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Insights on catheter-related bloodstream infections: a prospective observational study on the catheter colonization and multidrug resistance

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SUMMARY

Background: Central venous catheter-related bloodstream infection (CRBSI) is a huge public health concern with considerable impact on mortality and health costs.
Aim: A three-year observational study enrolling three tertiary hospitals located in Lisbon, Portugal, was designed to identify the major aetiological agents of CRBSI, their ability to colonize central venous catheters and their antimicrobial resistance profiles.
Methods: Aetiological agents of CRBSI were identified by Vitek 2. Whole-genome sequencing was used to confirm CRBSI by the most prevalent aetiological agents and characterize their resistome. Central venous catheter colonization (namely by biofilm assembly) was monitored by scanning electron microscopy.
Findings: Staphylococci were the most prevalent causative agent (36/58, 62.0%), with *S. aureus* and coagulase-negative *S. epidermidis* accounting for 24.1% and 36.2% of CRBSIs, respectively. Fifty-nine of 72 staphylococci isolates were meticillin resistant. Comparative genomic analysis of central venous catheters/haemoculture pairs of isolates revealed genomic matches for 35 of 36 pairs and a good correlation between antibiotic susceptibility phenotype and the presence of antimicrobial resistance genetic determinants. Biofilms were present on 48.6% of the central venous catheters; nevertheless, no statistically significant association was established between biofilm assembly and CRBSI, and the presence/absence of *ica* operon and *agr* groups did not correlate with biofilm phenotypes, highlighting the need for further studies to elucidate biofilms' role on this healthcare-associated infection.
Conclusion: Whole-genome sequencing was shown to be a valuable tool to confirm CRBSI. Although more than 42.3% of the central venous catheters were colonized by staphylococci, no statistically significant association was found between CRBSI and biofilms.

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Introduction

Central venous catheter-related bloodstream infection (CRBSI) is a healthcare-associated infection (HAI) responsible for high rates of morbidity and mortality, namely in critically ill patients in intensive care unit (ICU) [1,2]. The latest survey released by the European Centre for Disease Prevention and Control identified staphylococcus as the major aetiological agent of CRBSI, being coagulase-negative staphylococcus (*Staphylococcus epidermidis*) and *Staphylococcus aureus*, responsible for 23.6% and 12.0% of the cases, respectively [1]. The impact of extensive admission to ICU due to COVID-19 on CRBSI incidence has not been fully evaluated; nevertheless, an increase in CRBSI has been reported [3].

CRBSIs are determined by multiple factors such as patient's underlying health condition, catheter insertion site, dwelling time and catheter colonization by micro-organisms organized in biofilms [2—4]. Biofilms are thin layers of micro-organisms adhering to the surface of an organic/inorganic structure together with the secreted polymers [5]. Micro-organisms rarely exist as single units, persisting mostly as biofilms, which enhance resistance of micro-organisms to antimicrobials, and often induce recurrent infections [6]. As such, the assembly of mature biofilms within human hosts through medical devices, such as central venous catheters (CVCs), may mediate infections resistant to antibiotic treatment, interference with host immune response, and development of a chronic condition [5]. Certain genetic determinants have been linked to the regulation of cell density-dependent gene expression with implications on biofilm maturation and dispersion and probably in infection progression, such as the accessory gene regulator (Agr) system in staphylococci [7]. The intercellular adhesion (*ica*) operon is another example of biofilm-related genes in staphylococci that have been suggested to be more associated with nosocomial and invasive isolates [8].

In this study, the importance of CVC colonization in CRBSI was evaluated in patients admitted to three tertiary hospitals in Lisbon, Portugal, during a three-year period (2017—2020). For the most prevalent aetiological agent, staphylococci genome sequencing was applied to confirm the isogenicity of CVC/haemoculture (HC) isolate pairs and further characterize their genomic background and antimicrobial resistance (AMR) genetic signatures.

