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S. epidermidis Isolates from a Tertiary Care Portuguese Hospital Show Very High Antibiotic Non-Susceptible Rates and Significant Ability to Form Biofilms

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Abstract: Healthcare-associated infections (HAIs) have been increasing during recent decades, leading to long hospital stays and high morbidity and mortality rates. The usage of antibiotics therapy against these infections is enhancing the emergence of more multiple-drug resistant strains, in particular in *Staphylococcus epidermidis*. Hence, this study focused on the resistance pattern of *S. epidermidis* isolates from clinical settings and its association with phenotypic and molecular traits. Our results showed that HAIs were more prevalent among infants and older adults, and the most frequent type of HAI was central line-associated bloodstream infection. Half of the patients received antibiotic therapy before laboratory diagnosis. Preceding microbiological diagnosis, the number of patients receiving antibiotic therapy increased by 29.1%. Eighty-six per cent of the clinical isolates presented a multidrug resistance (MDR) profile, and a quarter were strong biofilm producers. Furthermore, polysaccharide intercellular adhesin (PIA)-dependent biofilms presented higher biomass production ($p = 0.0041$) and a higher rate of antibiotic non-susceptibility than PIA-independent biofilms, emphasizing the role of *icaABDC* operon in infection severity. Therefore, this study suggests that a thorough understanding of the phenotypic and molecular traits of the bacterial cause of the HAIs may lead to a more suitable selection of antibiotic therapy, improving guidance and outcome assessment.

Keywords: antibiotic non-susceptibility; biofilm formation; bacterial infections; coagulase-negative staphylococcus (CONS)

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1. Introduction

As part of the human skin microbiota, the coagulase-negative *Staphylococcus epidermidis* is now considered an opportunistic pathogen responsible for many healthcare-associated infections (HAIs), mainly those related to indwelling medical devices [1]. The length of catheterization and the overuse or improper use of antibiotics are associated with significant morbidity and mortality, mostly among immunocompromised, critically ill patients or very young and very old patients [2]. The last Portuguese national surveillance report on healthcare infections revealed an overall prevalence of 7.8% [3], with respiratory and bloodstream infections by coagulase-negative staphylococci, mainly *S. epidermidis*, being the most common occurrence [3,4]. Taking into consideration that multidrug resistance (MDR) among staphylococcal species is increasing [5–7], the study of such infections is of high importance. Worldwide, methicillin/oxacillin resistance which is encoded by the *mecA* gene has now been reported to range from 75% to 90% in many hospitals [8–10]. A study concerning *S. epidermidis* isolates and conducted in 2012 in a single-center Portuguese hospital

facility revealed a *mecA* prevalence of around 80%, similar to the reported *mecA* prevalence in *S. aureus* [11,12]. Although acquired antimicrobial resistance compromises the choice of adequate therapy [13], biofilm formation enhances this problem by affecting the efficacy of the administrated treatment, leading to persistent infections [14]. *S. epidermidis* strains are known to vary in their ability to form biofilms and several genes have been shown to influence this multifactorial process [15]. Among these, the most extensively studied is the *icaADBC* operon, responsible for the synthesis of polysaccharide intercellular adhesin (PIA) and considered the main factor mediating biofilm growth [16]. The *aap* (accumulation-associated protein) [17] and *bhp* (bap-homologous protein) genes [18] are also involved in and considered important genetic determinants of *S. epidermidis* biofilm development in a PIA-independent manner. Despite the high rates of antimicrobial resistance observed in Portugal and its clinical impact [12], there is a lack of available information relating to the molecular and phenotypic traits of *S. epidermidis* clinical isolates, especially those related to antibiotic resistance and biofilm formation. Given the importance of biofilm-associated infections and growing multidrug resistance (MDR) among staphylococcal species [19], this study aims at phenotypic and genetic evaluation of *S. epidermidis* isolates from a tertiary-care hospital of northern Portugal, namely determining the non-susceptible pattern of those, and assesses its association with the presence of biofilm-mediated genes and biofilm-forming capacity.

