REAexplAUR

RAPPORT NATIONAL 2022

VERSION 1

SOMMAIRE

1. CONTEXTE

Les mesures de prévention des bactériémies liées aux cathéters sont multiples.
Diminuer le risque de survenue de ces infections repose sur la mise en œuvre d'un ensemble de mesures, parmi lesquelles se distinguent tout d'abord des mesures **« basiques »** (Buetti N, 2020) :

- **avant l'insertion du cathéter**, le choix d'un cathéter adapté à son utilisation,
- **pour l'insertion du cathéter** : le fait de privilégier une insertion sous-clavière pour les CVCs, l'utilisation d'une checklist, le respect d'une asepsie rigoureuse lors de la préparation cutanée, l'utilisation chez l'adulte d'une solution de CHG alcoolique 2% pour la phase d'antisepsie avant l'insertion d'un cathéter central, ou d'un antiseptique alcoolique majeur pour les cathéters périphériques, l'utilisation de pansements occlusifs stériles et transparents permettant une inspection du site d'insertion au moins quotidienne,
- **pendant les soins** : l'évaluation régulière de la pertinence du maintien du cathéter et le retrait sans délai de tout dispositif devenu inutile, la réfection sans délai des pansements souillés, ou décollés, le respect d'une asepsie rigoureuse lors de toute manipulation du cathéter et/ou des lignes, la manipulation de la connectique avant tout accès, le fait de ne pas changer les lignes de façon systématique avec une fréquence <4 jours, et de les changer au moins tous les 7 jours.
- **au long cours** : la surveillance des bactériémies liées aux cathéters, l'observation des pratiques concernant la pose et les manipulations des dispositifs, la formation des professionnels en charge des cathéters.

Des mesures **« additionnelles »** font l'objet de recommandations plus ou moins consensuelles; en particulier :

- **avant l'insertion du cathéter** : le choix d'un cathéter imprégné par une substance antimicrobienne pour les services faisant face à un taux élevé d'incidence des B-div et pour lesquels les mesures correctives n'ont pas été suivies de l'amélioration attendue,
- **pour l'insertion du cathéter** (l'utilisation de pansements/éponges imprégnés de CHG et de systèmes de stabilisation du cathéter) ;
- **pendant les soins** : l'utilisation de verrous (antibiotiques, anticoagulants, ..), l'utilisation de connectiques coatés d'antimicrobiens, l'utilisation de pansements/éponges imprégnés de CHG, la toilette quotidienne des patients avec une solution de CHG, l'utilisation de pommade antibiotique au niveau du site d'accès pour l'hémodialyse.
- **au long cours**, la mise en place d'une équipe dédiée pour la pose et/ou la gestion des cathéters.

S. aureus est au centre de la problématique des bactériémies liées aux cathéters
En 2022, 1407 bactériémies associées aux soins ont été détectées dans les services de réanimation participant à la surveillance nationale (157 établissements de santé pour le secteur adulte, 7 en secteur pédiatrique et 34 en secteur néonatal), parmi lesquelles 178 ont été associées à *S. aureus* (B-Sau) (12,6%). La densité d'incidence des B-Sau est de 0.60/1000 JH ; 33,1% des B-Sau ont été liées à un cathéter (densité d'incidences 0.24/1000 JH), le plus souvent un CVC (47,5%), un cathéter artériel (15,2%) ou un cathéter de dialyse (5,1%). En 2022, selon les données de la surveillance nationale, 6,9% des *S. aureus* responsables de ces bactériémies liées à un cathéter en réanimation étaient résistantes à la méticilline.

REAexplAUR permet aux équipes des services de réanimation participant à la surveillance des bactériémies, aux responsables des CPias et à l'équipe SPIADI nationale:
- **de suivre la mise en œuvre des mesures de prévention des bactériémies liées à un cathéter,**
- **de connaître les caractéristiques des souches de *S. aureus* responsables de bactériémies, en particulier leur aptitude à résister aux antibiotiques et aux antiseptiques, leur appartenance à des clones particulièrement virulents et leur capacité à produire du biofilm,**
ces données pouvant être utilisées pour sensibiliser les professionnels aux mécanismes d'acquisition des bactériémies à *S. aureus* liées à un cathéter, et aux moyens innovants de prévention de ces infections.

2. PARTICIPATION DES ETABLISSEMENTS.

Au total, 27 services de réanimation ont participé à REAexplAUR en 2022 (tableau 1), soit 9% des services de réanimation participants à la surveillance nationale.

Tableau 1 : Participation à REAexplAUR.

	N ES	% des ES participants à la surveillance	N services	% des services participants à la surveillance
2021	53	29 %	65	23 %
2022	24	13 %	27	9 %

3. MISE EN ŒUVRE DES MESURES DE PREVENTION DES ILC.

Les résultats obtenus en 2021 et 2022 sont présentés au niveau du tableau 2.

Tableau 2 : Résultats du suivi de la mise en place des mesures de prévention des ILC.

Attendu	% de services répondant à l'attendu	
	2021	2022
Checklist et protocoles à disposition pour la pose des cathéters centraux et la gestion des lignes		
• checklist pour la pose des cathéters centraux	74 (48/65)	81 (22/27)
• protocole validé pour la pose des CVCs	95 (62/65)	96 (26/27)
• protocole validé pour la pose des PICClines	65 (42/65)	74 (20/27)
• protocole validé pour la pose des MIDlines	43 (28/65)	44 (12/27)
• protocole validé pour la réfection des pansements	92 (60/65)	100 (27/27)
• protocole validé pour les manipulations des lignes	92 (60/65)	96 (26/27)
Observation des pratiques (pose et/ou manipulations) depuis 12 mois	38 (25/65)	37 (10/27)
Formation (pose et/ou manipulations) depuis 12 derniers mois	49 (32/65)	37 (10/27)
Utilisation de la chlorhexidine alc. 2% pour la pose des voies centrales	66 (43/65)	67 (18/27)
Utilisation de pansements occlusifs, stériles et transparents	98 (64/65)	96 (26/27)
Toilette quotidienne avec la chlorhexidine	0 (0/65)	4 (1/27)
Pommade antibiotique appliquée au point d'insertion du cathéter	0 (0/65)	0 (0/27)
Utilisation de dispositifs innovants au cours des 12 derniers mois		
• cathéters imprégnés	0 (0/65)	0 (0/27)
• pansements imprégnés	8 (5/65)*	4 (1/27)*
• éponges imprégnées	8 (5/65)	0 (0/27)
• bouchons, connecteurs ou valves imprégnées	6 (4/65)	18,5 (5/27)
• systèmes de stabilisation suture-free	26 (17/65)	33 (9/27)
• verrous (antibiotiques, anticoagulant,…)	17 (11/65)	22 (6/27)

