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CLINICAL MICROBIOLOGY - RESEARCH PAPER

Coagulase-negative staphylococci in outpatient routines: the implications of switching from CLSI to BrCAST/EUCAST guidelines

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Abstract

Coagulase-negative staphylococci (CoNS) are frequently isolated in clinical specimens and are important reservoirs of resistance genes. In 2019, the Brazilian government set the BrCAST/EUCAST (Brazilian Committee on Antimicrobial Susceptibility Testing) guidelines as the national standard, resulting in changes in the interpretation of CoNS susceptibility tests. From outpatients, disk-diffusion susceptibility of 65 CoNS cultures were evaluated and compared using classification criteria from both CLSI and BrCAST/EUCAST. The isolates were identified using matrix assisted laser desorption ionization–time of flight (MALDI-TOF), and the presence of the *mecA* gene was determined. The most prevalent species were *Staphylococcus saprophyticus* (32.3%), *S. haemolyticus* (18.5%), and *S. epidermidis* (9.2%). Almost perfect agreement was seen between the guidelines, except concerning oxacillin and gentamicin, and the prevalence of multidrug resistant isolates increased with the use of BrCAST/EUCAST. Of all, 15 (23.1%) isolates, mainly *S. epidermidis* and *S. haemolyticus*, were positive for the *mecA* gene, and only three were detected when using CLSI or BrCAST/EUCAST disk-diffusion screening. This, using either guideline, could reveal the difficulty of determining oxacillin resistance. Using warning zones or molecular methods might well be indicated for CoNS. In conclusion, adoption of the BrCAST/EUCAST guidelines will result in certain artificial changes in epidemiological susceptibility profiles, and clinicians and institutions should be aware of the possible implications.

Keywords Antimicrobial susceptibility testing · Brazilian Committee on Antimicrobial Susceptibility Testing · European Committee on Antimicrobial Susceptibility Testing · Clinical Laboratory Standards Institute · Coagulase-negative staphylococci · Oxacillin resistance

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Introduction

Coagulase-negative staphylococci (CoNS), mainly in skin and mucous membranes, are a normal part of the human microbiota. However, for many years, the bacteria had been underrated as infectious agents, nowadays the pathogenic potential of CoNS is well established, and they have been associated with important opportunistic infections and multiple antibiotic resistance [1]. CoNS are a heterogeneous group consisting of at least 39 species divided phylogenetically into 14 cluster groups, where identification by classical phenotypic tests is difficult [2]. Although certain species remain susceptible to various antibiotics, some strains are frequently associated with resistance to multiple anti-staphylococcal agents. The increase of oxacillin resistant CoNS—as mediated by penicillin-binding protein (PBP2a) modifications, encoded by the *mecA* gene—has become a pressing concern [1, 3].

The Clinical and Laboratory Standards Institute (CLSI) M100 document (1986), originally from the former National Committee for Clinical Laboratory Standards (NCCLS), is worldwide one of the most used guidelines for interpretation of antimicrobial susceptibility testing (AST) [4]. In 2005, the Brazilian National Health Surveillance Agency (ANVISA) obtained permission from CLSI to distribute a translated version of M100-S15 to clinical laboratories [5], and henceforth CLSI - M100-S15 became the principal guide used in Brazil. Some laboratories, due to language barriers or lack of resources to purchase annually updated versions, still use older, outdated versions of M100-S15. In resource-poor settings (like Brazilian laboratories), there is a need for suitable and free AST guidelines. In 2019, after intense discussions by a group of experts contracted by the Brazilian Ministry of Health, the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST) guidelines were set as the new standard for AST in Brazil [6].

In 2013, various Brazilian scientific societies united to form BrCAST, aiming at consensus AST breakpoints for Brazil. This is because, at the time, there were no CLSI breakpoints for colistin or tigecycline and there were also reported emergences of KPC-2 producing *K. pneumoniae*. The BrCAST guidelines are based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, translated to Portuguese, and freely available on their website [7]. EUCAST bases its clinical breakpoints on epidemiological cut-off values and pharmacokinetic-pharmacodynamic properties. Unlike CLSI, the General Committee of EUCAST has representatives in several countries including Brazil, and the pharmaceutical industry retains only a consultative role [8].

