JAC-Antimicrobial Resistance

The MIC distribution of dalbavancin differs between different coagulase-negative staphylococci

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Background: CoNS constitute a significant part of the human microbiota of skin and mucous membranes. They can cause nosocomial infections, and have shown decreased susceptibility to several antibiotics. The few remaining treatment options include (lipo)glycopeptides such as dalbavancin. However, there is a lack of knowledge concerning whether susceptibility to lipoglycopeptides varies between different species of CoNS.

Objectives: To determine the susceptibility to dalbavancin in different species of CoNS.

Methods: We investigated 480 bacterial isolates from 10 CoNS species: *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Staphylococcus warneri*, *Staphylococcus pettenkoferi*, *Staphylococcus hominis*, *Staphylococcus sciuri* and *Staphylococcus simulans*. The isolates were randomly selected from different sources of infection, including blood isolates, as well as deep and superficial infections. Antibiotic susceptibility was tested with the gradient test method.

Results: There was a statistically significant difference (ANOVA; $P < 0.0001$) in the MIC distribution for dalbavancin between different CoNS species. *S. sciuri* was the least susceptible species, with 90% of the isolates having an MIC value for dalbavancin above the EUCAST breakpoint of 0.125 mg/L. The lowest MIC_{90} values were seen for *S. capitis*, *S. simulans* and *S. caprae* (all 0.032 mg/L).

Conclusions: This study demonstrated a difference in dalbavancin susceptibility between different CoNS species, suggesting that species-specific breakpoints for CoNS should be further investigated.

Introduction

CoNS are a heterogeneous group of Gram-positive bacteria, consisting of approximately 50 different species.[1] CoNS normally constitute a significant part of the microbiota of the skin and mucous membranes of humans, and are in most cases harmless and apathogenic. However, individuals can become predisposed to CoNS infections due to the presence of medical devices such as joint prostheses or artificial heart valves, or a compromised immune system following premature birth or the use of chemotherapeutic or immunosuppressive drugs.[1,2]

Treatment options are becoming limited because of the emergence of MDR CoNS strains, caused both by acquisition of mobile genetic elements such as *mecA* and by point mutations.[3] Glycopeptides such as vancomycin are in many cases one of few remaining options.[4] However, recent decades have also seen a decrease in CoNS antibiotic susceptibility toward glycopeptides,[5,6] demonstrated both by the presence of glycopeptide-intermediate susceptible phenotypes and by heterogeneous susceptibility profiles. The mechanisms are complex, and involve cell wall thickening,[7,8] as well as alterations in the cell wall metabolism.[9] This has contributed to an increased need for newer treatment options.

In addition to glycopeptides there is a new, closely related group of antibiotics, the lipoglycopeptides, that have the same mechanism of action (i.e. inhibition of cell wall synthesis) but

according to recent studies display significantly lower MIC values than glycopeptides against staphylococci.[10,11] Dalbavancin has an extraordinarily long half-life of 204 h and the terminal elimination half-life extends to 346 h,[12] and is approved for treating acute bacterial skin and skin structure infections either as a single infusion or as two doses 1 week apart.[12] Beyond the approved licensed dosing regimen, dalbavancin is also used off-label as a long-term treatment,[13] particularly for prosthetic joint infections.[14] Activity of dalbavancin against staphylococci in biofilm has been demonstrated *in vitro*.[11,15]

This long half-life, *in vitro* activity against biofilm, and high susceptibility of CoNS mean that dalbavancin may constitute a treatment option of infections caused by CoNS, especially foreign-body infections. However, there is a lack of knowledge regarding whether susceptibility to dalbavancin varies between different species of CoNS. The aim of the present study was to determine the MIC distribution for dalbavancin in 10 different CoNS species by using the gradient test method.