2. Materials and Methods

2.1. Isolation and Identification of Clinical Staphylococcus epidermidis Strains

A total of 86 *S. epidermidis* isolates were collected at different hospital wards from a 700-bed tertiary-care teaching hospital in Porto (Portugal) that handles about 35,000 inpatients admissions per year. Those *S. epidermidis* clinical isolates came from patients aged from 0 to 94 years old with a diagnosis of HAIs associated with device colonization, clinically and laboratory confirmed, following Infectious Diseases Society of America (IDSA) guidelines [20]. HAIs were defined as a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s). That condition occurred 48 h or more after hospital admission and was neither present nor incubating at the time of admission [21]. Patients' clinical and demographic data including medical comorbidities were collected under the approval of the Ethics Committee Board of Hospital de Santo António, Porto Hospital Centre (Reference 015/09: 014-DEFI/014-CES). Each isolate was identified at the species level using the commercially available VITEK® 2 identification system using the gram-positive ID card (BioMérieux) and subsequently by matrix-assisted laser desorption ionization-time of flight (BioMérieux), according to the manufacturer's instructions.

2.2. Antimicrobial Susceptibility Testing

Resistance to penicillin, clindamycin, erythromycin, daptomycin, fusidic acid, fosfomycin, gentamicin, levofloxacin, moxifloxacin, linezolid, rifampin, tetracycline, tigecycline, vancomycin, teicoplanin and trimethoprim-sulfamethoxazole was determinate by VITEK® 2 using the P619 panel (BioMérieux) and MIC values interpreted according to CLSI recommendations. *S. epidermidis* strains were considered as MDR if non-susceptible to at least one agent in three or more antimicrobial categories according to standardized international terminology [22]. Clinical isolates with an intermediate or resistant phenotype to a given antibiotic agent were considered non-susceptible.

2.3. Quantification of In Vitro Biofilm Formation

Biofilm cultures were performed in batch mode as previously described [23]. Furthermore, quantitative determination of in vitro biofilm biomass was performed as previously described by Stepanović [24] with some modifications. Briefly, after incubation time, the bacterial cells in suspension were carefully removed and each well was washed twice with 200 µL of 0.9% NaCl. Afterwards, 100 µL of 99.9% methanol (Fisher Scientific) was added to each well and let sit for 15 min to fix the biofilm. Methanol was then removed, and

the plate was left to air dry. The fixed bacteria biofilm cells were stained with 200 µL of 1% (*v*/*v*) crystal violet (Merck) per well, for 5 min. Excess crystal violet was removed by gently washing each well twice with distilled water and filled with 160 µL of 33% (*v*/*v*) glacial acetic acid (Fisher Scientific) to solubilize the crystal violet staining. The optical density (OD) was measured at 570 nm using a microtiter plate reader (Tecan) and set as a proxy for the amount of biomass present in a biofilm. Sixteen replicates of each isolate per biofilm assay were included and a minimum of three independent assays were carried out. Moreover, a biofilm producer (*S. epidermidis* ATCC 35984) and a non-biofilm producer (*S. epidermidis* ATCC 12228) were also included. The OD570 nm average of each isolate was compared with the mean OD570 nm of *S. epidermidis* ATCC 12228 (ODc = 0.1) and used to define the cutoff value to organize the clinical isolates into three main categories depending on whether they produced a strong and thick biofilm (SP), a moderate biofilm (MP) or weak/non-biofilm (NP). To simplify the data analysis, the level of biofilm production was classified as follows: $OD_C \geq OD < 2 \times OD_C$ (weak/non-producers), $2 \times OD_C \leq OD \leq 5 \times OD_C$ (moderate producers) $5 \times OD_C > OD$ (strong producers).