The aim of this study was to determine the CoNS species frequency in samples of an outpatient population using both the CLSI and the BrCAST/EUCAST AST guidelines and to then compare the susceptibility/resistance rates of selected antibiotics.

Materials and methods

The study included strains previously identified as CoNS from outpatients of a clinical laboratory in Porto Alegre, Southern Brazil, during the period of from March to August 2018. For fast bacterial identification, isolates were plated on Mueller-Hinton agar, and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) methodology was performed out in the Microflex LT (Bruker Corporation, USA) platform according to the manufacturer's specifications for direct colony identification. The spectra were analyzed using MALDI Biotyper 4.0 software and standard pattern matching with a default setting in accordance with previously studies [9].

For susceptibility testing, the disk diffusion method according to Kirby-Bauer was used [10]. Antimicrobial susceptibility for oxacillin using a cefoxitin 30 μg disk (CFO), ciprofloxacin 5 μg (CIP), norfloxacin 10 μg (NOR), gentamicin 10 μg (GEN), erythromycin 15 μg (ERY), clindamycin 2 μg (CLI), trimethoprim-sulfamethoxazole 1.25/23.75 μg (TMP/SMX), and chloramphenicol 30 μg (CLO) (SENSIDISC, DME, Brazil) was performed on Mueller-Hinton agar (TM Media, Titan Biotech, India) and using a suspension equivalent to MacFarland 0.5 from overnight cultures followed by incubation at 35 ± 1 °C for 20 and 24 h. Inhibition zone diameters were determined and interpreted according to the CLSI 2019, BrCAST/EUCAST 2019, and CLSI 2018 guidelines for norfloxacin (see supplementary file 1) [4, 11, 12].

Multiplexed PCR for detection of the *mecA* gene and determination of SCC*mec* type was performed for the isolates as previously described [13].

For statistical analysis, the percentage concordance rate between the two guidelines was compared. Cohen's Kappa coefficient was used to measure the guideline agreement rate for each antimicrobial; $p \leq 0.05$ was considered statistically significant, and all analyses were done using IBM SPSS version 20.0 for macOS (IBM Corporation, USA). Also, the research protocol was approved by an ethics committee.

Results

A total of 65 CoNS strains were included in this study, 90.7% of the isolates were obtained from urine, 6.2% from blood, and 3.1% from secretions. The average patient age was 41 ± 23.2 years (ranging from 9 to 89), and the majority of patients 52/63 (80%) were female.

Within the overall sample population, the MALDI-TOF methodology for rapid identification revealed the presence of at least seven CoNS species. Of all, 47 (72.3%) of the isolates were consistently identified at the species level. The remainder 18 (27.7%) did not fulfill the conditions for identification at the species level, being thus identified as *Staphylococcus* spp., of these, eight were best matched with *S. saprophyticus,* four with *S. haemolyticus,* three with *S. lugdunensis,* two with *S. epidermidis,* and one with *S. cohnii.* Table 1 summarizes the CoNS species identified according to sample origin.

In the antimicrobial susceptibility testing (AST), the specie-antibiotic susceptibilities were equal in both guidelines, agreement for each antibiotic ranged from 89.1 to 100%, with noted susceptibilities in both guidelines of 96.9% for CLO, 86.2% for CIP, 81.5% for CLI, 49.2% for ERY, 86.8% for NOR, and 63.1% for TMP/SMX. On the other hand, for GEN we observed a susceptibility of 93.8% with CLSI and 84.4% with BrCAST/EUCAST. For oxacillin, we observed a susceptibility of 93.8% with CLSI and of

Table 1 Species of CoNS species identified according to sample origin

Identification	Urine	Blood	Secretion	Total (%)
S. saprophyticus	21	0	0	21 (32.3)
S. haemolyticus	12	0	0	12 (18.5)
S. epidermidis	2	4	0	6 (9.2)
S. warneri	3	0	1	4 (6.2)
S. lugdunensis	2	0	0	2 (3.1)
S. caprae	1	0	0	1 (1.5)
S. condimenti	1	0	0	1 (1.5)
Staphylococcus spp.	17	0	1	18 (27.7)