*Pansements imprégnés de chlorhexidine

4. CARACTERISTIQUES MICROBIOLOGIQUES DE *S. AUREUS.*

Au total 90 souches de *S. aureus* en provenance de 29 services en 2021 (64 souches) et 15 services en 2022 (26 souches) ont été étudiées. Les résultats obtenus en 2021 et 2022 sont présentés au niveau des tableaux 3a-d. Toutes les informations concernant les méthodes utilisées sont présentées au niveau du protocole REAexplAUR, téléchargeable sur le site spiadi.fr.

Antibiorésistance. Les SARM ont représenté 8% des souches bactériémiques étudiées. Aucune souche n'a présenté une diminution de sensibilité vis-à-vis des glycopeptides. Les résistances aux antibiotiques ont été limitées et ont porté principalement sur l'érythromycine (43% des souches) et les fluoroquinolones (5,5%).

Résistance à la mupirocine et aux antiseptiques. La résistance à la mupirocine est exceptionnelle (2%), tout comme la résistance aux antiseptiques en lien avec la présence des gènes *qac* (2%).

Tableau 3a : Résistance aux antimicrobiens des souches de *S. aureus*.

	N de souches	
	2021	2022
N souches étudiées	64	26
Résistance aux antibiotiques		
oxacilline	4 (6%; 4/64; 4 *mecA*)	3 (11,5%; 3/26; 3 *mecA*)
gentamicine	0	0
kanamycine	1 (1,5%; 4/64; 1 SARM)	1 (4%; 1/26; 1 SARM)
lévofloxacine	2 (3%; 2/64; 2 SARM)	3 (11,5%; 3/26; 2 SARM)
érythromycine	31 (48%; 31/64; 2 SARM)	8 (31%; 8/26; 1 SARM)
clindamycine	3 (5%; 3/64; 1 SARM)	0
linézolide	0	0
tétracycline	1 (1,5%; 4/64; 1 SARM)	1 (4%; 1/26; 1 SARM)
acide fusidique	5 (8%; 5/64; 1 SARM)	0
rifampicine	0	0
triméthoprime/sulfamides	0	0
fosfomycine	1 (1,5%; 4/64; 1 SARM)	0
teicoplanine	0	0
vancomycine	0	0
Résistance à la mupirocine et aux antiseptiques		
mupA/B	2 (3%; 2/64) • 1 SARM *mupA* R FQ • 1 SASM *mupA* R Ery Lin Fuc	0
qacAB/C	2 (3%; 2/64) • 1 SARM *qacC* R FQ • 1 SASM *qacAB* R Ery	0

Gènes de virulence. Pour ce qui concerne les gènes de virulence, 12% des souches (toutes des SASM) ont porté le gène *tst* codant pour la toxine TSST-1, et les gènes codant pour la PVL ont été détecté pour un SARM en 2022 (1% des souches).

Tableau 3b : Virulence des souches de *S. aureus*.

	N de souches	
	2021	2022
N souches étudiées	64	26
Virulence		
tst	9 (14%; 9/64, 0 SARM)	2 (8%; 2/26; 0 SARM)
lukS-PV	0	1 (4%; 1/26 ; 1 SARM R K FQ Ery Te)

Diversité génétique des souches. Les souches sont diverses génétiquement, mais 5 complexes clonaux prédominent : CC398 (32% des souches), suivi de CC8 (15,5%), CC5 (12%), CC30 (12%) et CC45 (11%). Les 7 SARM se distribuent dans 4 clones différents : CC8 (3), CC5 (2), CC1 (1) et CC97(1).

Plusieurs particularités clonales ont été retrouvées :
- Le complexe CC398 est sur-représenté parmi les souches résistantes à l'érythromycine (97% pour les souches CC398 (28/29) vs 16% pour les autres complexes clonaux (10/61); p<0,001)
- Le gène *tst* est sur-représenté parmi les souches du CC30 (82% pour les souches CC30 (9/11) vs 2,5% pour les autres CCx (2/79); p<0,001)

Tableau 3c : Diversité génétique des souches de *S. aureus*, selon les résultats du MLST.

	N de souches	
	2021	2022
N souches étudiées	64	26
CC		
1	3 (5%; 3/64; 1 SARM)	1 (4%; 1/26)
5	4 (6%; 4/64; 1 SARM)	7 (27%; 7/26; 1 SARM R FQ)
8	10 (16%; 10/64; 2 SARM)	4 (15%; 4/26; 1 SARM R K FQ E Te *LukS-PV*)
15	3 (5%; 3/64)	0
22	3 (5%; 3/64)	0
30	9 (14%; 9/64)	2 (8%; 2/26)
45	8 (12,5%; 8/64)	2 (8%; 2/26)
59	1 (1,5%; 4/64)	0
97	0	1 (4%; 1/26; 1 SARM multi S)
121	0	2 (8%; 2/26)
398	22 (34%; 22/64)	7 (27%; 7/26)

Production de biofilm. En absence de stress (glucose, ou cloxacilline), la production de biofilm a été détectée pour 14% des souches de *S. aureus* étudiées. En présence d'un stress, la production de biofilm est plus fréquente, avec 54% des souches en présence de glucose 1% et 77% des souches après exposition à des concentrations sub-inhibitrices de cloxacilline ; 13% des souches de SASM montrent une résistance à la cloxacilline en conditions de biofilm. La résistance à la cloxacilline en conditions de biofilm des SASM est associée aux souches appartenant au CC398 (p=0,020).

Tableau 3d : production de biofilm des souches de *S. aureus*.