2.4. Gene Detection by Polymerase Chain Reaction (PCR)

One to five bacterial colonies of each isolate were inoculated from a TSA agar plate into 200 µL of nuclease-free water. The cells were lysed by heating at 95 °C for 10 min followed by 5 min on ice. Cellular debris was removed by centrifugation at maximum speed for 5 min. One µL of the collected supernatant was used as a template for PCR amplification. For single target amplification, the PCR was performed in an MJ Mini thermal cycler (Bio-Rad) with a final volume of 10 µL and containing 5 µL of DyNAzyme II PCR Master Mix 2x (Finnenzymes), 1 µL of primer mixture with a 10 µM concentration each and 2 µL of nuclease-free water. The primer sequences of the *mecA* gene and *icaA*, *aap* and *bhp* biofilm-mediating genes used in this study are listed in Table 1. To minimize PCR amplification bias and false-negative results, two sets of primers for each tested gene were used.

Table 1. Oligonucleotide sequences used in polymerase chain reaction (PCR) gene amplification.

Gene	Oligonucleotide Sequence (5′ to 3′)	PCR Product Size (bp)
PCR amplification of methicillin-resistance gene		
mecA set 1	Fw: CCG AAA CAA TGT GGA ATT GG Rv: TCA CCT GTT TGA GGG TGG AT	600
mecA set 2	Fw: GGC CAA TAC AGG AAC AGC AT Rv: CTG CAA CGA TTG TGA CAC G	425
PCR amplification of biofilm-mediated genes		
icaA set 1	Fw: TGC ACT CAA TGA GGG AAT CA Rv: TCA GGC ACT AAC ATC CAG CA	417
icaA set 2	Fw: TGC ACT CAA TGA GGG AAT CA Rv: TAA CTG CGC CTA ATT TTG GAT T	132
aap set 1	Fw: GCT CTC ATA ACG CCA CTT GC Rv: GGA CAG CCA CCT GGT ACA AC	617
aap set 2	Fw: GCA CCA GCT GTT GTT GTA CC Rv: GCA TGC CTG CTG ATA GTT CA	199
bhp set 1	Fw: TGG ACT CGT AGC TTC GTC CT Rv: TCT GCA GAT ACC CAG ACA ACC	213
bhp set 2	Fw: CGT TCC CTT GAT TGA GGT GT Rv: GTT ACG TGA ACG GGT CGA TT	404

The PCR program consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of DNA denaturation at 94 °C for 30 sec, primer annealing at 56 °C for 30 sec, and primer extension at 72 °C for 45 sec. After the last cycle, a final extension step at 72 °C for 10 min was added. Total PCR products were analyzed by gel electrophoresis with 2%

agarose (Bio-Rad) stained with Midori Green DNA stain (Nippon Genetics Europe GmbH, Germany) and visualized by GelDoc® 2000 (Bio-Rad). A 100-bp DNA ladder (NZYTech) was used as a marker. A mock PCR reaction lacking the DNA template was prepared and used as a negative PCR control. Besides, *S. epidermidis* ATCC 35984 was included as a positive PCR control. The *rpoB* gene was used as an internal control for each sample. *S. epidermidis* isolates were considered to harbor any of the tested genes if having at least one positive PCR result.

2.5. Statistical Analysis

Statistical analysis of the comparison of categorical variables was performed using the unpaired *t*-test, Fisher's exact test and Pearson's chi-squared test (χ^2). All analyses were performed using GraphPad Prism version 7 (Trial version, San Diego, CA, USA). The level of significance was set at p-value < 0.05 and all tests were two-tailed.