Methods

Study design and ethics

A prospective observational study was designed to identify: (i) the major aetiological agents of CRBSI, defined as a BSI occurring 48 h before or after CVC removal, and a positive culture of the same micro-organism from either a CVC, or blood, or pus from the insertion site or differential delay positivity of blood samples [1]; (ii) CVC colonization by micro-organisms; and (iii) their AMR profiles. The study took place in three tertiary hospitals from Lisbon, Portugal (hospitals A, B, and C with 149, 262, and 350 beds, respectively) during three years (March 2017 to February 2020) and was approved by the West Lisbon Hospital Centre Ethics Board in accordance with the World Medical Association Declaration of Helsinki. The micro-organisms isolated from HC and CVC of CRBSI patients were collected and preserved at −20 °C in trypticase soy broth (TSB)/20% glycerol. Briefly, a semiquantitative culture of CVCs was performed using the Maki reference method on blood agar, and a sample of peripheral blood was cultured on a Bact/Alert system (bioMérieux, Marcy l'Etoile, France) [9]. Vitek 2 or Vitek-MS (bioMérieux) was used to identify micro-organisms following the manufacturer's instructions. CVCs were washed with sterile saline solution and preserved in 4% paraformaldehyde (Merck, Darmstadt, Germany) in PBS at 4 °C protected from light until further processing. AMR profile was reported for the HC isolate.

Antimicrobial susceptibility testing

The antimicrobial activity of HC and CVC isolated *S. aureus* (28 isolates), *S. epidermidis* (42 isolates), and *S. haemolyticus* (two isolates) was assessed by a minimum inhibitory concentration (MIC) determination system (Vitek 2). Briefly, an inoculum of 1×10^5 bacteria/mL and an AST-P648 card (bioMérieux) were used, being the result interpreted according to EUCAST guidelines [10].

Scanning electron microscopy

The 35 CVCs preserved in fixative were washed twice in PBS for 10 min in a rotator at room temperature (RT) and post-fixed with 0.5% osmium tetroxide (EMS, Hatfield, PA, USA) in PBS overnight (ON) in the dark at 4 °C. The samples were then washed twice (10 min at RT) with PBS and deionized water. Dehydration was performed at RT in a rotator by using once 50%, 70%, and 95% ethanol (Merck) for 30 min and twice 100% ethanol for 30 min. Samples were allowed to dry at RT, mounted on top of double-sided carbon tape (EMS) in order to allow the observation of the inner and outer sides of the CVC, coated with 20-nm-thick gold—palladium film using a sputter coater QISOT ES (Quorum Technologies, Laughton, UK) and analysed under an electron microscope, JSM-7100F (JEOL, Tokyo, Japan).

DNA extraction and WGS

Five hundred microlitres of ON staphylococcus cultures (37 °C with aeration in TSB) were harvested by centrifugation (5 min, 8000 rpm), resuspended in 185 μL lysis buffer (20 Mm Tris buffer pH 8.0, 2 mM EDTA, 1.2% Triton X-100; all from Sigma, St Louis, MO, USA) and incubated ON at 4 °C. One hundred microgram each of lysostaphin (Sigma) and lysozyme (Sigma) were added, mixed using a vortex and incubated at 37 °C for 1 h. Then the manufacturer's instructions of the Qiagen DNeasy blood & Tissue kit (Qiagen, Hilden, Germany) were followed. DNA was subjected to Nextera XT library preparation (Illumina, San Diego, CA, USA) prior to paired-end sequencing (2×250 or 2×150 bp) on either a MiSeq or a NextSeq 550 instrument (Illumina), according to the manufacturer's instructions.

Comparative genomic analysis and resistome prediction

Genome sequences were assembled using INNUca v4.2.0 pipeline (https://github.com/B-UMMI/INNUca) [11]. Species confirmation and contamination screening were assessed using

Kraken v2 (with 8 GB database available at https://ccb.jhu.edu/software/kraken/) for both raw reads and final polished assemblies. For all isolates, assembly statistics are reported in Table S1. MLST prediction was determined using *mlst* v2.16.1 software (https://github.com/tseemann/mlst), with novel alleles identified upon query to the PubMLST database (http://pubmlst.org).

In order to compare the genome background of the same-patient pairs of isolates (CVC versus HC), HC quality-processed reads were mapped against the polished genome assembly obtained from the respective CVC isolate using Snippy v.4.5.1 (https://github.com/tseemann/snippy; mincov 10, minfrac 0.51, mapqual 20, basequal 20). All reported mutations were carefully confirmed and visually inspected using Integrative Genomics Viewer v2.9.4. For phylogenetic analysis, quality-improved reads (after Trimmomatic processing) of all isolates were individually mapped against Sau_B1-CVC-2017 and Sep_C24-CVC-2018 for *S. aureus* and *S. epidermidis*, respectively, using Snippy v4.5.1. (mincov 10, minfrac 0.7, mapqual 20, basequal 20). Core single nucleotide polymorphisms were then extracted using Snippy's core module (snippy-core), and phylogenetic trees were generated using Fasttree v2.1.10 software [12].