Materials and methods

Isolates

Ten CoNS species with clinical relevance were selected for the project: *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Staphylococcus warneri*, *Staphylococcus pettenkoferi*, *Staphylococcus hominis*, *Staphylococcus sciuri* and *Staphylococcus simulans*. Fifty bacterial strains from each species were used, except for *S. sciuri*, for which 30 were used (since only 30 strains were available). The isolates were obtained from different sources of infection, including blood isolates, as well as deep and superficial infections, and were randomly selected. These clinical isolates were saved as pure cultures according to routine procedures at the Department of Laboratory Medicine, Clinical Microbiology, University Hospital, Region Örebro County. Region Örebro County is geographically located in central Sweden and has approximately 300 000 inhabitants. The isolates were collected between 2002 and 2023, and were stored at −80°C in preservation medium consisting of trypticase soy broth supplemented with 0.3% yeast extract (BD Diagnostic Systems, Sparks, MD, USA) and 29% horse serum (Håtunalab AB, Håtuna, Sweden).

Antibiotic susceptibility testing

The antibiotic susceptibility test was performed using Mueller–Hinton agar 3.8% (w/v) plates (Oxoid, Basingstoke, Hampshire, England) and MIC Test Strip Dalbavancin (Liofilchem, Roseto degli Abruzzi, Italy).

Statistics

One-way ANOVA with Tukey's multiple comparison test was used to detect differences between CoNS species (GraphPad Prism, version 10.1.0). Statistical significance was defined as a *P* value of <0.05.

Ethics

The bacterial strains were used as pure cultures, and the samples were anonymized in a way that prevented their being traced to an individual patient. The samples did not contain any human genetic material. According to section 4§3 of the Swedish Act on Ethical Review (2003:460), ethical review is mandatory when using biological material obtained from a living patient that can be traced back to the patient.

Results

Antibiotic susceptibility testing

A total of 480 bacterial isolates were used, comprising 50 isolates from each of 10 CoNS species, except for *S. sciuri*, where only 30 isolates were available. The dalbavancin MIC of each isolate was determined by the gradient test, and MIC distributions were calculated for each species (Figure 1) to allow comparison between the different species (Figure S1, available as Supplementary data at *JAC-AMR* Online). Both MIC_{50} and MIC_{90} were calculated (Table 1).

Statistical comparison of MICs between the different species was performed using ordinary one-way ANOVA, showing a *P* value of <0.0001. Tukey's multiple comparison test was used to adjust for multiple testing (Table S1). Of the 45 comparisons, 29 showed statistically significant differences in the MIC distribution for dalbavancin. *S. sciuri* was statistically significantly different from all the other species. Moreover, the comparisons between *S. capitis* and *S. haemolyticus*, *S. caprae* and *S. lugdunensis*, and *S. haemolyticus* and *S. hominis* all showed *P* values of <0.0001 (Table S1).

The EUCAST breakpoint for dalbavancin for *S. aureus* is 0.125 mg/L (www.eucast.org, as at 4 March 2024) and is the same for Gram-positive organisms according to EUCAST guidance on when there are no breakpoints in breakpoint tables (www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Guidance_documents/When_there_are_no_breakpoints_20230630_Final.pdf). In our isolates, an MIC value above the breakpoint was found for 90% of *S. sciuri*, while no *S. epidermidis* had an MIC above the breakpoint. The percentages of isolates in each species with an MIC above the breakpoint are given in Table 1.

Discussion

The present study demonstrates that different CoNS species vary in their MIC distribution and thus their susceptibility to dalbavancin. All 10 investigated species showed MIC results with a normal distribution, indicating that the results correspond to the WTs. Only a few outliers could be found; for example, one isolate in the *S. hominis* group.