3. Results

3.1. Characterization of the Study Population and Antibiotic Therapy

Over 30 months, 86 clinical isolates of *S. epidermidis* were collected from patients with a diagnosis of device-related infection and treated in a tertiary-care hospital in Portugal's second major city (Porto). The studied patients represented a very heterogeneous group, comprising patients with distinct ages ranging from newborns to 94 years old, and near half (43.0%, n = 37) were female patients (Table 2). HAIs were more prevalent among infants and the elderly, being both considered the most susceptible group because of their long stays in hospitals and less effective immune systems [25]. Regarding the type of HAIs, central line-associated bloodstream infections (CLABIs) were by far the most frequently reported during the period under study, accounting for 60.5% (n = 52) of all clinical infections, while catheter-associated urinary tract infections (CAUTIs) and respiratory tract infections respectively accounted for 18.6% (n = 16) and 12.8% (n = 11). Furthermore, CLABIs were similarly distributed among the different age groups rather than CAUTIs, which were more prevalent among elders (68.8%). Surgical-site infections (5.8%, n = 5) and skin and soft tissue infections (2.3%, n = 2) were the less prevalent infections. Before laboratory diagnosis, 55.8% (n = 48) of the patients received antibiotic therapy, vancomycin being the most prescribed drug. Preceding microbiological diagnosis, the number of patients receiving antibiotic therapy increased by 29.1%, with major frequency in older adults, and among those, near half (48.0%) changed therapeutic after microbiological results.

Table 2. Patients' clinical and demographic data.

Patients' Clinical Parameters	
Demographic characteristics	
Age median (95% CI) in years	46.0 (10.5–75.3)
≤1 year (% of patients)	24.4
>65 years (% of patients)	39.5
Male gender (% of patients)	57.0
Average length of hospitalization from admission to *S. epidermidis* Isolation (Number of days)	25.0
Type of HAIs	
Central Line-Associated Bloodstream Infection (CLABIs)	60.5
Catheter-associated Urinary Tract Infections (CAUTIs)	18.6
Respiratory Tract Infections (RTIs)	12.8
Surgical Site Infections (SSIs)	8.1
Under antibiotic therapy (% of patients)	
Pre-culture	55.8
Post culture	84.9
Source (% of isolates)	
Blood	84.9
CVC	10.5
Other	4.7

3.2. Antibiotic Susceptibility Characteristics

S. epidermidis isolates' levels of non-susceptibility to at least one agent in three or more antibiotic categories, hence considered as MDR, were unexpectedly high (86%, $n = 74$) and of those, 91.9% carried the *mecA* gene, and were thus classified as methicillin-resistant *S. epidermidis* (MRSE). *S. epidermidis* isolates lacking *mecA* gene were considered as methicillin-susceptible *S. epidermidis* (MSSE), regardless of the oxacillin susceptibility results obtained. The analysis of antimicrobial susceptibility profiles revealed that all isolates were 100% susceptible to vancomycin, daptomycin and tigecycline (Figure 1). Additionally, linezolid (96.5%, $n = 83$) and fosfomycin (94.2%, $n = 81$) also demonstrated high levels of susceptibility to most of the clinical isolates tested. Seventy-two (83.7%) clinical isolates also showed a high rate of susceptibility to teicoplanin whereas the other 14 (16.3%) isolates were non-susceptible. Furthermore, the MDR phenotype was equally distributed among the different age groups and isolates with distinct biofilm phenotype and molecular traits.

