX, with a ratio of 1 to 5 (Figure 1.31), used to treat a variety of bacterial infections.

These two components act synergistically by simultaneously blocking two enzymes catalyzing successive reactions in the metabolic pathway of folinic acid, essential for the survival of many microorganisms. This association exercises *in vitro* bactericidal activity were appeared at low concentrations, for both components used separately, are only bacteriostatic. Due to this mode of action, the risk of development of resistance is minimized. It often remains active on resistant germs in one of its two components. Usually, the bacterial resistance mechanism is a production of new plasmid enzymes, resistant or partially resistant to TMP or SMX. It is currently used for UTI, septicemia, upper and lower respiratory tract infections, renal infection, gastrointestinal infections, SSTI and other infections caused by sensitive organisms. The most serious effects include the toxic epidermal Necrolysis (Lyell) and Stevens-Johnson syndrome. TMP-SMX alone is not a good alternative to treat MRSA infection [140]. However, TMP-SMX combined with another antibiotic is efficient to treat MRSA infection [122, 141].

Figure 1.31: Trimethoprim-sulfamethoxazole core structure.

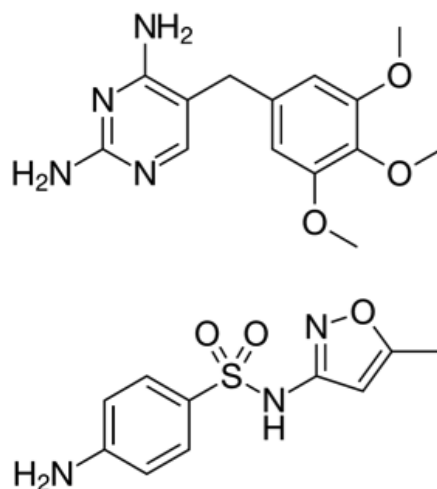
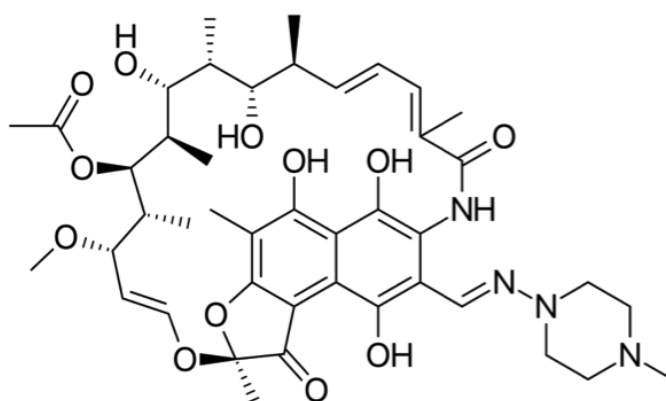


Figure 1.32: Rifampicin core structure.



1.8.4.4 Rifampicin

RMP is an antibiotic of the rifamycin family used to treat some bacterial infections including tuberculosis, leprosy, and legionella in combination with other antibiotics (Figure 1.32). RMP is efficient against MRSA, when it is combined with another antibiotic [141–146].

RMP is administered orally and intravenously. RMP was first isolated from *Amycolatopsis rifamycinica* in 1957 and has been marketed since 1971. This antibiotic is part of the essential drug list of the WHO. It works by blocking the synthesis of RNA in bacteria. RMP acts by specifically binding bacterial RNA polymerase and thereby inhibiting transcription of RNA messengers [147,148]. Data on crystallographic structures and biochemical data indicate that RMP binds to bacterial RNA polymerase on a site adjacent to the active center of RNA polymerase dedicated to the RNA synthesis. Resistances to RMP are due

to mutations in the RMP binding site of the RNA polymerase that result in a decreased affinity for RMP. Most of these resistant mutations are located in the *rpoB* gene of the RNA polymerase encoding the beta subunit. RMP is red-orange, causing similar colored urine and sweat of patients. It can cause liver problems or allergic reactions. RMP has hepatotoxicity as a main side effect. Patients treated with this antibiotic should carry out frequent tests of liver function to detect any lesions on the liver.

1.9 Precaution to prevent spread of MRSA

The Center for Disease Control and Prevention (CDC) has drafted guidelines to reduce the spread of MRSA within the health care facilities. There are 3 types of precautions: standard, contact and additional (www.cdc.gov/mrsa/healthcare/clinicians/precautions.html). The choice of the applied precautions depends on the local guidelines. In HUG, the standard precautions are used, except when the respiratory tractus is colonized/infected, they implement the contact precaution.

1.9.1 The standard precautions

- Hand Hygiene
- Gloving before possible contact with blood, potentially infected materials, mucous membranes, nonintact skin, or potentially contaminated intact skin
- Mouth, nose and eye protection during procedure and patient-care activities
- Gowing during procedure and patient-care activities
- Appropriate device handling of patient care equipment and instruments/devices
- Appropriate handling of laundry

1.9.2 The contact precautions

- Patient placement in single room or cohort-patient with the same MRSA in the same room or patient-care area when it is possible.
- Gloving whenever touching the patient's intact skin or surface
- Mouth, nose and eye protection during procedure and patient-care activities
- Gowing before entry in the room
- Patient transport limited
- Patient-care equipment and instruments/devices use disposal noncritical patient-care equipment or implement patient-dedicated use of such equipment

- Environmental measures
- Discontinuation of contact precautions

1.9.3 The additional precautions

- Patient placement
- Patient placement in dialysis facilities
- Group activities

1.10 Public health considerations

Since the end of the XXe century, the burden of MRSA is a public health concern. Only in the United States, MRSA is responsible for about several hundreds of thousands hospitalizations per years [149]. A major part of this infections are non-severe, however the Center for Disease and Control and Prevention (CDC) estimates that several thousand leads to death each year (<http://www.cdc.gov/drugresistance/threat-report-2013/> Mathematical models were created to control the spread of this pathogenic germ. As mentioned above for the UK and France, strategies combining screening and isolation of colonized and infected patients permitted to keep the control of the spread. In Netherland, they added a protocol of MRSA decolonization for the carriers before they were discharged from the hospital. This strategy permitted good control of the MRSA spread in the community. This is one of the reasons responsible for the low prevalence of MRSA in the Netherland [150], because loss of control occurs when colonized patients are discharged back into the community and then readmitted; when the number of colonized patients in the community reaches a certain threshold, the strategy is overwhelmed [151].

The burden of MRSA has an important economic impact. The cost of a nosocomial infection was ranged from 10'500 US dollars (US\$) per case (for bloodstream, urinary tract, or respiratory infections in immunocompetent patients) to 111'000\$ per case for bloodstream infection with resistant bacteria in patients with transplants. In Canada, the direct healthcare costs attributable to MRSA (management for infected and colonized patient and infrastructure) were estimated at 82 millions US\$ in 2004 until 129 millions US\$ in 2010) [152]. Concerning the CA-MRSA in the United States, they imposed an annual burden of about 478 millions US\$. The costs attributable to MRSA are considerable.

Recently, due to the huge burden induce by the problem of the antibiotic resistance in the world, the United Nations decided to react. They regrouped the global leaders during the assembly in New York in May 2015 to talk about the AMR (antimicrobial resistance). They spoke the high risk of AMR if no actions were taken. According to the report by the economist Jim O'Neill, he estimated that globally 700'000 deaths should be attributed to

AMR in 2015 and should be climb to 10 millions deaths until 2050 if no action are taken to reverse the trend.

The World Health Organisation (WHO) adopted the "Global action plan on antimicrobial resistance" during the World Health assembly to control and fight the spread of AMR.

The plan has 5 objectives:

- to improve awareness and understanding of antimicrobial resistance in policy-makers and professionals
- to strengthen surveillance and research;
- to reduce infection
- to encourage the rational use of medicines in human and animal health care
- to increase investment in developing new medicines, diagnostics and vaccines.

According to this plan, the WHO gave to its member States until May 2017 to adopt national actions and to achieve the 5 objectives.

Chapter 2

Comparative genomics of community-associated methicillin-resistant *Staphylococcus aureus* shows the emergence of clone ST8-USA300 in Geneva, Switzerland

"The world is a book and those who do not travel read only a page."

Saint Augustine

2.1 Introduction

The epidemiology of MRSA is very dynamic. First associated with healthcare institutions with an early identification in the 1960s, 30 years later, in the 1990s, some MRSA strains began to be spread outside the healthcare setting in the United States (see section 1.6.3.3). Several major clones of MRSA are responsible for community-associated infection with a worldwide distribution. While European countries were confronted with the ST80 clones coming from North Africa, the United States faced a massive spread of the USA300 clone [51, 153].

The genomes of these clones are genetically diverse, conserving some repertoire key accessory elements, including PVL, a pore-forming toxin that triggers recurrent SSTI or necrotizing pneumonia [154–156] (see section 1.6.2.1). The North Africa and the United States strains harbor an SCC mec IV element. In the United States, the USA300 clone has the specific SCC mec type IVa element. The USA300 strain belongs to the MLST 8 (ST8), carries the *agr* allele 1 and ACME locus (see section 1.6.2.2). Therefore, the acquisition of ACME by USA300 enhances the ability of the strain to colonize the human skin of healthy people and thus more efficiently disseminates in the community [51]. The genome

of the USA300 was sequenced in 2006 for the first time. It is composed of a single circular chromosome of 2.8 million bp and 2 560 genes. It also contains two plasmids. The genome contains genes encoding for the cytotoxins PVL and ACME which is known to have been acquired by the USA300 clone from *S. epidermidis* by horizontal transfer.

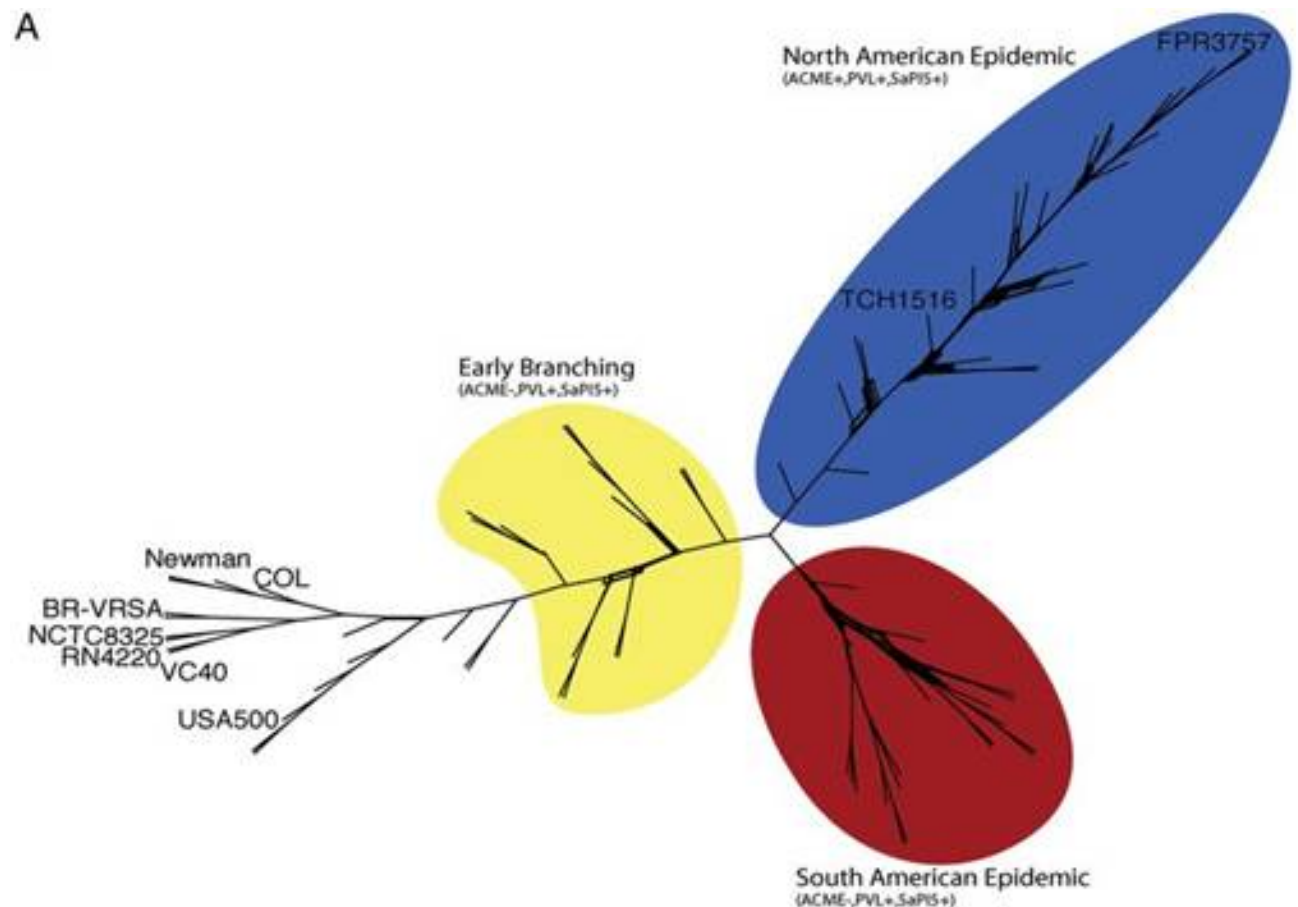
The first identification of USA300 was made in 1999 from an outbreak of infection in a prison in Mississippi [157]. In the following years, several additional outbreaks have been put in evidence, specifically in California, Colorado, Georgia, Pennsylvania and Texas. The outbreak took place in correctional facilities, military bases and day-care centers and sports teams. By 2004, the USA300 clone became the principal cause of SSTI widespread in the United States community and had replaced the possibly less virulent endemic clones [158]. In the past two decades, the USA300 clone has also been reported in others countries, and this clone has been found in 5 continents [159].

In 2005, a variant clone of USA300 was found [160,161]. It was named USA300 Latin-America Variant (USA300-LV). It replaced the prevalent MRSA clone (HA-MRSA clone, designated "Chilean/Cordobes") in the hospital and community in several countries like Colombia, Venezuela and Ecuador [161,162]. It became the endemic MRSA clone from North of the South America [162]. USA300-LV is responsible for the same type of infections as the North American USA300. Based on the standard molecular techniques, this variant of USA300 clone belongs to the same key genetic elements of the USA300 lineage (SCC mec IV and PVL elements) [161,162]. The significant difference between the USA300 North American clone and the USA300-LV is the absence of ACME in the latter [51,163], which is thought to be a major determinant of the USA300 clonal virulence [53, 54, 164]. The cassette harbored by the USA300-LV also differs from the North American variant, it has the SCC mec IVc in place of IVa (Figure 2.1) [161, 162, 165, 166]. The South America clone was observed six years later the North America clone, it was assumed that the USA300-LV likely disseminated southward from the North American clone. However, according to Planet et al. study, using whole-genome sequencing (WGS), showed that the South American outbreak is not an extension of the North American USA300 epidemic, but rather occurred simultaneously, with two lineages sharing a common ancestor before their spread (Figure 2.2) [157]. The common ancestor between the two clones was dated in 1975. The first North American variant appeared in 1989 and the first USA300-LV in 1985 [157]. The rapid multiplication of both clones suggests that they potentially share adaptations that enhance fitness and good capacity to spread [157].

2.2 Context

In Geneva (Switzerland), the first CA-MRSA PVL-positive clone was retrospectively identified in 1994; as the ST80 clone [167]. In 2004, due to the high prevalence of MRSA in Geneva, it was decided by our institution to implement an active surveillance and molec-

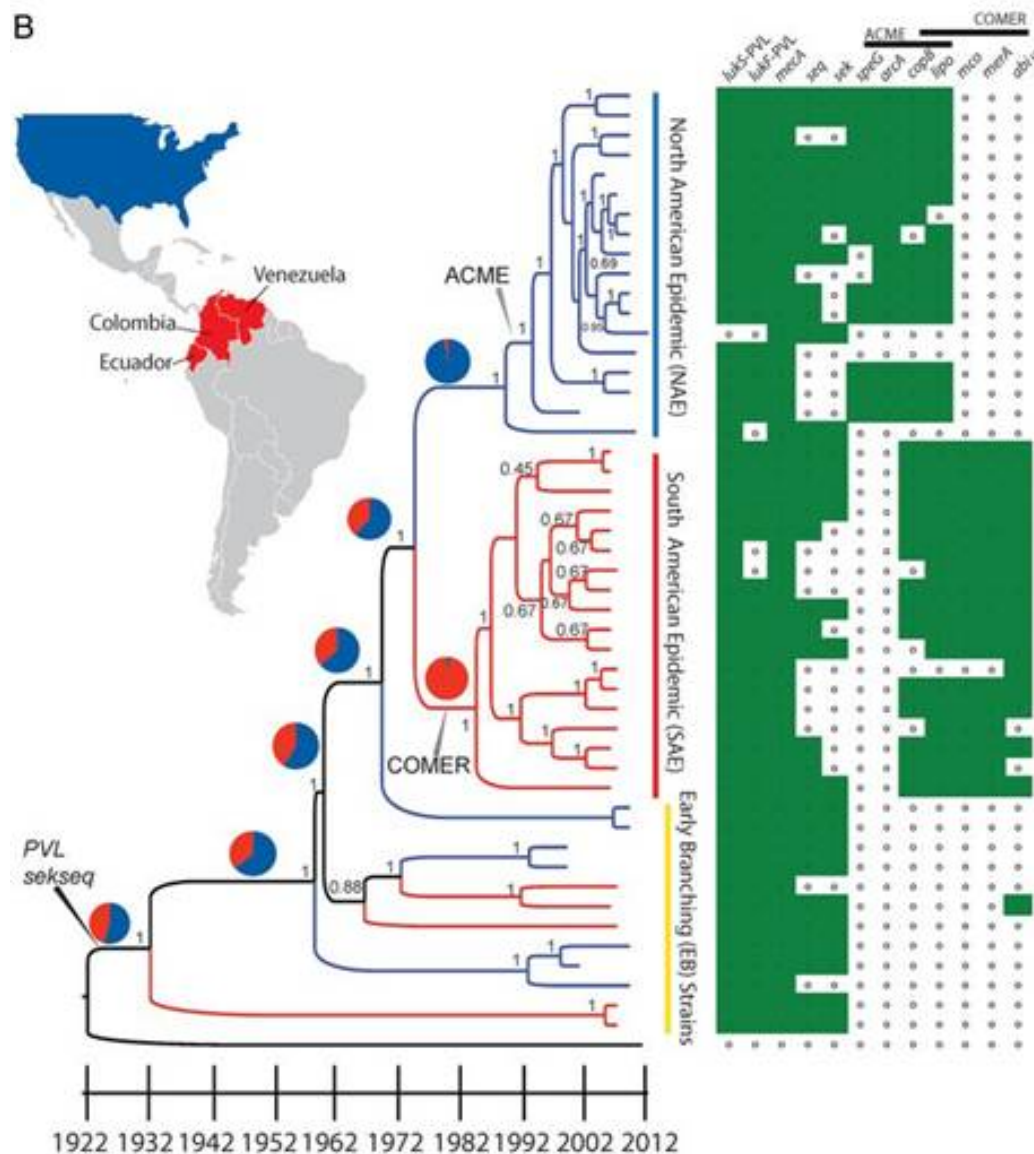
Figure 2.1: Phylogenetic analysis shows relationships between whole-genome *Staphylococcus aureus* isolates.



A, Phylogenetic network is based on 1000 maximum likelihood nonparametric bootstrap iterations for 88 *Staphylococcus aureus* genomes. Only sequence type 8 (ST8) strains are shown (8 genomes used as out-groups have been excluded for clarity). Areas of low support show higher amounts of reticulation. Note that the 2 epidemic lineages in the North American epidemic (NAE) and the South American epidemic (SAE) can be robustly distinguished from each other but exhibit high levels of reticulation internally. Whole genomes of BR-VRSA, COL, FPR3757, Newman, RN4220, TCH1516, USA500, and VC40 are shown for comparative purposes.

"Parallel Epidemics of Community-Associated Methicillin-Resistant *S. aureus* USA300 Infection in North and South America. Planet PJ, Diaz L, Kolokotronis SO, Narechania A, Reyes J, Xing G, Rincon S, Smith H, Panesso D, Ryan C, Smith DP, Guzman M, Zurita J, Sebra R, Deikus G, Nolan RL, Tenover FC, Weinstock GM, Robinson DA, Arias CA. *J Infect Dis.* 2015 Dec 15;212(12):1874-82.

Figure 2.2: Phylogenetic analysis shows relationships between whole-genome *S. aureus* isolates.



B, ChronoGram was constructed using core genome single-nucleotide polymorphisms from the 48 sequenced strains in a Bayesian phylogenetic analysis that the phylogenetic relationships among isolates, the times since the divergence of the major clades, and the rate of evolutionary change. We used a relaxed-clock model and a constant-size coalescent tree prior probability. Calendar dates of isolation were used to calibrate the clock. Branches are colored based on parsimony reconstruction of geographical location in North America (blue) or South America (red). Numbers on or near branches are Bayesian posterior probability support values. Ancestral branches with uncertain geographical reconstructions are black.

Pie charts show proportional likelihood reconstructed for each of the key branches based on the mkt1 model. Early branching (EB) strains are also indicated. Key evolutionary acquisition events (e.g., arginine catabolic mobile element [ACME] and copper and mercury resistance [COMER] element acquisition events) are indicated with arrows. The binary matrix shows presence (green; 1) and absence (white; 0) of selected genes associated with each lineage. Abbreviations: abi, abortive phage infection; arcA, arginine deiminase; copB, putative ATPase copper exporter; lipo, putative lipoprotein; lukSF-PVL, Pantone-Valentine leukocidin; mecA, PBP2a; mco, multicopper oxidase; merA, mercuric reductase; sek, enterotoxin K; seq, enterotoxin Q; speG, spermidine N(1)-acetyltransferase.

"Parallel Epidemics of Community-Associated Methicillin-Resistant *Staphylococcus aureus* USA300 Infection in North and South America. Planet PJ, Diaz L, Kolokotronis SO, Narechania A, Reyes J, Xing G, Rincon S, Smith H, Panesso D, Ryan C, Smith DP, Guzman M, Zurita J, Sebra R, Deikus G, Nolan RL, Tenover FC, Weinstock GM, Robinson DA, Arias CA. *J Infect Dis.* 2015 Dec 15;212(12):1874-82.

ular characterization of CA-MRSA [168]. In a previous study, covering a period between 1993-2005, we identified about 150 MRSA with an *SCC_{mec}* IV or V and PVL element. The European clone ST80 was identified in 35% of our isolates and was the predominant clone. The others clones were ST5 and ST8. Moreover, we identified many genetic backgrounds originating from importation events all around the world [121, 167, 168]. In 2003, in HUG, we identified for the first time a patient with a CA-MRSA type USA300. This patient had recently traveled to New York City. Since then, USA300 has been identified sporadically in HUG until 2012. However, in 2013, because of the institutional surveillance, we observed a sudden and worrisome increase of this clone (ST8, PVL-positive).

2.3 Objective

Due to the potential virulence of the USA300 clone and its high transmissibility, an investigation was initiated. The goal of this study was to identify the causes of its increased occurrence to guide public health intervention. This study attempted to provide a better understanding of the origin and the dynamics of this clone that is emerging in our population.

2.4 Methods

2.4.1 Design

To perform this investigation, we realized a molecular epidemiological study in which we combined epidemiological characteristics with the molecular data of the strains from each colonized or infected patient.

2.4.2 Population, strain, and definition

In HUG, for each new patient identified as a positive MRSA carrier, the strain was typed for its *SCC_{mec}* element, *agr* locus, and gene encoding exotoxins [169]. All MRSA strains identified in a patient at the admission or an outpatient clinic and carrying either *SCC_{mec}* IV or V and the gene encoding for the PVL have been defined as a CA-MRSA. No additional patients carrying CA-MRSA were identified during hospitalization.

Some departments in the hospital have a universal screening at the admission. Asymptomatic CA-MRSA carriers could be identified in these departments. Moreover, other asymptomatic patients could be detected during household screening after identification of an infected index patient. As for infected patients, they have been documented because of a positive clinical sample. USA300 (North American) isolates were defined as MRSA isolate harboring an *SCC_{mec}* IVa element, the PVL gene and showing the multilocus ST8. As for the USA300-LV, it carries the same markers except that their *SCC_{mec}* element is

of IVc type.

The study population corresponds to all patients diagnosed at HUG with a colonization or an infection due CA-MRSA positive MRSA strain in 2013.

2.4.3 Epidemiological data and statistical analyses

For each CA-MRSA isolate, a case report form with the patient's epidemiological and clinical data was completed. The following data were collected [169,170]:

- Demographic characteristics
- Sample and site characteristics
- Contact with health care settings
- Potential risk factors for acquisition of MRSA
- Comorbidities

In term of statistical analysis, the carriers of USA300 clone were compared to the patients with other CA-MRSA clones to distinguish these two groups of patients. For descriptive analysis, we used proportions for categorical variables, median values and interquartile range for continuous variables. For hypothesis testing and comparison of cases with and without USA300 clones, we performed the Student *t*-test for normal variables, the Wilcoxon signed-rank test for skewed distributions, and the Fisher exact or χ^2 test for the homogeneity of proportions for categorical data. Statistical analyses were performed using Stata 12 (Stata, College Station, Texas).

The confidentiality of the data was guaranteed by an anonymous database for the storage of all epidemiological, clinical and laboratory data. Ethical approval for CA-MRSA studies was obtained prior to the study from the institutional review board, waiving the need for an individual informed consent [121].

2.4.4 Molecular data, whole-genome sequencing, and molecular analyses

For each patient identified with a CA-MRSA, the strain was frozen and stored at the central bacteriological laboratory of the HUG, to conduct subsequent analysis of the strains.

2.4.4.1 Whole-genome sequencing

High-throughput sequencing was used to sequence the 23 genomes of USA300 isolates. Twenty-two strains were isolated in 2013 from colonized or infected patients. One strain was the first USA300 clone identified in HUG in 2003, the oldest of our collection [52]. The first sequenced USA300 from USA in 2003 is considered as the reference clone for the analysis. The DNA of each strain was purified, then sequenced on the Illumina HiSeq (Illumina, San Diego, California, United States) (Figure 2.3).