To assess the presence/absence of AMR genetic determinants, polished genome assemblies were queried against ResFinder v4.1 database (http://www.genomicepidemiology.org/) [13]. For *S. aureus*, PointFinder tool was also used to screen known AMR-associated mutations. In addition, for *S. epidermidis* and *S. haemolyticus* isolates, we searched for other genes/mutations potentially linked to AMR phenotypes by Snippy reference-based mapping [14,15]. For *S. aureus* isolates, agr specificity group typing was performed *in silico* using AgrVATE v1.0 (https://github.com/VishnuRaghuram94/AgrVATE) [16]. Additionally, for *S. aureus* and *S. epidermidis* isolates, presence and absence of *ica* operon was directly screened on polished assemblies, annotated using Prokka v1.14.6 [16].

All sequencing reads and genome assemblies generated in this study were deposited in the European Nucleotide Archive under BioProject accession no. PRJEB45360 (Supplementary Table S1).

Statistical analysis

Unadjusted association between the presence of biofilm on the CVC and the occurrence of CRBSI was evaluated by χ^2-test. $P < 0.05$ was considered statistically significant.

Results

CBRSI: identification and characterization of aetiological agents

During the period of the study, 58 cases of CRBSI were reported (Table I): 15 cases from hospital A (25.9%), 14 cases from hospital B (24.1%) and 29 cases from hospital C (50%). The majority of the CRBSIs had staphylococci (62.1%; 36/58) as aetiological agent. In hospital A, *S. aureus* was the most prevalent aetiological agent (6/15) followed by the coagulase-negative *S. epidermidis* (3/15). In hospitals B and C, *S. epidermidis* was the most prevalent aetiological agent, being responsible for 35.7% (5/14) and 44.8% (13/29) of the cases, respectively. One case by another coagulase-negative staphylococcus, *S. haemolyticus*, occurred in hospital B. *Klebsiella pneumoniae* was the second-most prevalent pathogen (10/58), being responsible for cases in all three hospitals (Table I).

Table I
Distribution of catheter-related bloodstream infections (CRBSIs) and aetiological agents by hospital

Aetiological agent(s)	CRBSIs			
	Hospital A	Hospital B	Hospital C	Overall
S. aureus	6 (40 %)	4 (28.5%)	4 (13.8%)	14 (24.1%)
S. epidermidis	3 (20%)	5 (35.7%)	13 (44.8%)	21 (36.2%)
S. haemolyticus		1 (7.1%)		1 (1.7%)
K. pneumoniae	3 (20%)	1 (7.1%)	6 (20.7%)	10 (17.2%)
P. aeruginosa			2 (6.9%)	2 (3.4%)
Enterococcus faecalis	1 (6.7%)			1 (1.7%)
Serratia marcescens	1 (6.7%)			1 (1.7%)
Candida glabrata			1 (3.4%)	1 (1.7%)
Candida parapsilosis		2 (14.3%)	1 (3.4%)	3 (5.1%)
K. pneumoniae, *S. epidermidis*			1 (3.4%)	1 (1.7%)
S. marcescens, *E. faecalis*		1 (6.7%)		1 (1.7%)
S. marcescens, *P. aeruginosa*	1 (6.7%)			1 (1.7%)
E. cloacae, *C. parapsilosis*			1 (3.4%)	1 (1.7%)
Total	15 (25.9%)	14 (24.1%)	29 (50.0%)	58 (100%)

Pseudomonas aeruginosa, *Serratia marcescens*, and *Enterococcus faecalis* were the other bacterial aetiological agents identified. Two species of fungi, *Candida parapsilosis* and *Candida glabrata*, were identified as aetiological agents of CRBSI. Finally, three co-infections by two bacterial species (*K. pneumonia*/*S. epidermidis*, *S. marcescens*/*E. faecalis*, *S. marcescens*/*P. aeruginosa*) and one by a bacterium and a fungus (*Enterobacter cloacae*/*C. parapsilosis*) were reported (Table I).

A high prevalence of AMR was observed for HC isolates. Among staphylococci, 64.3% (9/14) of *S. aureus*, 95.3% (20/21) of *S. epidermidis* isolates, and the *S. haemolyticus* were meticillin resistant. For *E. faecalis* and *S. marcescens* no antimicrobial resistance was reported. Resistance to third-generation cephalosporins was reported for 10% (1/10) of *K. pneumoniae* isolates. Resistance to third-generation cephalosporins and carbapenems was reported for 40.0% (4/10) of *K. pneumoniae* and 100% (2/2) of *P. aeruginosa* isolates.