96.9% using the BrCAST/EUCAST criteria. Kappa analysis presented perfect agreement for CLO and CLI (κ = 1), and almost perfect agreement for CIP (κ = 0.936), ERY (κ = 0.972), NOR (κ = 0.887), and TMP/SMX (κ = 0.968). Substantial agreement was observed with oxacillin using a CFO disk (κ = 0.652), and only moderate agreement was observed for GEN (κ = 0.458). Comparing the test results using both guidelines, we noted five minor divergences; in which CLSI classified isolates as intermediate and BrCAST/EUCAST classified isolates as resistant. Six major divergences were observed for GEN, being classified as susceptible by CLSI and yet resistant by BrCAST/EUCAST. For the oxacillin criteria, the results of two isolates; *S. haemolyticus* and *S. saprophyticus* were considered to be major errors, leading to classification by CLSI as resistant, and by BrCAST/EUCAST as susceptible. Table 2 summarizes the susceptibilities, agreement, and kappa statistics for both guidelines. Of the 65 CoNS tested, 10 (15.4%) - CLSI and 14 (21.9%) - BrCAST/EUCAST were resistant to at least three antimicrobial classes and were therefore classified as multidrug resistant (MDR) [9] with a concordance of 93.8% (Table 3).

Positives for the *mecA* gene were observed in 15 isolates (23%) (Table 4), three were classified as resistant by at least one guideline, and 12 (18.5%) were classified as susceptible, an error for both guidelines. Figure 1 presents the distributions of the CFO disk inhibition zone diameters observed in the study by criteria for *mecA* screening of staphylococci using CLSI and BrCAST/EUCAST. Inducible resistance to CLI was observed as positive for seven isolates, (two *S. saprophyticus,* two *S. warneri,* one *S. epidermidis,* one *S. haemolyticus,* and one *Staphylococcus* sp.), when using D test - (disk approximation test) detection.

Discussion

Due to the high association of CoNS with healthcare-acquired (nosocomial) infections [14, 15], there are few reports concerning epidemiology in outpatients. Our study did not include patients from healthcare facilities, and the samples were composed mainly of urine specimens. Indeed, some CoNS are frequent uropathogens, such as *S. saprophyticus* in young and middle-aged women [16]. Before MALDI-TOF became available, identification of CoNS at the species level was not routinely performed [14]. In urine specimens of a like population of patients in Ghana, a high prevalence of *S. haemolyticus* (75%), followed by *S. epidermidis* (13%) and *S. hominis*, and as in our study *S. warneri, S. lugdunensis,* and *S. condimenti* [17], was also observed, yet surprisingly *S. saprophyticus* was not reported. This might be indicative of a greater diversity of CoNS species in outpatients and of a possible rise of unfamiliar isolates such as *S. condimenti*, which was first reported as a pathogen in 2014 [18]. The data suggest that the *S. epidermidis*–like group, specially *S. haemolyticus*, seems to have an important role as gram-positive uropathogens in both patients and outpatients [14, 17, 19].

Table 2 Susceptibilities of CoNS to antibiotics, with concordance and kappa statistics for the CLSI and BrCAST/EUCAST guidelines

Antimicrobial agent	CLSI			BrCAST/EUCAST			Concordance %	Kappa	*P*
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)			
Chloramphenicol	62 (96.9)	0	2 (3.1)	62 (96.9)	0	2 (3.1)	100.0%	1	< 0.000
Ciprofloxacin	56 (86.2)	1 (1.5)	8 (12.3)	56 (86.2)	0	9 (13.8)	98.5%	0.936 (± 0.060)	< 0.000
Clindamycin	53 (81.5)	0	12 (18.5)	53 (81.5)	0	12 (18.5)	100.0%	1	< 0.000
Erythromycin	32 (49.2)	4 (6.2)	29 (44.6)	32 (49.2)	3 (4.6)	30 (46.2)	98.5%	0.972 (± 0.028)	< 0.000
Gentamicin	60 (93.8)	1 (1.6)	3 (4.7)	54 (84.4)	0	10 (15.6)	89.1%	0.458 (± 0.151)	< 0.000
Norfloxacin	33 (86.8)	1 (2.6)	4 (10.5)	33 (86.8)	0	5 (13.2)	97.4%	0.887 (± 0.100)	< 0.000
Oxacillin*	61 (93.8)	0	4 (6.2)	63 (96.9)	0	2 (3.1)	96.9%	0.652 (± 0.227)	< 0.000
Trimethoprim-sulfamethoxazole	41 (63.1)	2 (3.1)	22 (33.8)	41 (63.1)	1 (1.5)	23 (35.4)	98.5%	0.968 (± 0.031)	< 0.000