A 100 bp paired-end reads, and bar codes strategy were used according to the Nextera XT kit (Illumina) and following the manufacturer's guidelines.

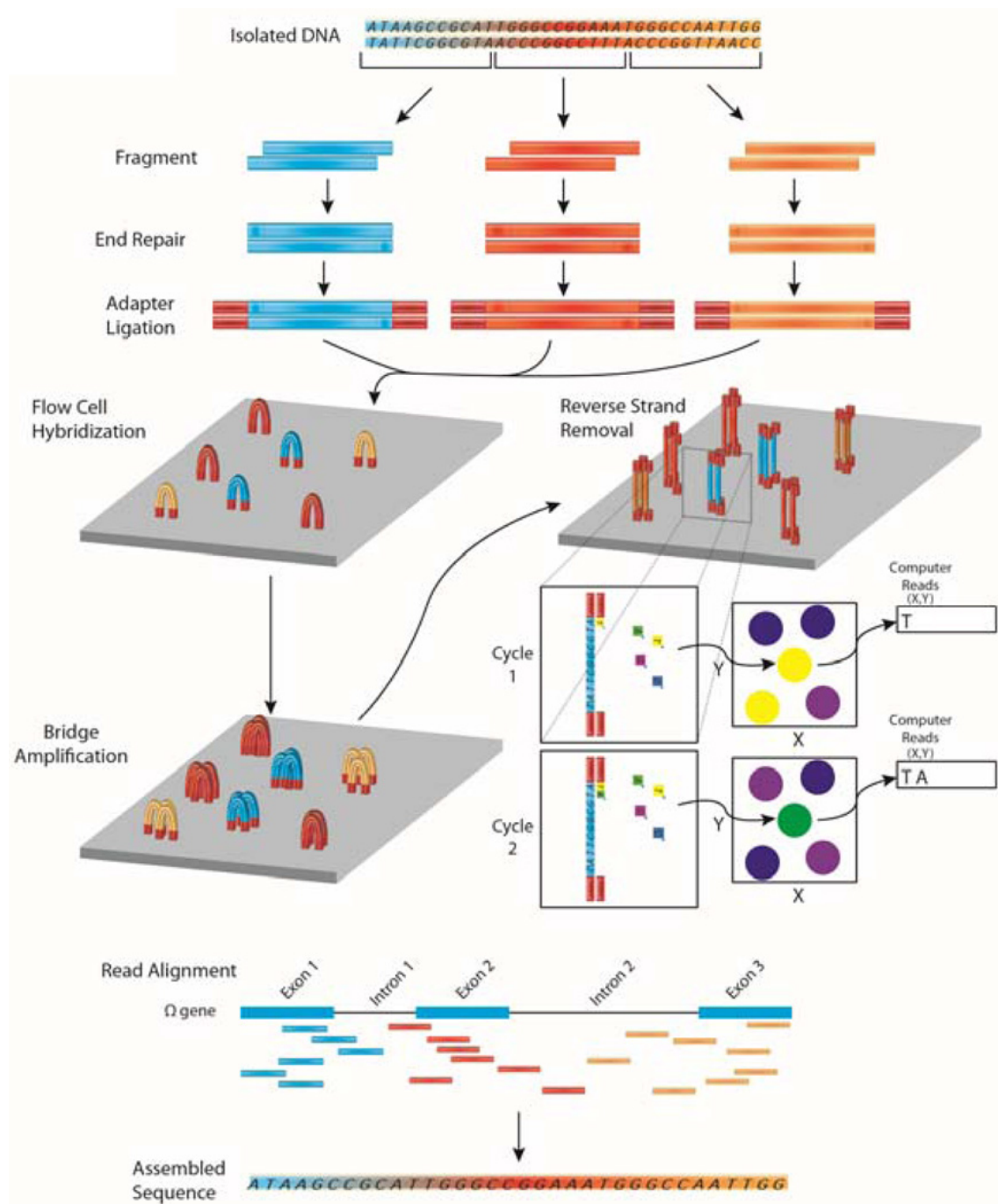
2.4.4.2 Sequence and genotyping analysis

Reading quality of the sequence was assessed with two programs: the Fastqc (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and the FastqMcf (<http://code.google.com/p/ea-utils/>) to filter. To assemble the genome, we performed an overlap of 63 bp using the Edena V3 assembler [170]. The following step is to annotate the genome, using the Prokka v1.10 program [171]. Once the genome was annotated, multilocus sequence analysis was performed on it and then submitted to the Center for Genomic Epidemiology database (<http://cge.cbs.ctu.dk/services/MLST>). The phylogenetic comparison of all sequences was made by genomic single-nucleotide polymorphism- (SNP) based analysis using as the reference genome *S. aureus* USA300_FPR3757 [51] with the Parsnp v1.0 program [172]. The presence of some genes involved in the phenotypes, evolution, and virulence of the strains have been evaluated by BlastP analysis. Moreover, 25 published genomes of *S. aureus* ST8 strains [173] from the European Nucleotide Archive database were used for genome comparison (Table 2.1).

2.4.4.3 Marker stability and microevolution

To perform marker stability analysis and microevolution rates, we compared SCC mec elements IVa and IVc and ACME locus between *S. aureus* and *S. epidermidis*. Genomics SNPs analysis from our isolates and the reference genome were assessed to identify the type of selection acting on each locus. The allelic and nucleotide diversity were calculated from a random selection of genes differing from the genomic reference sequence. For these analyses, the proportion of synonymous (ds) and nonsynonymous (dn) mutations have been calculated. The theoretical values for the different schemes of selection were compared to the next ratio dn/ds; a value of 0 explains a neutral evolution, while a value of 1 and -1 indicated a diversifying and purifying selection, respectively [174].

Figure 2.3: Overview of DNA sequencing using the Illumina platform.



In next generation DNA sequencing, DNA is first fragmented into smaller input-sized fragments by enzymes or by sonication. The ends of these fragments are repaired and specific adapters are ligated to the ends of the fragments, allowing hybridization to a flow cell to occur. A bridge amplification step is performed to create a "cluster" of fragments with the same sequence. One strand of DNA is removed and fluorescently labeled nucleotides are passed by each cluster. An image of the flow cells is recorded for the first cycle and a computer processes which nucleotide was incorporated at each cluster's co-ordinates. The fluorescent label is cleaved and a second round of fluorescently labeled nucleotides is passed by each cluster. Again (cycle 2) the nucleotide is recorded and each cycle leads to the sequence of each fragment. These reads are then aligned to a reference genome. By assembling reads (merging short reads together), it is therefore possible to reconstruct the unfragmented original sequence.

"Accuracy of multiplexed Illumina platform-based single-nucleotide polymorphism genotyping compared between genomic and whole genome amplified DNA collected from multiple sources. Paynter RA, Skibola DR, Skibola CF, Buffler PA, Wiemels JL, Smith MT. Cancer Epidemiol Biomarkers Prev. 2006 Dec;15(12):2533-6."

Table 2.1: List of published *S. aureus* genomes included in this study. Illumina read sequences of these isolates were retrieved from ENA database.

Isolates	ENA		FQ		ACME	Sa2int	SaPI5
	Accession	type	resistance	SCCmec			
1	ERS092759	colonizer	susceptible	IVc	0	1	1
2	ERS092768	environmental	susceptible	IVa	1	1	1
3	ERS092769	colonizer	resistant	IVa	1	1	0
4	ERS092770	clinical	resistant	IVg	0	1	0
5	ERS092779	clinical	resistant	IVa	1	1	0
6	ERS092798	clinical	susceptible	IVc	0	1	0
7	ERS092805	colonizer	susceptible	MSSA	1	1	1
8	ERS092806	environmental	susceptible	MSSA	0	0	0
9	ERS092809	colonizer	resistant	IVg	0	1	0
10	ERS092818	colonizer	susceptible	IVc	0	1	1
11	ERS092832	environmental	resistant	IVg	0	1	0
12	ERS092837	colonizer	susceptible	IVb	0	1	1
13	ERS092838	environmental	susceptible	IVb	0	1	1
14	ERS092851	colonizer	resistant	IVa	1	1	1
15	ERS092860	clinical	susceptible	IVb	0	1	1
16	ERS092952	clinical	susceptible	IVa	1	1	1
17	ERS092976	environmental	resistant	IVa	1	1	1
18	ERS092980	clinical	resistant	IVa	1	1	1
19	ERS092983	clinical	resistant	MSSA	0	1	1
20	ERS092987	colonizer	resistant	IVa	0	1	1
21	ERS093016	clinical	susceptible	IVc	0	1	1
22	ERS093036	clinical	resistant	IVa	1	1	1
23	ERS093076	colonizer	susceptible	IVb	0	1	1
24	ERS093089	clinical	susceptible	IVa	1	1	1
25	ERS093099	clinical	susceptible	IVc	0	1	0

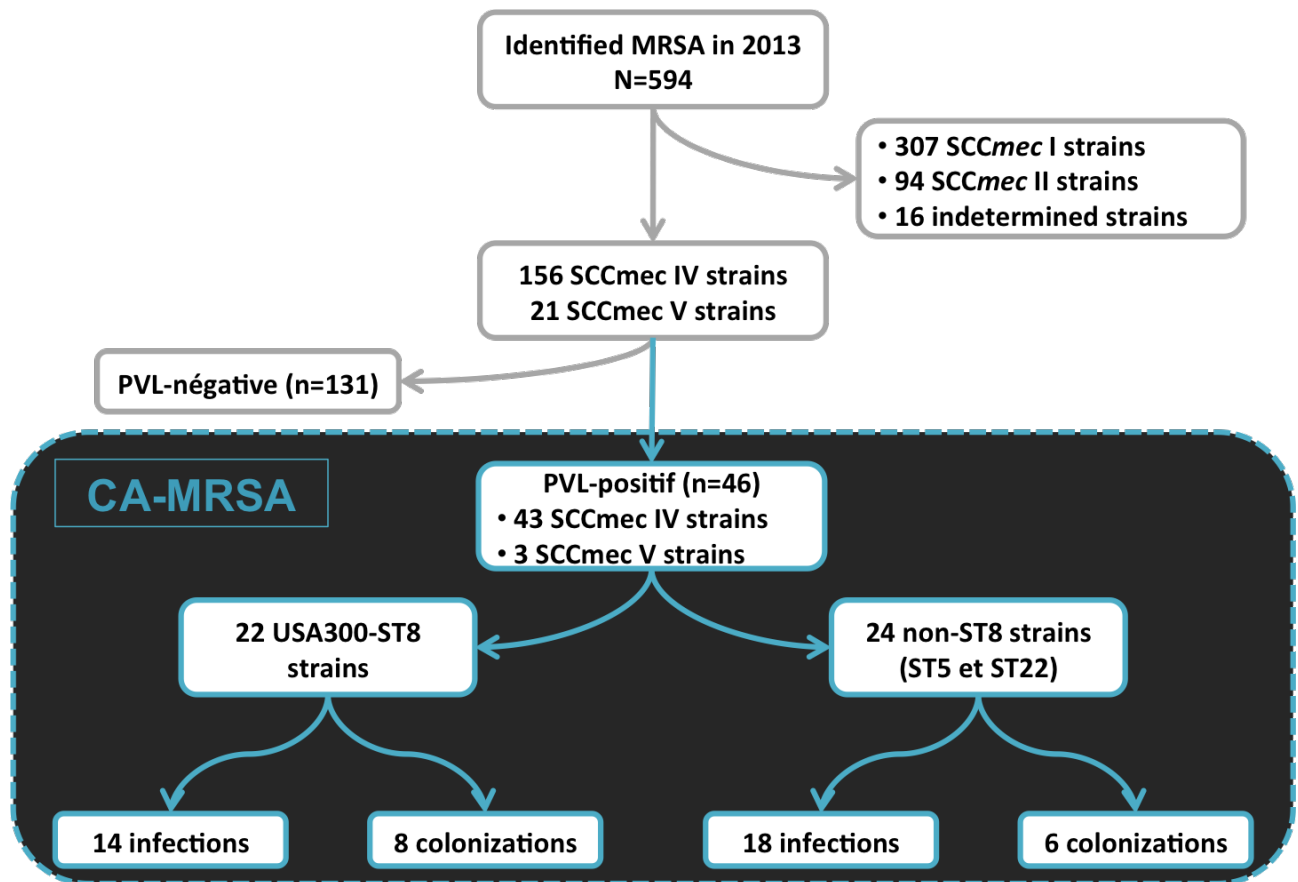
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2.5 Results

2.5.1 Epidemiological investigation

In 2012, the prevalence of the USA300 CA-MRSA strain was low in HUG. Only four isolates of ST8-USA300 or USA300-like clones were identified in infected or colonized patients. One year later, in 2013, the prevalence of these clones considerably increased. Among 594 MRSA isolates characterized, 307 were SCCmec I, 94 were SCCmec II, 156 were SCCmec IV, 21 were SCCmec V, and finally 16 were no type-able. Overall, 46 strains among 594

Figure 2.4: Study Flow-chart.



were PVL positive (7.7%) and the majority had an *SCCmec* IV ($n = 43$) (Figure 2.4).

In 2013, in HUG the isolation rates of CA-MRSA was 0.48 cases/100 admissions. Forty-six CA-MRSA were isolated, and only four were ST80. Thus, we detected a sudden increase of USA300 and USA300-like strains. Almost half of the cases belongs to this clone, with 22 isolates (48%). These 22 isolates were identified from 14 clinical infections and 8 cases of asymptomatic colonization. The remaining clones belonged to ST5 and ST22. Figure 2.5 shows the distribution of the sites of infection of the CA-MRSA across the body. The diagram shows that the sites of isolation of USA300 or USA300-like clones were distributed across the body rather than being localized to a particular body area (Table 2.2).

We decided to investigate the possibility of a cantonal outbreak, considering the place of living of each USA300 carrier in Geneva and close to Geneva (Figure 2.6). No evidence of a specific geographical clustering was identified on this map, and no single location was shared by USA300 carriers in collective housing as a place of treatment, location of work, similar school or nursing and retirement home (Figure 2.6).

Figure 2.5: Human body diagram of identification of infected sites.

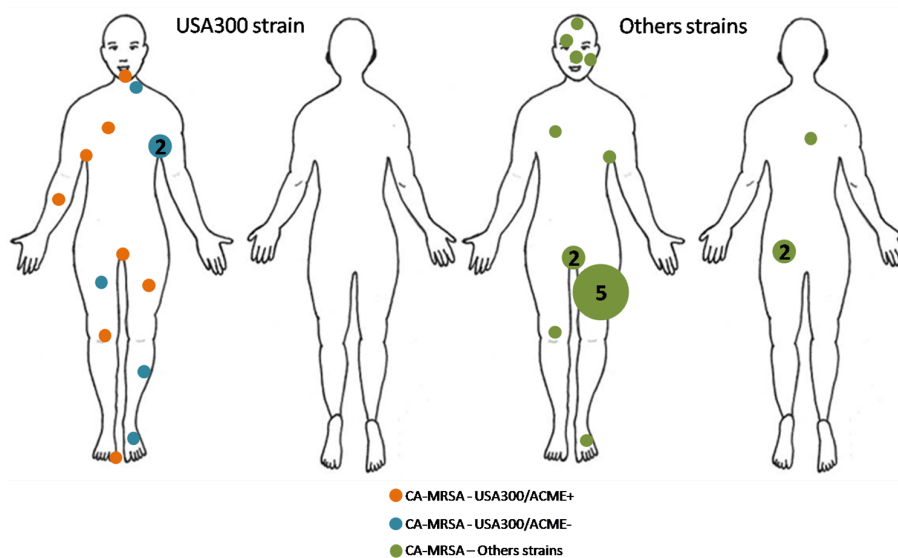


Table 2.2: Sites of identification of infecting isolates.

Infected site, n (%)	All patients, with CA-MRSA (N=46)	Patients with USA300 or USA-like strains (N=22)	Patients with other CA-MRSA strains (N=24)	P value
Groin	2 (4.3)	1 (4.5)	1 (4.2)	0.478
Axillary	4 (8.7)	3 (13.6)	1 (4.2)	0.336
Arm	1 (2.2)	1 (4.5)	0 (0.0)	1.000
Neck	1 (2.2)	1 (4.5)	0 (0.0)	0.478
Thigh	7 (15.2)	2 (9.1)	5 (20.8)	0.478
Knee	2 (4.3)	1 (4.5)	1 (4.2)	1.000
Chin	1 (2.2)	1 (4.5)	0 (0.0)	0.478
Foot	2 (4.3)	1 (4.5)	1 (4.2)	1.000
Thorax	2 (4.3)	1 (4.5)	1 (4.2)	1.000
Tibia	1 (2.2)	1 (4.5)	0 (0.0)	0.478
Hallux	1 (2.2)	1 (4.5)	0 (0.0)	0.478
Buttock	2 (4.3)	0 (0.0)	2 (8.3)	0.490
Nose	1 (2.2)	0 (0.0)	1 (4.2)	1.000
Back	1 (2.2)	0 (0.0)	1 (4.2)	1.000
Cheek	1 (2.2)	0 (0.0)	1 (4.2)	1.000
Eyelid	1 (2.2)	0 (0.0)	1 (4.2)	1.000
Scalp	1 (2.2)	0 (0.0)	1 (4.2)	1.000
Vulva	1 (2.2)	0 (0.0)	1 (4.2)	1.000

Table 2.3: Characteristics of patients with CA-MRSA infection or carriage identified at HUG in 2013.

Characteristics	All patients (n=46)	Patients with		P values ^a
		ST8-USA300 (n=22)	CA-MRSA non-ST8 (n=24)	
Male sex	23 (50.0)	13 (59.1)	10 (41.7)	0.238
Age, years	31 (7-50)	32 (15-43)	30.5 (7-53.5)	0.768
Employment status				
Employed	15 (32.6)	10 (45.5)	5 (20.8)	0.075
Unemployed	9 (19.6)	4 (18.2)	5 (20.8)	0.821
Retired	6 (13.0)	2 (9.1)	4 (16.7)	0.667
Student	16 (34.8)	6 (27.3)	10 (41.7)	0.306
Nationality				
Switzerland	22 (47.8)	9 (40.9)	13 (54.2)	0.369
Other European countries	5 (10.9)	3 (13.6)	2 (8.3)	0.659
North America	0 (0.0)	0 (0.0)	0 (0.0)	1.000
South America	8 (17.4)	7 (31.8)	1 (4.2)	0.020
Africa	8 (17.4)	3 (13.6)	5 (20.8)	0.702
Asia	3 (6.5)	0 (0.0)	3 (12.5)	0.235
People living in the same household				
Adults	3.5 (2-4)	3 (2-4)	4 (2-4)	0.525
Children	2 (2-2)	2 (2-2)	2 (2-2)	0.663
	1 (0-2)	1 (0-2)	2 (0-2)	0.506
Newly identified case of CA-MRSA	41 (89.1)	20 (90.9)	21 (87.5)	0.711
Type of sample				
Clinical sample from				
Infected site	32 (69.6)	14 (63.6)	18 (75.0)	0.403
Abscess specimen	24 (75.0)	8 (57.1)	16 (88.9)	0.040
Other	8 (25.0)	6 (42.9)	2 (11.1)	0.040

Data are number (%) of subjects or median value (interquartile range).

^a Comparison between patients colonized or infected by ST8-USA300 vs non-ST8 CA-MRSA strains.

Table 2.3 (continued): Characteristics of patients with CA-MRSA infection or carriage identified at HUG in 2013.

Characteristics	All patients (n=46)	Patients with ST8-USA300 (n=22)	Patients with	P values ^a
			CA-MRSA non-ST8 (n=24)	
MRSA screening swab	14 (30.4)	8 (36.4)	6 (25.0)	0.403
Hospital stay in the past 12 months	12 (26.1)	4 (18.2)	8 (33.3)	0.242
Outpatient care in the past 12 months	35 (76.1)	7 (77.3)	18 (75.0)	0.857
Professional contact with patients	1 (2.2)	1(4.5)	0 (0.0)	0.478
Potential risk factors for CA-MRSA acquisition in past 12 months				
Contact with family member with similar lesions	3 (6.5)	2 (9.1)	1 (4.2)	0.600
Stay in collective housing	3 (6.5)	0 (0.0)	3 (12.5)	0.235
Travel outside Switzerland	39 (84.8)	19 (86.4)	20 (83.3)	0.775
Place of travel				
<i>North America</i>	8 (20.5)	6 (31.6)	2 (10.0)	0.127
<i>South America</i>	13 (33.3)	11 (57.9)	2 (10.0)	0.002
<i>Colombia</i>	6 (42.9)	6 (50.0)	0 (0.0)	0.473
<i>Ecuador</i>	2 (14.3)	2 (16.7)	0 (0.0)	1.000
<i>Bolivia</i>	1 (7.1)	1 (8.3)	0 (0.0)	1.000
<i>Brazil</i>	2 (14.3)	1 (8.3)	1 (50.0)	0.275
<i>Uruguay</i>	2 (14.3)	1 (8.3)	1 (50.0)	0.275
<i>Africa</i>	7 (17.9)	2 (10.5)	5 (25.0)	0.407
<i>Asia</i>	11 (28.2)	0 (0.0)	11 (55.0)	0.001
Recent use of antibiotics	13 (28.3)	6 (27.3)	7 (29.2)	0.887
Team or contact sports	3 (6.5)	2 (9.1)	1 (4.2)	0.600

Data are number (%) of subjects or median value (interquartile range).

^a Comparison between patients colonized or infected by ST8-USA300 vs non-ST8 CA-MRSA strains.

Table 2.3 (continued): Characteristics of patients with CA-MRSA infection or carriage identified at HUG in 2013.

Characteristics	All patients (n=46)	Patients with		P values ^a
		ST8-USA300 (n=22)	CA-MRSA non-ST8 (n=24)	
Sexual preferences				
<i>Heterosexual</i>	29 (63.0)	14 (63.6)	15 (62.5)	0.936
<i>Homosexual/bisexual</i>	2 (4.3)	2 (9.1)	0 (0.0)	0.223
<i>Not applicable or unknown</i>	15 (32.6)	6 (27.3)	9 (37.5)	0.460
Presence of any comorbidity	5 (10.9)	2 (9.1)	3 (12.5)	1.000
<i>Immunosuppression</i>	2 (4.4)	1 (4.5)	1 (4.2)	1.000
<i>Diabetes mellitus</i>	4 (8.7)	1 (4.5)	3 (12.5)	0.609

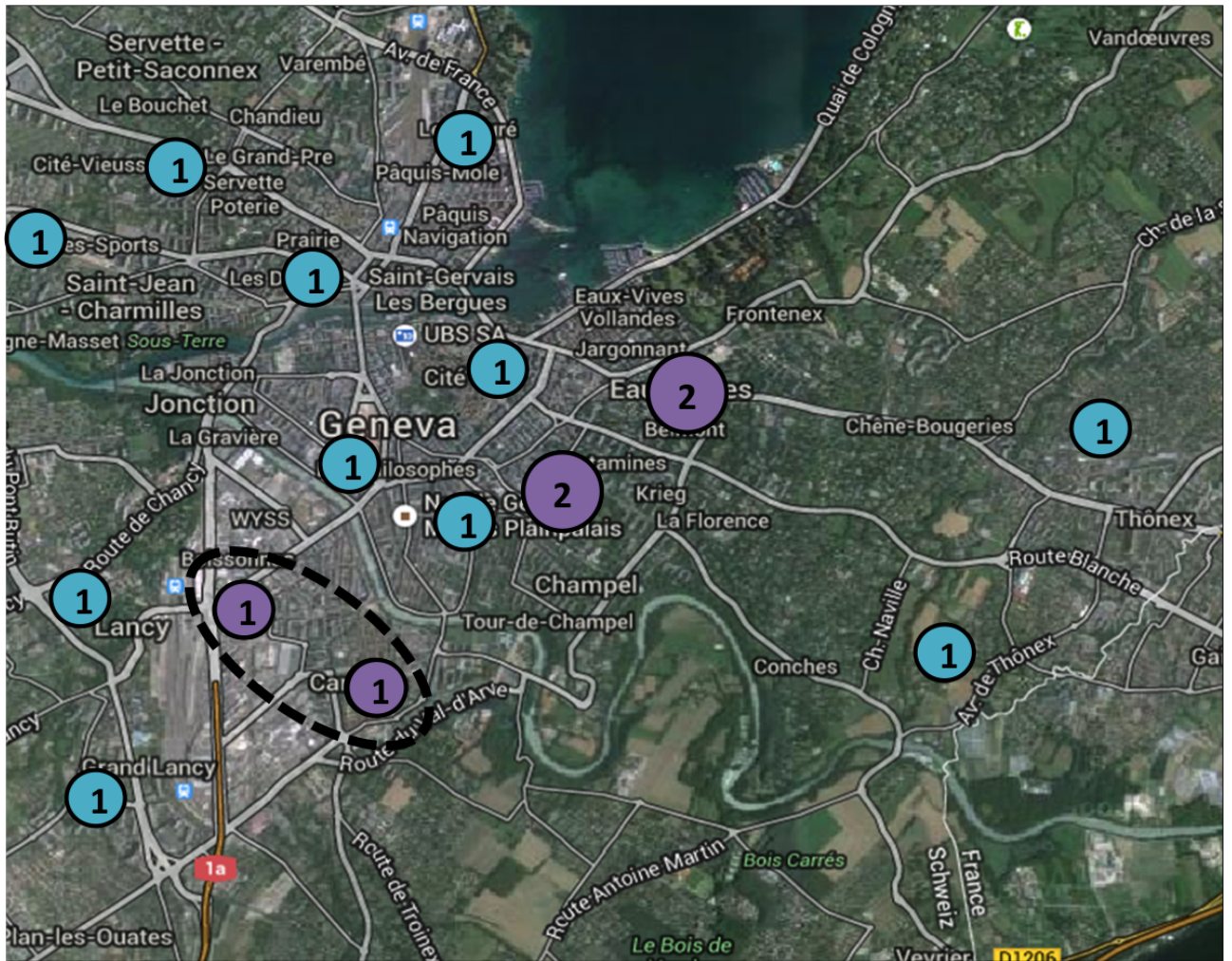
Data are number (%) of subjects or median value (interquartile range).