For the co-infection cases, different AMR profiles were reported. In the pair *E. faecalis*/*S. marcescens*, AMR was reported only for *S. marcescens* (resistant to third-generation cephalosporins). The second pair includes a meticillin-resistant *S. epidermidis* (MRSE) and a *K. pneumoniae* resistant to third-generation cephalosporin and carbapenems. *E. cloacae* paired with *C. parapsilosis* was resistant to third-generation cephalosporins and piperacillin–tazobactam combination. The last pair includes a *P. aeruginosa* reported as

intermediate to third-generation cephalosporins and carbapenems and a penicillin-resistant *S. marcescens*.

Staphylococci CVC/HC comparative genomics and AMR genotype–phenotype associations

Bacterial WGS was applied to confirm the isogenicity of CVC/HC isolate pairs, and to further characterize the genomic background and AMR genetic signatures of CRBSI causative agents. This detailed analysis was performed for HC and CVC isolates of the most common causative agent of CRBSI (staphylococci). Genomic analysis enrolled 21 CVC/HC pairs of coagulase-negative staphylococci (20 *S. epidermidis* and one *S. haemolyticus*) and 15 pairs of *S. aureus*.

For *S. epidermidis*, a genetic match between CVC and HC isolates for 19/20 pairs ($\leq$9 mutations between the two genome sequences) was found, thus confirming a CVC-mediated bacteraemia. Considering that WGS was performed over cultured isolates (selected after colony picking and short culture passaging), we made no inference on the link between the detected mutations and the pathogen ability to infect or its microevolution on the course of BSI. Noteworthy, pair Sep_C28 had genetically divergent CVC and HC isolates (>500 SNPs). Even though they belonged to the same ST2, the detailed genomic analysis revealed that the *S. epidermidis* strain isolated from CVC was not the causative agent of BSI. This was further corroborated by the detection of rather distinct AMR profiles, i.e. HC and CVC isolates were resistant to seven and to 11 antibiotics, respectively, being the only *S. epidermidis* isolate resistant to daptomycin and vancomycin (Figure 1A). Ten distinct STs were observed for *S. epidermidis* (19 isolate pairs), with ST2/ST2-like being predominant (9/19 pairs) and detected in hospitals B and C. ST87 (detected in hospitals B and C) and ST731 were associated with two distinct pairs of isolates, whereas all other STs were only detected once (Figure 1A). Regarding AMR profiles, all isolates (except Sep_C31 pair) were meticillin resistant, harbouring *mecA* gene. Multidrug resistance (MDR $\geq$5 antibiotics) was observed across all MRSE isolates regardless of the ST. For most antibiotics, the AMR phenotype was linked to specific genetic determinants (Figure 1A).

Genetic identity for *S. haemolyticus* isolate pairs in hospital C (presenting 12 SNPs between isolates) was confirmed. These ST25 isolates were also MDR (Table S1B).

Regarding *S. aureus*, a genetic match was observed between CVC and HC isolates for all 15 pairs ($\leq$12 mutations between the paired genome sequences, except pair Sau_B13 that presented 30 mutations, although these were supported by low coverage), thus confirming the CVC-mediated bacteraemia. Seven *S. aureus* STs (ST5, ST22, ST30, ST45, ST72, ST97 and ST105) were detected among the 15 studied CRBSI. ST22 was predominant, being linked to seven CRBSI and detected in all hospitals (Figure 1B). *S. aureus* ST22 caused all CRBSI in hospital C. Besides ST22, only ST5 and ST30 were detected more than once, each one originating from different hospitals. Of note, six distinct STs were detected among the seven CRBSIs from hospital A. This might reflect the main hospital activity, since hospital A receives patients from multiple hospitals, and is thus more likely to import higher genetic diversity. Five pairs of isolates were meticillin-susceptible *S. aureus* (MSSA) and eight were meticillin-resistant *S. aureus* (MRSA), as assessed by antibiotic susceptibility testing (phenotypic assays) and supported by the presence/absence of *mecA* gene (Figure 1B) [17]. For the remaining tested antibiotics, we highlight the detection of resistance to fluoroquinolones (levofloxacin and moxifloxacin), clindamycin, erythromycin, benzylpenicillin and fusidic acid, all supported by detection of respective known genetic determinants (Figure 1B) [13]. In particular, MDR phenotypic/genotypic profiles were detected for all ST22 isolates, one ST105 and ST5 pairs. Notably, the two ST5 pairs from the same hospital revealed a remarkably different AMR signature, with Sau_A7 presenting resistance to seven antibiotics, and Sau_A3 being only resistant to benzylpenicillin. Only ST9 isolates were susceptible to all tested antibiotics. For two pairs of isolates (Sau_A10-2019 and Sau_C11-2017), discordant AMR phenotypic profiles were observed, despite the identical AMR genetic determinants. Although intriguing, other studies have identified *mecA* gene in MSSA and its absence in MRSA, as we observed for Sau_C11_CVC and Sau_A10_HC, respectively [18–20].