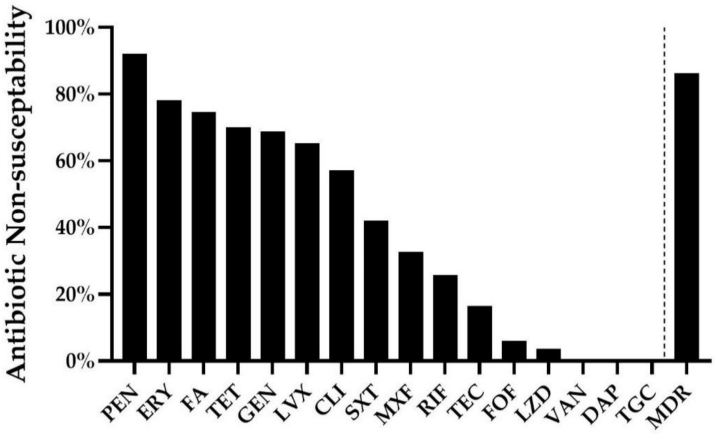

Figure 1. Antibiotic non-susceptible profile of the *S. epidermidis* isolates. PEN, penicillin; ERY, Erythromycin; FA, fusidic acid; TET, tetracycline; LVX, levofloxacin; GEN, gentamicin; CLI, clindamycin; SXT, trimethoprim/sulfamethoxazole; MXF, moxifloxacin; RIF, rifampicin; TEC, teicoplanin; FOF, fosfomycin; LZD, linezolid; VAN, vancomycin; DAP, daptomycin; TGC, tigecycline; MDR, multidrug resistance.

3.3. Phenotypic and Virulence-Associated Genetic Traits

Regarding the biomass production quantified and used to define the biofilm-forming capacity of the clinical isolates, 24.4% ($n = 21$) and 61.6% ($n = 53$) were, respectively, strong and moderate producers, while 14.0% ($n = 12$) were considered weak/non-biofilm producers.

Furthermore, the carriage of *icaA*, *aap* and *bhp* biofilm-mediating genes was assessed. The molecular determination of *icaA*, *aap* and *bhp* genes revealed that *aap* was the most prevalent gene, detected in 90.7% ($n = 78$) of the isolates, followed by *icaA* accounting for 64.0% ($n = 55$) and *bhp* for 44.2% ($n = 38$) of the isolates. Of interest, none of the clinical isolates was characterized by the presence of just a *bhp* virulence-associated gene. Only 5% ($n = 4$) of the isolates were negative for all tested genes ($icaA^{-}aap^{-}bhp^{-}$). Regarding the genetic combinations, the most frequently observed was $icaA^{+}aap^{+}bhp^{-}$ (39.5%, $n = 34$), followed by $icaA^{-}aap^{+}bhp^{+}$ (22.1%, $n = 19$) and by the carriage of the three-gene combination ($icaA^{+}aap^{+}bhp^{+}$, 19.8%, $n = 17$). Moreover, all clinical isolates that were $icaA^{+}aap^{+}bhp^{+}$ were related with both the MRSE and MDR phenotypes while only 89% of the $icaA^{-}aap^{+}bhp^{+}$ isolates demonstrated an MRSE and an MDR phenotype. A significant difference was found between the amount of biomass formed by PIA-dependent and PIA-independent clinical isolates [OD570 nm of 0.63 ± 0.07 vs. 0.32 ± 0.02, $p = 0.0041$ unpaired *t*-test, respectively].

3.4. Analysis of Antibiotic Non-Susceptibility Against Biofilm-Forming Capacity and Biofilm-Mediating Genes

The impact of the biofilm thickness against the antibiotic efficacy was considered in this study. As expected, the higher rates of non-susceptibility obtained seemed to be related to strong biofilm producers (Figure 2a). Remarkably, independently of the biofilm formation capacity and genetic background concerning the biofilm-mediating studied genes, most of the *S. epidermidis* isolates seemed to be resilient to fusidic acid. Gentamicin was demonstrated to have a higher impact in reducing moderate rather than strong biofilms ($p < 0.05$, Fisher's exact test).