^a Comparison between patients colonized or infected by ST8-USA300 vs non-ST8 CA-MRSA strains.

Table 2.3 presents the epidemiological and clinical data of patients with ST8-USA300/USA300-like and non-ST8-USA300 CA-MRSA strains. Among patients with CA-MRSA, there was an equal distribution of gender, with 50% of male for all CA-MRSA carriers and 59.1% and 41.7% for ST8-USA300/USA300-like and non ST8-USA300 CA-MRSA from patients respectively. The patients were relatively young with a median age at 31 years (range from 0 to 87 years) as often with CA-MRSA unlike patients carrying HA-MRSA. No particular statistical difference was found between the two groups in term of employment status, people living in the same household, newly identified CA-MRSA, link with a healthcare environment, new antibiotics use, practiced team or combat sports, sexual preference or comorbidities (immunosuppression and diabetes mellitus). Approximately, 70% of patients developed a clinical infection, with a majority of the abscesses in the group of the non ST8-USA300 CA-MRSA carriers.

Few CA-MRSA carriers had previous exposure to healthcare. However, travel outside of Switzerland in the last year was extremely frequent in both groups. Investigating the place of the journey of these patients, we highlighted a significant difference between the two groups (Figure 2.7). More patients traveled to South America in the ST8-USA300 group (57.9% versus 10.0%, P-value=0.002). Conversely, a majority of patients in the non-ST8-USA300 group traveled in Asia, specifically in the Philippines (55.0% versus 0.0%, P-value=0.001). Moreover, 31.6% of the ST8-USA300 group and 10.0% of the non ST8-USA300 group were in North America in the past 12 months. Many of the patients in the ST8-USA300 group who have traveled in South America also have a South American nationality (31.8% versus 4.2%, P-value=0.020).

Figure 2.6: Place of living of USA300 MRSA carriers (Geneva map).



Place of living of USA300 MRSA carrier outside of Geneva:

- Switzerland: 1 carrier in Lausanne.
- France: 3 carriers in Viry, Annecy and Fillings.
- USA: 1 carrier in New York.

Figure 2.7: Place of travel in the previous 12 months.



2.5.2 Genotyping and main genomic content of isolates

All CA-MRSA, USA300 types sequenced were deposited in the DDBJ/EMBL/GenBank database, under the following numbers:

MRSA1: LFUU00000000
MRSA2: LFUV00000000
MRSA4: LFUW00000000
MRSA5: LFUX00000000
MRSA7: LFUY00000000
MRSA8: LFUZ00000000
MRSA9: LFVA00000000
MRSA10: LFVB00000000
MRSA11: LFVC00000000
MRSA12: LFVD00000000
MRSA13: LFVE00000000
MRSA14: LFVF00000000
MRSA15: LFVG00000000
MRSA17: LFVH00000000
MRSA18: LFVI00000000
MRSA19: LFVJ00000000
MRSA20: LFVK00000000
MRSA21: LFVL00000000
MRSA22: LFVM00000000
MRSA23: LFVN00000000
MRSA24: LFVO00000000
MRSA26: LFVP00000000
MRSA28: LFVP00000000

According to this WGS, for each isolate, between 7'638'322 and 9'605'888 paired reads were produced and the average genome coverage was around 270x. All strains, their gene contents, and WGS analysis are presented in Table 2.4.

In this table, for each isolate is specified the origin or travel exposure, the date of isolation, the clinical presentation (infection or colonization), its genome size, the percentage of GC content, the SCC*mec* type, and the presence of *mecA*, ACME, Sa1int, Sa2int, SaPI5, and Sa3int. Genome assembly produced between 21 and 40 contigs per sequenced genome (Table. 2.5).

For the 23 sequenced isolates, the genome size varied from 2.83 to 2.95 Mb, with a percentage of GC content from 32.6% to 32.7%. The sequencing confirmed the presence of *mecA*, *pvlS* and *pvlF* in each ST8-USA300 or USA300 like clones. One or 2 circular plasmids were detected from 8 CA-MRSA strains with a size between 3.22kb and 20.52kb (Table 2.5).

Table 2.4: Epidemiologic, clinical, and genomic characteristics of ST8-USA300 or USA300-like CA-MRSA isolates subjected to WGS.

Strains	Origin or Travel exposure ^a	Isolation date	Clinical presentation	Genome size, Mb	GC content %	SCCmec		SaPI5	Sa2int	Sa1int	Sa3int	
						mecA	type					
S1	Columbia	Jan 2013	Infection	2861	32.66	+	IVc	-	+	-	+	+
S2	Columbia	Jan 2013	Colonization	2863	32.66	+	IVc	-	+	-	+	+
S4	Switzerland	Feb 2013	Colonization	2881	32.66	+	IVa	+	+	-	+	+
S5	US	Feb 2013	Infection	2890	32.65	+	IVa	+	+	-	+	+
S7	Ecuador	Apr 2013	Infection	2894	32.70	+	IVc	-	+	-	-	+
S8	Cuba	Apr 2013	Colonization	2885	32.65	+	IVa	+	+	-	+	+
S9	Columbia	Apr 2013	Colonization	2861	32.66	+	IVc	-	+	-	+	+
S10	Switzerland	Apr 2013	Infection	2913	32.67	+	IVa	+	+	-	-	+
S11	Ecuador	Apr 2013	Colonization	2894	32.69	+	IVc	-	+	-	-	+
S12	Brazil	Jun 2013	Colonization	2913	32.67	+	IVa	+	+	-	+	+
S13	Bolivia	Jun 2013	Infection	2852	32.67	+	IVc	-	+	-	+	+
S14	Uruguay	Jun 2013	Infection	2837	32.68	+	IVc	-	+	-	-	+
S15	Columbia	Jul 2013	Infection	2831	32.66	+	IVc	-	+	-	+	+
S17	Columbia	Aug 2013	Infection	2908	32.69	+	IVc	-	+	-	+	+
S18	US	Aug 2013	Infection	2882	32.65	+	IVa	+	+	-	+	+
S19	Columbia	Aug 2013	Infection	2831	32.66	+	IVc	-	+	-	+	+
S20	US	Apr 2013	Colonization	2947	32.71	+	IVa	+	+	-	+	+
S21	Cameroon	Sep 2013	Infection	2908	32.63	+	IVa	+	+	-	+	+
S22	Switzerland	Oct 2013	Infection	2929	32.66	+	IVa	+	+	-	+	+
S23	Sierra Leone	Oct 2013	Colonization	2853	32.67	+	IVa	+	+	-	+	+
S24	US	Nov 2013	Infection	2853	32.67	+	IVc	-	+	-	+	+

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Abbreviations: -, absent in the genome; +, present in the genome; ACME, arginine catabolic mobile element; mecA, methicillin resistance gene; SCCmec, staphylococcal cassette chromosome mec.

^a Data denote the country where the patient was originally from or where they traveled during the year before CA-MRSA isolation.

Table 2.4 (continued): Epidemiologic, clinical, and genomic characteristics of ST8-USA300 or USA300-like CA-MRSA isolates subjected to WGS.

Strains	Origin or Travel exposure ^a	Isolation date	Clinical presentation	Genome size, Mb	GC content %	mecA	SCCmec		Sa1int	Sa2int	SaP15	Sa3int
							type	ACME				
S26	US	Nov 2013	Infection	2848	32.67	+	IVc	-	-	+	+	+
S28	US	Dec 2013	Infection	2923	32.67	+	IVa	+	-	+	+	+

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Abbreviations: -, absent in the genome; +, present in the genome; ACME, arginine catabolic mobile element; mecA, methicillin resistance gene; SCCmec, staphylococcal cassette chromosome mec.

^a Data denote the country where the patient was originally from or where they traveled during the year before CA-MRSA isolation.

Table 2.5: List of the 23 *S. aureus* sequenced genomes.

Strains	Number of contigs	Number n:N50	Mean contig size (kb)	Mean contig size (Mb)	Genome coverage (x)	Number plasmids
MRSA_S1	25	4	114.46	2.861	250.3	1 (20.51 kb)
MRSA_S2	25	3	114.50	2.863	253.5	1 (20.51 kb)
MRSA_S4	40	8	72.03	2.881	245.9	-
MRSA_S5	28	3	103.22	2.890	279.5	2 (4.54 kb ; 3.22 kb)
MRSA_S7	28	4	103.35	2.894	277.1	-
MRSA_S8	34	3	84.85	2.885	272.3	-
MRSA_S9	30	4	95.37	2.861	259.8	-
MRSA_S10	33	5	88.28	2.913	248.6	-
MRSA_S11	26	4	111.31	2.894	262.0	-
MRSA_S12	29	4	100.44	2.913	239.6	-
MRSA_S13	30	4	95.06	2.852	275.6	-
MRSA_S14	25	3	113.48	2.837	281.8	-
MRSA_S15	27	3	104.87	2.831	267.1	2 (20.51 kb : 4.54 kb)
MRSA_S17	27	4	107.70	2.908	281.1	-
MRSA_S18	24	3	120.10	2.882	277.3	-
MRSA_S19	25	3	113.23	2.831	291.4	2 (20.51 kb : 4.54 kb)
MRSA_S20	31	3	95.08	2.947	275.0	1 (16.62 kb)
MRSA_S21	36	4	80.77	2.908	285.2	-
MRSA_S22	31	4	94.49	2.929	275.2	-
MRSA_S23	21	3	135.86	2.953	281.3	-
MRSA_S24	26	3	109.71	2.852	266.6	-
MRSA_S26	26	3	109.55	2.848	274.2	1 (20.52 kb)
MRSA_S28	29	3	100.80	2.923	271.3	-

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According to the Table 2.6, all isolates contained the core adhesion genes, *clfA* (except strain MRSA_10), *clfB* (except strain MRSA_9), *fnbA*, *mapW*, *sdrC*, and *spa*, the accessory genes *ebpS*, *fnbB*, *sdrB*, *sdrD*, and *sdrE* (except strain MRSA_9) and the hemolysin genes *hly*, *hlyB*, *hlyD* and *hlyG*. However, the three toxin genes *eta*, *etb* and *tsst-1* and the core adhesion gene *cna* were absent from these isolates. The core adhesion gene *fnbA* and the accessory adhesion gene *fnbB* are known to contribute to the interaction with fibrinogen or fibronectin. All isolates also harbored a functional *agr* locus.

The genome comparison highlighted two diverse groups of isolates: one first group with the presence of the full ACME cluster integrated into the genome (11 strains - ACME-positive group) and the second group lacking the ACME locus (12 isolates - ACME-negative group) (Table 2.6 and Figure 2.8).

Table 2.6: Investigation of toxin, adhesion, and hemolysin genes in all MRSA isolates.

ACME group	Strain name	Toxin genes			Core adhesion genes						Accessory adhesion genes				Hemolysin genes			
		<i>eta</i>	<i>etb</i>	<i>tst-1</i>	<i>clfA</i>	<i>clfB</i>	<i>fbaA</i>	<i>mapW</i>	<i>sdnC</i>	<i>spa</i>	<i>cna</i>	<i>ebpS</i>	<i>fnbB</i>	<i>sdnD</i>	<i>sdnE</i>	<i>hly</i>	<i>hnb</i>	<i>hld</i>
	S1	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S2	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S7	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S9	-	-	-	+	-	+	+	+	-	+	+	+	-	+	+	+	+
	S11	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
ACME -	S13	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S14	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S15	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S17	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S19	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S24	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S4	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S5	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S8	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S10	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S12	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
ACME +	S18	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S20	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S21	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S22	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S23	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	S26	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+

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According to the comparison between the locus ACME present in USA300 strain and *S. epidermidis*, it was found to be highly conserved in both strains with a similarity 99%. Moreover, the ACME locus is flanked by transposase genes (*rev*), suggesting a separate acquisition of the locus in this mobile genetic element (MGE) (Figure 2.9).

In contrast, when we compare the peptide transporter operon (*opp* locus) close to the ACME locus, we identified a high variability with 97% nucleotide identity and 144 SNPs between both species. It proves the two different acquisition events in *S. aureus*: one for the ACME locus and the second one for the *opp* locus (Figure 2.10).

According to the various levels of polymorphisms in both loci, we can conclude that the ACME locus is highly conserved compared to the *opp* locus. Moreover, the dn/ds values of the ACME genes are very low whereas the dn/ds values of the *opp* locus are higher, as the latter is subject to diversifying selection (Figure 2.10).

Indeed, all isolates in the ACME-negative group are susceptible to ciprofloxacin and erythromycin, whereas the strains of the ACME-positive group were resistant except for two isolates susceptible to erythromycin and one to ciprofloxacin. For the resistance profile, all isolates were susceptible to vancomycin, fosfomicin, fusidic acid, teicoplanin, linezolid, TMP-SMX and mupirocin (Table 2.7).

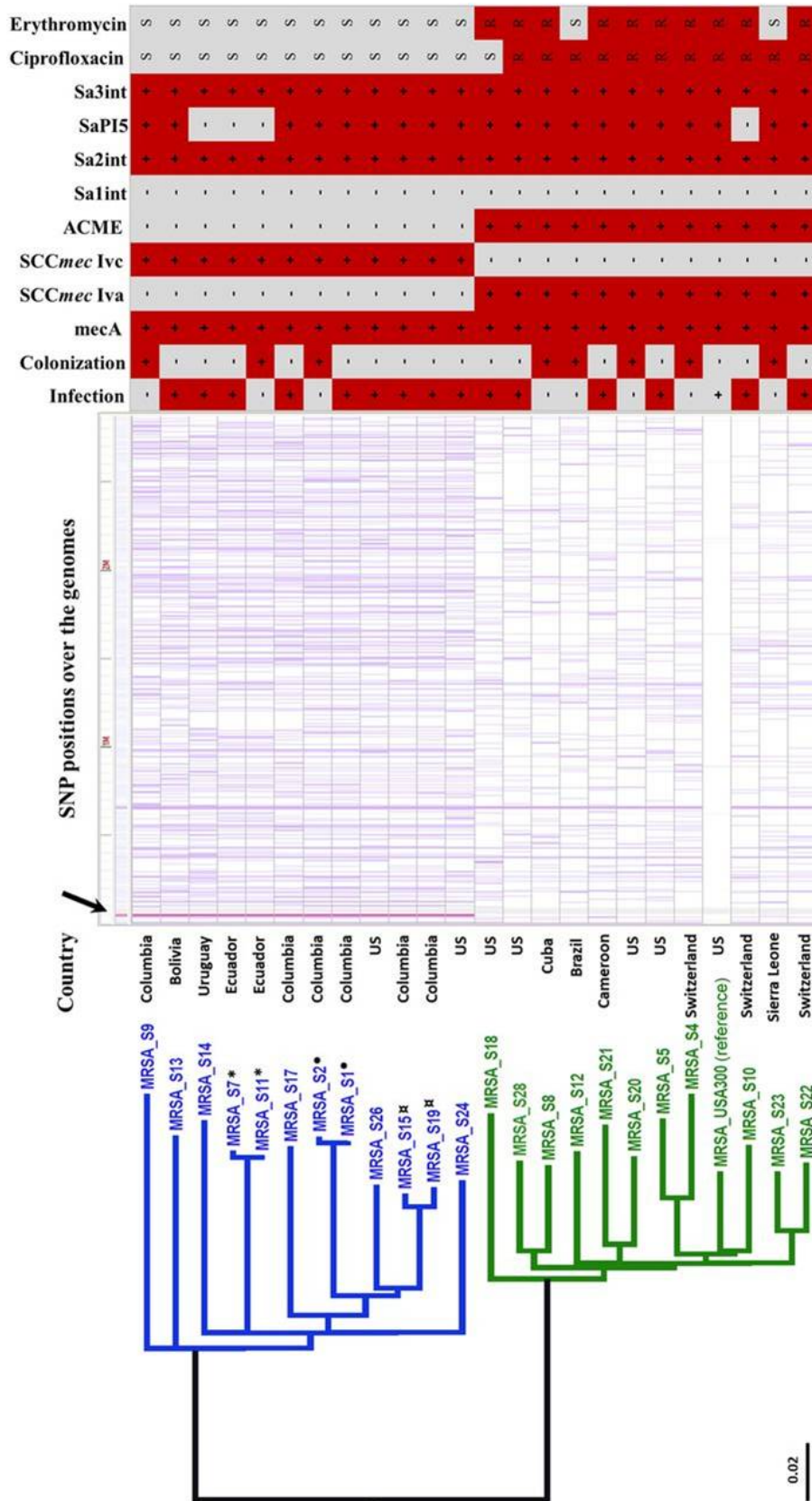
2.5.3 SNPs between whole genomes

All CA-MRSA ST8-USA300 isolated in 2013 were compared to the reference genome of USA300_FPR3757 in terms of SNPs in the conserved genome core. These comparisons revealed two groups of isolates, separated according to the number of SNPs identified in their respective genome and showing similar distances to the root of the tree (Figure 2.8). These two groups of isolates in terms of SNPs were similar to the ACME-positive and -negative groups. A phylogenetic tree has been constructed according to the number of SNPs; it was not consistent with a grouping of clones according to their potential country of origin or travel exposure. Figure 2.11 shows that the number of SNPs in the ACME-positive group (94-157 SNPs) was lower than in the ACME-negative group (243-393 SNPs).

We also performed a pair-wise comparison between genomes within each cluster (Figure 2.12), showing that the ACME-positive group has significantly more SNPs than the ACME-negative group (186 versus 140 SNPs, P value <0.05).

The analysis of SNPs in coding regions shows that open-reading frames were 10-fold less likely to present SNPs than in intergenic regions. A hot spot of mutation has been identified by detailed SNPs analysis that correspondes to the highly variable open reading frame: 1788 base pairs, 34,45% GC (Figure 2.8). This sequence encodes a conserved hypothetical protein upstream of the *SCCmec* cassette.

Figure 2.8: Phylogenetic comparison of strain collection.

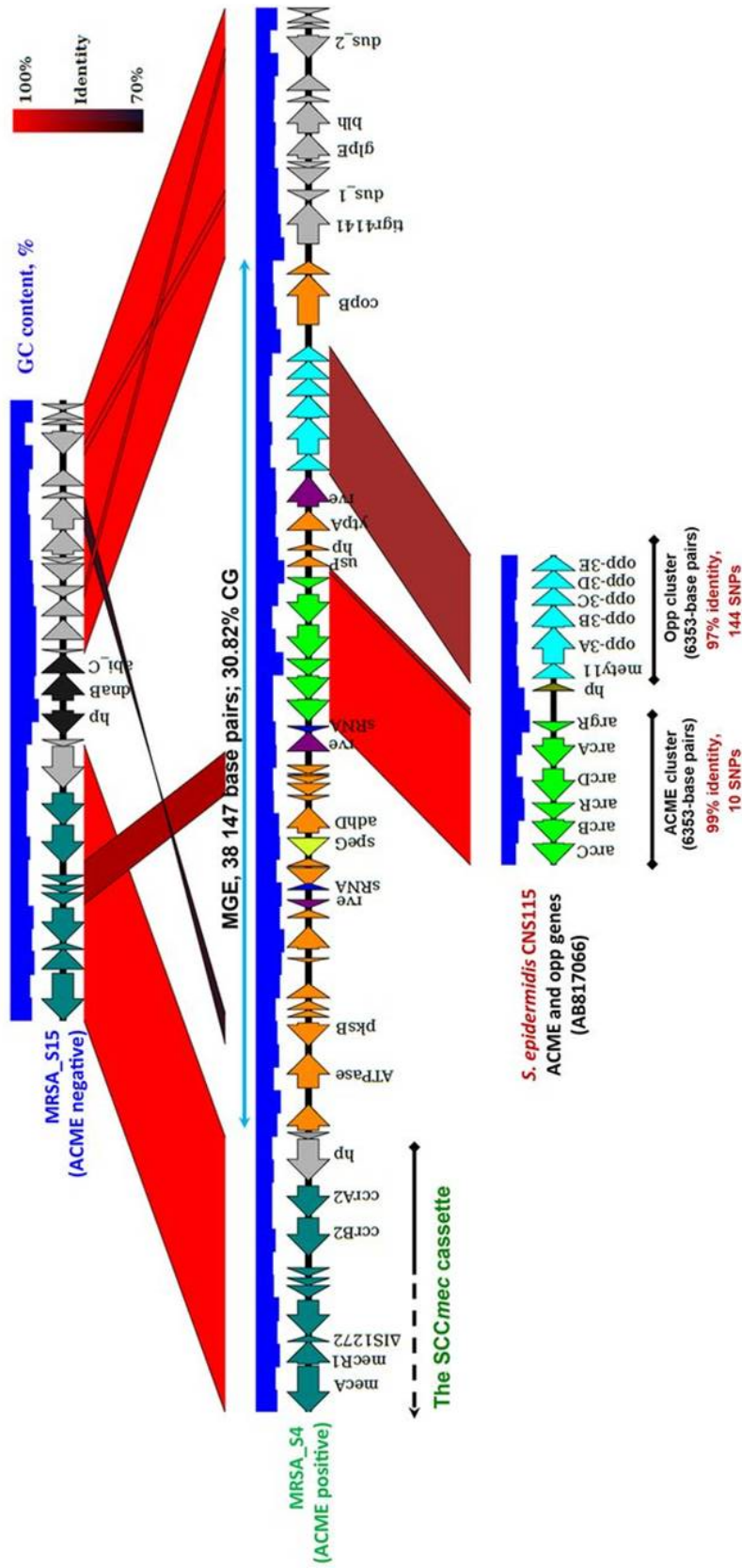


Loci known to be a contributor of the bacterial virulence [173] have been identified in a majority of strains (Table 2.4). For example, the pathogenicity island *S2int* and *Sa3int* were present in all isolates, whereas *SaPI5* was found in 19 isolates. *SaPI5* was absent in one strain from the ACME-positive group and from three strains from the ACME-negative group.

The resistance profile of both ACME-positive and -negative groups was relatively different, particularly for ciprofloxacin and erythromycin (Figure 2.8). The USA300 genome is linearized horizontally, and each vertical bar represents a locus containing nucleotide poly- morphism, compared with the reference strain (methicillin-resistant *Staphylococcus aureus* [MRSA] USA300 FPR3757). The country where the patient was originally from or where they had traveled during ≤ 1 year before strain isolation is shown. Two clear clusters describing arginine catabolism mobile element (ACME)-negative (upper cluster) and ACME-positive (lower cluster) strains are visible. Transmission events are indicated by symbols (*, *bullet*, and \square). Abbreviations: -, negative; +, positive; R, resistant; S, susceptible; SCC*mec*, staphylococcal cassette chromosome *mec* ; SNP, single-nucleotide polymorphism.

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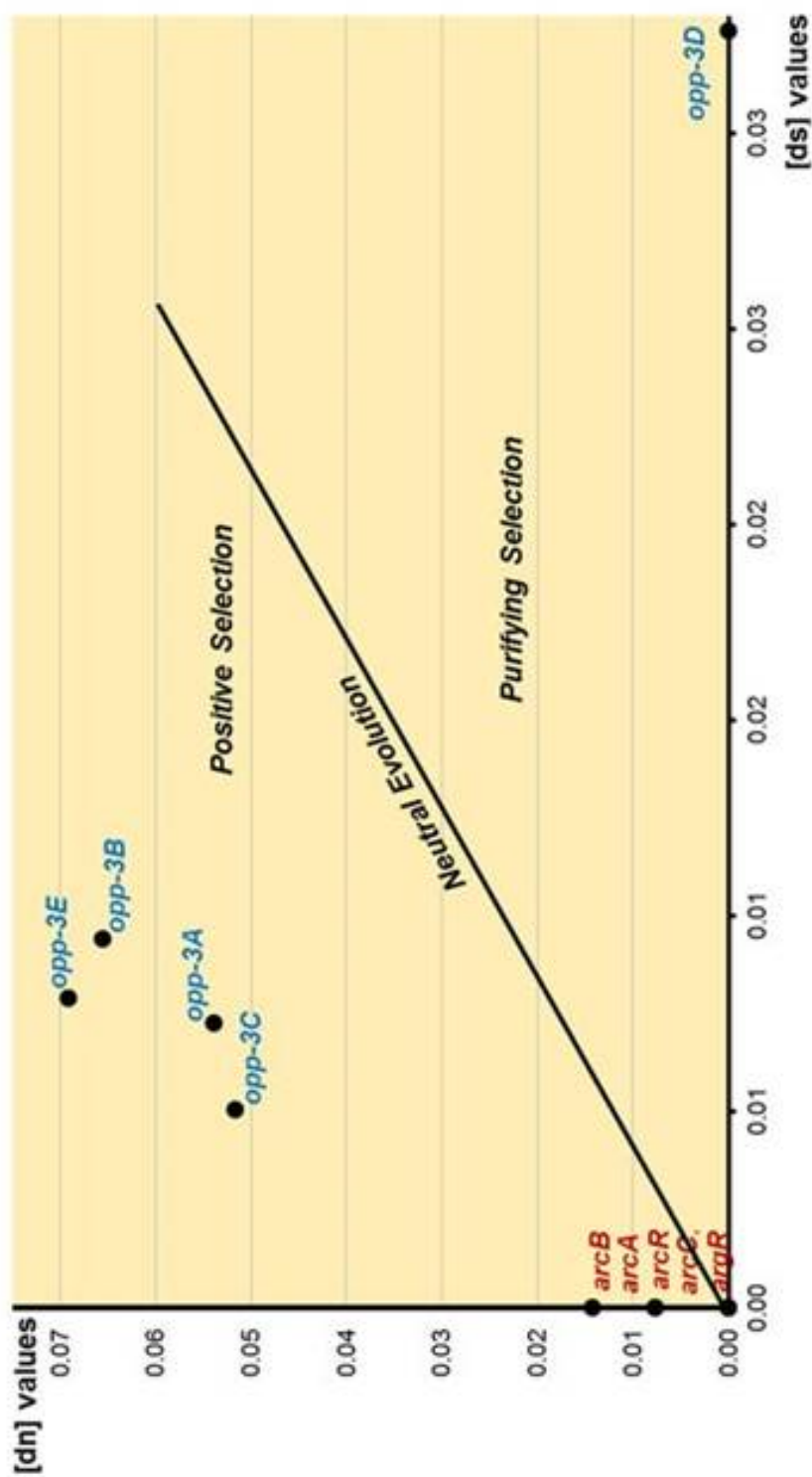
Figure 2.9: Evolution scheme: genomic comparison of ACME locus between MRSA, MSSA and *S. epidermidis*.