Biofilms assembled on CVCs

SEM was used to detect biofilms on 35 CVCs (inner and outer surfaces). Biofilm assembly was observed for 48.6% (17/35) of all staphylococci, including 50% (10/20) of *S. epidermidis*, 42.8% (6/14) of *S. aureus* and the *S. haemolyticus* sample. Neither the presence/absence of *ica* operon (*ica*ABCD present in all isolates) nor *agr* specificity group typing (10 isolates were classified as type I, three as type II and two as type III) correlated with the observed biofilm phenotypes for *S. aureus* (Table S1C). No association between biofilm formation by *S. epidermidis* isolates and the presence/absence of *ica* operon was found (Table S1A).

Biofilms were assembled mostly inside the CVC (Figure 2A, B, and D) – it was possible to visualize extracellular matrix surrounding the bacteria and host components such as red blood cells. On the CVC's outer surface, a different result was observed with mostly adherent staphylococci present (Figure 2C). Biofilms assembled on the CVC outer surface had higher bacterial density and a lower amount of extracellular matrix than the biofilm assembled on the CVC lumen. Nevertheless, with only one positive sample for biofilms on both sides of the catheter we cannot extrapolate. No statistically significant correlation was found between biofilm assembling (either by coagulase-negative staphylococcus or *S. aureus*) on the CVC and CRBSI in this sample ($\chi^2 = 0.305$; $P = 0.581$).

Discussion

The development of medical care had a positive impact on patients' survival prognosis but led to more invasive practices. Indwelling devices commonly used in medical practice are also associated with HAI, such as CRBSI, catheter-associated urinary tract infection and ventilator-associated pneumonia [21]. Most studies and the European Centre for Disease Prevention and Control (ECDC) CRBSI surveillance focus on ICU data, although these infections also occur in other hospital units [1,22]. As such, in this study, we focused on CRBSI detected in three tertiary hospitals to determine whether catheter colonization by biofilms affects the onset of this HAI. Additionally, bacterial