I intermediate, *R* resistant, *S* sensible

*Cefoxitin disk

Table 3 Susceptibilities profiles of MDR CoNS according to CLSI and BrCAST/EUCAST guidelines

Species	CLSI		BrCAST/EUCAST	
	Number of isolates	Antimicrobial resistance profiles	Number of isolates	Antimicrobial resistance profiles
S. epidermidis	3	OXA - CIP - TMP/SMX CLI - ERY - CIP CLI - ERI - CIP - TMP/SMX	4	OXA - CIP - GEN - TMP/SMX CLI - ERY - CIP CLI - ERI - CIP - TMP/SMX GEN - TMP/SMX - NOR
S. haemolyticus	2	CLI - ERY - GEN - NOR ERY - GEN - TMP/SMX	5	CLI - ERY - GEN - NOR ERY - GEN - TMP/SMX ERY - CIP - GEN - TMP/SMX OXA - ERY - GEN CIP - GEN - TMP/SMX - NOR
S. warneri	2	CLI - ERY - TMP/SMX CLI - ERY - TMP/SMX	2	CLI - ERY - TMP/SMX CLI - ERY - TMP/SMX
S. saprophyticus	1	OXA - CLI - ERY - TMP/SMX	1	CLI - ERY - TMP/SMX
Staphylococcus spp.	2	ERY - CIP - TMP/SMX -NOR CLI - ERY - TMP/SMX	2	ERY - CIP - GEN- TMP/SMX -NOR CLI - ERY - TMP/SMX

CLI clindamycin, *CIP* ciprofloxacin, *ERY* erythromycin, *GEN* gentamicin, *NOR* norfloxacin, *OXA* oxacillin, *TMP/SMX* trimethoprim-sulfamethoxazole

Though CoNS present fewer virulence factors than *S. aureus,* they still maintain an active role in antimicrobial resistance, serving as reservoirs of many resistance genes and contributing to the spreading of multiresistance between staphylococci [20]. That being said, the determination of an accurate CoNS susceptibility profile should carefully pursued. CLSI, despite its cost and remaining untranslated to Portuguese, has been the standard guideline in Brazilian laboratories for interpreting AST. With AST, it is critical for guidelines to be up to date, free, and readily available. Since 2019, the Brazilian Ministry of Health has adopted BrCAST/EUCAST as the standard guideline for Brazilian laboratories [6], yet CLSI and BrCAST/EUCAST methods lead to differing disk diffusion breakpoints and consequently divergent epidemiological susceptibility profiles.

CLSI and EUCAST susceptibility profiles have been compared in studies, and significant changes have been reported concerning certain pathogens [21–26]. Due to its more restrictive breakpoints, lower susceptibility rates have been observed for EUCAST [21]. However, the impact of this change on CoNS susceptibility profiles has not been fully explored, and in our comparison study, most CoNS-antibiotic combinations presented perfect or almost perfect agreement (except for GEN and Oxacillin).

Several countries have been progressively moving to implement EUCAST as a standard AST guideline, and for *S. aureus,* (excluding use of aminoglycosides) some studies evaluating the impact of this change in staphylococci show excellent correlations between both guidelines. The use of EUCAST criteria for minimal inhibitory concentration (MIC) results in a significant reduction in GEN susceptibility in *S. aureus* [22–24]. One study with CoNS observed the same for *S. haemolyticus* but not for *S. lugdunensis.* A plausible explanation is the lower number of resistance mechanisms observed in *S. lugdunensis* [25]. The contrast observed for GEN is due to the more stringent BrCAST/EUCAST breakpoint, which leads to a higher resistance rate for staphylococci. The CLSI breakpoint for GEN for staphylococci is ≥

Table 4 Distribution of *SCCmec* types according to CoNS species

Species	Number of *mecA*-positive isolates	*SCCmec* type		
		III	IV	V
S. epidermidis	5	0	3	2
S. haemolyticus	5	1	3	1
S. saprophyticus	1	0	1	0
Staphylococcus spp.	4	1	1	2
Total	15	2 (13.3%)	8 (53.3%)	5 (33.3%)