S. epidermidis and *S. aureus*. Integration site of the mobile genetic element (MGE) containing the ACME and opp cluster genes in the MRSA isolates and comparison with *S. epidermidis* CNS115. From left to right, genes are as follows: mecA, penicillin-binding protein PBP 2a; mecR1, methicillin resistance mecR1 protein; ΔIS1272, transposase DDE domain protein; ccrB2, cassette chromosome recombinase B2; ccrA2, cassette chromosome recombinase A; ATPase, transporter; rve, resolvase; sRNA, small RNA; arcC, carbamate kinase; arcB, ornithine carbamoyltransferase; arcR, HTH-type transcriptional regulator; arcD, arginine ornithine antiporter; arcA, arginine deiminase; argR, arginine repressor; usf, universal stress protein family protein; hp, hypothetical protein; ytpA, phospholipase; mety11, tRNA (mo5U34)-methyltransferase; opp-3A, peptide ABC transporter peptide-binding protein; opp-3B, oligopeptide permease; opp-3C, oligopeptide permease; opp-3D, ABC transporter ATP-binding protein; and opp-3E, ABC transporter ATP-binding protein. Abbreviation: SCCmec, staphylococcal cassette chromosome *mec*.

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Figure 2.10: Evolution scheme: proportions of synonymous and non-synonymous mutations in the ACME locus and the flanking peptide transporters between *S. aureus* and *S. epidermidis*.



The aligned sequences of each locus were used to calculate the proportion of synonymous (ds) or nonsynonymous (dn) mutations and for plotting dn/ds ratios for each gene of the ACME and the opp loci. The ratio of dn/ds indicates a diversifying selection for the opp genes, whereas ACME genes showed similar results than housekeeping genes. Abbreviation: SCCmec, staphylococcal cassette chromosome mec.

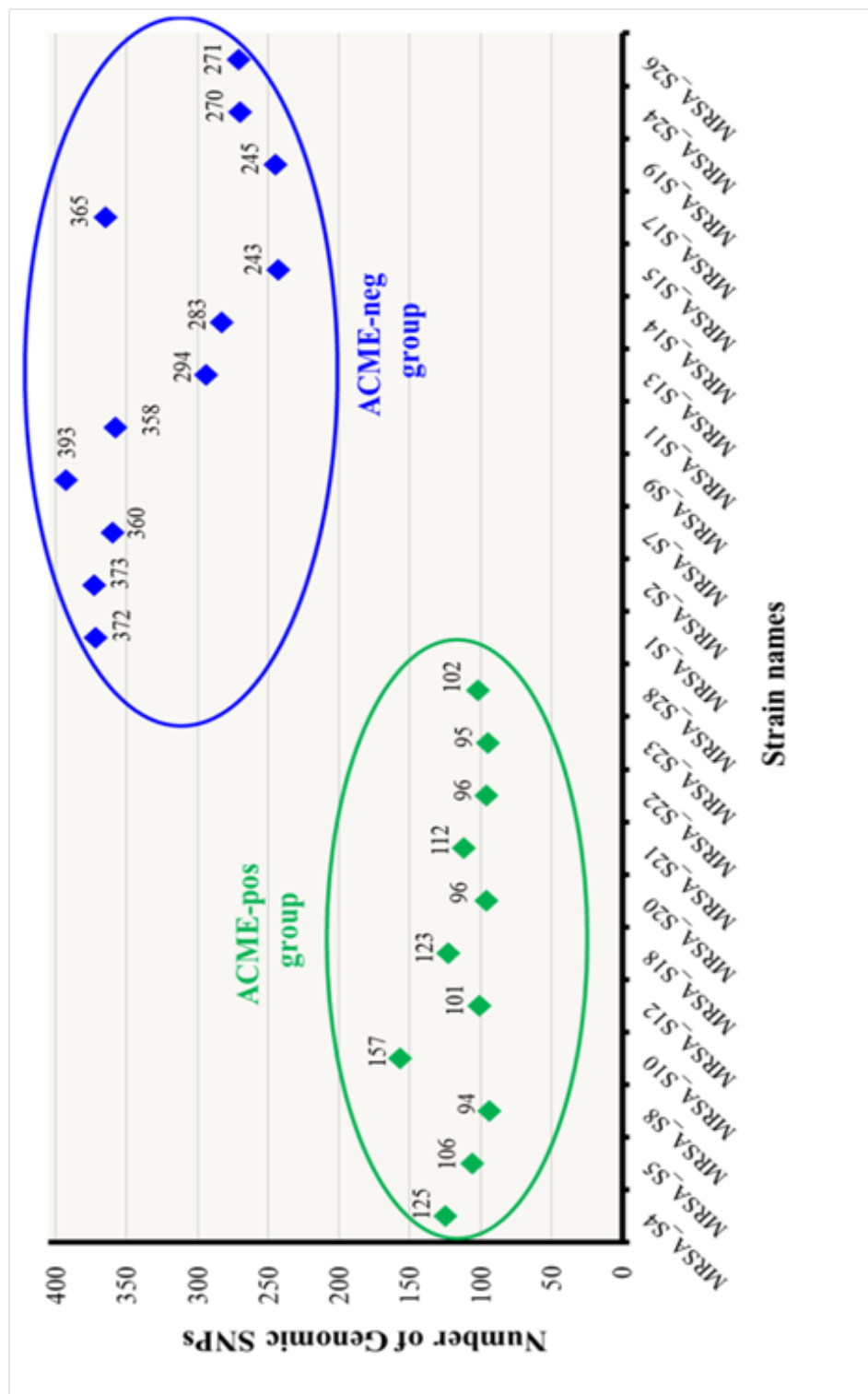
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Table 2.7: Antibiotic resistance patterns of the MRSA isolates.

ACME group	Strains	PEN G	INN/OXA	GEN	CIP	CLI	ERY	FUS	CTZ	FOS	RIF	TET	VAN	TEC	LZD	MUP	
ACME -	S1	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	
	S2	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	
	S7	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	
	S9	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	
	S11	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	
	S113	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	
	S14	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	
	S15	R	R	S	S	S	S	S	S	S	S	R	S	S	S	S	
	S17	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	
	S19	R	R	S	S	S	S	S	S	S	S	R	S	S	S	S	
	S24	R	R	S	S	S	S	R	S	S	S	S	S	S	S	S	
	ACME +	S4	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S
		S5	R	R	S	R	S	R	S	S	S	S	R	S	S	S	S
		S8	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S
S10		R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	
S12		R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	
S18		R	R	S	S	S	R	S	S	S	S	S	S	S	S	S	
S20		R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	
S21		R	R	S	R	R	R	S	S	S	S	R	S	S	S	S	
S22		R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	
S23		R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	
S26		R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	

Penicillin G (PEN G); Flucloxacillin/Oxacillin (INN/OXA); Gentamicin (GEN); Ciprofloxacin (CIP); Clindamycin (CLI); Erythromycin (ERY); Fucidic acid (FUS); Cotrimoxazole (CTZ); Fosfomicin (FOS); Rifampicin (RIF); Tetracycline (TET); Vancomycin (VAN); Teicoplanin (TEC); Linezolid (LZD); Mupirocin (MUP). R, Resistant; S, Susceptible.

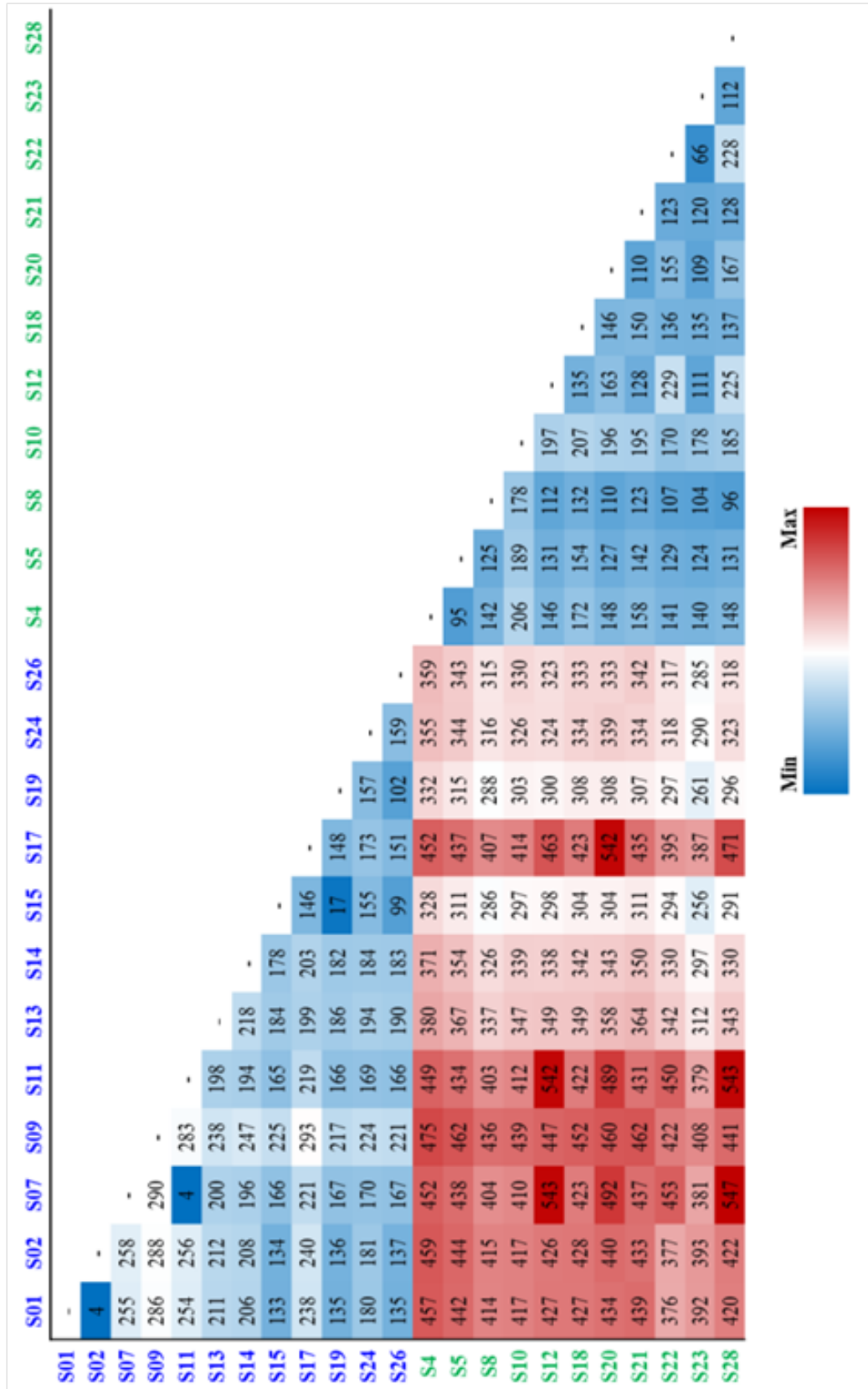
Figure 2.11: Analysis of SNPs within ACME-positive and ACME-negative clusters: number of genomic SNPs.



The number of genomic SNPs, based on the reference strain, is lower among ACME-positive isolates (green), compared with ACME-negative isolates (blue). These values are determined relative to the reference genome of the reference USA300_FPR3757 strain.

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Figure 2.12: Analysis of SNPs within ACME-positive and ACME-negative clusters: pairwise comparisons.



Pairwise comparisons confirmed this trend among the 2 clusters of isolates by identifying lower SNP numbers in the ACME group.

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The pair-wise comparisons show that the ACME-negative group of strains included three pairs of closely related clones (strains S1/S2, S7/S11, S15/S19). According to the epidemiological information, we were able to reveal that each pair of strains was isolated in members of the same family (mother/daughter, wife/husband and cousins working together). The number of SNPs for these three pairs were 4 SNPs (S1/S2), 4 SNPs (S7/S11) within a period of 4 and 14 days respectively, and 17 SNPs (S15/S19) identified in a period of 5 weeks. Except for these three family transmission events, all other clones appeared quite distantly related to each other (66 SNPs between the two closest clones). We conclude that no other direct relatedness, even between strains originating from the same country, was identified. All of these three family transmission events involved the ACME-negative clone. In the ACME-negative group, the majority of the clone was responsible for infection (n=9/12), while in the AMCE-positive group, infection were less frequent (n=6/11).

2.5.4 Genetic relatedness and distance of isolation

Genomes from previous US studies [173] have been compared to our genome ST8-USA300 (Figure 2.13 and Table 2.1). We constructed a phylogenetic structure, and we observed both clusters (Figure 2.13), one with ACME locus and presenting an *SCCmec* IVa element distinctly, separate from a second group without ACME locus and harboring an *SCCmec* IVc element (Figure 2.11). The strains allowing the *SCCmec* element IVc are closer to the root of the phylogenetic tree and harbored a slightly higher diversity than the cluster of ACME-negative clones (greater distance within the cluster). Moreover, the strains harboring the *SCCmec* element IVb appear more distantly related to both groups of isolates but less than the strains harboring *SCCmec* element IVg, which are clearly the most distantly related.

2.5.5 Evolution scheme and genetic event in ST8-MRSA

A low level of plasticity has been revealed in a population of homogeneous isolates according to a sequence analysis at the SNP level. Among both groups, the ACME-positive group with *SCCmec* element IVa and the ACME-negative group with *SCCmec* element IVc, we identified a high degree of conservation without sequence alteration by comparison of the common part (Table 2.8).

Table 2.8: Single nucleotide polymorphism (SNP) comparison of the ACME locus and opp gene clusters from *S. aureus* and *S. epidermidis*.

Gene name	Function	Site (bp)	SNPs	Non-syn
<i>arcC</i>	Carbanate kinase	930	0	0
<i>arcB</i>	Ornithine carbamoyltransferase	999	3	0
<i>arcR</i>	HTH-type transcriptional regulator	690	1	0
<i>arcD</i>	Arginine ornithine antiporter	1422	0	0

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<i>arcA</i>	Arginine deiminase	1236	2	0
<i>argR</i>	Arginine repressor	447	0	0
<i>Opp-3A</i>	Peptide ABC transporter peptide-binding protein	1554	26	9
<i>Opp-3B</i>	Oligopeptide permease, channel-forming protein	957	21	7
<i>Opp-3C</i>	Oligopeptide permease, channel-forming protein	768	12	3
<i>Opp-3D</i>	ABC transporter ATP-binding protein	768	20	20
<i>Opp-3E</i>	ABC transporter ATP-binding protein	636	13	4

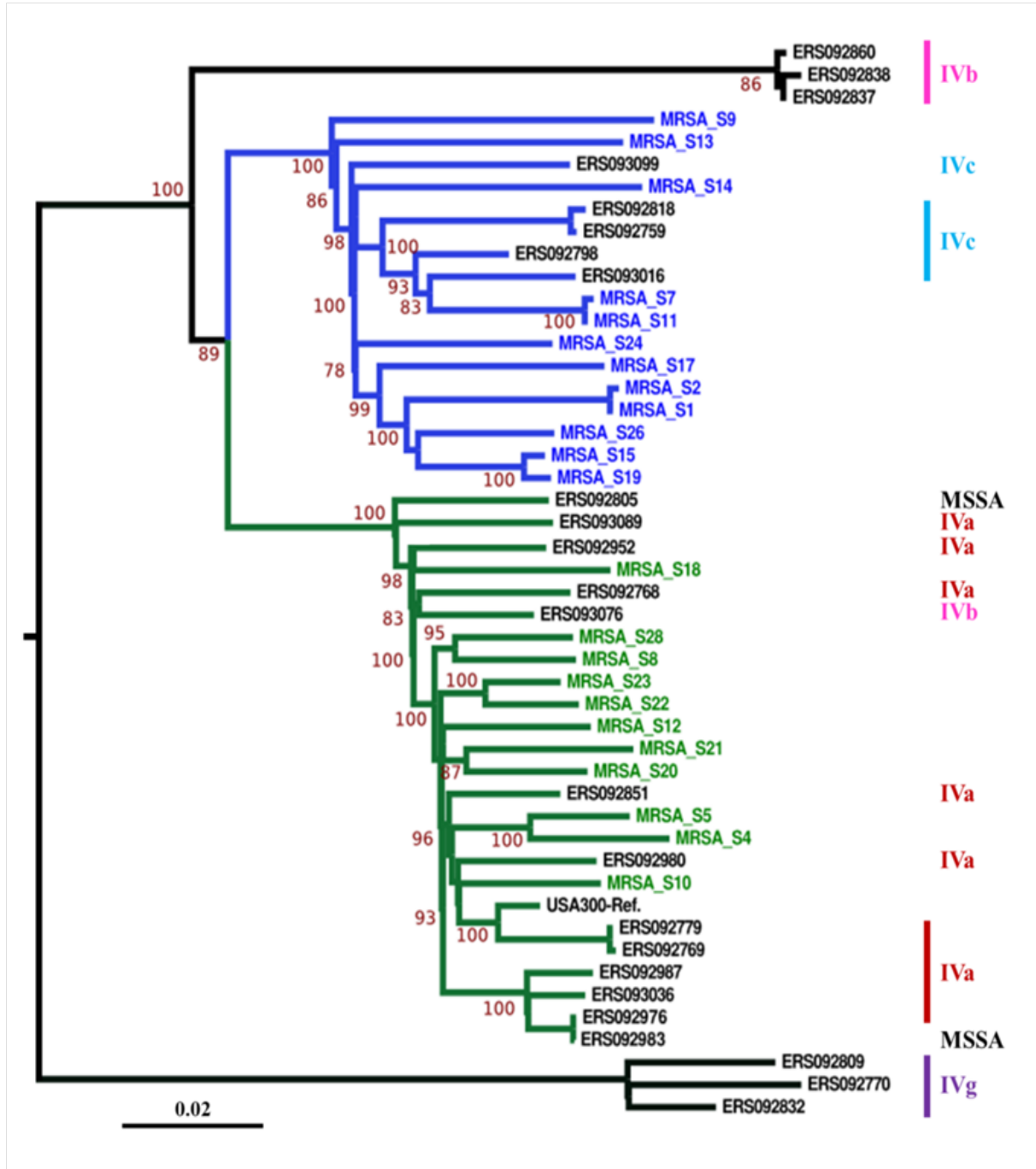
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2.6 Discussion

According to this epidemiological and molecular study, several interesting findings can be highlighted. First, compared to the past years, we identified a significative increase of ST8-USA300 and USA300-like clones in Geneva in our community in 2013. This is explained by several separate repeated importation events from North and South America predominantly. Secondly, WGS allowed to reveal that no clonal spread of one successful strain was identified in our community. Thus, no cantonal outbreak was identified. WGS is the only method available capable of distinguishing between both hypotheses, a very crucial tool for epidemiological surveillance. Thirdly, the tree transmission events in our study occurred between family members. Fourth, genomic SNPs analysis showed a higher heterogeneity in the strains with the ACME locus compared to the strain without, despite the genomes in both groups having similar contents of MGE. Fifth, the acquisition of different MGE colonized in the circular genome, such as the SCC*mec* element, the ACME locus with the *opp* genes flanking it, probably happened during different acquisition events. Finally, we observed more frequently clinical infection in the ACME-negative group. However, the number of cases is too small to make any conclusion.

The exact explanation for the recent and worrisome increase of ST8-USA300 and USA300-LV isolates in the Geneva region remains unclear. According to our epidemiological and molecular analysis, we found a strong link between affected patients and a possible past travel or origin in the Americas, suggesting the absence of local CA-MRSA USA300 and USA300-LV spread in our Geneva community. In particular, our data doesn't support the hypothesis of one single clone being responsible for this small outbreak. Moreover, the patterns of immigration rules between Switzerland and Americas didn't change during the last five years. Thus, the only way to explain this increase of dissemination in Geneva is a successful spread of the CA-MRSA USA300 clone in South America, that was then detected in travelers or immigrants coming from South America to HUG due to infection or colonization. Our results have been confirmed by other recent European studies also showing increased rates of USA300 isolated in hospital populations [175, 176]. Planet and al. performed a Pan-American genome study with 51 MRSA clinical isolates collected in the United States, Colombia, Venezuela and Ecuador between 1999 and 2012 [157]. This

Figure 2.13: Phylogenetic tree comparison.



Publicly available genomic sequences of several strains (8) were obtained and compared to those of our strains. Distances between ACME-positive and ACME-negative strains are consistent with the structure of the staphylococcal cassette chromosome *mec* element. Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.
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study confirms our hypothesis about survival fitness and transmissibility of the strains. However, the genome of these isolates was not publicly available at the time of analysis. Thus it was not possible for us to assess their relatedness with our strains. However, we were not able to detect the presence of new copper and mercury loci identified by Planet and al. in our isolates.

We noted that our 22 CA-MRSA USA300 isolates were comparable to strains identified during an extensive comparative genomic study of >387 of ST8 CA-MRSA clones during a surveillance network in New York [173]. For example, the comparison of these strains with our isolates identified some common features, such as the presence of ACME locus for the North American isolates and the absence of ACME element for the strains from South America. Moreover, the *SCC_{mec}* element and the susceptibility to fluoroquinolone were also similar according to the place of origin (Table 2.1 and 2.4).

Investigating the cause of this sudden worrisome increase of ST8-USA300 clones in the Geneva region by using epidemiological data and conventional molecular analysis without WGS (i.e., MLST, *SCC_{mec}* element typing and detection of PVL) could have led to an inappropriate conclusion that the prevalence of ST8-USA300 clones currently increases in our area. Only the WGS was able to elucidate the difference. Except for three family transmission events, ST8-USA300 strains identified in our region were the consequence of multiple importation events. Moreover, thanks to the epidemiological data, we know that only a few patients were in contact with the health care system in the past 12 months prior to their CA-MRSA detection, so we could reasonably exclude a potential health-care associated contamination or transmission of CA-MRSA, *de facto* exceedingly rare in our setting [177].

Two different groups were identified among our collection of isolates: the first one with the ACME locus and *SCC_{mec}* IVa element and the second one without the ACME locus and with *SCC_{mec}* IVc element, also named USA300-LV [166]. Symptomatic infection was caused by a majority of ACME-negative strains (9/12) compared to the ACME-positive strain where only one-half of those containing the ACME locus were responsible for infection (5/11). The ACME locus could contribute to the virulence of the strain [50, 178], although this hypothesis was not supported by all investigators. Our results did not confirm the hypothesis that the ACME locus plays a unique role in strain virulence, although we cannot draw any firm conclusion due to the limited number of isolates. A study of Liu and al. suggested that the ACME locus allowed adaptation to specific niches [179]. Moreover, it is interesting to note that among the three transmission events recorded in our study, all of them belong to the ACME-negative group. Among these three transmission events, it has been recorded only 4-17 SNPs at the genome scale on more than, on > 2.5 million nucleotides in the circular genome. As we know the date of isolation of each isolate thanks to the epidemiological data, it was possible to calculate an approximate rate of spontaneous mutations of about 1-2 SNPs per week [176]. Uhlemann and al. recently published a paper on a large number of isolates in which they established a cutoff of 23

SNPs to consider an epidemiological link between strains, a number that agrees with our observation [173]. According to the WGS results, the minimum of SNPs found between isolates without epidemiological link is 66 SNPs. The number of SNPs identified in both ACME-positive and -negative groups, especially among the most distant clones, is also in full agreement with the observation of Planet and al., in which the common ancestor of USA300 and USA300-LV was evaluated to appear in the mid-1970s [157].

The adaptation and spread of *S. aureus* was possible due to the transmission of MGE, contributing to increasing the virulence of the bacteria [180,181]. According to our molecular analysis, we showed that both groups harbored the same pathogenicity islands: SaPI5, Sa2int and Sa3int. Furthermore, we assumed that the ACME locus as an MGE was transferred by *S. epidermidis* to MRSA [52,182]. Contrary, the flanking oligopeptide transporter *opp* seems to be subject to a different selective pressure or to be the result of a different insertion event. We also observed differences between both groups in terms of antibiotic resistance, specifically against erythromycin and ciprofloxacin. These differences could be explained by a difference in antibiotic selection pressure between South and North American countries and other extrinsic features, leading to a different evolution and expansion of the fluoroquinolone resistance in the USA300 clone.

To summarize, the epidemiology of MRSA is evolving in the Geneva area. In approximately one decade the prevalent European ST80 clone in community-acquired MRSA infections decreased significantly [176,183]. While it was absent from the past epidemiological studies, the USA300 clone is currently the commonly identified clonotype of CA-MRSA in Geneva. The use of WGS allows superior discriminatory power to identify transmission events within an apparently clonal population of isolates. Due to the absence of close relatedness identified in our collection of isolates, we suggest that our region is clearly exposed to numerous individual importation events, rather than to clonal spreading of a prevalent clone. Thanks to the active surveillance of CA-MRSA in Geneva, we were able to demonstrate the presence of these isolates known to be particularly prevalent in the Americas and perhaps limit the spread of the ST8-USA300 clone.

This work was accepted for publication in September 2015 and published in *The Journal of Infectious Disease* in May 2016 (Appendix 1).

