Aus dem Institut für Medizinische Mikrobiologie und Krankenhaushygiene
der Heinrich- Heine-Universität Düsseldorf
Direktor: Univ.-Prof. Dr. med Klaus Pfeffer

Teicoplanin-Resistant Coagulase-Negative Staphylococci: Current Susceptibility Testing Methods are Discordant in the Detection of this Elusive Phenotype

Dissertation

zur Erlangung des Grades eines Doktors der Medizin (Dr. med)
der Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

vorgelegt von
Adriana D. Balasiu
2023

Dean: Prof. Dr. med. Nikolaj Klöcker

First Professor Dr. med. Colin R. Mackenzie

Second PD Dr. med. Dr. rer. nat. Sören Twarock

This thesis is dedicated to my family, to my constant support Natasa
and to my shining star Mia

Parts of this work have been published [1]

Balasiu AD, MacKenzie CR. Teicoplanin-Resistant Coagulase-Negative Staphylococci: Do the Current Susceptibility Testing Methods Reliably Detect This Elusive Phenotype? Antibiotics (Basel). 2023;12(3). 10.3390/antibiotics12030611.

Summary/ Zusammenfassung

Coagulase-negative staphylococci (CoNS), members of the skin commensal microbiota, are increasingly associated with local or systemic infections due to a shift in patient populations in recent decades. Subsequently, more CoNS strains have been subjected to antibiotic susceptibility testing (AST), thus leading to the increased detection of teicoplanin resistance. However, data concerning teicoplanin resistance among CoNS strains remain limited, heterogeneous, and inconclusive.

We collected 162 consecutive CoNS strains identified as teicoplanin-resistant by routine diagnostic testing and re-tested them with a range of AST methods. The results of standard and high inoculum broth microdilution (sBMD; hBMD), agar dilution (AD) after 24 h and 48 h incubation, standard and macrogradient diffusion strip (sGDT, MET), screening agar, and disc diffusion were compared to assess their robustness and to establish a diagnostic algorithm to detect teicoplanin resistance.

sBMD was used as the reference method, and the lowest number of strains were teicoplanin-resistant using this method. Compared with sBMD, AD-24 h generated the lowest number of false teicoplanin-resistant strains, followed by hBMD, AD-48 h, and Vitek-2 (bioMérieux, Marcy l'Etoile, France). sGDT, a fast, easy, affordable method in diagnostic settings, generated the highest rate of false teicoplanin-susceptible strains. Vitek-2 testing produced the highest number of teicoplanin-resistant strains. Only in two strains was the initial Vitek-2 teicoplanin resistance confirmed using five other AST methods.

In conclusion, the different AST methods generated inconsistent, inconclusive, and discrepant results, thus making it difficult to establish a diagnostic algorithm for suspected teicoplanin resistance. Teicoplanin testing proved to be challenging and easily influenced by technical factors. This study aimed not only to raise awareness of teicoplanin resistance testing but also of the need for future studies focusing on the clinical efficacy of teicoplanin in relation to its susceptibility results.

Summary/ Zusammenfassung

Koagulase-negative Staphylokokken (CoNS), Bestandteile der kommensalen Mikrobiota der Haut, werden aufgrund von Änderungen in den Patientenpopulationen in den letzten Jahrzehnten zunehmend mit lokalen oder systemischen Infektionen in Verbindung gebracht. Infolgedessen werden mehr CoNS-Stämme einer Antibiotika-Empfindlichkeitsprüfung (AST) unterzogen, was zu einem vermehrten Nachweis von Teicoplanin-Resistenzen führte. Die bisherigen Daten zur Teicoplanin-Resistenz bei CoNS-Stämmen sind jedoch nach wie vor begrenzt, heterogen und nicht schlüssig.