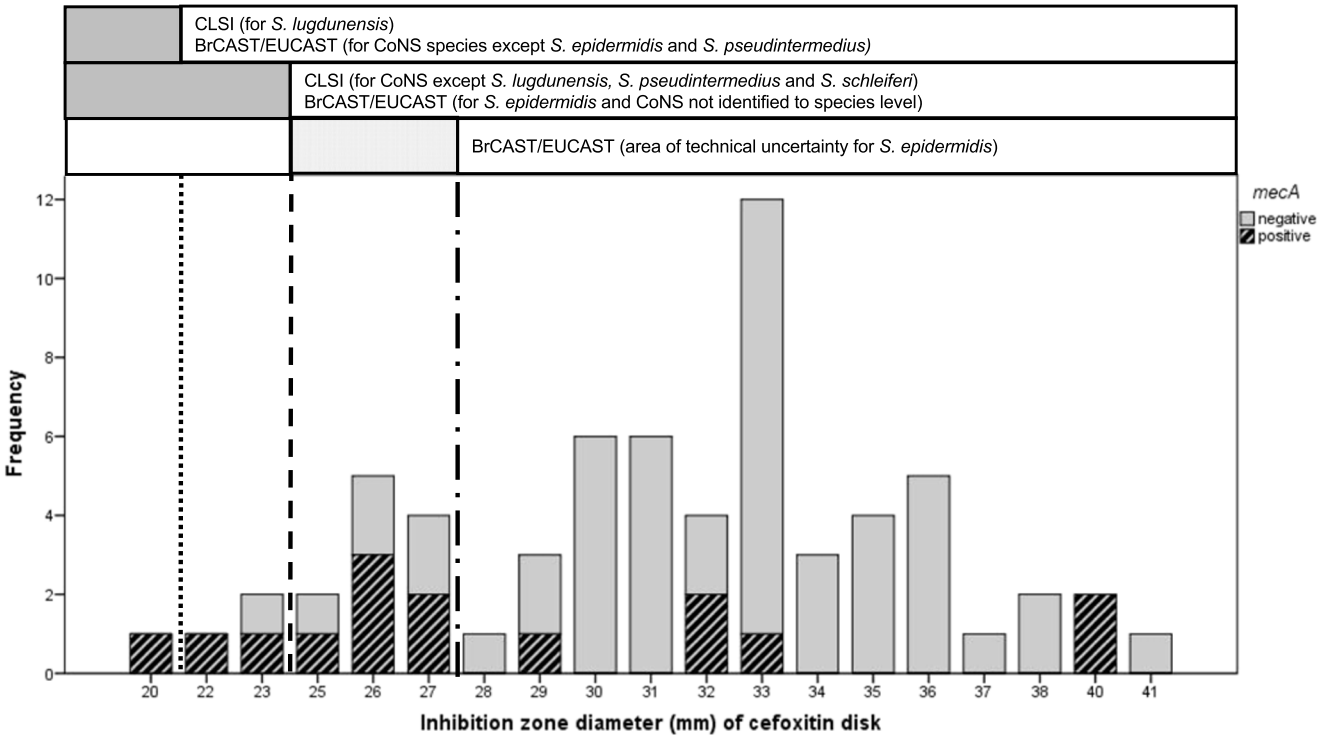

Fig. 1 Distribution of inhibition zone diameters for CoNS and screening criteria for *mecA* gene by CLSI and BrCAST/EUCAST

15 mm [4], conversely, the BrCAST/EUCAST breakpoint for CoNS is ≥ 22 mm. Currently, BrCAST/EUCAST has a less rigorous GEN breakpoint for *S. aureus* [12], and a review of CoNS breakpoints might be in order to prevent false, BrCAST/EUCAST-related epidemiological profile changes in Brazil.

Organizing disagreements into minor and major divergences, we found five minor divergences between the guidelines. In each case, CLSI classified the isolate as intermediate, whereas BrCAST/EUCAST classified the isolate as resistant. In three cases, this resulted in an MDR classification. Disagreement for ERY as an *S. haemolyticus* isolate resistant to oxacillin was observed, and a different *S. haemolyticus* presented disagreement for CIP. Discordances were also noted for GEN and NOR against another *S. epidermidis*. Reclassification to MDR can change regional epidemiological profiles and influence antimicrobial selection by clinicians.