In 2015, Planet et al. described the emergence of two parallel epidemics of USA300; the first one, from 1989 in North-America and the second one from 1985 in South America. They showed that the emergence of these two strains coincide with the independent acquisition of ACME in the North American clone and a novel copper and mercury resistance mobile element (COMER) in the South American clone [157]. After the publication of our article, Planet et al. published the following article "Global Spread of the Community-Associated Methicillin-Resistant *Staphylococcus aureus* USA300 Latin American Variant" [184]. In this publication, they criticized the fact that we did not seek the presence of COMER in our strains. To this, we replied that the sequence of the COMER

element was not available at the time of publication of our manuscript. We could not identify this sequence in our isolates, as discussed in the discussion part of our article (Appendix 2). They used our publically available genomes from our study to look for the presence of the COMER cassette. As expected, they found the presence of the COMER element in our strain from South America; this confirms their previous results about the acquisition of the COMER element in the South America USA300 clone [184].

Chapter 3

Determinants of successful methicillin-resistant *Staphylococcus aureus* decolonization

"Great love and great achievements involve great risks."

Dalai Lama

3.1 Introduction

MRSA colonization is known to be associated with a high risk of suffering a MRSA infection [185]. Patients carrying MRSA can play the role of reservoirs and vectors for new exogenous cross transmissions [186]. Infection prevention and transmission control can be achieved by decolonization with antiseptics [187, 188]. Decolonization treatment regimens already exist, such as chlorhexidine, sometimes in association with mupirocin, which then can be successful in decreasing MRSA carriage rates [189–191]. However, due to the widespread use of these agents, the emergence of resistance against these treatments have considerably increased [192, 193]. As such, new alternative agents for MRSA decolonization are needed.

Case reports of successful MRSA decolonization with polyhexanide treatment were published [194, 195]. Despite these cases, the real efficacy of the treatment was not yet evaluated for MRSA decolonization; this is why an RCT was performed at HUG between January 2011 and July 2014 to compare polyhexanide (Prontoderm[®]) versus a placebo for topical MRSA decolonization. A double-blind, placebo-controlled randomized clinical trial was performed. The treatment was applied for ten days on the skin and in the nose of previously identified MRSA carriers, then a control swab was taken from the skin of the perineal/inguinal region and the nose according to the local practices and other evidence [196]. A full description of this study was reported elsewhere [197] (Appendix 3). The results of the RCT were recently reported and demonstrated the failure of polyhexanide

to eliminate MRSA carriage [197] (Appendix 3). The decolonization efficacy was 31.5% on average for the entire population with a slightly higher success rate of 33.8% (24/71) in the polyhexanide group and 29.3% (22/75) in the placebo group (P-value=0.56). This RCT suggested that under real-life conditions, a single polyhexanide decolonization course is ineffective for MRSA decolonization.

3.2 Objective

Topical decolonization to eradicate MRSA carriage is currently used infrequently in settings with endemic MRSA because of a variable chance of success, risk of resistance and logistical challenge [198]. During the RCT, polyhexanide was not efficient for MRSA decolonization. However, a spontaneous decolonization rate of about 30% was observed among all patients of the trial, which is relatively high. Few studies have described determinants of successful MRSA decolonization among patients with or without a topical regimen of decolonization treatment [187, 199–201, 201]. We decided to use the data of the previous RCT comparing polyhexanide versus placebo to assess possible determinants of successful MRSA decolonization, independent of the decolonization regimen.

3.3 Methods

3.3.1 Settings, study design and study population

The study was conducted between January 2011 and July 2014 at HUG, a Swiss tertiary hospitals with about 1900 beds and 48'000 annual admissions. During the study period, the approximate annual rate of new patients identified with MRSA carriage decreased from 1.1 cases to 0.32 cases per 100 admissions in 2014 [122].

The RCT on which the study is based was an investigator-initiated, double-blind, randomized and placebo-controlled superiority clinical trial. It was conducted according to the Declaration of Helsinki and approved by the institutional review board in Geneva, Switzerland. Each patient included signed an informed consent, and the trial was registered at the ISRCTN (registry on the number ISRCTN02288276). Potential participants were identified based on the daily review of positive MRSA culture results provided by the Central Microbiology Laboratory at HUG. The study was proposed to all patients, that fulfilled the inclusion and exclusion criteria (see below).

Patient inclusion criteria:

- Age \geq 18 years
- Microbiologically documented MRSA carriage at any site, without any signs and symptoms of active MRSA infection
- Written informed consent

Patient exclusion criteria:

- Active MRSA infection
- Chronic ulcers and deep-seated wounds colonized by MRSA
- Presence of tracheostomy
- Presence of external fixator colonized with MRSA
- Unavailability of adequate help if subject is unable to self-administer the investigational product
- Concurrent treatment with antimicrobial agents with anti-MRSA activity at the time of enrollment
- Participation in another prospective clinical trial
- Previous enrollment in the proposed study
- Inability to understand or to follow the study protocol
- Planned cardiac or orthopedic implant surgery
- Known or suspected hypersensitivity or allergy to any of the study drugs
- Known hypersensitivity to chlorhexidine
- Pregnancy or breastfeeding
- Current or planned treatment with other agents that are topically applied to the skin or the nares

- Critically ill patients hospitalized in the intensive care unit

If the patients fulfilled all the criteria and agreed to participate, they were randomly assigned to one of the treatment or placebo groups in a 1:1 ratio. Bacteriological exams were made at baseline and repeated after completion of therapy 28 days after the end of the treatment. The clinical and microbiological data were recorded prospectively.

Using data from this RCT, we performed a prospective, unmatched case-control study. We identified as potential subjects for this study all patients from the RCT with a MRSA-positive swab at the inclusion time and with a control sample at the end of follow-up (at day 28). Cases were defined as a patient with an MRSA-negative swab at day 28. The control group consisted of all patients still MRSA-positive at the end of the study. The potential variables implicated in MRSA decolonization were selected according to literature and expert advice [187,198–203]. Possible determinants of successful MRSA decolonization included several treatments and patient characteristics (see above). Dependent patients were defined as patients with a partial or total inability to perform the activities of daily life without the help of another person.

The following data were recorded prospectively:

Patient characteristics

- Gender
- Age
- Body mass index (BMI)
- Absence of comorbidities
 - ★ Cardiovascular disease
 - ★ COPD
 - ★ Chronic renal failure
 - ★ Chronic liver disease
 - ★ Malignancy
 - ★ Diabetes mellitus
 - ★ Immunodeficiency
- Degree of dependence
- McCabe score
- Presence of wound or skin damage

- Presence of invasive device
- Antibiotic treatment during the study
- Length of stay since the start of treatment

Colonization characteristics

- MLST sequence type
- Colonized body site
- MRSA quantity at baseline
- MRSA status

Treatment characteristics

- Treatment
- Place of treatment

3.3.2 Study medication and procedures

Each included patient received either the active or placebo solution for ten days after randomization. The active treatment (Prontoderm[®] solution; B. Braun Medical AG, Sempach, Switzerland) was composed by a mix of polyhexanide, allantoin, a cationic component, surfactant and purified water. The active and placebo solutions were applied to the hair and scalp and left in place for about 3 minutes, followed by washing with a shampoo, then, after the shower, the solution was applied to the entire skin without rinsing off. For the nose, the active treatment was a mix of polyhexanide, glycerol, cellulose polymer and purified water (Prontoterm[®] Gel Light; B. Braun Medical AG, Sempach, Switzerland). The active or placebo gel was applied three times daily in the anterior nares by applying a sufficient amount of about 0.5-1ml. For both products (solution and gel), the placebo was similar in appearance with the active treatment. Infection control measures were set up according to the principles of contact precautions routinely performed in our institution for MRSA carriers [204] (Appendix 3).

3.3.3 Microbiological evaluation

For the microbiological evaluation two control swabs were taken, one in the nares and the other one on the perineal/inguinal region. We used sterile Darcon-tipped swabs, premoist-

ened with a sterile saline solution on day 0 and at the end of study 28 days after the end of the MRSA decolonization regimen.

As described in Cherkaoui et al. [205], chromogenic agar plates (MRSAid®; Biomérieux, Marcy l'Etoile, France) was used to perform the MRSA identification. Moreover, a colistin-salt backup broth was cultured with both Copan swabs immediately after inoculating the agar plates. After 24h, if suspect colonies were absent, the backup broth was inoculated onto a second chromogenic plate and read 24 hours later. In the presence of MRSA, the laboratory technician identified the quantity of bacterial colonies on the plates; positive broth and "+" indicating low quantity, "++" indicating medium quantity and "+++" a high quantity. After identification, suspect colonies were confirmed by picking one colony and identifying the presence of *femA_SA* (*S. aureus*-specific gene target) and *mecA* by polymerase chain reaction (PCR) [206]. The MRSA-positive culture was defined by the presence of one colony harboring the proper color and morphology on a chromogenic plate and the detection of both *mecA* and *femA_SA* by PCR from the same colony. Then, molecular typing of the MRSA clone was performed using MLST [207] and the multiple-locus variable number of tandem repeat analysis assay.

MRSA strains were tested for their antibiotic susceptibility according to the Vitek 2 automatic susceptibility testing cards for Gram-positive bacteria (Biomérieux). After identification, all MRSA strains were frozen in skimmed milk for further determination. At the end of patient inclusion, the susceptibilities to polyhexanide and chlorhexidine were assessed for 27 pairs of MRSA strains (at day 0 and day 28) showing MRSA decolonization treatment failure. A macrodilution method was realized to determine MIC values. Several serial dilutions ranging from 0.25 to 2 mg/L for polyhexanide and 0.25 to 16mg/L for chlorhexidine were tested on fresh bacterial cultures, following CLSI recommendations. No resistance was found among our strains for polyhexanide and chlorhexidine (subject to separate molecular investigation).

3.3.4 Statistical analyses

Data recorded for case and control patients were described by frequencies for categorical variables and by the mean and standard deviation (SD) for continuous variables. Case and control groups comparisons were performed using the Student-*t* test for continuous variables and Pearson's χ^2 test and Fischer's exact test for categorical variables when appropriate.

Regression analyses were conducted on all variables with a P-value ≤ 0.25 with descriptive analyses.

Before being included in the regression models, the continuous variables were tested for log-linearity and when not fulfilled, the variable was converted into a categorical variable. The category "Age" was divided into 4 block of 18 years. The category "Length of stay

since the start of treatment" into 2 block according to its median value

Initially, we performed an univariate logistic regression on the data. This analysis was conducted to obtain an odds ratio (OR), commonly used to assess associations between exposure and outcome [208]. However, in recent public health and medical literature, it has been shown that when the outcome is common (incidence $\geq 10\%$), it is more appropriate to use a relative risk (RR), because the OR tends to overstate RR [209,210]. Moreover, there is a tendency to interpret the OR as if it were the RR [209,211,212]. Despite this some still argue, that the OR should be used in all cases even when the outcome is common [213]. We nevertheless decided to perform a second log-binomial regression analysis on the data to calculate RRs. In the log-binomial model, all the included variables have to be categorical. Consequently, the continuous variables have been categorized. Two different types of regression analyses have been performed on the data.

If an increased likelihood of MRSA decolonization with a P-value ≤ 0.25 was found in the univariate logistic regression, the variables were included in the multivariate logistic regression model. The same approach was followed with the multivariate log-binomial regression.

Correlation was checked for each variable before being included in the multivariate model. The correlation between the continuous variables and binary variables were tested calculating a point-biserial correlation coefficient. In binary variables, the correlation was checked using the phi coefficient (ϕ). A pair of variables was considered as highly correlated if coefficient ≥ 30 [214]. If two variables were considered to be correlated, one variable was entered into the multivariate model, and then the effect of placing the correlated variable in the same multivariate model was evaluated.

The multivariate logistic regression and log-binomial regressions were created using a forward stepwise procedure and then checked using a backward stepwise procedure.

The products of the interaction between variables included in the multivariate model were tested. If the product was significant, it was integrated into the model.

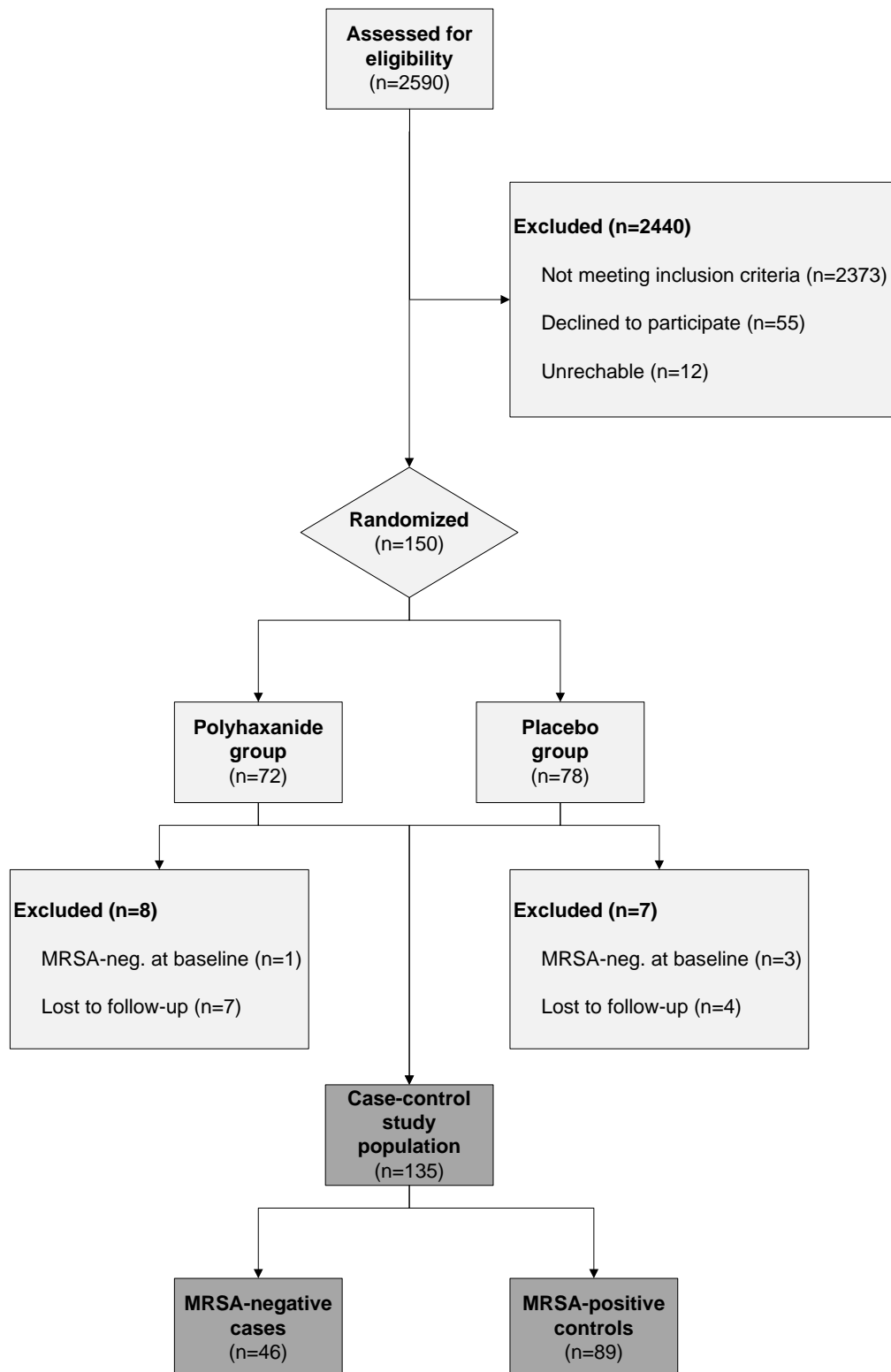
The goodness-of-fit of the multivariate logistic regression model was assessed using the Hosmer-Lemeshow test.

All statistical analyses were performed using Stata version 12 (Stata Corporation, College Station, Texas, USA).

3.4 Results

In the RCT, 150 patients were included. Among these patients, 135 fulfilled the criteria to participate in the case-control study (Figure 3.1). Fifteen patients were excluded from

Figure 3.1: Case-control study flow-chart.



the RCT, 8 from the polyhexanide group and seven from the placebo group. Among these excluded patients, 4 subjects had an MRSA-negative sample at baseline and 11 patients were lost to follow-up at the end of the study. The case-control study population was composed by 135 patients and at the end of the study there were 46 MRSA-negative cases (34.1%) and 89 MRSA-positive controls (65.9%) (Figure 3.1).

All variables have been described according to the characteristics of cases or controls described in Table 3.1.

Table 3.1: Patient, colonization and treatment characteristics.

Variables	MRSA-neg. cases (N = 46)	MRSA-pos. controls (N = 89)	P Value
Patient characteristics			
Gender, No. (%)			0.824
<i>Male</i>	27 (58.7)	54 (60.7)	
<i>Female</i>	19 (41.3)	35 (39.3)	
Age (years), (\pm SD)	61.9 (\pm 16.07)	66.8 (\pm 14.86)	0.078
BMI (kg/m ²), (\pm SD)	27.61 (\pm 6.37)	27.39 (\pm 6.80)	0.857
Presence of at last 1 comorbidity, No. (%)	34 (73.9)	68 (76.4)	0.750
Absence of comorbidities, No. (%)			
<i>Cardiovascular disease</i>	23 (50.0)	41 (46.1)	0.664
<i>COPD</i>	41 (89.1)	70 (78.7)	0.131
<i>Chronic renal failure</i>	38 (82.6)	72 (80.9)	0.808
<i>Chronic liver disease</i>	40 (87.0)	81 (91.0)	0.464
<i>Malignancy</i>	42 (91.3)	71 (79.8)	0.086
<i>Diabetes mellitus</i>	38 (82.6)	77 (86.5)	0.545
<i>Immunodeficiency</i>	34 (73.9)	64 (71.9)	0.805
Degree of dependence, No. (%)			0.006
<i>Dependant or semi-dependant</i>	11 (23.9)	43 (48.3)	
<i>Independent</i>	35 (76.1)	46 (51.7)	
McCabe score, No. (%)			0.329
<i>Non-fatal</i>	44 (95.7)	81 (91.0)	

BMI, body mass index; COPD, chronic obstructive pulmonary disease; MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSA-neg., MRSA-negative; MRSA-pos., MRSA-positive; SD, standard deviation

^a Invasive devices: central venous catheter, peripheral venous catheter, implantable venous access device, or urinary catheter.

^b Anti-MRSA therapy: vancomycin, linezolid, trimethoprim-sulfamethoxazole, or rifampicin.

^c Endemic MRSA clone at the study site (mupirocin- and chlorhexidine-resistant).

^d Other non-endemic clones (mupirocin-susceptible).

Table 3.1 (continued): Patient, colonization and treatment characteristics.

Variables	MRSA-neg. cases (N = 46)	MRSA-pos. controls (N = 89)	P Value
<i>Ultimately fatal</i>	2 (4.3)	8 (9.0)	
<i>Rapidly fatal</i>	0 (0.0)	0 (0.0)	
Presence of wound or skin damage, No. (%)	17 (37.0)	33 (37.1)	0.989
Presence of invasive device ^a , No. (%)	5 (10.9)	21 (23.6)	0.076
Antibiotic treatment during the study, No. (%)	9 (19.6)	20 (22.5)	0.697
<i>Fluoroquinolone</i> , No. (%)	2 (4.3)	5 (5.6)	1.000
<i>Cephalosporin</i> , No. (%)	3 (6.5)	5 (5.6)	1.000
<i>Anti-MRSA agent</i> ^b , No. (%)	2 (4.3)	3 (3.4)	1.000
Length of stay since the start of treatment (days), (±SD)	13.37 (±25.93)	23.58 (±58.84)	0.078
			0.046
>3 weeks, No. (%)	8 (17.4)	30 (33.7)	
≤3 weeks, No. (%)	38 (82.6)	59 (66.3)	
Colonization characteristics			
MLST, No. (%)	n=35	n=80	0.878
<i>ST228</i> ^c	24 (68.6)	56 (70.0)	
<i>ST5 - ST8 - ST22 - ST105 - ST225</i> ^d	11 (31.4)	24 (30.0)	
Colonized body site, No. (%)			
<i>Nose</i>	34 (73.9)	75 (84.3)	0.148
<i>Groin</i>	31 (67.4)	69 (77.5)	0.203
Colonized sites, No. (%)			0.023
<i>2 (nose and groin)</i>	19 (41.3)	55 (61.2)	
<i>1 (nose or groin)</i>	25 (58.7)	34 (38.2)	
MRSA quantity at baseline, No. (%)			0.380
<i>Low level of carriage</i>	18 (39.1)	25 (28.1)	
<i>Medium level of carriage</i>	16 (34.8)	33 (37.1)	
<i>High level of carriage</i>	12 (26.1)	31 (34.8)	

BMI, body mass index; COPD, chronic obstructive pulmonary disease; MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSA-neg., MRSA-negative; MRSA-pos., MRSA-positive; SD, standard deviation

^a Invasive devices: central venous catheter, peripheral venous catheter, implantable venous access device, or urinary catheter.

^b Anti-MRSA therapy: vancomycin, linezolid, trimethoprim-sulfamethoxazole, or rifampicin.

^c Endemic MRSA clone at the study site (mupirocin- and chlorhexidine-resistant).

^d Other non-endemic clones (mupirocin-susceptible).

Table 3.1 (continued): Patient, colonization and treatment characteristics.

Variables	MRSA-neg. cases (N = 46)	MRSA-pos. controls (N = 89)	P Value
MRSA status, No. (%)			0.697
<i>Newly identified MRSA carriage</i>	30 (65.2)	55 (61.2)	
<i>Known MRSA carriage</i>	16 (34.8)	34 (38.2)	
Treatment characteristics			
Treatment, No. (%)			0.425
<i>Polyhexanide</i>	24 (52.2)	40 (44.9)	
<i>Placebo</i>	22 (47.8)	49 (55.1)	
Place of treatment, No. (%)			0.366
<i>Hospital</i>	20 (43.5)	46 (51.7)	
<i>Home</i>	26 (56.5)	43 (48.3)	

BMI, body mass index; COPD, chronic obstructive pulmonary disease; MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSA-neg., MRSA-negative; MRSA-pos., MRSA-positive; SD, standard deviation

^a Invasive devices: central venous catheter, peripheral venous catheter, implantable venous access device, or urinary catheter.

^b Anti-MRSA therapy: vancomycin, linezolid, trimethoprim-sulfamethoxazole, or rifampicin.

^c Endemic MRSA clone at the study site (mupirocin- and chlorhexidine-resistant).

^d Other non-endemic clones (mupirocin-susceptible).

Regarding the patient's characteristics, only two variables had a statistically significant P-value ≤ 0.05 to predict successful MRSA decolonization at the end of follow-up. The first one, was the "Degree of dependence" with more patients independent in the case group compared to the control group (76.1% and 51.7%, P-value=0.006). The second significant variable was "Length of stay since the start of treatment." 82.6% of patients in the case group and 66.6% in the control group (P-value=0.046) had a length of stay ≤ 3 weeks. For the colonization characteristics, only one variable was significant, the "Colonized site." Within the case group 41.3% and 58.7% were found with two and one colonized sites respectively while 61.2% and 38.2% in the control group (P-value=0.23). No variable associated to the treatment was significant. Other variables such as age, absence of COPD, absence of malignancy, presence of invasive devices, colonized body sites were not significant but had a P-value ≤ 0.25 . Consequently, the univariate logistic regression were performed on these variables, and they were included in the forward multivariate logistic regression model. The remaining variables were not included in the univariate and multivariate logistic regression: gender, BMI, presence of at least one comorbidity, absence of comorbidities such as cardiovascular disease, chronic renal or liver failure, diabetes mellitus and immunodeficiency.

ciency, McCabe score, presence of wound or skin damage, antibiotic treatment during the study, MLST, MRSA quantity at baseline, MRSA status, type of treatment and place of therapy (Table 3.1).

For the univariate and multivariate logistic regression analyses, the log-linearity of the continuous variable was verified to allow their use as continuous variables in the logistic regressions. According to Figures 3.2 and 3.3, we noted that the log-linearity was respected for the "Age" variable but not for the "Length of stay since the start of treatment variable", It has therefore been categorized in two categories $>$ and ≤ 3 weeks for the logistic regression analysis.

According to the univariate logistic regression, three variables were significant and associated with a successful MRSA decolonization (Table 3.2).

Table 3.2: Univariate logistic regression.

Variables	OR	95% CI	P Value
Patient characteristics			
Age, per 1-year increment	0.98	0.96 - 1.00	0.079
COPD	2.23	0.77 - 6.41	0.119
Malignancy	2.66	0.84 - 8.40	0.073
Degree of dependence			
<i>Dependant or semi-dependant</i>	1.00	...	
<i>Independent</i>	2.97	1.34 - 6.59	0.005
Presence of invasive device ^a	0.39	0.14 - 1.13	0.065
Length of stay since the start of treatment (days)			
<i>>3 weeks</i>	1.00	...	
<i>≤3 weeks</i>	2.42	1.00 - 5.82	0.040
Colonization characteristics			
Colonized body site			
<i>Nose</i>	1.89	0.79 - 4.52	0.155
<i>Groin</i>	1.67	0.76 - 3.69	0.208
Colonized sites			
<i>2 (nose and groin)</i>	1.00	...	
<i>1 (nose or groin)</i>	2.30	1.11 - 4.75	0.023

CI, confidence interval; COPD, chronic obstructive pulmonary disease; OR, odds ratio

^a Invasive devices: central venous catheter, peripheral venous catheter, implantable venous access device, or urinary catheter.

The first one is the degree of dependence with an OR for the independent status at

Figure 3.2: Log-odds by age category.