Wir haben 162 aufeinanderfolgende CoNS-Stämme gesammelt, die in der Routinetestung als Teicoplanin-resistent identifiziert wurden und sie mit einer Reihe von AST-Methoden getestet. Die Ergebnisse der standard- und hoch Inokulum Bouillon - Mikrodilution (sBMD; hBMD), der Agardilution (AD) nach 24- und 48-stündiger Inkubation, des standard- und makrogradienten-Diffusionsstreifens (sGDT, MET), des Screening-Agars und der Disc Diffusion wurden verglichen, um ihre Zuverlässigkeit zu bewerten und einen diagnostischen Algorithmus zum Nachweis von Teicoplanin-Resistenzen zu erstellen.

sBMD wurde als Referenzmethode verwendet dabei zeigte sich in dieser Methode die geringste Anzahl an Teicoplanin-resistenten Stämmen. Im Vergleich dazu erzeugte AD-24 h die wenigsten falsch Teicoplanin-resistenten Stämme, gefolgt von hBMD, AD-48 h und Vitek-2 (bioMérieux, Marcy l'Etoile, France). sGDT, eine schnelle, einfache und kostengünstige Methode in diagnostischen Einrichtungen, lieferte die höchste Rate falscher Teicoplanin-empfänglicher Stämme. Der Vitek-2-Test detektierte zuletzt die höchste Anzahl von Teicoplanin-resistenten Stämmen. Nur bei zwei Stämmen wurde die ursprüngliche Vitek-2-Teicoplanin-Resistenz durch fünf andere AST-Methoden bestätigt.

Zusammengefasst lieferten die verschiedenen AST-Methoden widersprüchliche, nicht schlüssige und voneinander abweichende Ergebnisse, was die Erstellung eines diagnostischen Algorithmus für eine vermutete Teicoplanin-Resistenz erschwerte. Die Teicoplanin-Testung erwies sich als herausfordernd, da sie leicht durch technische Faktoren zu beeinflussen ist. Diese Studie sollte nicht nur die Aufmerksamkeit auf Teicoplanin-Resistenztests erhöhen, sondern auch auf die Notwendigkeit künftiger Studien hinweisen. Dabei sollte der Fokus auf die klinische Wirksamkeit von Teicoplanin, im Verhältnis zu seinen Empfindlichkeitsergebnissen gesetzt werden.

Abbreviations

ACME	Arginine catabolic mobile element
AD	Agar dilution
AST	Antibiotic susceptibility testing
AtlE	Surface autolysin AtlE
BMD	Broth micro-dilution BMD
C	Cytosine C
CA	Categorical agreement
CFU	Colony forming units
CIED	Cardiovascular implantable electronic device
CLABSI	Central-line-associated bloodstream infections
CLSI	Clinical & Laboratory Standards Institute
CoNS	Coagulase negative staphylococci
CoPS	Coagulase positive staphylococci
DNA	Deoxyribonucleic acid
Dnase	Deoxyribonuclease
EA	Essential agreement
ECDC	European Centre for Disease Prevention and Control
ECOFF	Epidemiological ECOFF
EMA	European Medicines Agency
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FBRI	Foreign body-related infection
G	Guanine
GA	Glycopeptide antibiotics
hBMD	High inoculum broth micro-dilution
ICPN	International Code Product Number
IS	Insertion sequence
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
ME	Major error
mE	Minor error
MET	Macrogradient test
MIC	Minimal inhibitory concentration
MLST	Multilocus sequence typing
NaCl	Sodium chloride
PAP	Population analysis profiles
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
PIA	Polysaccharide intercellular adhesins

RAPD-PCR	Polymorphic DNA random amplification polymerase chain reaction
REP-PCR	Repetitive element polymerase chain reaction
sBMD	Standard inoculum broth micro-dilution
SCC*mec*	Staphylococcal cassette chromosome *mec*
SCV	small colony variants
sGDT	Standard gradient test
SSI	Surgical site infections
vME	Very major error