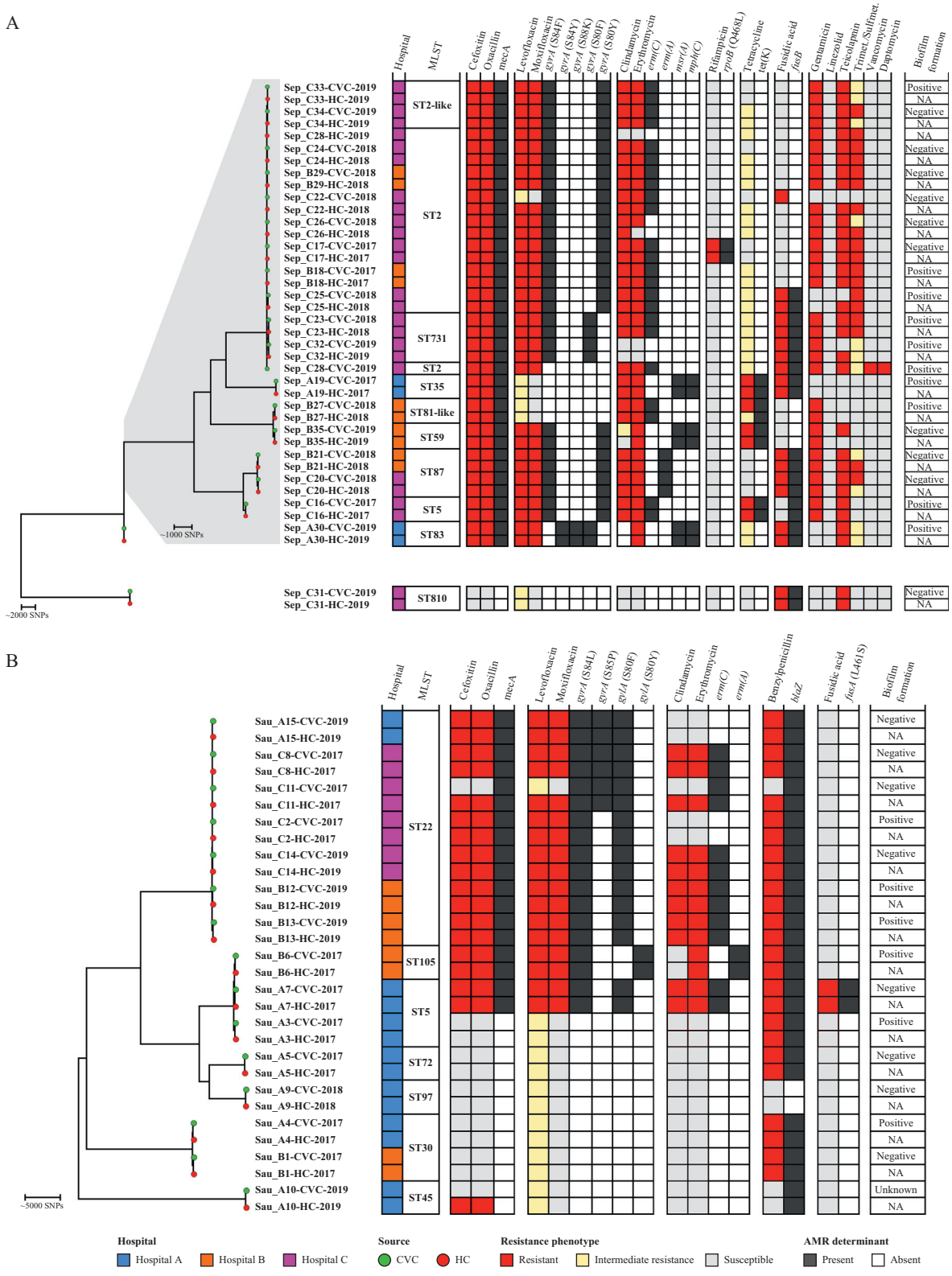

Figure 1. Distribution of the presence/absence of antibiotic resistance determinants for all (A) *S. epidermidis* and (B) *S. aureus* surveyed isolates, respective antimicrobial resistance and biofilm formation phenotypes assessed by scanning electron microscopy. Tree nodes were coloured according to the isolates' source. In panel (A), the grey box corresponds to a zoom-in on the phylogenetic tree for visualization purposes. Details are presented in Table S1A and S1C. MLST, multi-locus sequence type; ST, sequence type; CVC, central venous catheter; HC, haemoculture; NA, not applicable; unknown: this CVC was not available for biofilm analysis.