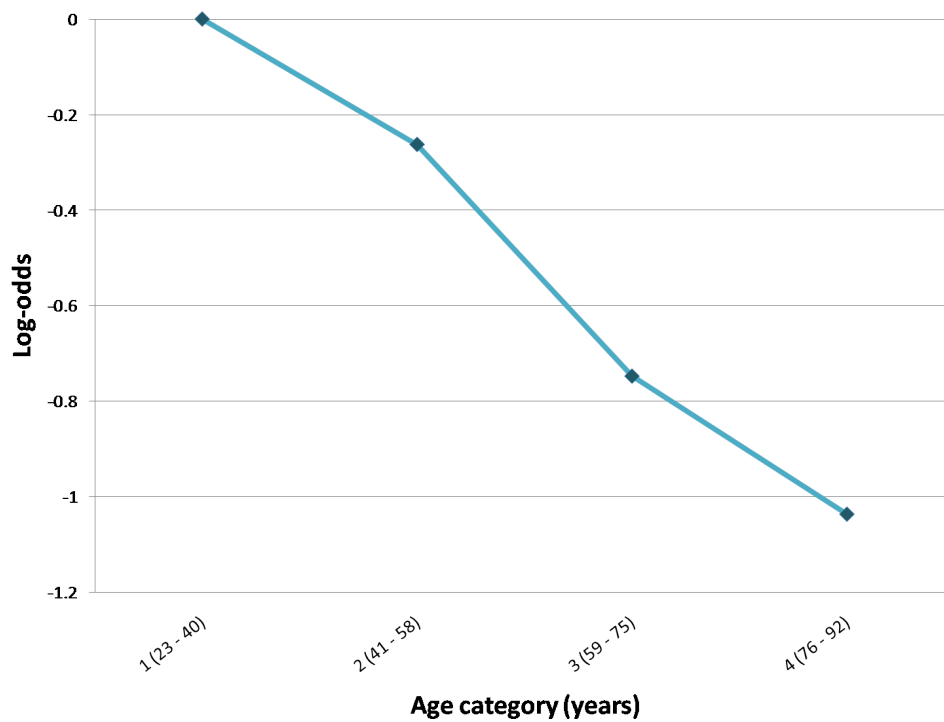
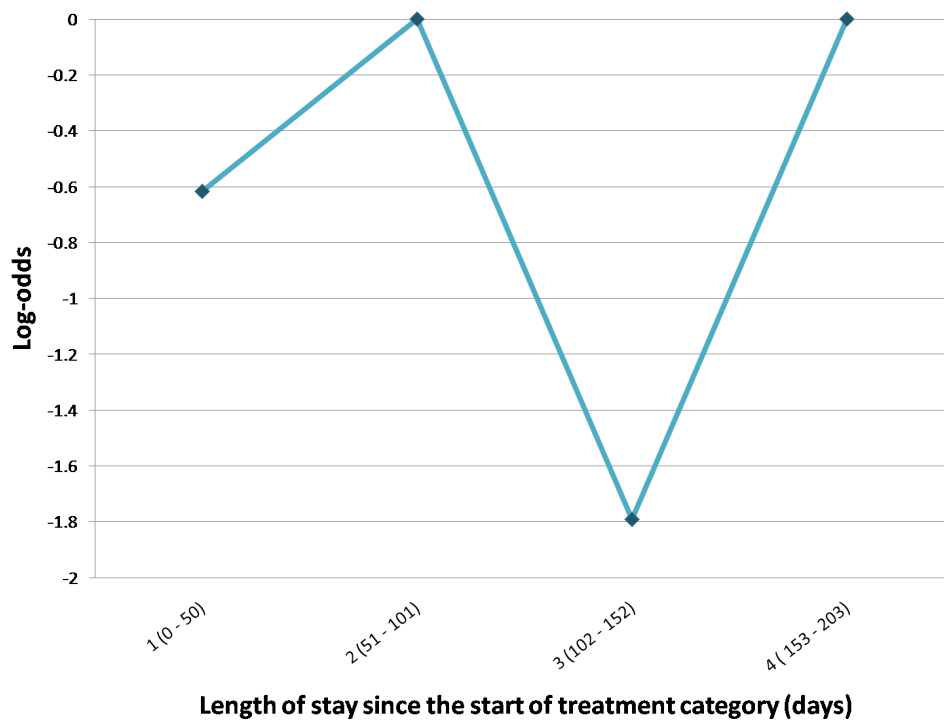


Figure 3.3: Log-odds by length of stay since the start of treatment category.



2.97 and a 95% confidence interval (CI) of [1.34-6.59] (P-value=0.005). The second one is the length of stay since the start of treatment with an OR=2.42 and a 95% CI [1.00-5.82] for a length of stay ≤ 3 weeks (P-value=0.040). The last one is the number of colonized sites; for one colonized site, OR=2.30 and a 95% CI [1.11-4.75] (P-value=0.023). The other variables with a P-value ≤ 0.25 in Table 3.1 (descriptive variables) were not significant in the univariate logistic regression (age, absence of COPD, absence of malignancy and the presence of invasive devices). According to the univariate logistic regression analysis, cases were more likely to have only the groin or nose colonized (and not both), a shorter length of stay after treatment and living without assistance. Moreover, the MRSA-negative patients also tended to be younger, without COPD, malignancy, and invasive equipment.

For the log-binomial regression, the same result was obtained except for the length of stay since the start of treatment, which was not statistically significant anymore (P-value=0.067) (Table 3.3).

Table 3.3: Log-binomial regression.

Variables	RR	95% CI	P Value
Patient characteristics			
Age			
<i>23-40 years</i>	1.00	...	
<i>41-58 years</i>	0.87	0.43 - 1.75	0.696
<i>59-75 years</i>	0.64	0.34 - 1.23	0.181
<i>75-92 years</i>	0.52	0.25 - 1.09	0.082
COPD	1.77	0.78 - 4.01	0.169
Malignancy	2.04	0.82 - 5.12	0.127
Degree of dependence			
<i>Dependant or semi-dependant</i>	1.00	...	
<i>Independent</i>	2.12	1.18 - 3.80	0.012
Presence of invasive device ^a	0.51	0.22 - 1.16	0.111
Length of stay since the start of treatment (days)			
<i>>3 weeks</i>	1.00	...	
<i>≤ 3 weeks</i>	1.86	0.96 - 3.61	0.067
Colonization characteristics			
Colonized body site			
<i>Nose</i>	1.48	0.90 - 2.44	0.125
<i>Groin</i>	1.38	0.85 - 2.23	0.187
Colonized sites			

CI, confidence interval; COPD, chronic obstructive pulmonary disease; OR, odds ratio

^a Invasive devices: central venous catheter, peripheral venous catheter, implantable venous access device, or urinary catheter.

Table 3.3 (continued): Log-binomial regression.

Variables	RR	95% CI	P Value
2 (nose and groin)	1.00	...	
1 (nose or groin)	1.72	1.07 - 2.78	0.021

CI, confidence interval; COPD, chronic obstructive pulmonary disease; OR, odds ratio

^a Invasive devices: central venous catheter, peripheral venous catheter, implantable venous access device, or urinary catheter.

For both variables that remained statistically significant in the log-binomial regression, we found for the independent status a RR=2.12 with a 95%CI [1.18-3.61] (P-value=0.012) and for one colonized site RR=1.72 and 95% CI [1.07-2.78] (P-value=0.021).

Before being included together in the multivariate model, the variables were checked for potential correlations between each other (Table 3.4).

Three pairs of variables showed a high correlation coefficient (coeff ≥ 0.35) between them; the independent status and the length of stay since the start of treatment (coeff=0.43), the colonized nares and the number of colonized sites (coeff=0.54) and finally the colonized inguinal region and the number of colonized sites (coeff=0.65). These variables were included separately in the multivariate model. The length of stay since the start of treatment, the colonized nares and the colonized inguinal region were not significant in the multivariate logistic regression.

Table 3.5 and Table 3.6 shows the multivariate logistic regression and the log-binomial regression models, respectively. The result of the multivariate analysis was the same using forward or backward procedures. According to these techniques, two determinants were associated with a successful MRSA decolonization. According to the logistic regression model, the two determinants were the independent status with an adjusted odd ratio (aOR)=2.83 and 95% CI [1.26-6.34] (P-value=0.011) and the only 1 MRSA-colonized site at baseline, with aOR=2.16 and 95% CI [1.03-4.56] (P-value=0.042). The results of the multivariate log-binomial regression model for the independent status lead to an adjusted relative risk (aRR)=2.04 and 95% CI [1.14-3.63] (P-value=0.016) and the only 1 MRSA-colonized site at baseline had an aRR=1.64 and 95% CI [1.03-2.61] (P-value=0.037).

The product of interaction between both variables was not significant, aOR=1.87 (95% CI [0.37-9.42]; P-value=0.447) and aRR=1.32 (95% CI [0.41-4.31]; P-value=0.640). It was not included in our multivariate model. The final multivariate logistic regression model fit was validated by the Hosmer-Lemeshow test with a $\chi^2=0.58$ and a P-value=0.748).

Table 3.4: Correlation coefficient between independent variables of the multivariate model.

Variables	Age	Absence COPD	Absence malignancy	Independent status	Presence invasive device	Length stay since start treatment	Colonized nares	Colonized plis inguinal
Absence of COPD	-0.2201 ^a							
Absence of malignancy	-0.0397 ^a	-0.0571 ^b						
Independent status	-0.3349 ^a	-0.0949 ^b	-0.0901 ^b					
Presence of invasive device	0.0539 ^a	-0.0306 ^b	0.1914 ^b	0.1380 ^b				
Length stay since start treatment	-0.2679 ^a	0.0536 ^b	0.1698 ^b	0.4304 ^b	0.1120			
Colonized nares	0.0434 ^a	0.0797 ^b	0.0897 ^b	0.2147 ^b	0.0473	0.0702		
Colonized inguinal region	-0.1367 ^a	0.0098 ^b	0.0780 ^b	0.3105 ^b	0.1175	0.2199	0.2889	
Number of colonized sites	-0.0860 ^a	0.0718 ^b	0.0024 ^b	0.1033 ^b	0.0660	0.1380	0.5379	0.6516

^aPoint biserial correlation coefficient^bPhi coefficient

3.5 Discussion

This case-control study was based on the data of a previous clinical trial which took place at the HUG. It compared polyhexanide treatment versus a placebo for the decolonization of MRSA carriers.

In the univariate logistic regression, three determinants were significant for an association with a success of MRSA-decolonization; independent status, the length of stay since the start of treatment ≤ 3 weeks and a single-body-site colonized (nares or groin) at baseline, while in the univariate log-binomial regression, only two determinants were significant; independent status and a single-body-site colonized (nares or groin) at baseline.

Table 3.5: Multivariate logistic regression.

Variables	aOR	95% CI	P Value
Patient characteristics			
Degree of dependence			
<i>Dependant or semi-dependant</i>	1.00	...	
<i>Independent</i>	2.83	1.26 - 6.34	0.011
Colonization characteristics			
Colonized sites			
<i>2 (nose and groin)</i>	1.00	...	
<i>1 (nose or groin)</i>	2.16	1.03 - 4.56	0.042

CI, confidence interval; aOR, adjusted odds ratio

Table 3.6: Multivariate log-binomial regression.

Variables	aRR	95% CI	P Value
Patient characteristics			
Degree of dependence			
<i>Dependant or semi-dependant</i>	1.00	...	
<i>Independent</i>	2.04	1.14 - 3.63	0.016
Colonization characteristics			
Colonized sites			
<i>2 (nose and groin)</i>	1.00	...	
<i>1 (nose or groin)</i>	1.64	1.03 - 2.61	0.037

CI, confidence interval; aRR, adjusted risk relatif

According to the results of the multivariate model two determinants were systematically associated with a MRSA decolonization success: the independent status of the colonized patient and a single-body-site which was MRSA-positive. A short length of stay since the start of treatment was not significant in the multivariate model. The results were consistent with the study performed previously in which a higher number of MRSA-colonized sites were associated with an increased risk of MRSA-decolonization failure [199]. Concerning the other determinants highlighted in the multivariate model, the independent status in daily activities, had never been reported to be associated with higher likelihood of an MRSA decolonization success. However, Ammerlaan et al. showed in a previous study that a dependent status leads to a poor compliance to an MRSA decolonization treatment and, consequently, a higher likelihood of MRSA-decolonization failure [187]. Unfortunately, patient compliance in the initial RCT was not tested, so these variables were not included in our univariate and multivariate models to potentially confirm this hypothesis. Based on a literature review, other risk factors were associated with an MRSA-decolonization failure in previous studies such as an older age, a recent antibiotic use, especially fluoroquinolones, a immunosuppressive treatment, the presence of skin wound or a central venous catheter and finally a pulmonary disease [187, 198, 200, 201]. Although these variable were available in our dataset and included in the univariate and multivariate analysis, no positive association between their absences and an MRSA-decolonization success was found. These results could be explained by the flow frequency of these exposures in our case-control study.

Our study presents some limitations. The first one was the reduced number of the eligible patients included in the initial RCT, excluding the sickest patients and those receiving systemic antibiotics treatment against MRSA infection. Consequently, the study population may not be representative of all potential MRSA carriers. Moreover, a few determinants known to be associated with MRSA decolonization failure were not tested in our analysis due to the absence of these data in the initial RCT, e.g. colonization at other body sites (eg, axilla, throat) and the MRSA carriage among household contacts [187, 198, 200, 202, 203]. Despite those limitations, the prospective design and data collection remains a strength of this case-control study

Thanks to the results of this study, we can now better understand the MRSA decolonization process and potentially target the patients for topical MRSA decolonization to improve chances of success. By choosing independent patients with only one body site MRSA colonized, we can increase the effectiveness of decolonization treatment and by this way limit the emergence of resistance to topical decolonization agents [198].

This work was accepted for publication in January 2016 and published in the *Infection Control & Hospital Epidemiology* journal in June 2016 (Appendix 4).

Chapter 4

Cost-effectiveness analysis of linezolid versus trimethoprim-sulfamethoxazole plus rifampicin for the treatment of MRSA infection: a health care provider perspective

"Rêver est une chose douce qui ne coûte rien et qui rassure."

Tahar Ben Jelloun

4.1 Introduction

Invasive infection caused by MRSA represents an important therapeutic challenge. A prolonged course of vancomycin or daptomycin is the most frequently recommended treatment [215]. Vancomycin treatment may increase healthcare costs due to the extended hospital stay and can thus expose patients to complications associated with renal toxicity, venous lines and the need for a therapeutic drug monitoring. Alternative antibiotic treatments available for oral administration have been proposed such as fluoroquinolone and rifampicin for MSSA and LZD for MRSA [216,217]. The use of older antibiotics such as minocyclin or TMP-SMX combined with RMP or fusidic acid may represent a particularly interesting treatment alternative for both HA- and CA-MRSA infections [215,218,219].

Between January 2009 and December 2013, we performed a randomized, non-inferiority clinical trial to evaluate the efficacy and the safety of a treatment based on TMP-SMX plus RMP versus LZD to treat MRSA infections [122] (Appendix 5). In this study, we showed that among the 150 included patients, 59/75 (78.7%) in the TMP-SMX plus RMP group 56/75 (74.7%) in the LZD group experienced a clinical success (risk difference=4%, 95%CI [-9.7% to 17.6%]. Compared to LZD, a treatment of TMP-SMX plus RMP is non-inferior

for the treatment of MRSA infections. Moreover, there were no statistical differences between the treatments in terms of a total adverse event (AE), serious adverse event (SAE) and adverse drug reaction (ADR) [122]. A full description of this RCT was published elsewhere [122].

Furthermore, generic agents are available for TMP-SMX and RMP. Consequently, these regimen may offer an attractive cost advantage over other agents such as daptomycin and LZD [220]. As the launch of a generic agent for LZD was postponed in several countries for the end of 2016 and new oxazolidinone agent such as tedizolid will be patent-protected against generic erosion for still many years, the off-patent combination of TMP-SMX and RMP seems to be an attractive alternative for oral treatment options against MRSA infection, though still underused because of safety concerns. This combination of agents may still generate substantial indirect costs due to rare, costly and potentially severe ADR.

4.2 Objective

For the reasons described in the previous section, we decided to perform a cost-effectiveness analysis (CEA) using data from the RCT to elucidate the role and evaluate the economic impact of both treatment regimens from the perspective of a health-care provider

4.3 Methods

4.3.1 Trial design

This trial was a non-inferiority, single-centre, open-label, investigator-initiated, randomized control trial to estimate the efficacy and safety of a combined treatment of TMP-SMX plus RMP versus LZD in patients (allocation ratio 1:1) requiring antibiotics treatment for MRSA infection. The study protocol was approved by the Swiss agency for the therapeutic product (SwissMedic no. 2008DR4305) and the local institutional review board (no. 08-56). It has been registered with www.ClinicalTrials.gov (NCT00711854). All participants gave their written consent.

4.3.2 Setting and participants

We conducted the study at the HUG, which is a Swiss tertiary care center with among 1'900 beds and 48'000 yearly admissions. In 2011, the antibiotics susceptibility revealed that MRSA isolates from clinical culture were 97%, 97%, 100% and 100% susceptible to TMP-SMX, RMP, LZD and vancomycin, respectively.

The patients were enrolled between January 2009 and December 2013. The patients were adults (≥ 18 years) and able to give informed consent. They had clinical symptoms and signs of MRSA infection according to the result of a clinical culture (MRSA had to be

the predominant or unique microorganism culture). The isolated MRSA strain had to be susceptible to all antibiotics of the study. For MRSA identification and antimicrobial susceptibility testing, standard microbiology methods were used like genotypical (*femA* and *mecA* determination, using the BD GeneOhm MRSA assay) and phenotypical methods (using disc diffusion method, interpreted with CLSI breakpoints). Daily surveys and an electronic alert of all MRSA-positive cultures generated by the clinical bacteriology laboratory allowed us to identify potentially eligible patients [221].

The patients presenting the following criteria were excluded from the study: an antibiotic treatment against MRSA ≥ 72 h prior to study inclusion (mostly vancomycin), pregnancy or breastfeeding, known hypersensitivity to TMP-SMX, RMP or LZD, impairment of hepatic function, thrombocytopenia ($< 50\,000$ platelet/ μL), haemodialysis, chronic MRSA osteomyelitis without surgical debridement, severe sepsis or septic shock due to MRSA bacteraemia [222], a superinfected indwelling foreign body kept in place, left-sided endocarditis, an history of phaeochromocytoma, untreated hyperthyroidism or carcinoid syndrome, no oral contraception or being receipt of serotonergic agents. All types of infections were included in the study except the infections mentioned in the exclusion criteria above.

4.3.3 Interventions

All patients who were randomized in the TMP-SMX plus RMP arm received intravenous infusion (with a suggested maximum duration of 5 days) or an oral therapy of 160mg of TMP and 800mg of SMX three times per day and 600mg of RMP once per day. Patients randomized to the LZD arm received intravenous infusion or an oral therapy of 600mg twice a day. Any modification or discontinuation of the antimicrobial treatment was left to the judgment of the treating physicians. Systemic antibiotic treatment without anti-MRSA activity could be added in case of polymicrobial infection not covered by the study drugs, if it was considered to be clinically indicated.

4.3.4 Outcomes

Assessments of clinical symptoms and signs of infection, bacteriological results, as well as laboratory and clinical safety evaluation, were made at study baseline and the end of study treatment. A study physician made the final study assessment at 6 weeks (± 1 week) after the patient received the last dose of the study drug. In case the patient could not be reached for the final assessment, a telephone contact and an evaluation by the general practitioner in charge were considered sufficient. The study outcome was the clinical resolution of MRSA infection at 6 weeks after the end of the study drug. It was defined as the resolution of all the clinical symptoms and signs of the infection that were previously present at the beginning of the study [216]. Failure was defined as a deterioration or no improvement in the clinical condition or a change of the allocated treatment at any time of the study or the death of the patient. The outcome was determined using all available

information including microbiology results, subsequent radiographic or surgical intervention and the patient's clinical course.

AE, SAE, and ADR were monitored for all included patients. ADR was defined as harm causally related to the infusion or administration of the study drug at a standard dosage. Toxicity was considered to be related to the study drug if it began when the treatment were first administered, abated after the discontinuation of it and was not clearly attributable to other causes.

4.3.5 Randomization

Once the antimicrobial susceptibility testing results were available, and with all the enrollment criteria were fulfilled, the patients were contacted by the investigators. Patients meeting all the inclusion criteria (including the absence of any exclusion criteria) were randomly assigned by sealed, numbered, opaque envelopes to one of the two intervention arms. After signing informed consent, the sequence was generated using an internet-based randomization generator with a block size of 30.

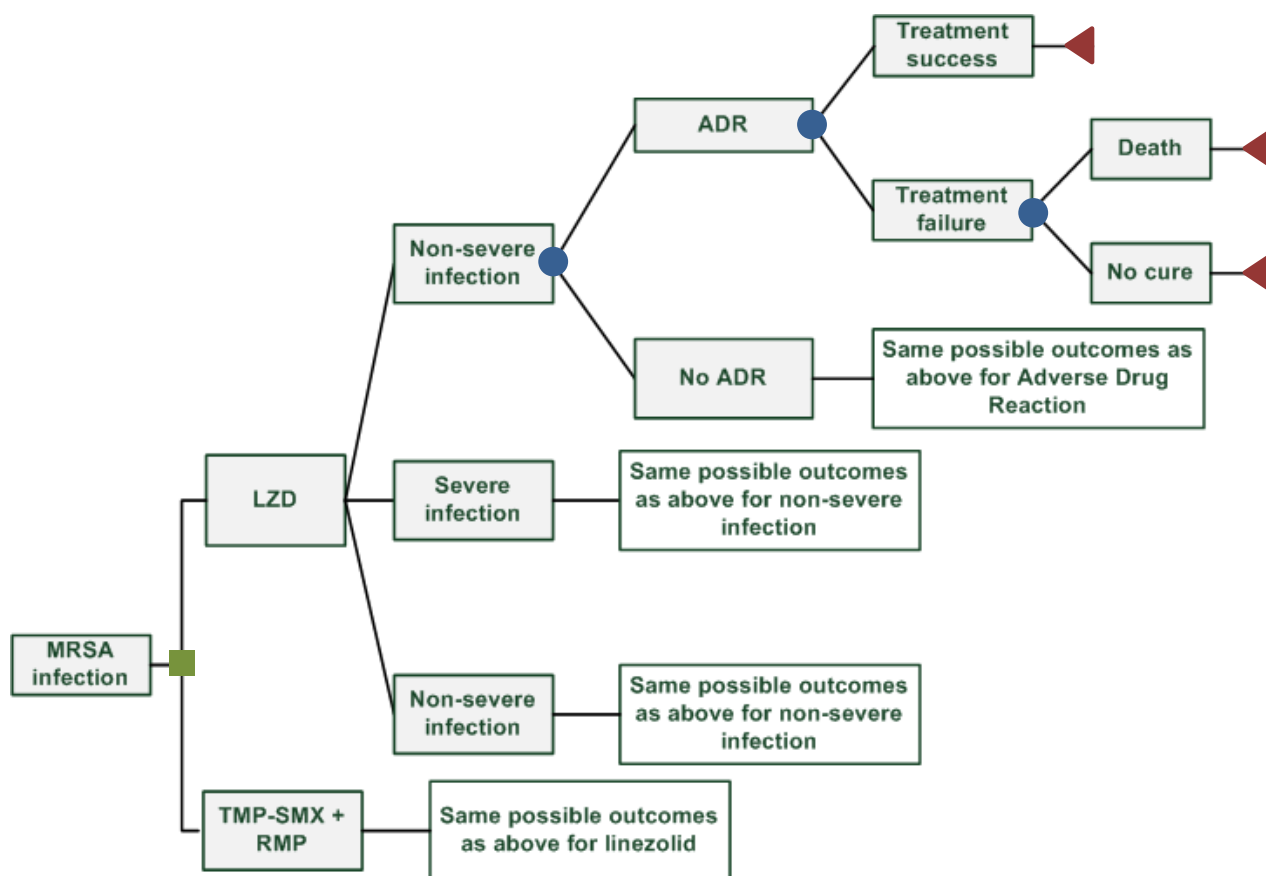
4.3.6 Sample size

This non-inferiority trial has been designed to test whether the clinical efficacy of treating MRSA infections with TMP-SMX plus RMP is comparable to LZD. The 95% CI for the difference in success rate (success rate at 6 weeks after the end of treatment in the TMP-SMX plus RMP arm minus success rate cure at 6 weeks after the end of therapy in the LZD arm) was calculated base on the normal approximation to the binomial distribution [223]. The non-inferiority test was based on the lower boundary of the 95% CI for a clinically relevant difference in success rates and settled within the non-inferiority margin of 20% and the upper limit containing 0%. A difference of at least 20% in the clinical success rate of the two treatment regimens would justify the use of one over the other. We based this margin on acceptable differences in cure rates but also on considerations of treatment costs and study feasibility. For a statistical power of 80% and a one-sided significance of 0.025, and assuming a 75% success rate in both treatment groups [130, 217, 224], we estimated that 90 evaluable patients needed to be enrolled in each intervention group to test the null hypothesis that the treatment efficacy would differ by >20%.

4.3.7 Analyzed population

For the analysis, we used the ITT population which including all patients who have been randomized. The patients lost to follow-up (n=13), were assigned to the outcome "failure."

Figure 4.1: Decision tree model.



4.3.8 Cost-effectiveness analysis (CEA)

We decided to conduct a CEA and more specifically a cost-utility analysis to compare the two interventions using a decision tree. A decision-analytic computer simulation model was constructed from a health-care provider perspective. All the simulation and analyses were performed using TreeAge pro 2015 (TreeAge Software, Williamstown, Massachusetts, USA). The decision tree of this CEA based on the design and data of the previously published RCT comparing TMP-SMX plus RMP versus LZD for the treatment of MRSA infection (Figure 4.1).

The base case scenario was defined by the following conditions:

$$\begin{aligned} \text{Incremental cost (CHF/€)} &= (\text{TMP-SMX} + \text{RMP cost}) - (\text{LZD cost}) \\ \text{Incremental effectiveness (QALYs)} &= (\text{TMP-SMX} + \text{RMP effectiveness}) - (\text{LZD effectiveness}) \\ \text{Incremental cost-effectiveness ratio (ICER)} &= \text{Incremental cost} / \text{Incremental effectiveness} \end{aligned}$$

Table 4.1: Model input data for the base-case scenario.