	N de souches		
	2021	2022	2021-2022
N souches étudiées	64	26	90
Production de biofilm en conditions statiques (24 h)			
sans supplémentation (24h)	6/64 (9%) • 1/4 SARM (25%) • 5/60 SASM (8%)	7/26 (27%) • 2/3 SARM (67%) • 5/23 SASM (22%)	13/90 (14%) • 3/7 SARM (43%) • 10/83 SASM (12%)
avec glucose 1% (24h)		14 (54%) • 2/3 SARM (67%) • 12/23 SASM (52%)	
avec glucose 1% et cloxacilline (subinhib.) (48h)		20 (77%) • 3/3 SARM (100%) • 17/23 SASM (74%)	
MBEC (minimum biofilm eradication concentration of cloxacillin) > 64		6 • les 3 SARM • 3/23 SASM (13%)	

5. ORIGINE DES BACTERIEMIES ET CARACTERISTIQUES MICROBIENNES.

La documentation de l'origine de la bactériémie a été rapportée pour les 86 bactériémies (tableau 4).

Les sources principales ont été l'origine pleuro-pulmonaire (46,5%) et les cathéters (24%). Ces résultats sont concordants avec ceux de l'ensemble des bactériémies à *S. aureus* détectées en réanimation pour 2021-2022.

Aucun lien n'a été retrouvé entre un complexe clonal et une porte d'entrée particulière.

Le décès dans les 7 jours suivant le diagnostic de la bactériémie a été retrouvé pour 20% des bactériémies documentées. Aucun lien n'a été retrouvé entre un complexe clonal et un taux élevé de mortalité à J7 du début de l'épisode bactériémique.

Tableau 4 : Origine des bactériémies et diversité clonale (2021-2022).

Diversité génétique (MLST)												
	N	Porte d'entrée de la bactériémie*										Décès J7 (%)
CC		C ou D1	A3	A9	A1	A5	A10	A4	A2	A13	NR	
1	4	1	1		1					1		0
5	11	2	5	1			1			2		2 (18%)
8	14	4	7			2				1		2 (17%)
12	1	1										0
15	3		2							1		0
22	3	1	1								1	0
30	11	3	5				1			1	1	0
45	10	2	5		1			1		1		4 (40%)
59	1	1										0
97	1		1									0
121	2		1						1			0
398	29	6	12	2	4					3	2	10 (37%)
Toutes les souches												
	90	21	40	3	6	2	2	1	1	10	4	17/84 (20%)
%		24	46,5	3,5	7	2	2	1	1	12		

* les codes portant sur l'origine des bactériémies sont ceux utilisés pour la surveillance.

6. SYNTHESE ET PERSPECTIVES.

Evaluation de la mise en œuvre des mesures de prévention des ILC. L'analyse des résultats permet d'identifier les besoins en termes de protocoles, d'observations de pratiques, et/ou de formation selon les cas. Des outils sont à la disposition sur le site spiadi.fr pour répondre à ces besoins; en particulier :

- les fiches synthétisant les recommandations pour la pose des cathéters centraux et la manipulation des lignes sont à disposition au niveau de l'onglet « outils »;
- l'outil OBSERVA4 qui permet d'identifier les écarts entre les pratiques et les recommandations actuelles lors de la pose des cathéters, des pansements et des manipulations des lignes, et d'obtenir l'indicateur de suivi de la Stratégie nationale concernant la pose des cathéters centraux.
- l'outil pédagogique VALV'FRICTION pour l'amélioration de l'utilisation des valves bidirectionnelles pour des formations courtes ou en autonomie.

Volet microbiologique. Les résultats sont rassurants pour ce qui concerne les marqueurs de résistance aux antimicrobiens (antibiotiques, antiseptiques, mupirocine), avec des SARM peu fréquents et l'absence de souches de sensibilité diminuée aux glycopeptides.

Nous confirmons la prédominance du clone CC398. Ce clone est décrit de façon croissante chez l'homme ; il est associé à une mortalité importante et responsable d'infections sévères (bactériémies, endocardites et infections ostéo-articulaire). Pour notre étude, les souches CC398 ont été associées à des bactériémies principalement d'origine pulmonaire (44%) ou associées à un cathéter (22%). Elles ont été retrouvées dans 2 des 3 cas d'endocardites. La mortalité dans les 7 jours suivant le début de l'épisode infectieux est de 37%. De plus, en conditions de biofilm, elles montrent une résistance à la cloxacilline alors que ce sont des SASM.

7. VALORISATION.

Les résultats de REAexplAUR ont fait l'objet
- d'une publication dans la revue scientifique Microorganisms. Vous pouvez accéder à la publication avec ce lien https://www.mdpi.com/2076-2607/10/9/1857 et en annexe du rapport.
- d'une communication orale lors du congrès de la SFAR 2022 (Paris).

ANNEXE

LISTE DES ETABLISSEMENTS PARTICIPANTS EN 2022

CH BLOIS
CHU NICE HOPITAL PASTEUR
CHU NICE HOPITAL DE L'ARCHET
CHIC ALENCON-MAMERS
HOPITAL DE LA CROIX SAINT SIMON
CH SAINT-LO
CH SAINT BRIEUC
CHIVA ST JEAN DE VERGES
CHI EPINAL
CH GRASSE
HNFC SITE TREVENANS
HOPITAL FONDATION A DE ROTHSCHILD
CHR ORLEANS
CH SAINT NAZAIRE
NCT+ ST GATIEN ALLIANCE, TOURS

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Article

Strong Biofilm Formation and Low Cloxacillin Susceptibility in Biofilm-Growing CC398 *Staphylococcus aureus* Responsible for Bacteremia in French Intensive Care Units, 2021

Nathalie van der Mee-Marquet [1,*] , Sandra Dos Santos [1], Seydina M. Diene [2] , Isabelle Duflot [1], Laurent Mereghetti [3], Anne-Sophie Valentin [1] , Patrice François [4] and on behalf of the SPIADI Collaborative Group [†]

1 Centre d'Appui pour la Prévention des Infections Associées aux Soins (CPias) de la Région Centre Val de Loire, Hôpital Bretonneau, Centre Hospitalier Régional Universitaire, 37044 Tours, France
2 Faculté de Pharmacie, Microbes Evolution Phylogeny and Infections, IHU-Méditerranée Infection, Aix-Marseille Université, 13005 Marseille, France
3 Service de Bactériologie, Virologie et Hygiène, Hôpital Trousseau, Centre Hospitalier Régional Universitaire, 37044 Tours, France
4 Department of Medicine, University of Geneva Hospitals, 1205 Geneva, Switzerland
* Correspondence: n.vandermee@chu-tours.fr
† SPIADI Collaborative Group are listed in acknowledgments.