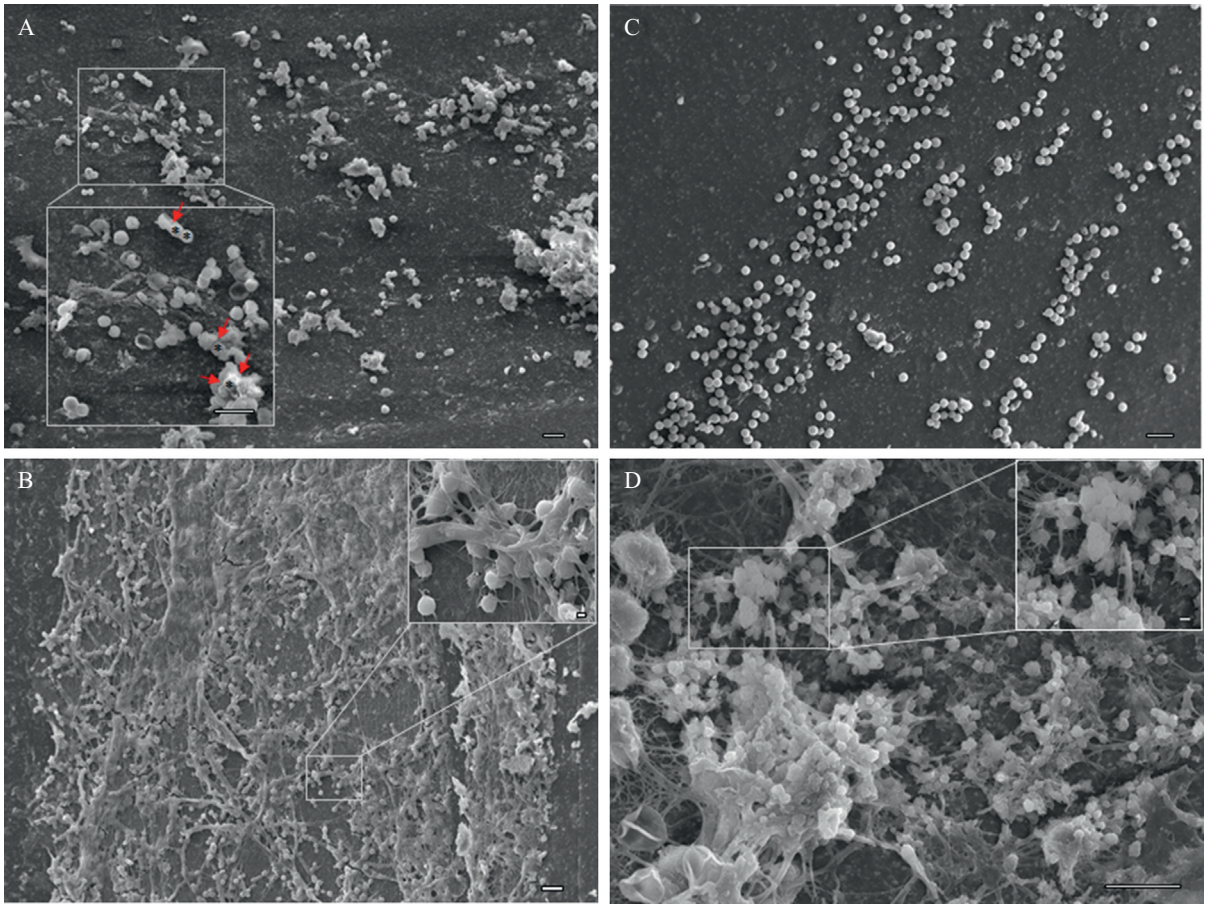

Figure 2. Staphylococci biofilms on central venous catheters (CVCs). Representative micrographs of biofilms assembled by coagulase-negative staphylococci [(A) *S. haemolyticus* Sha_B36-CVC-2019 and (B) *S. epidermidis* Sep_C28-CVC-2019] on the inner surface of polyurethane CVCs are shown. For the coagulase-positive *S. aureus*, biofilms assembled on the outer (C) and inner (D) surfaces of the CVC are shown (isolates Sau_B12-CVC-2019 and Sau_B13-CVC-2019, respectively). The inlets allow the observation of extracellular matrix surrounding the staphylococci units, as well as the presence of host factors. In panel (A), extracellular matrix and staphylococci are highlighted by red arrows and (*), respectively. Scale bars: 10 μm except for (B) and (D) inlet that is 1 μm.

WGS was performed to confirm the CVC-mediated infection and characterize isolates of the most common CRBSI aetiological agent (staphylococci).

The 58 CRBSIs were caused by either single Gram-positive (staphylococci and *E. faecalis*), Gram-negative (*K. pneumoniae*, *P. aeruginosa*, and *S. marcescens*) and non-*C. albicans* species or by combinations of micro-organisms (co-infection). Four co-infection cases were detected (4/58, 6.8%), which is in agreement with previous reports of polymicrobial CRBSI [22—24]. Although this number could be considered low, this scenario might represent a huge challenge for treatment, due to pathogens' synergism by modulating the host response to infection, drug—drug interaction, and emergence of AMR [25—27]. Notably, *C. parapsilosis* and *C. glabrata* accounted for 6.8% of CRBSIs in accordance with the fact that candidaemia is strongly associated with catheterization and the concerns raised by *C. auris* outbreaks in healthcare units [28,29].