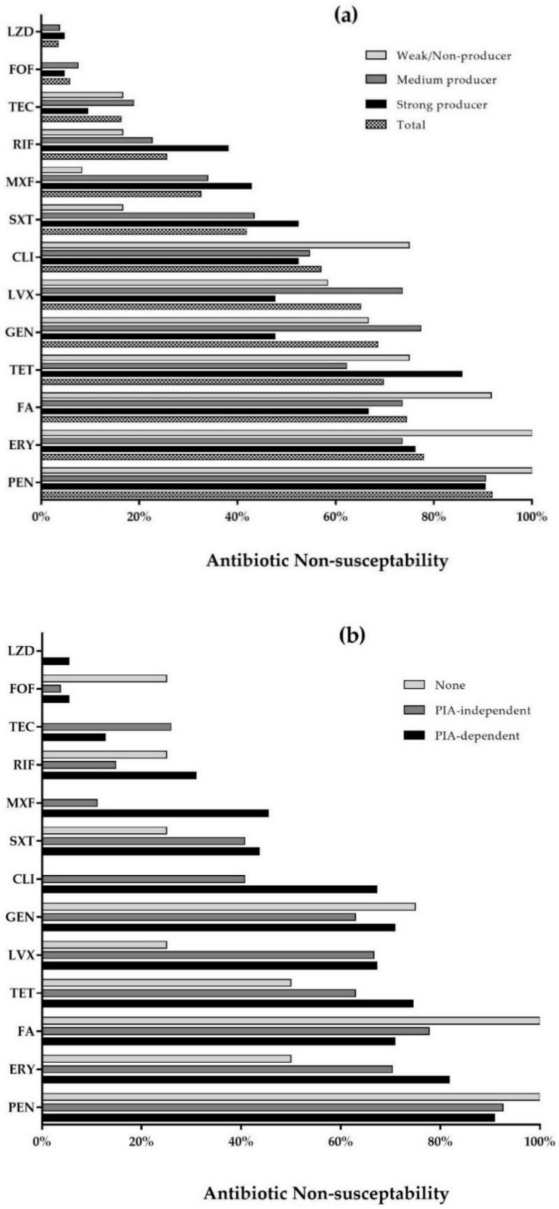

Figure 2. Relation between antibiotic non-susceptible profile of the *S. epidermidis* isolates and: (**a**) their biofilm-forming capacity; (**b**) their genetic profile grouped into two major classes (PIA-dependent—*icaA*$^+$, and PIA-independent—*icaA*$^-$). PEN, penicillin; ERY, Erythromycin; FA, fusidic acid; TET, tetracycline; LVX, levofloxacin; GEN, gentamicin; CLI, clindamycin; SXT, trimethoprim/sulfamethoxazole; MXF, moxifloxacin; RIF, rifampicin; TEC, teicoplanin; FOF, fosfomycin; LZD, linezolid. None, negative for all genes (*icaA*$^-$*aap*$^-$*bhp*$^-$).

S. epidermidis isolates were grouped in two classes for the analysis of their genetic profile: PIA-dependent (positive for *icaA*, independent of being positive or negative for *aap* and/or *bhp*—*icaA*$^+$*aap*$^+$*bhp*$^+$; *icaA*$^+$*aap*$^+$*bhp*$^-$; *icaA*$^+$*aap*$^-$*bhp*$^+$ and *icaA*$^+$*aap*$^-$*bhp*$^-$) and PIA-independent (positive only for *aap* and/or *bhp* not *icaA*—*aap*$^+$*bhp*$^-$; *aap*$^-$*bhp*$^+$ and *aap*$^+$*bhp*$^+$). The relation of this classification of isolates to their non-susceptible profile demonstrated that the biofilm-mediated gene *icaA* seemed to be the main gene responsible for the antibiotics' failure against the *S. epidermidis* isolates in this study (Figure 2b). Additionally, among all antibiotics tested, moxifloxacin ($p = 0.0026$) and clindamycin ($p = 0.0317$) were revealed to be more efficient against *S. epidermidis* isolates that did not carry the *icaA* gene.

4. Discussion

Portugal has one of the highest rates of antimicrobial resistance and antibiotic consumption across the European Union [26]. Despite the high prevalence of HAIs in Portugal (7.8% in 2017; [3]), very limited information is available regarding those caused by biofilm-forming species, coagulase-negative staphylococci and *S. epidermidis* in particular [27], which reinforces the importance of local studies in gathering and analyzing data to provide local and detailed information regarding opportunistic pathogens. Therefore, this study was conducted to provide a picture of the non-susceptible patterns of *S. epidermidis* clinical isolates from a major tertiary-care hospital and its relationship with phenotypic and/or molecular traits.