Abstract: A prospective 3-month study carried out in 267 ICUs revealed an *S. aureus* nosocomial bacteremia in one admitted patient out of 110 in adult and pediatric sectors, and in one out of 230 newborns; 242 *S. aureus* bacteremias occurred during the study, including 7.9% MRSA-bacteremias. In one ICU out of ten, the molecular characteristics, antimicrobial susceptibility profiles and biofilm production of the strains responsible for *S. aureus* bacteremia were studied. Of the 53 strains studied, 9.4% were MRSA and 52.8% were resistant to erythromycin. MLST showed the predominance of CC398 (37.7% of the strains) followed by CC8 (17.0%), CC45 (13.2%) and CC30 (9.4%). The *lukF/S* genes were absent from our isolates and *tst-1* was found in 9.4% of the strains. Under static conditions and without exposure to glucose, biofilm production was rare (9.4% of the strains, without any CC398). The percentage increased to 62.3% for strains grown in broth supplemented with 1% glucose (including 7 out of 9 CC8 and 17 out of the 20 CC398). Further study of the CC398, including whole genome sequencing, revealed (1) highly frequent patient death within seven days after CC398 bacteremia diagnosis (47.4%), (2) 95.0% of the strains producing biofilm when exposed to sub-inhibitory concentrations of cloxacillin, (3) a stronger biofilm production following exposure to cloxacillin than that observed in broth supplemented with glucose only ($p < 0.001$), (4) a high minimum biofilm eradication concentration of cloxacillin (128 mg/L) indicating a low cloxacillin susceptibility of biofilm-growing CC398, (5) 95.0% of the strains carrying a φSa-3 like prophage and its particular evasion cluster (i.e., yielding *chp* and *scin* genes), and (6) 30.0% of the strains carrying a φMR11-like prophage and yielding a higher ability to produce biofilm. Our results provide evidence that active surveillance is required to avoid spreading of this virulent staphylococcal clone.

Keywords: *Staphylococcus aureus*; intensive care; CC398; bacteremia; biofilm; cloxacillin; antibiotic tolerance

1. Introduction

S. aureus possesses numerous virulence factors facilitating tissue colonization, immune evasion and tissue destruction. One of its defense mechanisms is the capacity to form biofilms. Bacteria embedded in biofilms are resistant to host immune response and difficult to eradicate with antibiotic [1,2]. Biofilm-forming capacity is a virulence determinant in the development of catheter-related infections, and the effective treatment of staphylococcal infections that share such features has become a challenge [3].

Described initially in livestock, *S. aureus* of clonal complex 398 (CC398) are increasingly responsible for bacteremia in humans living in animal-free environments [4–7]. Whole

genome sequencing analysis of CC398 strains have demonstrated that livestock-associated and emerging strains differ by their prophage content [8,9]. The emerging strains usually carry φSa-3 and φMR11-like prophages, which have been shown to be involved in immune escape, adhesion to host cells and extracellular matrix components, along with epithelial cell invasion, i.e., factors contributing to an increased ability of the bacteria to colonize and infect the host [10].

Enhanced biofilm formation ability was recently described in emerging CC398 strains in China [11]. Facing the epidemiological changes observed with this clone in human clinical settings, epidemiological data remain scarce. We conducted a prospective incidence-study of nosocomial bacteremia in 267 intensive care units (ICUs), and analyzed the molecular characteristics and antimicrobial susceptibility profiles of the *S. aureus* strains responsible for bacteremia in one ICU out of ten. We sequenced the genome of the CC398 strains, analyzed their prophage content, studied their ability to produce biofilm following exposure to sub-inhibitory concentrations of cloxacillin, the first line treatment for patients suffering from MSSA bacteremia, and determined their cloxacillin susceptibility in biofilm, where applicable.

2. Materials and Methods

2.1. Nosocomial Bacteremia Survey, Data Collection and Analysis

In each participating ICU, a 3-month surveillance of nosocomial bacteremia was carried out between 1 January and 15 August 2021. A protocol derived from that of the ECDC HAI light surveillance protocol was used (https://www.ecdc.europa.eu/sites/default/files/documents/HAI-Net-ICU-protocol-v2.2_0.pdf; accessed on 1 January 2021). During the survey period, each positive blood culture was analyzed to determine whether it was associated with a nosocomial bacteremia. The origin of the bacteremia was determined using clinical and biological data. Data were collected (HCF data, patient characteristics (sex, age, birth weight for newborns, the severity index, the immune status, the type of cancer where applicable, and death within 7 days of bacteremia diagnosis), characteristics of the bacteremia (origin (skin (primary cutaneous form or superinfection of a skin wound), surgical site, lungs, urinary tract, intravascular device, intra-abdominal or digestive tract) and microorganisms). Data processing, validation of the database and data analysis were carried out by the national team using R software (version 3.6.1 on Ubuntu; General Public Licence; Vienna, Austria). For the variables studied, percentages were calculated from the numbers, without taking missing data into account. Incidence rates were generated according to patient-days and admitted patients. Here, we report the data regarding *S. aureus* bacteremias.