Variables	NSInf		SInf		DFInf		Ref.
	Mean	SD	Mean	SD	Mean	SD	
Probabilities							
LZD treatment (N=75)	0.36	0.06	0.41	0.06	0.23	0.05	Beta [122]
Presence of ADR	0.04	0.04	0.13	0.06	0.00	0.00	Beta [122]
Treatment failure	0.19	0.07	0.29	0.08	0.29	0.11	Beta [122]
Death among treatment failure	0.00	0.00	0.67	0.16	0.40	0.22	Beta [122]
TMP-SMX + RMP treatment (N=75)							
Presence of ADR	0.47	0.06	0.29	0.05	0.24	0.05	Beta [122]
Treatment failure	0.14	0.06	0.06	0.04	0.06	0.05	Beta [122]
Death among treatment failure	0.14	0.06	0.23	0.09	0.33	0.11	Beta [122]
Death among treatment failure	0.00	0.00	0.60	0.22	0.33	0.19	Beta [122]
Duration of treatment (days)							
LZD treatment (N=75)							
IV administration	0.63	1.84	0.97	2.95	1.65	3.46	Gamma [122]
PO administration	7.11	3.37	10.98	4.56	28.71	10.74	Gamma [122]
TMP-SMX + RMP treatment (N=75)							
IV administration	0.03	0.17	0.73	2.98	4.83	9.86	Gamma [122]
PO administration	7.89	2.18	12.00	4.27	32.28	28.64	Gamma [122]
Costs							
LZD IV treatment (600mg)	Price, by drug unit^b, CHF / €						
	92.23/84.77						

DFInf: infections associated with deep-seated foci; Distrib.: distribution; NSInf: non-severe infections; SInf: severe infections; Ref.: References; SInf: severe infections

^a Cost are adjusted to December 2016

^b <http://www.listedesspecialites.ch/FederalDepartmentofHomeAffairs-FederalOfficeofPublicHealth-Listofspecialities> [cited 2016 December].

^c The price of this kit is 5.75 CHF, provided by the pharmacy of the Geneva University Hospitals. According to the local recommendations, the peripheral venous catheter has to be changed every 4 days, representing a daily price of this supply for intravenous administration of 1.44 CHF, which needs to be added to the daily cost of the intravenous antibiotic treatment.

Table 4.1 (continued): Model input data for the base-case scenario.

Variables	NSInf	SInf	DFInf	Ref.
LZD PO treatment (600mg)		94.14/86.53		^c
TMP-SMX IV treatment (800/160mg)		5.08/4.67		^c
TMP-SMX PO treatment (800/160mg)		0.67/0.62		^c
RMP IV treatment (600mg)		3.48/3.20		^c
RMP PO treatment (600mg)		37.6/34.56		^c
RMP IV treatment (600mg)		3.48/3.20		^c
ADR due to LZD treatment	0.00/0.00	10.09/9.27	0.00/0.00	^c
ADR due to TMP-SMX + RMP treatment	20.24/18.60	0.00/0.00	42.77/39.31	^c
ADR due to LZD treatment (mean)		1.44/1.32		^c
QALY				
Death	0.00	0.00	0.00	[225–227]
Cure	1.00	1.00	1.00	[225–227]
No cure				
<i>LZD</i>	0.96	0.90	0.86	[225–227]
<i>TMP-SMX + RMP</i>	0.95	0.89	0.82	[225–227]
<i>ADR</i>	1.00	1.00	1.00	[225–227]

DFInf: infections associated with deep-seated foci; Distrib.: distribution; NSInf: non-severe infections; Ref.: References; SInf: severe infections
^a Cost are adjusted to December 2016

^b <http://www.listedesspecialites.ch/FederalDepartmentofHomeAffairs-FederalOfficeofPublicHealth-Listofspecialities>
[cited 2016 December].

^c The price of this kit is 5.75 CHF, provided by the pharmacy of the Geneva University Hospitals. According to the local recommendations, the peripheral venous catheter has to be changed every 4 days, representing a daily price of this supply for intravenous administration of 1.44 CHF, which needs to be added to the daily cost of the intravenous antibiotic treatment.

4.3.9 Probabilities and duration of study drugs

All the effectiveness probabilities used in the model are presented in Table 4.1 and are based on the previous RCT. These probabilities are the efficacy of the study drugs, the rate of ADR and the cumulative incidence of death observed in each study arm of treatment. Data surrounding duration (days) of each treatment were stratified by mode of administration per os (PO) versus intravenous (IV) and obtained from the RCT.

4.3.10 Costs

In this CEA, direct costs in Swiss francs (CHF) and euro (€) (1 CHF = 0.92€, December 2016) have been used for the study drug and the ADR costs (Table 4.2). The official Swiss medication agency's website was used to collect drug costs; they were converted in CHF (Table 4.2). If the drug was sold at different unit prices, the most expensive unit price of the drug was used in the model. Concerning the studied antibiotic drug, no discount was offered to our institution, and none was considered for the base case scenario. Some micro costs, specifically related to the use of IV equipment, both for the study drug and those needed for ADR treatment were added in the model. Other ADR-related costs were included in the model were those related to the lab testing required for the investigation of the ADR as well as additional drugs. The prices charged to HUG (adjusted to December 2016) were included in the model under the laboratory costs. No additional medical exam or hospital stay extension was recorded in our study related to our ADR.

4.3.11 Quality-adjusted life years

The effectiveness outcome from our CEA was the quality-adjusted life year (QALY) (Table 4.1). QALY is a generic measure of disease burden, including quality and quantity of life lived and which is commonly used in health-related economic evaluation such as the CEA. A utility weight that typically ranges from 0 (death) to 1 (perfect health) were applied to estimate QALYs. In our model, a QALY value of 1 was attributed if the patient fully recovered and a QALY of 0 if the patient died during the study. In the case of a patient experiencing a treatment failure during the study not leading to a death, we attributed a utility related to the severity of the MRSA infection developed by the patient [225]. The type of infection was stratified into three categories (severe, associated with deep-seated foci and severe infection) and was determined by the duration of the therapy and the site of infection, as defined in the RCT [122]. The utilities attributed to each category of infection was derived from the Health-Related Quality-of-Life (HQORL) score using the EuroQol 5D Health Domains (with United Kingdom scoring) [226, 227]. The QALYs were calculated by weighting the utilities by the average duration of MRSA infection: in the RCT (7/8 days for non-severe infections, 13 days for severe infections and 30/38 days for infections associated with deep-seated foci, mainly osteomyelitis, for LZD and TMP-SMX plus RMP respectively. The same process was performed to attribute QALYs for patients who developed an ADR during the study.

Table 4.2: Adverse Drug Reaction costs during RCT.

N°	Treatment	Type	Name	Treatment administered	Adm.	Dosage	Num.	Laboratory test	Costs
								Type	Num.
1	TMP-SMX + RMP	Neurological			∅			∅	0\$
2	LZD	Tongue discoloration			∅			∅	0\$
3	TMP-SMX + RMP	Dermatological	clemastine fumarate	IV	2mg	2		∅	8.03\$
			prednisone	PO	20mg	2		∅	
4	TMP-SMX + RMP	Gastrointestinal	metoclopramid	PO	10mg	1		∅	44.46\$
			ondansetron	PO	8mg	4		∅	
			domperidone	PO	10mg	2		∅	
5	LZD	Nephrological	NaCl	IV	0.9% - 1.5l	1		creatinine	3 34.79\$
								urea	3
6	TMP-SMX + RMP	Nephrological			∅			∅	0\$
7	LZD	Haematological			∅			∅	0\$
8	TMP-SMX + RMP	Gastrointestinal	domperidone	PO	10mg	1		∅	0.50\$
9	TMP-SMX + RMP	Dermatological	clemastine fumarate	IV	2mg	4		∅	14.06\$
10	LZD	Gastrointestinal	domperidone	PO	10mg	1		∅	6.98\$
		ondansetron	PO	4mg	1			∅	
11	TMP-SMX + RMP	Gastrointestinal	domperidone	PO	10mg	2		pregnancy test	1 25.68\$
			ondansetron	PO	4mg	1		∅	
12	TMP-SMX + RMP	Nephrological			∅			∅	0\$
13	TMP-SMX + RMP	Gastrointestinal	ondansetron	PO	8mg	3		∅	58.36\$
			ondansetron	PO	4mg	4		∅	

Adm.: administration; IV: intravenous; LZD: linezolid; Num.: number; PO: per os; RMP: rifampicin; TMP-SMX: trimethoprim-sulfamethoxazole

4.3.12 One-, two-, and three-way sensitivity analyses

Sensitivity analyses were performed to evaluate how the variation in one, two or three variables can potentially affect the model results. A couple of key parameters were altered to test potential differences in a real world setting, including LZD efficacy, ADR cost and LZD prices (see below for full list).

4.3.13 Probabilistic sensitivity analyses

A probabilistic sensitivity analysis was conducted using a Monte Carlo (MC) simulation in order to allow for simultaneous variation of all variables [228]. Concerning the variable of the duration of treatment, we assumed that it follows a gamma distribution, due to its continuous nature. An MC simulation was performed to sample randomly from those distributions, comparing plausible ICERs over 10'000 iterations. An incremental cost-effectiveness plane was created to facilitate interpretation of the results with a built 95% confidence ellipse. We also calculated the cost-effectiveness acceptability curve (CEAC) to summarize the information and support decision-making under differing perceptions of potential risk and benefits.

4.3.14 Generic linezolid cost

In many countries, generic LZD was made available by 2016. Consequently, simulations of our CEA were performed with plausible whole-sale prices of generic LZD. The generic LZD price can be 10% - 60% less expensive than the originator LZD price, according to the Swiss Federal Authorities and depend on sales volume of the drug [229]. In Switzerland, the price of LZD was recently fixed with a 10% discount compared to the originator. The reduction can be as much as 50% in Italy and Germany. Altering the LZD price in line with the different possible price levels, we conducted a sensitivity analysis.

4.3.15 Linezolid efficacy

A couple of RCTs on LZD efficacy to treat MRSA infections have already been published. Using each of these studies to extract the various frequency of LZD efficacy to treat MRSA infection, a literature review was performed (Table 4.3). We identified 20 different clinical trials, with an efficacy of LZD against MRSA infection ranging from 0.37 to 1.00, with a median of 0.75 and a weighted average of 0.69 (weighted by the number of patients included in the study). We incorporated these range of values and the weighted average from the literature, in a triangular distribution in the sensitivity analyses.

4.3.16 Serious adverse drug reaction

In our RCT, the sample size of patients included was relatively small and can explain that rare and serious ADR due to the study treatment did not occur during the study and was

Table 4.3: Literature review on the efficacy of linezolid to treat MRSA infection, generate by RCTs.

Authors	Year	Title	n (success)	Total N	Linezolid efficacy (%)
Rubinstein [129]	2001	Linezolid (PNU-100766) versus vancomycin in the treatment of hospitalized patients with nosocomial pneumonia a randomized, double-blind, multicenter study	15	23	0.65
Stevens [130]	2002	Linezolid versus vancomycin for the treatment of methicillin-resistant Staphylococcus aureus infections.	59	98	0.60
Wible [230]	2003	Linezolid versus cefadroxil in the treatment of skin and skin structure infections in children.	13	14	0.93
Kaplan [131]	2003	Linezolid versus vancomycin for treatment of resistant Gram-positive infections in children.	15	18	0.83
Yogev [231]	2003	Linezolid for the treatment of complicated skin and skin structure infections in children.	9	10	0.90
Wunderink [132]	2003	Linezolid vs vancomycin: analysis of two double-blind studies of patients with methicillin-resistant Staphylococcus aureus nosocomial pneumonia.	36	75	0.48
Lipsky [232]	2004	Treating foot infections in diabetic patients: a randomized, multicenter, open-label trial of linezolid versus ampicillin-sulbactam/amoxicillin-clavulanate.	13	18	0.72
Weigelt [133]	2004	Linezolid eradicates MRSA better than vancomycin from surgical-site infections.	26	30	0.87
Sharpe [134]	2005	Clinical and economic outcomes of oral linezolid versus intravenous vancomycin in the treatment of MRSA-complicated, lower-extremity skin and soft-tissue infections caused by methicillin-resistant Staphylococcus aureus.	15	30	0.50
Weigelt [135]	2005	Linezolid versus vancomycin in treatment of complicated skin and soft tissue infections.	125	176	0.71
Kohno [136]	2007	Linezolid versus vancomycin for the treatment of infections caused by methicillin-resistant Staphylococcus aureus in Japan.	22	60	0.37
Wunderink [137]	2008	Early microbiological response to linezolid vs vancomycin in ventilator-associated pneumonia due to methicillin-resistant Staphylococcus aureus. ¹³	23	0.57	
Wilcox [233]	2009	Complicated skin and skin-structure infections and catheter-related bloodstream infections: noninferiority of linezolid in a phase 3 study.	42	48	0.88

Table 4.3 (continued): Literature review on the efficacy of linezolid to treat MRSA infection, generate by RCTs.

Authors	Year	Title	n (success)	Total N	Linezolid efficacy (%)
Itani [234]	2010	Efficacy and safety of linezolid versus vancomycin for the treatment of complicated skin and soft-tissue infections proven to be caused by methicillin-resistant <i>Staphylococcus aureus</i> .	223	276	0.81
Craft [235]	2011	A randomized, double-blind phase 2 study comparing the efficacy and safety of an oral fusidic acid loading-dose regimen to oral linezolid for the treatment of acute bacterial skin and skin structure infections.	37	37	1.0
Covington [236]	2011	Randomized, double-blind, phase II, multicenter study evaluating the safety/tolerability and efficacy of JNJ-Q2, a novel fluoroquinolone, compared with linezolid for treatment of acute bacterial skin and skin structure infection.	25	29	0.86
Wunderink [237]	2012	Linezolid in methicillin-resistant <i>Staphylococcus aureus</i> nosocomial pneumonia: a randomized, controlled study.	102	186	0.55
Noel [238]	2012	A randomized, evaluator-blind, phase 2 study comparing the safety and efficacy of omadacycline to those of linezolid for treatment of complicated skin and skin structure infections.	30	32	0.94
Prokocimer [239]	2013	Tedizolid phosphate vs linezolid for treatment of acute bacterial skin and skin structure infections: the ESTABLISH-1 randomized trial.	77	90	0.86
Chavanet [138]	2013	The ZEPHYR study: a randomized comparison of linezolid and vancomycin for MRSA pneumonia.	95	165	0.58
Harbarth [122]	2015	Randomized non-inferiority trial to compare trimethoprim/sulfamethoxazole plus rifampicin versus linezolid for the treatment of MRSA infection.	56	75	0.75

Table 4.4: Serious adverse drug reaction simulation.

Treatment	Type of ADR	Incidence maximum	Cost by ADR	Average cost by patient treatment by the study drug	QALYs
RMP	Liver failure requiring liver transplantation	Rare 1/1'000 ^a	197'471\$ ^b	197\$	0.69 [240]
TMP-SMX	Lydell, Stevens-Johnson syndrome	Very rare 1/10'000 ^a	32'935\$ ^b	3\$	0.66 [241]
TMP-SMX	Renal failure with dialysis	Very rare 1/10'000 ^a	72'800\$ ^b (Average duration, 21 years)	153\$	0.70 [242]

ADR: adverse drug reaction; LZD: linezolid; QALYs: quality-adjusted life years; RMP: rifampicin; TMP-SMX: trimethoprim-sulfamethoxazole

^a Real incidence is unknown, the maximum incidence found in the Swiss drug information has been applied

^b Average cost charged at Geneva University Hospitals in 2015

thus not accounted for in the base-case scenario. However, since rare and serious ADR can be extremely expensive and could increase the cost of treatment per patient. The risk could not be ignored. A literature review was performed on prescribing manual's and the pharmaco-vigilance reference standards to identify some of the serious ADR described in the past. Those considered relevant were added in the analysis. For each rare serious ADR, once the full cost that would be charged on the patient's bill at HUG was determined, the incidence was included in the model and the sensitivity analyses were updated (Table 4.4). For serious ADR, QALYs were constructed using data from published literature. Based on the available drug information, we choose to include in our analysis one additional serious ADR due to RMP (acute liver failure requiring liver transplant) and two due to TMP-SMX (acute renal failure requiring dialysis and Toxic Epidermal Necrolysis (TEN)) [240–242].

4.4 Results

According to the decision tree (Figure 4.1) and using the base case scenario with the mean value of each probability and all duration treatments (Table 4.2), the CEA showed that the combined treatment of TMP-SMX plus RMP (159CHF/146€ and 0.916 QALY) was less costly and more effective than the LZD treatment (2758CHF/2535€ and 0.881 QALY). The combine of old treatment is dominant compare to LZD therapy to treat MRSA infection. The treatment combining of TMP-SMX and RMP has a cost by one QALY gain of 174CHF/160€ compared to 3130CHF/2877€ (Table 4.5).

Table 4.5: Base-case scenario by types of infections.

	AllInf	NSInf	SInf	DFInf
LZD treatment				
Cost	2536€	1338€	2066€	5248€
Effectiveness (QALY)	0.881	0.992	0.796	0.860
ACER (€/QALY)	2877	1348	2595	6105
TMP-SMX + RMP treatment				
Cost	146€	44€	97€	406€
Effectiveness (QALY)	0.916	0.993	0.846	0.851
ACER (€/QALY)	160	44	115	477
Incremental cost	-2390€	-1294€	-1969€	-4842€
Incremental effectiveness (QALY)	0.035	0.001	0.050	-0.008
ICER (€/QALY)	Dominant	Dominant	Dominant	631883

ACER: average cost-effectiveness ratio; AllInf: all infections DFInf: infections associated with deep-seated foci; ICER: incremental cost-effectiveness ratio; NSInf: non-severe infections; SInf: severe infections

By type of infection (respectively, non-severe, severe and deep-foci associated), the average cost-effectiveness ratio (ACER) were 44, 115 and 477€/QALY for TMP-SMX plus RMP and 1348, 2595 and 6105€/QALY for LZD.

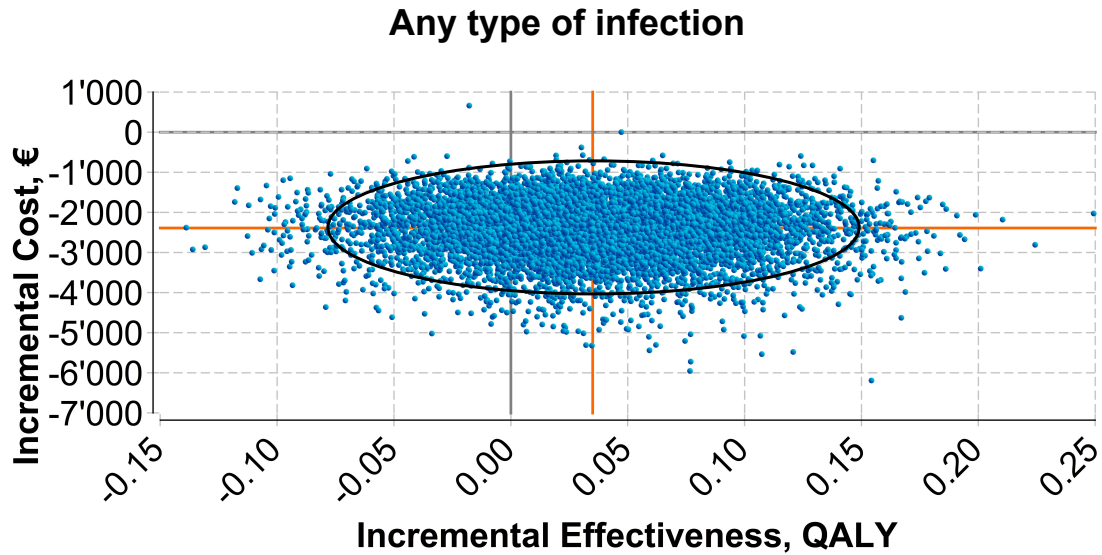
We decided to conduct a probabilistic sensitivity analysis using a MC simulation to create an incremental cost-effectiveness plane and a CEAC in which all parameters of the model were simultaneously varied (Figure 4.2). According to the MC simulation, for 78% of the 10'000 simulations the TMP-SMX plus RMP is dominant compared to LZD (7'771 iterations). In 0% of cases the TMP-SMX plus RMP is dominated by the LZD (1 iterations) while in 22% of cases the TMP-SMX plus RMP treatment is less expensive and more effective (2'227 iterations). Finally in 0% of cases the TMP-SMX plus RMP is more expensive and more effective (1 iterations) (Figure 4.2.A). The CEAC showed that with a willingness-to-pay threshold of 0€, 50'000€ and 200'000€, TMP-SMX plus RMP was cost-effective in 100%, 96% and 85% of cases respectively (Figure 4.2.B). Figure 4.3 shows the results of the MC simulation by type of infection.

Figure 4.4, we conducted a one- and two-way sensitivity analyses with different values of the LZD efficacy (0.37 to 1.00), the ADR cost attributed to TMP-SMX plus RMP (0€ to 320€) and discount on LZD prices (0% to 100%). In all one-way sensitivity analyses performed, TMP-SMX plus RMP dominated LZD even in the extreme scenario where the LZD was efficacy fixed at 1.0 (ACER (LZD) = 2536€/QALY), or with a maximum ADR cost attributed to TMP-SMX plus RMP (ACER for TMP-SMP + RMP=506€/QALY), or the highest known discount on the LZD price of 50% (Avg CE for LZD=1439€/QALY) (Figure 4.4.A). Moreover, the one-way sensitivity analysis suggested that a 95% discount on the price of LZD would need to be applied for it to become competitive versus TMP-SMX plus RMP. Two-way sensitivity analyses confirmed these results. In the two-way sensitivity analyses presented in Figure 4.4.B, the TMP-SMX plus RMP combined of treatment were dominant compared to LZD treatment.

Finally, we performed three-way sensitivity analyses, in which the TMP-SMX plus RMP is always dominant compared to LZD (Figure 4.5). For this scenario, we conducted probabilistic sensitivity analyses (MC simulation) to produce CEAC with maximum assumed ADR costs attributed to TMP-SMX plus RMP (320€), a LZD efficacy modeled through a triangular distribution. Each iteration was associated with a different value of the discount LZD prices (0% to 90%) to create ten different CEAC (Figure 4.6). The results showed that the therapy with TMP-SMX plus RMP is cost-effective compared to LZD. Even in an extreme case scenario with a willingness-to-pay set at 200'000€ per QALY gained, the TMP-SMX plus RMP remains cost-effective in 81%, 80%, 79%, 79%, 77%, 78%, 77%, 76%, 76%, and 75% of iterations, respectively for a discount rate of 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90%.

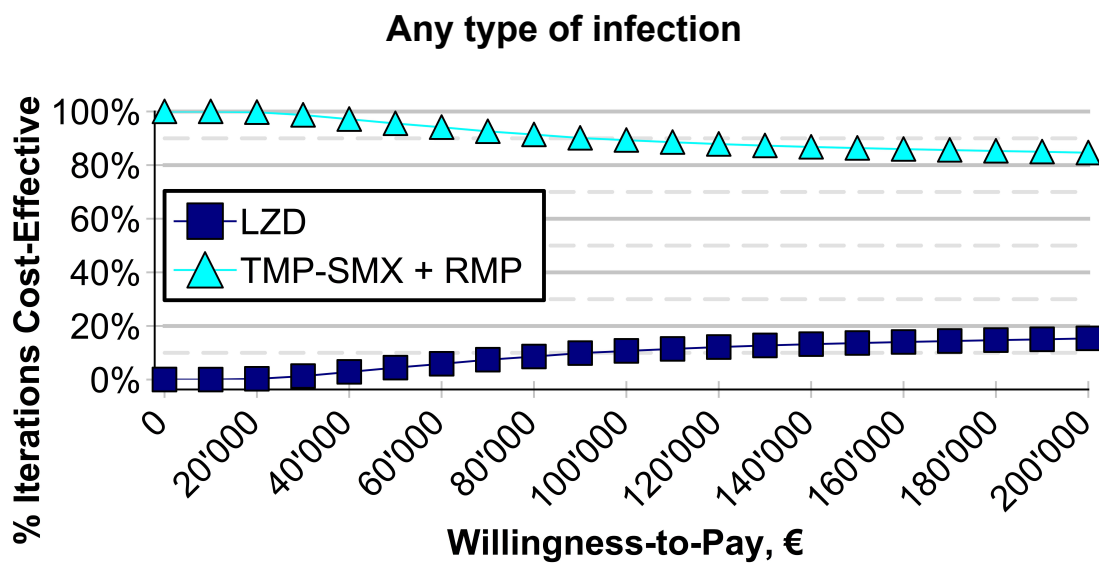
Figure 4.2: Incremental cost-effectiveness planes and table, and cost-effectiveness acceptability curves (CEAC) on study inputs.

A.