Even though there is perfect agreement observed between the guidelines in the literature for oxacillin MICs in staphylococci [24–26], the disk diffusion method remains the standard tool for detecting oxacillin resistance and the changes between the guidelines are relevant. Oxacillin resistance is used as a predictor for the presence of the *mecA* gene and consequently of resistance to beta-lactams, (penicillins, most cephalosporins, and carbapenems), this may well affect patient management. CLSI requires differing conditions according to species, with incubation of 16–18 h and using a cefoxitin 30-μg disk with a cut-off of ≤ 21 mm for *S. lugdunensis*; for *S. epidermidis* and other CoNS, it requires an incubation of 24 h and cut-off of ≤ 24 mm (except *S. pseudintermedius* and *S. schleiferi*). An oxacillin 1-μg disk with cut-offs of ≤ 17 mm is mandatory for *S. pseudintermedius* and *S. schleiferi* yet can also be used for *S. epidermidis* [4]. On the other hand, for all CoNS species except *S. epidermidis* and *S. pseudintermedius* (e.g. *S. capitis, S. cohnii, S. haemolyticus, S. hominis, S. hyicus, S. lugdunensis, S. saprophyticus, S. schleiferi, S. sciuri, S. simulans, S. warneri* and *S. xylosus*), BrCAST/EUCAST requires incubations of 18 ± 2 h, and a cefoxitin 30-μg disk with a cut-off of < 22 mm. For *S. epidermidis* and non-species level identified CoNS, BrCAST/EUCAST requires a cut-off of < 25 mm. For *S. pseudintermedius* screening, BrCAST/EUCAST requires use of a 1-μg oxacillin disk and a cut-off of < 20 mm [12].

In our results, four CoNS were classified as oxacillin resistant by CLSI and two by BrCAST/EUCAST, revealing only partial agreement. Despite the specific conditions for *S. epidermidis* in BrCAST/EUCAST, other species included in the *S. epidermidis*–like group, such as *S. haemolyticus*, are not covered in this criteria, and one *mecA*-positive isolate with a borderline inhibition zone detected by CLSI was classified as oxacillin susceptible by BrCAST/EUCAST. In fact, *S. haemolyticus* is frequently MDR [20, 25] developing resistance to beta-lactams. In our sample, 41.7% harbored *mecA*, three of these (with inhibition zones of 26 mm, 26 mm, and 29 mm) were not detected using the disk diffusion screening criteria of either guideline. In a previous study with Brazilian isolates, approximately 60% of the community isolates and 100% of the blood isolates were positive for *mecA* [27].

CLSI and BrCAST/EUCAST have different breakpoints for oxacillin MIC and disk diffusion in *S. saprophyticus* (respectively 0.5 mg/L and ≤ 24 mm; and 2.0 mg/L and < 22 mm,) [4, 12]. One *mecA*-negative isolate was classified as resistant by CLSI, and one *mecA*-positive isolate was classified as susceptible by both guidelines. For *S. saprophyticus,* oxacillin resistance needs to be careful evaluated since previous studies have observed differing results from the guideline criteria for MIC, disk diffusion, and *mecA* presence [27, 28].

The presence of the *mecA* gene in *S. epidermidis* was high (83%), but only one of five isolates was detected using the screening criteria for disk diffusion. The isolates classified as oxacillin susceptible presented large CFO disk inhibition zones (27 to 40 mm), and only one isolate met the suggested BrCAST/EUCAST confirmation criteria [12].

In resource-poor laboratory settings, CoNS are frequently not identified at the species level due to the limitations of classical phenotypic tests [14]. In our sample, even with the use of MALDI-TOF, 27.7% of isolates were not confidently identified at the species level, thus the importance of evaluating the CLSI and BrCAST/EUCAST guidelines for determination of oxacillin resistance for *Staphylococcus* spp. According to CLSI, using the CFO disk is an acceptable method for *Staphylococcus* spp. or oxacillin MIC. However, the MIC may emphasize resistance, and results between 0.5 and 2 mg/L must be tested for *mecA* [4]. BrCAST/EUCAST indicates using the CFO disk with no recommendations of MIC breakpoints for CoNS not identified at species level [12]. Of 18 CoNS not identified at the species level, four (22%)—best matched to *S. haemolyticus*—harbored the *mecA* gene, and both guidelines failed in screening for this resistance mechanism.