2.2. Microbiological Study

Local infection control teams were asked to send the *S. aureus* strains isolated from bacteremia to the central lab using transport swabs (Copan Italia SPA, Brescia, Italy). The strains were tested for antimicrobial drug susceptibility using the AST-P631 vitek card (oxacillin, cefoxitin, gentamicin, tobramycin, ofloxacin, erythromycin, linezolid, teicoplanin, vancomycin, tetracycline, nitrofurantoin, fusidic acid, rifampicin, trimethoprim-sulfamethoxazol, fosfomycin; bioMérieux, France), Etest® (daptomycin, mupirocine; bioMérieux, Marcy-l'Étoile, France) and Eucast guidelines (http://www.eucast.org/; accessed on 1 January 2021). The presence of *mecA/C*, *mupA/B*, *qacAB/C*, *tst-1*, and *lukF/S* genes was determined using PCR. Strain genomic diversity was studied using MLST (https://pubmlst.org/organisms/staphylococcus-aureus; accessed on 1 January 2021). Purified genomic DNA for CC398 strains was sequenced on the Illumina HiSeq (Illumina, San Diego, CA, USA) using 100 base-pair paired-ends read and bar code strategy according to the Nextera XT kit (Illumina, San Diego, CA, USA), following the manufacturer's recommendations [12]. The ability to produce biofilm was assessed under static conditions using the method of Christensen [13]. Bacteria were grown at 37 °C in Tryptic Soy broth (TSB) or TSB supplemented with 1% D-(+)-glucose (Sigma Aldrich), or TSB supplemented with 1% D-(+)-glucose and cloxacillin

at a concentration of MIC/4. After 48 h of growth, the plates were washed three times with Phosphate-buffered saline, prior to staining with a 0.4% crystal violet solution. Each strain was tested three times. A biofilm-positive phenotype was defined as an optical density at 595 nm twice that of the negative control, and the strains were divided into four categories: no biofilm producer, weak, moderate and strong producers [14]. To assess a decrease in susceptibility to cloxacillin, bacteria in biofilm were tested using the Calgary biofilm device (CBD, Innovatech Inc., Edmonton, Alberta, Canada) to determine the minimum biofilm eradication concentration of cloxacillin (MBEC) following the manufacturer's recommendations [15]. The biofilm inhibitory concentrations were defined as the lowest concentrations of drug resulting in an OD650 nm difference of ≤10% of the mean of two positive control well readings.

2.3. Statistical Analysis and Ethics Approval

For categorical variables, we used Pearson's chi-squared test to compare groups. All analyses were two-tailed and a $p < 0.05$ was considered significant. We used Stata version 10.0 software (Stata Corp., College Station, TX, USA) for statistical analysis.

Ethics approval for the survey was obtained from the Réseau national de Prévention des Infections Associées aux soins (REPIAS), Santé Publique France national agency. Written informed consent was exempted, since the study focused on bacteria and patient intervention was not required.

3. Results

3.1. Nosocomial bacteremia 3-Month Survey

The survey was carried out in 267 ICUs at 212 French hospitals. Monitoring focused on 3668 beds (i.e., 52% of French ICU beds) and covered 313,891 patient-days (PDs). A total of 1931 nosocomial bacteremias occurred during the study in the ICUs, and out of these bacteremias, 242 were *S. aureus* bacteremias (12.5%), including 7.9% MRSA-bacteremias (19/240; 2 nk). An *S. aureus* bacteremia was detected in one admitted patient out of 110 in adult and pediatric sectors, and in one out of 230 newborns (Table 1).

Table 1. *S. aureus* bacteremia incidence rates.

ICU Sector	Bacteremia Incidence (Average Value (Median; Standard Deviation))		
	All Bacteremias	*S. aureus* Bacteremias	
	/1000 patient-days	/1000 patient-days	/100 admitted patients
Adult	5.79 (5.01; 4.63)	1.58 (0.39; 11.01)	0.88 (0.29; 1.53)
Pediatric	3.05 (2.26; 3.38)	0.24 (0.00; 0.55)	0.88 (0.00; 2.88)
Neonatal	2.50 (2.50; 3.78)	0.37 (0.00; 0.76)	0.45 (0.00; 1.02)

The characteristics of the infected patients and their bacteremias are presented in Table 2. The 242 patients suffering *S. aureus* bacteremia had frequent immunodepression (12.3%), cancer (13.6%), and COVID-19 infection (61.5%). In adults, the IGS II severity score was 40.0. The major sources of the *S. aureus* bacteremias were the lungs (50.0%) and intravascular-catheters (62; 25.6%). Death within the first week following infection diagnosis was notified in 69 cases (28.8%).

3.2. Microbiological Study

Of the 267 ICUs participating in the survey, 30 took part in the microbiological study (11.2%). Despite this modest ICU participation in our study, the number of *S. aureus* bacteremias detected in these 30 ICUs represented 22.7% of the whole group of 242 bacteremias. In addition, whereas 55 *S. aureus* bacteremias occurred during the study in these 30 ICUs, 53 *S. aureus* strains were available (96.4%). As there were no significant differences regarding the source of the 53 bacteremias compared with those of the 242 from the national level, we considered the 53 strains representative of the national set of *S. aureus* bacteremias (Table 2).

Table 2. Characteristics of the patients suffering from bacteremia.

Patient Characteristics		Patient with Nosocomial Bacteremia					
		All	%	With *S. aureus* Bacteremia			
				All	%	With Studied Strains %	
	N	1931		242	12.5	53	
HFC category	univ./region/army	728	37.7	89	36.8	17	31.1
	general	928	48.1	118	48.8	33	62.3
	short stay clinics	262	13.6	31	12.8	3	5.7
	oncology centers	13	0.7	4	1.7		
Patient category	adults	1736	89.9	221	91.3	50	94.3
	children	34	1.8	4	1.7	1	1.9
	newborns	161	8.3	17	7.0	2	3.8
Sex	males (nk [1])	1316 (20)	68.9	151 (1)	62.7	37	74.0
Age (yr) [2]	adults	63.4 (66.0)		63.7 (66.0)		63.8 (64.5)	
	children	2.6 (<1.0)		0.3 (<1.0)		14 (14)	
Neonatal	birth weight (g) [2]	1011.2 (840.0)		981.2 (770.0)		1820.0 (1820)	
Neonatal	gestational age (Wk) [2]	27.6 (27.0)		27.3 (26.0)		31.5 (31.5)	
Immunosuppression [3] (nk [1])		295 (62)	16.9	30 (9)	13.6	10 (1)	19.2
Cancer (nk [1])		195 (175)	12.0	26 (19)	12.3	6 (8)	13.3
Severity score IGS II (adults) [2]		45.9 (43.0)		44.4 (40.0)		40.0 (40.0)	
COVID-19 status (nk [1])		992 (186)	56.8	136 (21)	61.5	32 (7)	69.6
Patient death [4] (nk [1])		533 (27)	28.0	69 (2)	28.8	15 (1)	28.8
Bacteremia source							
	catheters	538	27.9	62	25.6	14	26.4
	pneumonia	529	27.4	121	50.0	24	45.3
	urinary tract	115	6.0	1	0.4	1	1.9
	digestive tract	139	7.2	3	1.2		
	other	200	10.3	26	10.7	5	9.4
	not known	410	21.2	29	12.0	9	17.0