A previous study similarly showed that antimicrobial susceptibility to dalbavancin differed between different CoNS species.[10] There were 46/5088 CoNS isolates that had MICs of >0.125 mg/L, or 99.1% susceptible based on the EUCAST breakpoints for *S. aureus* (www.eucast.org as at 4 March 2024). The corresponding percentage in the present study was 90.4%. In addition, there were species-specific differences too; for example, 0% of *S. haemolyticus* strains in the study of Sader *et al.*[10] displayed an MIC value above the EUCAST breakpoint, compared with 18% of *S. haemolyticus* strains in the previous study. Moreover, our study included a larger number of species, since we added *S. caprae*, *S. pettenkoferi* and *S. sciuri* and excluded *Staphylococcus saprophyticus*. We also were able to report statistically significant differences in the MIC distribution between dalbavancin-susceptible species of CoNS.

The most deviant of the 10 species was *S. sciuri*, where 90% of the MIC values were higher than the EUCAST breakpoint. There were no clear outliers within this species, indicating that this pattern was due to intrinsic attributes of the WT rather than acquired
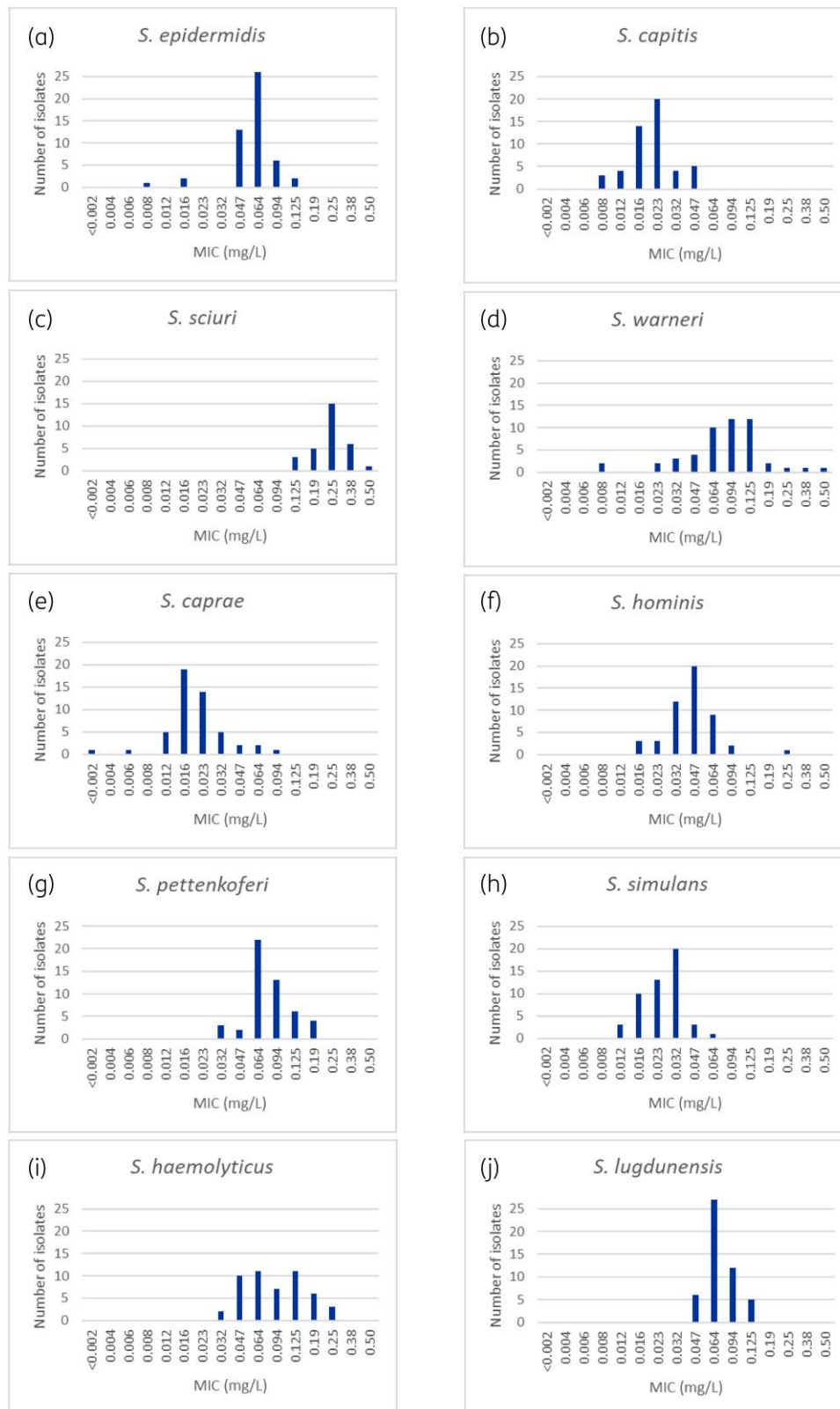
Figure 1. MIC distribution determined by gradient test for dalbavancin in (a) *S. epidermidis* (*n*=50), (b) *S. capitis* (*n*=50), (c) *S. sciuri* (*n*=30), (d) *S. warneri* (*n*=50), (e) *S. caprae* (*n*=50), (f) *S. hominis* (*n*=50), (g) *S. pettenkoferi* (*n*=50), (h) *S. simulans* (*n*=50), (i) *S. haemolyticus* (*n*=50) and (j) *S. lugdunensis* (*n*=50).