It is also important to note that the oxacillin 1-μg disk is recommended by CLSI for *S. pseudintermedius* and *S. schleiferi* and by BrCAST/EUCAST for *S. pseudintermedius* (cut-off ≤ 17 mm and < 20 mm, respectively) to screen for *mecA*. CLSI also recommended oxacillin MIC as an acceptable methodology for all CoNS [4, 12]. Despite this consideration, in our sample we did not identify these two species.

The vast majority of clinical CoNS possess the mobile genetic element *SCCmec* that harbors the *mec* gene [2]. Detection of oxacillin resistance in these isolates is a huge challenge since agreement between phenotypic and molecular methods oscillates among strains. Pinheiro et al. [27] attributes this to resistance heterogeneity, with borderline resistance being a very common phenomenon. In 2019, BrCAST/EUCAST introduced a warning zone (25–27 mm) for *S. epidermidis* called the Area of Technical Uncertainty (ATU) indicating the need of confirming susceptibility categorization, by using an alternative test [12]. The adoption of the ATU warning for all CoNS might increase oxacillin resistance detections in our study, especially in *S. haemolyticus* isolates.

MDR strains frequently acquire resistance to macrolides and related antibiotics. Indeed, some *SCCmec* harbor genes for resistance to ERY and others antibiotics [2]. Macrolides are not used for first-line treatment of urinary infections, and the high rates of acquired ERY resistance observed could indicate a change in the human microbiota due to the overuse of these agents in the community. The mutation in 23S rRNA mediated by the *erm* gene is the most frequently noted mechanism of resistance to ERY, and the presence of these genetic elements generally results in a phenotype of resistance to macrolides, lincosamides, and streptogramin B (MLS), which can be either constitutive (cMLS) or inducible (iMLS). As other mechanisms, efflux pumps encoded by the *msrA* gene mediate resistance to MLS, and drug modification is encoded by the *lnu* gene [29]. The iMLS phenotype occurs in strains that carry *erm* genes but are susceptible to CLI in AST. Clindamycin treatment in patients with iMLS resistance may lead to therapeutic failure [30], and the disk approximation test, or D test, is used to detect this phenotype. Of the isolates resistant to macrolides, 7/30 (23.3%) were positive to phenotype iMLS. For the cMLS phenotype, 3/30 (10%) were positive, with 20/30 (66.6%) of the isolates being resistant only to macrolides. All isolates resistant to CLI were also resistant to ERY. The prevalence of macrolide-resistant phenotypes in CoNS might vary according to regions and populations that use macrolides; in fact, our prevalence for iMLS was greater than previous studies around the world, 11.8 to 15.2% [29–31].

There are few studies evaluating the use of CLSI and EUCAST guidelines for CoNS, and our study presents some limitations. First, the limited sample size in a single laboratory may interfere in the epidemiology. The AST comparison results are limited to antibiotics where both guidelines indicated the same disk concentrations. And also, we did not evaluate nitrofurantoin (important in treating urinary infections), penicillin, ampicillin, or linezolid. Our purpose was to evaluate the implications of switching AST guidelines and not to design an experiment establishing which presents better performance in determining susceptibility to oxacillin. However, evaluation of oxacillin disk performance, or the oxacillin MICs for all CoNS, could be important data for analysis of this issue. Finally, we did not use molecular typing tools such as MLST or PFGE to evaluate clonality.

CoNS are often underestimated as etiological agents of human infections, especially in outpatients. The introduction of MALDI-TOF led to recognition of the great species diversity; and that *S. haemolyticus* seems to have an important role as urinary pathogen with the ability to accumulate antibiotic resistant determinants. Our research revealed acceptable concordance between CLSI and BrCAST/EUCAST except when using aminoglycosides. We note that determination of oxacillin susceptibility in CoNS has many pitfalls for both guidelines. When investigating oxacillin resistance, the use of a warning zone (ATU) for all CoNS, more than one

susceptibility test, or a specialized molecular methodology may well be indicated. In conclusion, when adopting the BrCAST/EUCAST guidelines and comparing data during and after implementation, clinicians and institutions should be aware of the implications of the change.

Compliance with ethical standards

Conflict of interest The author d'Azevedo PA is member of the General Committee of BrCAST in 2019–2020.

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