[1] nk not known; [2] average value (median value); [3] the presence of immunosuppression is defined according to several criteria: 1- either in the presence of true aplasia with <500 circulating polynuclear; 2- either the patient is on immunosuppressive treatment (chemotherapy, radiotherapy, immunosuppressants, long-term and high-dose corticosteroid therapy dose, e.g., for >30 days, recent corticosteroid therapy at a dose >5 mg/kg of Prednisolone for >5 days); 3- either the patient is HIV positive with CD4 < 500/mm^3, or 4- either the patient has leukemia or lymphoma with PNN < 500/mm^3. [4] Patient death within 7 days after bacteremia diagnosis.

Of the 53 *S. aureus* strains, 5 were MRSA (9.4%) and 28 were resistant to erythromycin (52.8%) (Table 3). MLST revealed 20 CC398 strains (37.7%), 9 CC8 (17.0%), 7 CC45 (13.2%), 5 CC30 (9.4%), and 12 in 6 other CCs. *mupA*, *qacAB*, and *qacC* were carried by 2 CC8, 1 CC398, and 1 CC8 strain, respectively; *tst-1* was found in 5 strains (9.4%); the *lukF/S* genes encoding PVL were absent from our isolates. An association was found (1) between CC8 and methicillin resistance (p = 0.039), fusidic acid resistance (p < 0.001), and mupirocin resistance (p = 0.026), (2) between CC398 strains and erythromycin resistance (p < 0.001), and (3) between CC30 strains and the *tst-1* gene (p < 0.001).

Table 3. Antimicrobial susceptibility profiles, virulence genes and biofilm production of the 53 *S. aureus* strains, bacteremia source and patient death within 7 days after bacteremia diagnosis, according to sequence type obtained by MLST.
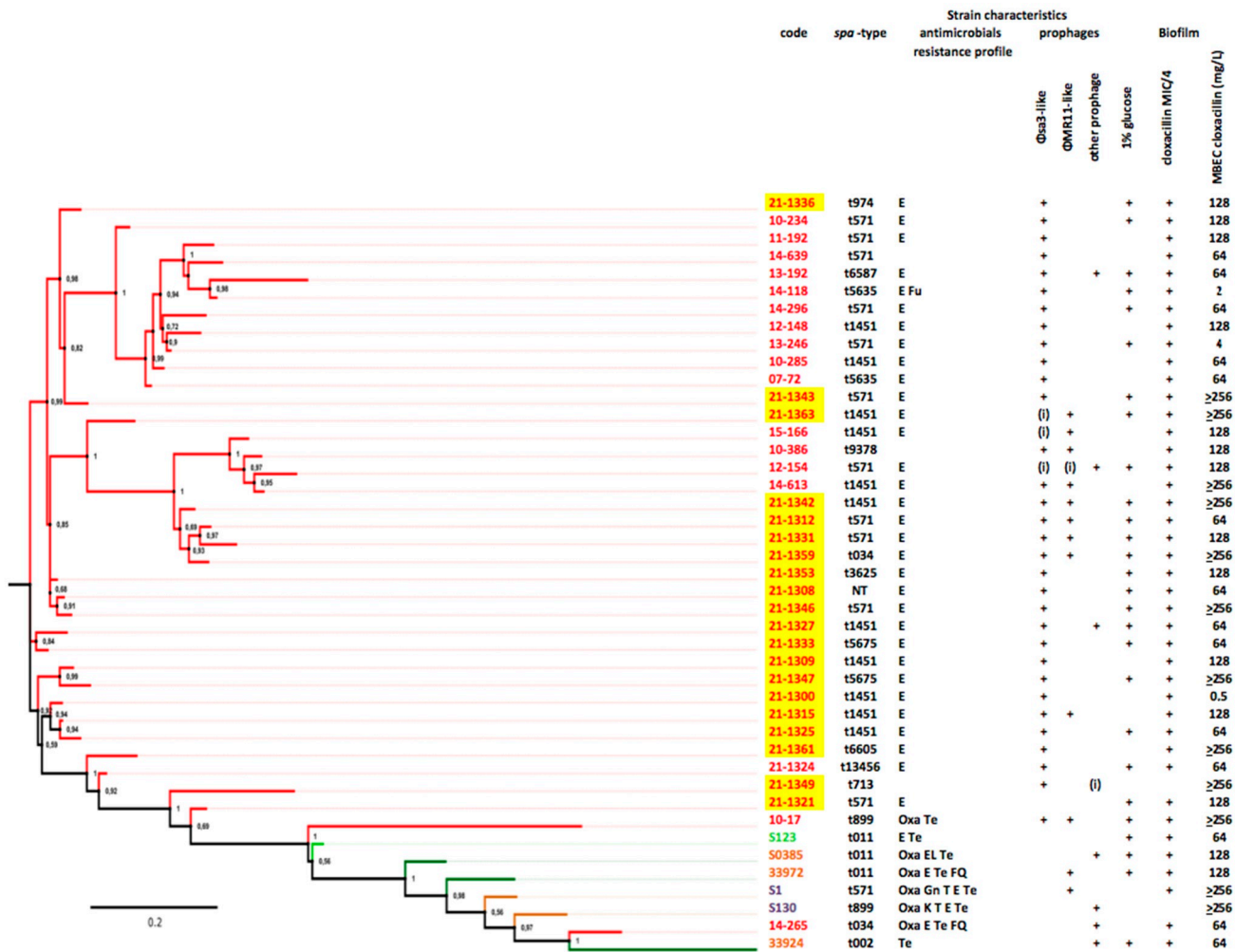
Strain Characteristics	According to MLST										All Strains
	1	5	8	12	15	22	30	45	59	398	
N	2	3	9	1	3	2	5	7	1	20	53
Antimicrobial susceptibility											
FOX resistance	1	1	3								5
erythromycin resistance	1		5				2		1	19	28
fusidic acid resistance	1		4								5
fluoroquinolone resistance			2								2
fosfomycin resistance			1								1
tetracycline resistance	1										1
kanamycin resistance	1										1
mupA			2								2
qac			1							1	2
Virulence genes											
tst-1							3	2			5
lukF/S											0
Biofilm production											
without glucose 1%	2	1	1					1			5
strong producers								1			1
with glucose 1%	2	2	7		2			2	1	16	32
strong producers	2		1							3	6
with glucose 1% and cloxacillin MIC/4 [1]										19	
strong producers										17	
MBEC cloxacillin [1,2] (mg/L)										128	
Bacteremia source											
catheters	1		3	1			3	2	1	3	14
pneumonia		3	4		2	1	1	4		9	24
urinary tract			1								1
digestive tract											
endocarditis										2	2
skin and soft tissue										3	3
not known	1		1		1	1	1	1		3	9
Death [3] (nk [4])			1	2						3	9 (1)