Table of content

1 Introduction

1.1 *Staphylococcus* Taxonomy and classification

The name *Staphylococcus* was proposed by Ogston [2] and derives from Greek language, the prefix *staphyle* stands for bunch of grapes and *kokkus* for grain [3]. *Staphylococcus* species have undergone multiple taxonomic changes after Rosenbach at the end of nineteenth century described "*Staphylococcus pyogenes aureus*" involvement in soft tissue infections [4]. These taxonomy revisions were mainly based on the species growth attributes and not on their phylogenetic relatedness. In the 1940s Fairbrother [5] initiated the use of coagulase production as the main feature to characterise *Staphylococcus* species. Free- coagulase is an extracellular protein without enzyme activity which forms a proteolytic complex with prothrombin thus prompting the conversion of fibrinogen to insoluble fibrin. Certain *Staphylococcus* strains produce coagulase hence the name "coagulase positive" (CoPS), whereas the others are coagulase negative staphylococci (CoNS). This identification tool used initially in diagnostic setting to assign *Staphylococcus* strains, later translated into the clinical practice were CoPS prominently represented by *S. aureus,* the more virulent species, overshadowed the CoNS strains, which were long considered as only contaminants.

Subsequently new molecular aspects and phylogenetic analyses have led to a new classification and species re-assignment within the *Staphylococcus* genus. The genus *Staphylococcus* belongs to Staphylococaccae family [6] encompassing 85 *Staphylococcus* species from which 70 are validated under International Code Product Number (ICPN) and 30 subspecies. The allocation of the strains is done mainly by phylogenetic analysis of the 16S rRNA gene [3]. This classification has its own limitation, therefore Lamers et al. [7] extended the number of genes used for *Staphylococcus* species identification. Their results confirmed the existing classification but showed new relationships among *Staphylococcus* species. Even if the genetic analysis has generated a lot of new information about *Staphylococcus* species, the coagulase classification has persisted over time being the best known, most clinically useful, and easily

applied criteria to differentiate *Staphylococcus* strains in both the diagnostic and clinic setting as well as in the literature.

1.2 Epidemiology

The CoNS represent in humans and in animals a large part of the skin and mucous membrane commensal microbiota. In the last few years CoNS species have shifted from being only a skin colonizer to an important cause of nosocomial infections especially among patients with short- or long-term indwelling devices. CoNS colonize areas either in a permanent or an intermittent manner and have a predisposition for certain types of skin areas. The CoNS bacterial load can vary from 10^3 to 10^6 CFU (colony forming units) / cm^2 in moist habitats [8]. While *S. epidermidis* prefers places with higher humidity (e.g., axillae and groin) [9], *S. haemolyticus* und *S. hominis* tend to occupy the apocrine glands (e.g., pubic areas) [9] and *S. capitis* mainly sebaceous areas like the forehead [10]. *S saprophyticus* colonizes not only specific areas like the gastrointestinal tract and cervix, but also differs according to host age and also in a season-dependent manner [11].

Given the increasing clinical importance of CoNS the current strain identification has become insufficient and thus different genotyping methods have been used to analyse CoNS epidemiology. These studies have found that CoNS strains have either a high degree of genetic diversity or clonal relatedness, that their origins is either in the community or the hospital, that the reservoir may be human or animal, and a number of putative transmission patterns has become apparent [12, 13]. When using typing methods, results can vary greatly not only on account of the method itself but also because of the selected isolates. Thus, repetitive element PCR (REP-PCR) or polymorphic DNA random amplification PCR (RAPD-PCR) shows low CoNS discrimination resolution [14], whereas when subjected to pulsed field gel electrophoresis (PFGE), *S. epidermidis*, the most common CoNS, shows a higher genetic diversity than, for example, S. *haemolyticus* and other CoNS species [15, 16]. These methods are useful for short-term CoNS epidemiology within a single location, but phylogenetic and long-term information can be obtained using multilocus sequence typing (MLST),

a method that analyses the polymorphism of certain housekeeping genes, providing better information over a long period of time and from different locations [17].