Table 1. MIC_{50} and MIC_{90} for dalbavancin and percentage with $MIC > 0.125$ mg/L for 10 different CoNS species

Species (no. tested)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Percentage (%) of MIC > 0.125 mg/L
S. epidermidis (50)	0.064	0.094	0
S. caprae (50)	0.016	0.032	0
S. warneri (50)	0.094	0.125	10
S. capitis (50)	0.023	0.032	0
S. hominis (50)	0.047	0.064	2
S. sciuri (30)	0.250	0.380	90
S. lugdunensis (50)	0.064	0.094	0
S. pettenkoferi (50)	0.064	0.125	8
S. simulans (50)	0.023	0.032	0
S. haemolyticus (50)	0.094	0.190	18

resistance mechanisms. According to a previous study, an increased cell wall thickness in *S. sciuri* is a possible cause of β-lactam resistance.[16] This could perhaps explain the high percentage of decreased susceptibility to dalbavancin among *S. sciuri* strains.

The present study has several limitations. Our use of the gradient test method is a limitation, since broth microdilution is the reference method recommended by EUCAST (www.eucast.org as at 4 March 2024). However, a recent study concluded that the gradient diffusion method is an acceptable alternative to broth dilution for dalbavancin.[17] Therefore, our results could still be considered as useful regarding knowledge of differences in susceptibility to dalbavancin between different species of CoNS, even though not produced by the reference method. The MIC Test Strip from Liofilchem has, however, not been evaluated for staphylococci other than *S. aureus*. Staphylococci were determined to species level by MALDI-TOF from 2014, but before that the biochemical method of analytical profile index (API) was used. Furthermore, only 30 isolates of *S. sciuri* were available to compare with 50 isolates of the other species.

In conclusion, a difference in dalbavancin susceptibility between different CoNS species can be demonstrated, even among species that are fully susceptible. The results from this study suggest that the WT of *S. sciuri* is distributed beyond the breakpoint for dalbavancin. This raises the question of whether the EUCAST breakpoint for dalbavancin should be the same for all CoNS species.

Funding

Transparency declarations
B.S. has been a member of an advisory board at ADVANZ PHARMA, and has also received speaker's fees from Correvio Pharma Corp and ADVANZ PHARMA. All other authors: none to declare.

Author contributions
S.S. and P.H. collected and analysed the data. B.S. designed and supervised the work. S.S. and P.H. wrote the manuscript draft, and B.S. made critical revisions. All authors have read and approved the final manuscript.

Data availability
The datasets generated and analysed in the current study are available from the corresponding author upon reasonable request.

Supplementary data
Figure S1 and Table S1 are available as Supplementary data at *JAC-AMR* Online.

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