[1] carried out with the 20 CC398 strains; MIC/4 indicates a concentration of cloxacillin equal to the strains' MIC value for cloxacillin divided by 4; [2] median value; [3] patient death within 7 days after bacteremia diagnosis; [4] nk not known.

Under static conditions and without exposure to glucose, a biofilm production was observed in 5 strains (9.4%), including one strong producer. The percentage increased to 60.4% for strains grown in broth supplemented with 1% glucose, including 6 strong producers (18.7%). No association was found between any clone and a particular bacteremia source. Patient death within seven days after bacteremia diagnosis was most frequent with bacteremia associated with a CC398 or a CC45 strain (47.4% and 42.9%, respectively) compared to bacteremia associated with strains from other CCs (11.5%; NS).

The 20 CC398 strains were sequenced and studied with a previously studied collection of 23 CC398 strains (i.e., six livestock-associated strains and 17 emerging strains; Figure 1) [16]. Among the 20 strains recovered during the present study, 19 carried a φSa-3 prophage (95.0%) and its particular evasion cluster (8). The φSa-3 prophages were inserted in 18 cases into the virulence gene *hlb*, and in the remaining strain, in *ebh*, a gene encoding for the giant surface anchored protein Ebh that has been associated with *S. aureus* complement resistance [17]. Six strains carried a φMR11-like prophage (30.0%), all inserted into the *smpB* virulence gene. Two

carried other prophage features (10.0%), and one strain, responsible for a case of endocarditis in a 74 year-old female, did not carry any prophage elements.

code	*spa*-type	antimicrobials resistance profile	Φsa3-like	ΦMR11-like	other prophage	1% glucose	cloxacillin MIC/4	MBEC cloxacillin (mg/L)
21-1336	t974	E	+			+	+	128
10-234	t571	E	+				+	128
11-192	t571	E	+				+	128
14-639	t571		+				+	64
13-192	t6587	E	+		+	+	+	64
14-118	t5635	E Fu	+			+	+	2
14-296	t571	E	+			+	+	64
12-148	t1451	E	+				+	128
13-246	t571	E	+			+	+	4
10-285	t1451	E	+				+	64
07-72	t5635	E	+				+	64
21-1343	t571	E	+			+	+	≥256
21-1363	t1451	E	(i)	+		+	+	≥256
15-166	t1451	E	(i)	+			+	128
10-386	t9378		+	+			+	128
12-154	t571	E	(i)	(i)	+	+	+	128
14-613	t1451	E	+	+			+	≥256
21-1342	t1451	E	+	+		+	+	≥256
21-1312	t571	E	+	+		+	+	64
21-1331	t571	E	+	+		+	+	128
21-1359	t034	E	+	+		+	+	≥256
21-1353	t3625	E	+			+	+	128
21-1308	NT	E	+			+	+	64
21-1346	t571	E	+			+	+	≥256
21-1327	t1451	E	+		+	+	+	64
21-1333	t5675	E	+			+	+	64
21-1309	t1451	E	+				+	128
21-1347	t5675	E	+			+	+	≥256
21-1300	t1451	E	+				+	0.5
21-1315	t1451	E	+	+			+	128
21-1325	t1451	E	+			+	+	64
21-1361	t6605	E	+				+	≥256
21-1324	t13456	E	+			+	+	64
21-1349	t713		+	(i)				≥256
21-1321	t571	E	+			+	+	128
10-17	t899	Oxa Te	+	+		+	+	≥256
S123	t011	E Te				+	+	64
S0385	t011	Oxa EL Te			+	+	+	128
33972	t011	Oxa E Te FQ		+		+	+	128
S1	t571	Oxa Gn T E Te		+			+	≥256
S130	t899	Oxa K T E Te				+		≥256
14-265	t034	Oxa E Te FQ				+	+	64
33924	t002	Te		+		+	+	64

Figure 1. Phylogeny of CC398 strains, prophage content, biofilm production, and MBEC of cloxacillin. Emerging strains (i.e., those from human bacteremia contracted in an animal-free environment) are shown in red, whereas the remaining strains are livestock-associated (animal colonization in light green, animal infection in dark green, and human colonization in yellow). Prophages are indicated by "+" when complete and by "(i)" when incomplete. Note that the emerging strains isolated in 2021 are highlighted in yellow.

Biofilm production of CC398 strains was further studied in the presence of sub-inhibitory concentrations of cloxacillin. Whereas glucose-induced biofilm production was demonstrated in 85.0% of the CC398 strains, all but one strains produced biofilm in broth supplemented with 1% glucose and cloxacillin at a concentration of MIC/4 (95.0%; NS). Strong producers were more frequent following exposure to sub-inhibitory concentrations of cloxacillin (18/19; 94.7%) than in the case of strains grown with glucose only (3/17; 17.6%; $p < 0.001$), suggesting that cloxacillin at sub-inhibitory concentration may have increased biofilm production in CC398 strains.