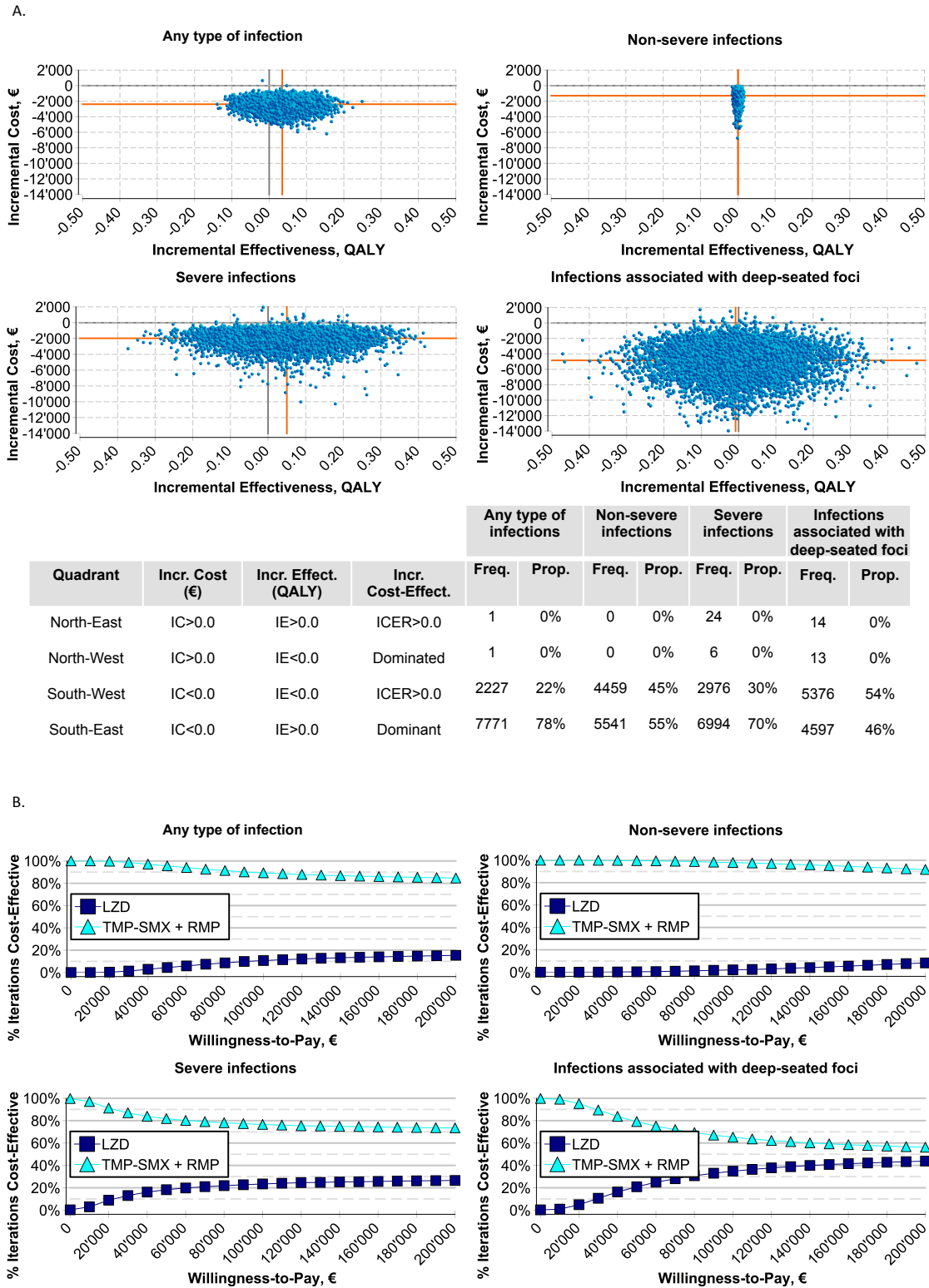
Quadrant	Incr. Cost (€)	Incr. Effect. (QALY)	Incr. Cost-Effect.	Freq.	Prop.
North-East	IC>0.0	IE>0.0	ICER>0.0	1	0%
North-West	IC>0.0	IE<0.0	Dominated	1	0%
South-West	IC<0.0	IE<0.0	ICER>0.0	2227	22%
South-East	IC<0.0	IE>0.0	Dominant	7771	78%

B.



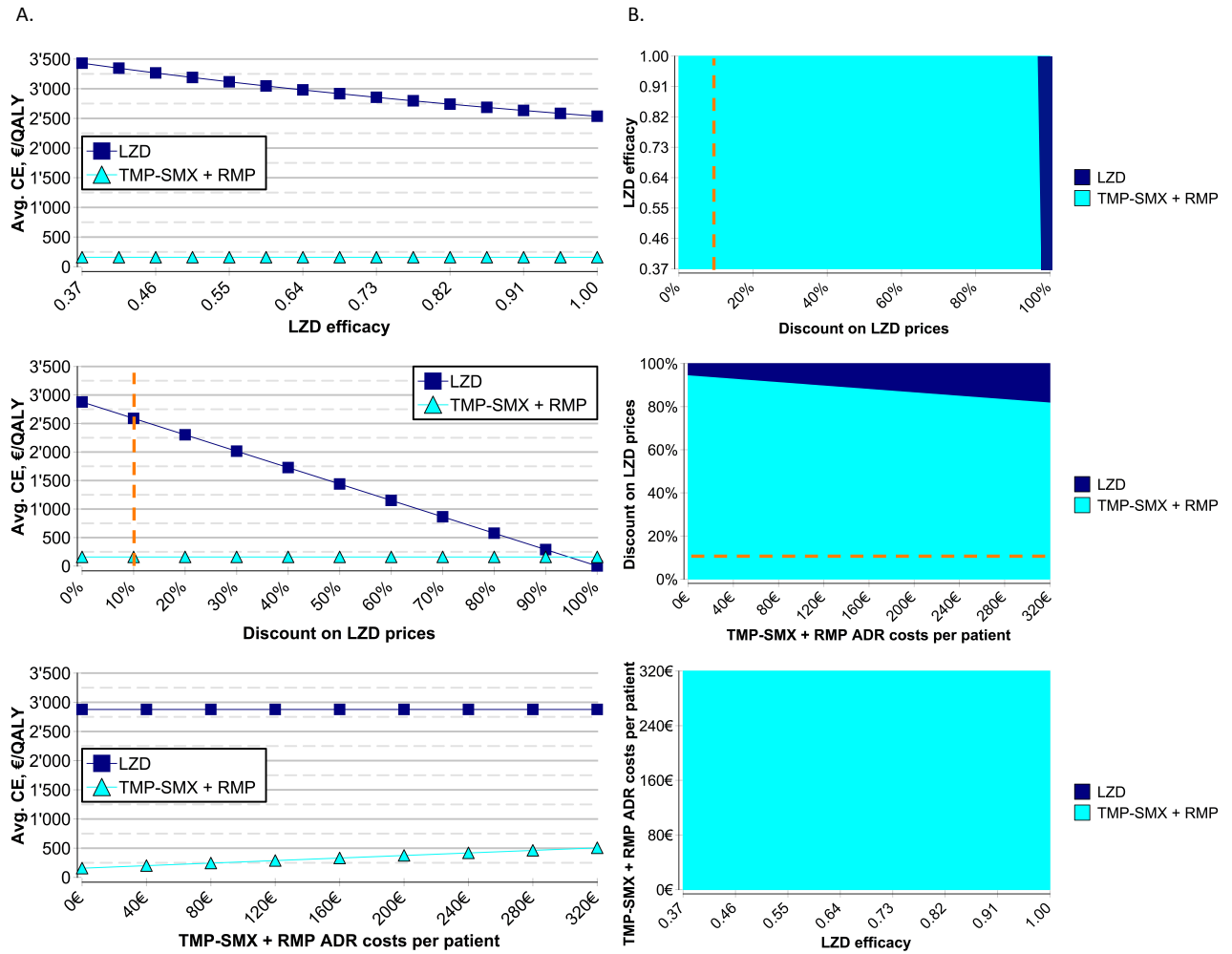
Incr. Cost: incremental cost; Incr. Eff: incremental effectiveness; Incr. cost-Effect.: Incremental cost-effectiveness.
 A. Monte Carlo simulation. Each blue spot represents one of the 10'000 iterations. The two orange lines represent the base-case scenario. B. Cost-effectiveness acceptability curves.

Figure 4.3: Incremental cost-effectiveness planes and table, and cost-effectiveness acceptability curves (CEAC) on study inputs by types of infections.



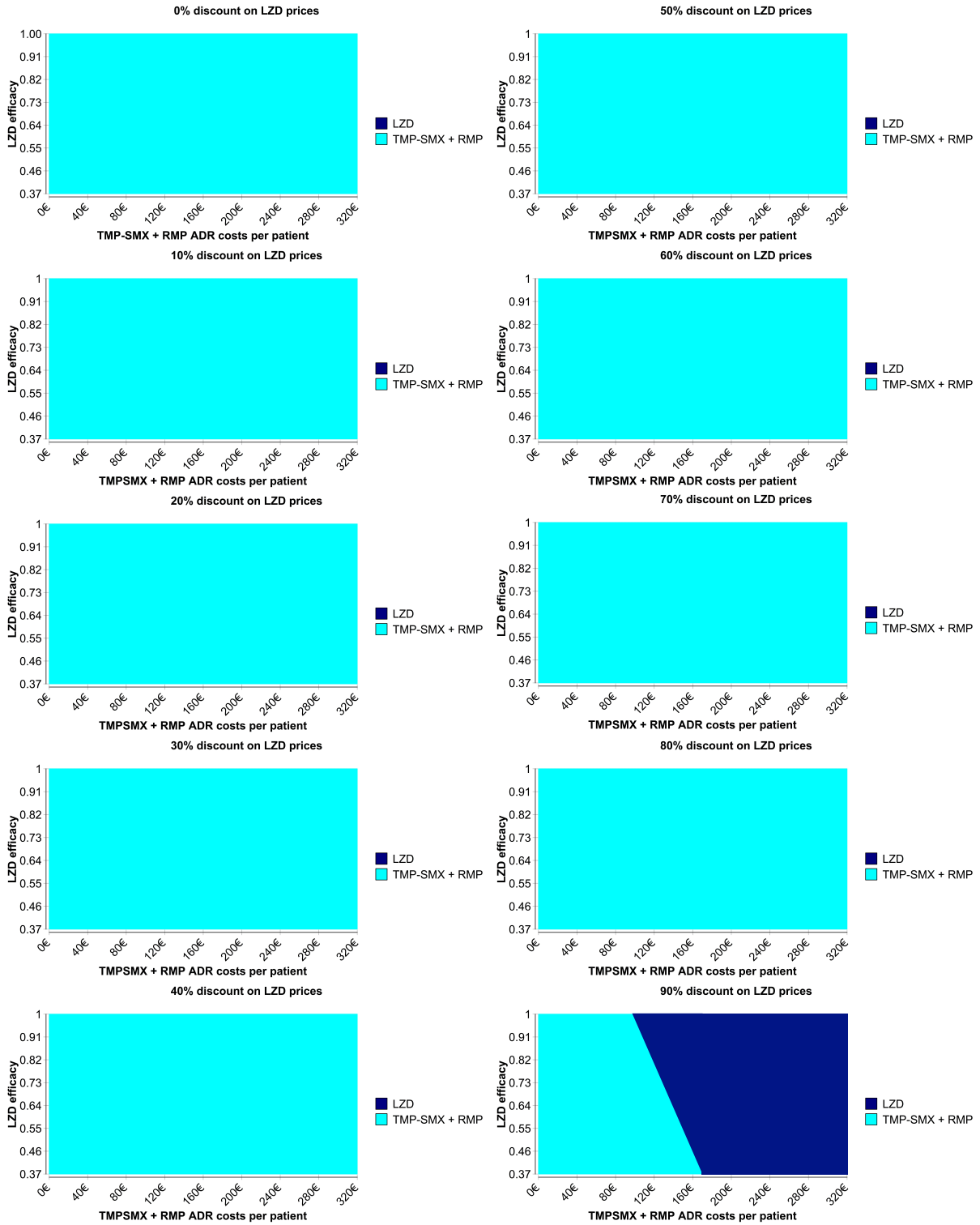
Incr. Cost: incremental cost; Incr. Eff.: incremental effectiveness; Incr. cost-Effect.: Incremental cost-effectiveness.
 A. Monte Carlo simulation. Each blue spot represents one of the 10'000 iterations. The two orange lines represent the base-case scenario. B. Cost-effectiveness acceptability curves.

Figure 4.4: One-way, and two-way sensitivity analyses on assumed inputs.



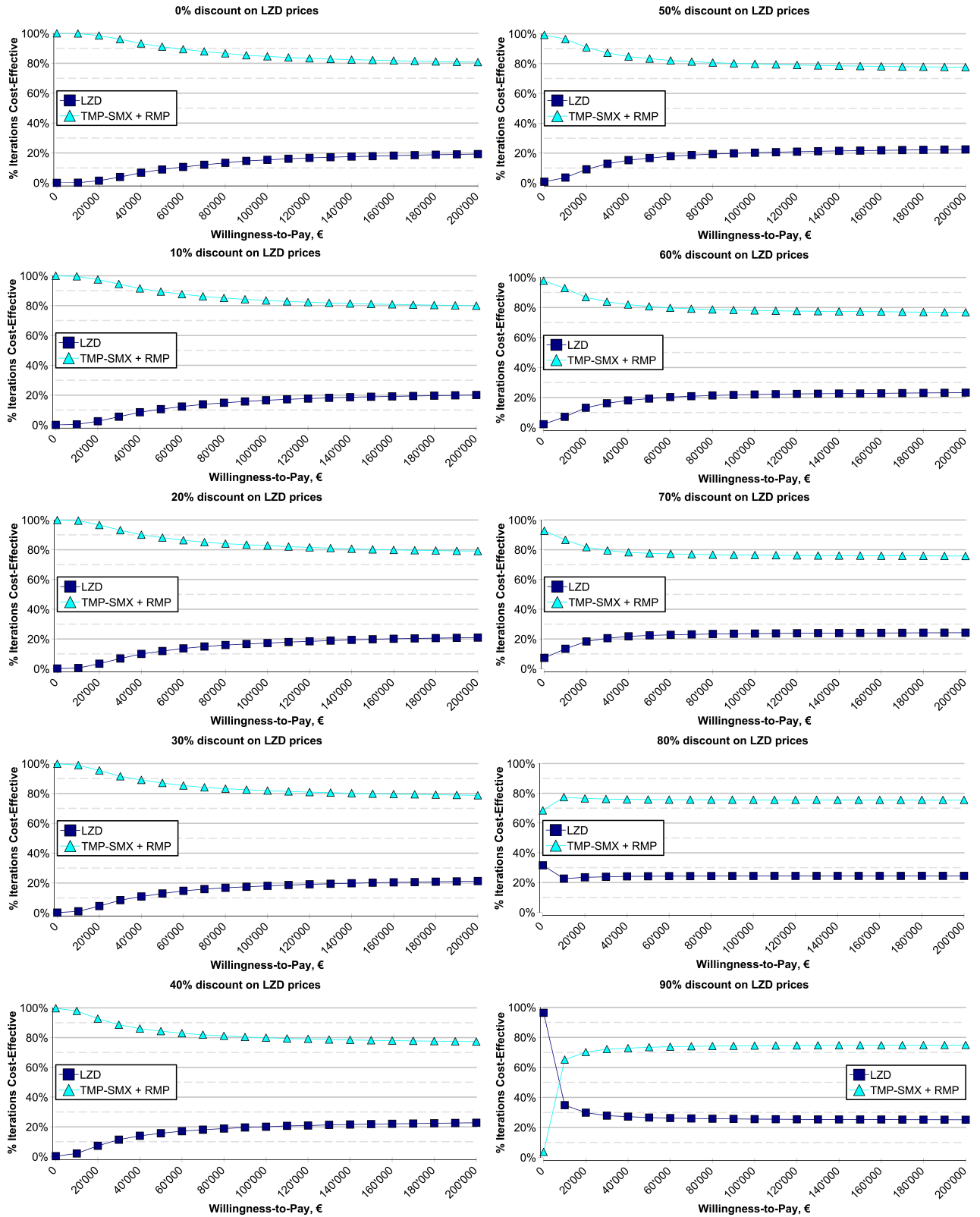
A. One-way sensitivity graph: the cost by QALY gained is represented for each treatment according to the value for the variable tested. B. Two-way sensitivity analysis is an analysis in which two variables of interest are simultaneously varied over a range of plausible values while holding all other variables constant (according to the base case scenario). In these types of graphs the most cost-effective interventions according to the value for the variables tested are represented according to their colors. The orange line represents the 10% discount on generic LZD price applied in Switzerland.

Figure 4.5: Three-way sensitivity analyses on assumed inputs, considering various discounted pricing of generic linezolid.



Three-way sensitivity analysis is an analysis in which two variables of interest are simultaneously varied over a range of plausible values while holding a third variable with a determinate value and all other variables constant (according to the base case scenario). In these types of graphs the most cost-effective intervention according to the value for the variables tested is represented according to their colors.

Figure 4.6: Cost-effectiveness acceptability curves (CEAC) on assumed inputs, considering various discounted pricing of generic linezolid.



4.5 Discussion

According to our previous RCT, we showed that anti-MRSA therapy with a combined treatment of older antibiotics (TMP-SMX plus RMP) is non-inferior to a treatment of LZD in terms of safety and efficacy [122]. However, the use of new versus old drugs and one versus two independently marketed antibiotics can generate cost differences. In an effort to investigate different health-economic scenarios linked to the use of TMP-SMX plus RMP versus LZD to treat MRSA infection, we performed a CEA whose principal findings were: (i) the combination of TMP-SMX plus RMP (159CHF/146€ and 0.916 QALY) was less costly and more effective than the LZD treatment (2758CHF/2535€ and 0.881 QALY); (ii) probabilistic sensitivity analyses using MC simulations confirmed that the combination of old antibiotics is dominant in the majority of iteration; (iii) even in extreme scenarios with assumed high costs of ADRs for TMP-SMX plus RMP treatment, high LZD efficacy and a discount rate (50%) applied to LZD prices, the combination of old antibiotics remains dominant.

The use of old antibiotics such as TMP-SMX plus RMP could be an effective and interesting strategy to cure MRSA infection [215, 218, 219], especially due to the emergence of intermediate resistance against vancomycin or LZD [243]. Moreover, with the increasing incidence of CA-MRSA and knowing that these community-acquired strains are often more susceptible to antibiotics than HA-MRSA, in particular to the older antibiotics [46, 244], the use of TMP-SMX plus RMP could again be an interesting alternative strategy. In addition the fact, the oral administration is available for such drugs can considerably reduce the intra-hospital costs by enabling a faster hospital discharge.

Several industry-sponsored CEAs have been already conducted for LZD. In the majority of them, it has been showed that, compared to vancomycin, LZD is more cost-effective in the treatment of MRSA infection [245–252]. In contrast, our CEA shows that even with a willingness-to-pay fixed at 50'000€ par QALY gained (a commonly used threshold for determining value-for-money of new healthcare interventions) [253], the strategy using combination of old antibiotics remains mostly dominant over LZD. However, a limitation of this treatment combination could be the lower compliance among patients due to the increased the frequency and number of treatment administrations, the patient compliance may become lower [254, 255]: 4 pills on 3 administrations for TMP-SMX plus RMP versus two pills and administrations for LZD.

Our CEA study has several strengths. First, this is the first industry-independent study evaluating the economic impact of the two anti-MRSA strategies. A higher quality CEA was possible thanks to the randomized controlled design with the prospective collection of data. Moreover, both the efficacy and the burden of the study treatment were taken into account by the use of QALYs as an effectiveness measure. We conducted a couple of sensitivity analyses, in which all relevant variables of interest were varied to evaluate sources of potential uncertainty. The results remain stable within all sensitivity analyses, suggesting

a high probability that our findings could be observed in a real normal practice. Finally, with a cost adjusted to December 2016 and one-, two- and three-way sensitivity analyses conducted on potential discounts to simulate LZD generic prices, this study informs us on a future comparison between both treatments, once LZD loses patent protections.

Our CEA study also has some limitations, mostly related to the previous RCT design. First, as already mentioned previously, the condition under which the previous non-inferiority RCT was carried out might differ from real life due to the fact that the trial was confined to a selected population from a single Swiss hospital with a distinct endemic HA-MRSA strain [256], possibly limiting the external validity of the analyses. Second, the sample size of the study was too small to be able to capture all potential treatment-related ADRs that may occur. We had to simulate the financial impact of related health-economic adverse outcome and missing ADRs in the CEA. By consequence, we chose to conservatively overestimate ADR costs and incidences, mostly increasing the potential economic impact of ADRs for the old combination of antibiotics. The ADR costs were derived from an average of DRG (Diagnosis-related group) costs charged to patients presenting similar pathologies or symptoms at HUG. For few rare pathologies (e.g., Stevens-Johnson Syndrome), the average was generated from a small number of episodes, making them potentially less representative.

To conclude, the result of the CEA suggests that, on cost-effectiveness grounds, treatment with a combination of TMP-SMX and RMP is dominant over a LZD therapy to treat MRSA infection from the perspective of the health-care provider.

This work was accepted for publication in February 2017 and published in *Clinical Microbiology Infection* journal in September 2017 (Appendix 6).

Chapter 5

Conclusion

"I hear and I forget. I see and I remember. I do and I understand"

Confucius

Antibiotic resistance represents a real clinical challenge leading to many attributable deaths and enormous additional healthcare costs. For several decades, multiresistant *S. aureus* (or MRSA) has been a major public health threat around the world, since for many years there were few antibiotics available to fight these resistant bacteria. Importantly, preventive and therapeutic measures for HA-MRSA versus CA-MRSA are not the same, due to the different places of propagation, host features and molecular characteristics of these strains. Thus, many approaches exist to fight against MRSA, targeting different stages: prophylactic measures to avoid individual infection and prevent between human spread and contamination, as well as appropriate and cost-effective antibiotic therapies to treat symptomatic infection.

However, several important issues related to the control and management of MRSA remain a subject of debate in public health and clinical medicine. First, although the prevalence of hospital-associated MRSA has decreased in the last few years, there is a lack of adequate early alert tools for new and virulent MRSA strains that are emerging, particularly within the community setting (i.e. CA-MRSA). Second, there is ongoing uncertainty about optimal methods to decolonize chronic MRSA carriers and which patient profile is more suitable for topical decontamination treatment. Finally, from a larger perspective, it remains unclear which MRSA treatment options offer the best value for the money, considering many different clinical and health-economic uncertainties.

Therefore, the overarching objective of this thesis was to improve understanding and provide innovative tools for fighting against MRSA carriage and infection. This work addresses both preventive approaches and therapeutic measures using three different and complementary studies. It covers various types of statistical and epidemiological methods and clinical research study designs (including epidemiological, molecular and cost-effectiveness studies).

We showed in the first study that carefully retrieved epidemiological data combined with WGS results are a suitable method to understand the origin of a local CA-MRSA cluster. We excluded local dissemination of a virulent CA-MRSA strain, mainly imported from the Americas by multiple patients. Moreover, this process is now becoming less expensive and allows investigating an outbreak adequately and quickly. We conclude here that, in terms of public health, WGS linked to epidemiological investigations is an appropriate technique to improve the investigation of large-scale outbreaks of emerging bacteria in the community.

The second part of this thesis provides a clarification of how MRSA decolonization works. This could allow better targeting of patients to be treated with topical anti-MRSA decolonization regimens in order to maximize the effectiveness of treatment. Indeed, regarding public health, we hope with our work to individualize MRSA decolonization, limit the often too rapid local occurrence of resistances to these topical treatments and allow their sustainable use over the longer term.

Finally, we explored an old antibiotic treatment combination as an alternative to a more recent and more expensive anti-MRSA therapy. We showed that the old treatment is non-inferior in term of efficacy and safety. Once the effectiveness proven, we demonstrated the considerable economic benefit of its use. Therefore, we would encourage leaving the new antibiotic only as a last resort. Moreover, in terms of public health, the money saved by the use of these less expensive antibiotics could then be used elsewhere for preventive purposes or investment into the R&D of truly novel classes of antibiotics.

Antibiotic resistance will remain a real public health threat for the upcoming years, despite the relative decrease of nosocomial MRSA transmission. In response to this matter of urgency, The United Nations organized a high-level meeting on the problem of antimicrobial resistance on September 21, 2016, at the United Nations General Assembly to enhance national multi-sectoral efforts and accelerate global commitments to combat antimicrobial resistance. The World Health Organization adopted the "Global action plan on antimicrobial resistance" to control and fight the spread of multi-resistant organism (MRO). The struggle is far from finished; but useful lessons can be learned and applied already from various sources and investigations, including the present work.

*"Et puis il n'avait plus su quoi lui dire. Et puis il le lui avait dit.
Il lui avait dit que c'était comme avant, qu'il l'aimait encore,
qu'il ne pourrait jamais cesser de l'aimer,
qu'il l'aimerait jusqu'à sa mort."*

Marguerite Duras

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Titre: *Staphylocoque doré* méticilline résistant (SARM) : prévention et lutte contre ce pathogène

Mots clés: *Staphylococcus doré* résistant à la méticilline (SARM), USA300, épidémiologie, décolonisation, analyse coût-efficacité, QALYs

Résumé: Le *Staphylocoque aureus* (*S. aureus*) est une bactérie pouvant causer des infections d'origine communautaire ou hospitalière. En plus des personnes pouvant être infectées par *S. aureus*, de nombreux individus environ 20-25% parmi les adultes en bonne santé, portent ce pathogène sur leurs corps (ils sont dits "colonisés"). Bien qu'asymptomatique et souvent bénigne, la colonisation à *S. aureus* peut rarement impliquer un risque d'infection secondaire grave mais représente un risque d'être transmise à une tierce personne. A l'hôpital, le risque d'infection et de transmission apparaît comme étant particulièrement haut pour la souche de *S. aureus* résistante à la méticilline (SARM). La prévention et la lutte contre le SARM est un problème de santé publique important. Les Nations Unies se sont récemment engagés dans cette lutte en créant le "Global Action Plan on Antimicrobial Resistance". Ce travail de thèse traite de trois différentes études dans la cadre de la prévention et du contrôle de ce pathogène.

Title: Methicillin-resistant *Staphylococcus aureus* prevention and fight against this pathogen

Keywords: methicillin-resistant *Staphylococcus aureus* (MRSA), USA300, epidemiology, decolonization, cost-effectiveness analysis, QALYs

Abstract: *Staphylococcus aureus* is a bacterium that can cause blood infections that can have a community or hospital origin. Among people infected with *S. aureus*, about 20-25% is healthy adults that carry the pathogen on their bodies, without suffering any symptom (they are said "colonized"). Although asymptomatic and often benign, colonization with *S. aureus* involves a risk of secondary infection and represents a serious risk of transmission to a third party. In hospitals, the risk of infection and transmission appears to be unusually high for methicillin-resistant *S. aureus* (MRSA) and complications can be very severe. Prevention and the fight against MRSA is a major public health concern. The United Nations recently engaged in this struggle by creating the "Global Action Plan on Antimicrobial Resistance". This work is about three different studies in the prevention and control of this pathogen.