Single aetiological agent CRBSIs were mostly ESKAPE pathogens (*E. faecalis*, *Staphylococcus* sp., *K. pneumoniae*, and *P. aeruginosa*). The high prevalence of *K. pneumoniae* and *P. aeruginosa* AMR is in line with the latest AMR ECDC report [30]. In the latest ECDC report on BSI, *Klebsiella* spp. and *P. aeruginosa* accounted for 21.1% and 19.2% of the cases in Portugal, being the most prevalent aetiological agents followed by coagulase-negative staphylococci with 10.8% of the cases [1]. In the present study, the reverse was observed, with coagulase-negative staphylococci being the most prevalent aetiological agent followed by *S. aureus* and *K. pneumoniae*. The study focus (i.e. including fewer healthcare units but not limited to ICU) could explain this difference, as the prevalence of staphylococci varies depending on the healthcare facility. Notwithstanding, our study corroborates *S. epidermidis* as an emergent aetiological agent of hospital-acquired bacteria, in agreement with previous observations [1,31]. This highlights the duality of commensal/pathogenic bacteria that are simultaneously part of the human microbiota while being responsible for life-threatening infections [32,33].

In this context, as WGS could be a valuable tool to confirm the link between the pathogens isolated from a CVC and BSI, we used this approach to better understand CRBSI and characterize the causative staphylococci. MDR was observed across the 19/20 MRSE isolates, with ST2, ST5 and closely related STs being the most predominant lineages. These globally spread lineages have indeed been associated with AMR [32,34]. The

S. aureus population was less homogeneous in terms of AMR, with 60% of MRSA. This difference in meticillin resistance among staphylococci species might be partially explained by the implementation of specific measures to prevent MRSA spread in hospital units and general infection prevention protocols [35,36]. In addition, the majority of *S. aureus* isolates belonged to ST22 lineage; it is tempting to hypothesize that the dispersion of the isolates per different STs results from patient colonization. Unfortunately, the design of the present study does not allow us to confirm this hypothesis and recent studies have associated ST22 with both oral colonization and BSI [34,37]. Of note, besides ST22, all other STs found in our study (with the exception of ST72 and ST105) were also implicated in BSIs in a previous large prospective study [38].

For all staphylococci, except two *S. epidermidis* pairs, the strains isolated from the same patient belong to the same ST lineage, as expected for microbiologically confirmed CRBSI [1]. The lack of isogenicity observed for the Sep_C28 pair reflects the increased discriminatory power of WGS for strain discrimination, and suggests the existence of same-species co-infection, as reported previously for *S. aureus* and other pathogens [39—41]. One of the concerns raised by co-infections is that different isolates could have different AMR profiles with direct implications for treatment success. For patient C28, different resistance phenotypes for daptomycin and vancomycin were observed: the CVC isolate was resistant and the HC isolate susceptible. For patients A10 and C11, inconsistencies between meticillin resistance phenotype and *mecA* gene presence were observed (Figure 1B). Absence of *mecA* in MRSA has been previously reported, suggesting the existence of alternative resistance mechanisms to meticillin [16,17]. Another possible explanation is the existence of a heterogeneity in the mono-species staphylococci population (subpopulations). This could be due to co-infection by two strains of the same species, existence of subpopulations with borderline meticillin resistance phenotypes, or to differentiation of the isolates by interaction with each other, for instance within a biofilm [42]. Indeed, biofilms promote horizontal gene transfer with survival advantages for the micro-organisms [43]. Biofilms were not present on the CVC extracted from patient C11 and CVC was not available for patient A10, hampering any speculation on the role of biofilms on this specific result. Biofilms were present in 17 of the 35 CVC tips, most often in the CVC's lumen. Biofilm assembly is a surface phenomenon determined by the characteristics of the surface (e.g. CVC) and the micro-organisms. All CVCs were made of polyurethane but its surface could have been modified by host components that would vary from patient to patient. Specific host factors (such as plasma components) influence biofilm assembly by staphylococci and this might explain the occurrence of biofilms within CVCs [44,45]. Shape is another factor that could account for the observed preference for biofilm formation on the CVC's lumen. Although both coagulase-negative staphylococci and *S. aureus* were able to assemble biofilms, no statistically significant correlation was found between biofilm assembly and CRBSI. Although *ica* operon has been linked to biofilm formation, we found no association between the presence/absence of these genes and the presence of biofilm on CVCs, for both *S. aureus* and *S. epidermidis* [8,45]. In addition, no association between *agr* types and biofilm formation was found.

In conclusion, staphylococci are main CRBSI aetiological agents with concerning rates of AMR. The presented data show that WGS could be a key tool to confirm the linkage between CVC and BSI, while providing important insight on the genetic diversity and AMR signature of CRBSI aetiological agents. The ability of these micro-organisms to colonize CVCs might contribute to their success, but more prospective in-vitro and in-vivo studies are required to clarify the role of biofilms on CRBSI and other foreign-body-related infections.

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Conflict of interest statement
None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2022.01.025.

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