In the present study, CLABIs were the most common type of infection among our patients' group. This is not surprising, since *S. epidermidis* is considered the leading cause of BSI episodes related or not to a secondary site of infection [26]. One of the risk factors associated with BSIs seems to be linked to previous antibiotic consumption [28,29], which is often administrated before microbiological sampling analysis. This exposure leads to antibiotic resistance and subsequent resistant infections yield higher rates of morbidity and mortality [30]. The levels of antibiotic consumption prior to microbiology diagnosis were very high in this hospital (55.8%), as well as the levels of MRSE (91.9%) (Table 2), somewhat similar to those obtained in an earlier study conducted in a single-center hospital in the Portuguese capital, Lisbon (79.8% versus 87.2%) [31]. These numbers suggest that the spread of antibiotic non-susceptibility by the accumulation of resistance genes (i.e., *mecA*) is high and might be mostly due to the selective pressure exerted by the overconsumption of antibiotics among the Portuguese community outside/within healthcare facilities [4,32]. Similar observations were made in other countries [33–35]. In fact, numerous ecological studies have shown a clear association between the emergence of antibiotic resistance and the growing antibiotic overuse and misuse [36]. *S. epidermidis*, in particular MRSE strains, are considered reservoirs of antimicrobial resistance genes and prone to accumulate these genes [36,37], which is a cause of major concern as it is often associated with a higher risk of therapeutic failure. Vancomycin alone or in combination was the drug of choice, as recommended as a first-line treatment for infections caused by MRSE [38,39], and it remains a valued choice as all *S. epidermidis* isolates were susceptible to vancomycin. Additionally, the susceptibility rates to daptomycin and tigecycline were also high (Figure 1), a fact consistent with other studies [40,41]; reinforcing that those antibiotics remain important alternatives for antibiotic therapy against *S. epidermidis* infections at least in this hospital's service community. Linezolid and fosfomycin also demonstrated high levels of efficacy against *S. epidermidis* isolates. It was already reported that linezolid exhibits excellent activity against staphylococci species, being the only oxazolidinone approved for clinical use. Although linezolid resistance among *S. epidermidis* remains uncommon worldwide, it has increasingly been reported among some European countries, consequently its usage should be carefully evaluated [35]. Conversely, very high levels of non-susceptibility were found among the β-lactam antibiotics such as penicillin (91.9%), but also erythromycin (77.9%) and tetracycline (69.8%). While of great concern, this fact was therefore expected as many reports conveyed high and growing rates of resistance among those antibiotics [34,42].

Since propensity for biofilm formation has an enormous clinical impact on bacteria resilience and consequently drug resistance [43,44], the *in vitro* biofilm-forming capacity and the carriage of *icaA*, *aap* and *bhp* biofilm-mediated genes of each clinical isolate was evaluated. Similar to previous studies [45,46], the majority of the studied clinical isolates of the *S. epidermidis* study population exhibited biofilm-forming *in vitro* capacity, and among those 27.9% formed thick biofilm. *S. epidermidis* strains are known to vary in their ability to form biofilm and several genes are known to impact this multifactorial process. The *icaA* gene is known to play a major role in the formation of more dense/robust biofilms and thus commonly related to infection severity and inefficacy of drug treatment [47,48]. Among the studied clinical isolates, 64.0% presented in their genome the *icaA* gene, and its presence was mainly among strong biofilm producers. These results are in agreement with previous studies confirming that *icaA* is a key element in the biofilm formation process and has major relevance in the pathogenesis of *S. epidermidis* [42,49].