Biofilm eradication assay of CC398 strains. The minimum biofilm eradication concentration (MBEC) of cloxacillin, determined for the 20 CC398 strains, ranged from 0.5 to >256 mg/L according to the strain (Figure 1), with a median value of 128. Strains carrying a φMR11-like prophage more frequently presented a MBEC > 64 mg/L rather than strains lacking this prophage (1/5 vs. 6/14; NS), suggesting an impact of lysogeny by φMR11-like on the susceptibility to cloxacillin of sessile CC398 bacteria.

4. Discussion

To our knowledge, this is the first multicenter study depicting the incidence of CC398 *S. aureus* bacteremia in ICUs. The survey revealed one nosocomial bacteremia for 230 newborns, and twice more in adult and pediatric patients, a situation close to the one described in a French ICU in 2011, where a 5-month study revealed 1 case of CC398 nosocomial bacteremia in 89 patients [7]. We observed one third of bacteremias associated with a CC398 strain. This prevalence, obtained in the ICU setting, was higher than that shown in a recent study carried out in all the departments of 17 Spanish hospitals (i.e., medical, surgical, and ICU) [18], in which CC398 strains represented 4.3% of the *S. aureus* recovered from bacteremias during a 6–12-month period in 2018–2019. Due to the similarity of the characteristics of both infected patients and bacteremia in the 30 ICUs and in the 267 ICUs that participated in the French survey, we believe our results may reflect the general situation. Further studies should be carried out, both inside and outside the ICU, to investigate the current incidence of the CC398 clone in hospitals worldwide.

As usual in Europe [18,19], the CC398 strains responsible for bacteremia were mostly of *spa*-types t1451 and t571, methicillin and tetracycline-susceptible and resistant to erythromycin. The lack of MRSA is reassuring, as is the rarity of strains carrying a *qac* gene, as recent studies mostly carried out in Asia have described an increasing incidence of severe infections caused by livestock-independent MRSA [11,20,21].

A high mortality rate was associated with CC398 bacteremias (47.4%), a concordant result with previous studies [22]. However, in contrast to the strains recently described in China [23] and Australia [24], none of the 20 CC398 studied carried the *lukF/S* or *tst-1* genes in their genome. Patient risk factors such as extreme age, immunosuppression (16.7%), and cancer (15.0%), or other unknown compounds present on mobile genetic elements and that should be carefully studied, might play a major role in infection outcome. Note that the number of events is moderate and would need analysis of a larger number of strains.

WGS confirmed typical prophage content of the CC398 emerging strains, with φSa-3 in all but one isolate, and φMR11 in one third of these [8,9]. Consequently, the strains carried the putative virulence genes *chp* and *scn* in φSa-3 encoding the chemotaxis inhibitor protein (CHIPS) and the staphylococcal complement inhibitor (SCIN), respectively, and *seb* encoding a putative superantigen similar to enterotoxin B in φMR11. As previously demonstrated, CC398 lysogens carrying these prophage genes may benefit from the production of the three immune-modulating proteins, CHIPS, SCIN, and SEB, when exposed to conditions favoring prophage induction [25,26]. Analysis of the insertion sites of the prophages identified three different loci in the bacterial genomes studied, located in all cases in bacterial virulence genes (i.e., *hlb*, *smpB*, or *ebh*). As shown with a prophage favoring *S. aureus* colonization of diabetic feet [27], lysogeny of the strains with ß-converting prophages may limit *S. aureus* virulence and favor colonization, and thus bacteremia [28]. In the ICU, where risk factors of infections are numerous, whether patient-related or healthcare-related, it would seem likely that in *S. aureus*, the acquisition of such prophages could contribute to a further increase of the ability to colonize human flora and devices and infect humans.

The ability of *S. aureus* to form biofilm is a significant factor that enhances the pathogenicity of this species [1,3,29]. Concordant with previous studies [3], a minority of our strains was capable of biofilm development under standard conditions in TSB, but enhanced biofilm formation was obtained when strains were grown in broth supplemented with 1% glucose [30]. We confirmed a strong biofilm formation associated with CC8 [31], one of the major lineages associated with catheter-related bacteremia [32]. To our knowledge, our study is the first showing that the CC398 lineage also has a high ability to produce biofilm.

Due to slow diffusion into the biofilm, biofilm bacteria are exposed to sub-inhibitory concentrations of antibiotics [33]. Previous studies have demonstrated that biofilm formation is increased when *S. aureus* are cultured in the presence of sub-inhibitory concentrations of antibiotics [34], and especially oxacillin, i.e., the antibiotic of choice in the course of severe MSSA infections [35]. Our study is the first to show a strong production of biofilm

by CC398 strains following exposure to sub-inhibitory concentrations of cloxacillin. Biofilm helps bacteria to escape immune response, contributes to bacterial persistence in the environment [36–39], and increases phenotypic resistance to antimicrobials [40]. Biofilm-grown microorganisms have an inherent lack of susceptibility to antibiotics, whereas planktonic cultures of the same organism do not [2,33,41–43]. We demonstrated extremely high minimal biofilm eradication cloxacillin concentrations of the CC398 MSSA studied. The resulting tolerance of microbial biofilms to in principle adequate antibiotic therapy may lead to problems in their eradication and in the management of infected patients. Our preliminary data, showing cloxacillin-induced strong production of biofilm by CC398 strains, and a tolerance of *S. aureus* to cloxacillin should be explored further.

The determinants carried by the φSa-3 and φMR-11 prophages have previously been associated with high levels of transmissibility [10], and high potential to persist and disperse in the hospital environment [7]. In this study, the strains recovered from a same ICU were genetically distant, allowing us a priori to exclude intra-ICU transmission. The design of our study did not allow us to investigate the mechanisms of acquisition of the *S. aureus* strains. The genetic diversity observed in our study argues for (but does not prove) dissemination of the CC398 human-adapted subpopulation in human, and nosocomial bacteremia likely caused by multiple strains colonizing the patients before the hospital stay or acquired during hospital stay. Our results suggest the spread of a virulent clone in the hospital settings, confirming the need to investigate the mechanisms involved with the epidemiological success of CC398 in humans.

DETULLIO (CH Trevenans); Marion LECURU (Hôpital Foch, Suresnes); Malcie MESNIL (Hôpital Fondation de Rothschild, Paris); Fabienne GUASP (Hôpital privé de Provence, Aix-en-Provence).

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