Therefore, other genes are known to also be involved in biofilm formation, such as *aap* and *bhp*, and their expression seems to be related to the formation of more thinner and proteinaceous biofilms—known as PIA-independent [27,50]. In accordance, these genes, alone or in combination, were present in 31.4% of clinical isolates and were mainly related to moderate biofilm-formation capacity, therefore confirming their importance in *S. epidermidis*-related infections. Despite *app* gene being the most common virulent gene in *S. epidermidis* infection, strains carrying only *aap* gene, demonstrated a diminished potential to form *in vitro* biofilms. Since none of the clinical isolates presented only the *bhp* gene, its role could not confirm any link to biofilm accumulation, also confirming other observations [15].

The analysis of antibiotic efficiency against *S. epidermidis* biofilms revealed that the non-susceptibility to penicillin and erythromycin is widely spread among strains in this hospital, and the action/non-action of those antibiotics seems to be independent of the biofilm thickness (Figure 2a). Similar results were found in a study conducted by Cabrera-Contreras et al., wherein 245 *S. epidermidis* strains isolated from nosocomial infections were analyzed, and which found high rates of resistance among both biofilm and non-biofilm producers (penicillin ≈97%, erythromycin ≈70%) [51]. In contrast, fosfomycin and linezolid seemed to be effective against *S. epidermidis*-related infections, affecting near to 97–98% of strong and moderate producers and yet presenting high susceptibility rates. Remarkably, linezolid and fosfomycin in combination demonstrated a synergetic effect against *S. epidermidis* isolates in a recent study [52,53], suggesting that these antibiotics can still be used in combination (at reduced concentrations) for treatment when monotherapy is not possible. Within the biofilm producers, 82.5% of isolates presented an MDR phenotype, which is a major red flag warning to surveillance and should prompt the design of more efficient programs to effectively tackle and block the rise of antibiotic resistance in this local hospital.

The relationship between the antibiotics' non-susceptibility and the carriage of biofilm-mediating genes was also explored (Figure 2b). Overall, it seems that PIA-independent biofilms are more susceptible to all tested antibiotics than PIA-dependent biofilms, a result in agreement with others [54,55] and expected, since lack of the *icaA* gene leads to the development of less thick biofilms [56], which in turn allows more effective penetration of the antibiotic in the biofilm structure. However, this rate of higher non-susceptibility in PIA-dependent biofilms seemed to be remarkable for moxifloxacin ($p = 0.0026$) and clindamycin ($p = 0.0317$) comparatively to PIA-independent strains. Rifampicin seems to also have a higher inhibitory effect against *S. epidermidis* isolates forming PIA-independent biofilms. Therefore, it was already pointed out that rifampicin has the broadest range of action against *S. epidermidis* isolates' biofilms, alone or in combination with clindamycin or with gentamicin, as it penetrates the biofilm structure, more easily reaching the bacteria within [57]. However, the efficiency of the antibiotic combination will be therefore dependent on the molecular and phenotypic traits of the *S. epidermidis* strains.

5. Conclusions

Portugal has some of the highest rates of HAIs, antibiotic consumption and antimicrobial resistance across the European Union [3]. Despite this high prevalence [4], limited information is available concerning HAIs' determinants and etiological agents associated with these types of infections, more specifically concerning *S. epidermidis*—known as a major nosocomial pathogen. To our knowledge, this is the first study addressing clinical, microbiological and molecular information of *S. epidermidis*-causing HAIs associated with medical devices in Portugal. This report reinforces the urgent need for knowledge to fight against HAIs associated with medical devices and caused by biofilm-forming species and suggests that the screening of biofilm-mediated genes may lead to a more suitable selection of antimicrobial therapy, thus reducing the overuse/misuse of antibiotics and MDR spread in alignment with Sustainable Development Goals 2030.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki. Study protocol and access to medical records by C. V. (clinician and co-author of this study) was approved by the Ethics Committee Board of Hospital de Santo António, Porto Hospital Centre (Reference number: 015/09: 014-DEFI/014-CES).

Informed Consent Statement: Patient individual informed consent was not necessary. All patient data was de-identified at the source and anonymized prior being accessed.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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

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