Although genotypic methods have their drawbacks; mainly used in research and not in the diagnostic setting, are time-consuming and costly and are easily influenced by interpretation criteria and definitions. They do reveal the existence of certain clonal complexes within the hospital environment, which are: a. different from those in the community [18] and are adapted to the environment, b. are resistant to antibiotics, and c. carry more virulence factors and thus may cause infections more often than others. Furthermore, using MLST, it has been shown that CoNS evolves rapidly through frequent gene transfer rather than by point mutations as described so far for *S. aureus* [19].

1.3 Microbiology

1.3.1 Culture

Staphylococci are nonmotile, non-spore-forming, facultative anaerobe, non-fstidious bacteria. Microscopically they are gram-positive with a diameter from 0.5 to 1 µm showing aggregation pattern either in pairs, tetrads, or grape-like clusters [10]. CoNS strains grow on Columbia agar 5% sheep blood usually within 18h to 24h, displaying circular, raised, or depressed, smooth, glistering, translucent to opaque, white, or grayish colonies with diameter from 4 to 9 mm.

Many of the CoNS show gamma haemolysis (non-haemolytic) on blood agar, whereas some strains (e.g., *S. haemoliyticus*) can display hazy or even beta-haemolysis (complete haemolysis) [20]. Moreover, the haemolysis degrees might differ depending on the blood used for agar (e.g., bovine vs. sheep). Most CoNS are non-pigmented, but some strains produce a yellow or brownish pigment.

Some CoNS strains have a mucoid appearance, while other more rarely have a pin-point growth, the so-called small colony variants (SCV). CoNS SCV require a longer incubation time of 48 to 72 hours and sometimes retain this despite

repeated subculturing. The appearance and auxotrophic characteristic of SCV are the consequences of induced changes in cell metabolism [21, 22].

1.3.2 Identification

Laboratory identification of CoNS includes the colony morphology and additional biochemical tests. Most CoNS strains do not produce free coagulase and are catalase positive. *S. intermedius*, unusual among clinically detected strains, belongs to CoPS because it produces coagulase. Most CoNS are deoxyribonuclease (Dnase)-negative nevertheless; some strain can show weak Dnase activity on DNase test agar. CoNS are haloduric growing well at NaCl concentration up to 7.5%, are lysostaphin susceptible and resistant to lysozyme. Guanine and cytosine (G+C) content in the CoNS DNA ranges from 30-38%. The cell wall contains peptidoglycan and teichoic acids [10]. Teichoic acid is built up from different polymers such as glycerol, glucose or glucosamine which alternate one after the other [23]. The cell wall peptidoglycan and teichoic acid composed mainly from glycerol and glucose, or glucosamine. The novobiocin susceptibility testing has previously been used as an identification tool for *S. saprophyticus* especially relevant in urine samples as *S. saprophyticus* is an important cause of urinary tract infection.

The morphological and biochemical features were previously used to identify and differentiate CoNS from *Micrococcaceae*, but are time consuming, challenging and not always accurate from current state of knowledge. Moreover, these tests where not always conclusive since different *Staphylococcus* species can share the same features. Therefore, many of these tests have been abandoned, but some have been improved and are still applicable in a cost-efficient manner as rapid tests within a clinical laboratory. Currently, besides the rapid tests, species identification is performed either by manual or automated test systems available commercially (e.g., Vitek 2 bioMérieux, Marcy l'Etoile, France) or by matrix-assisted laser desorption ionization-time of light mass spectrometry (MALDI-TOF).

Genes encoding antibiotic resistance (e.g., SCC*mec*), the *ica* operon involved in biofilm formation, arginine catabolic mobile element (ACME) and the IS element were proposed as marker for the diagnosis of more virulent CoNS strains causing

infection [18, 24, 25]. Their detection by means of PCR would indicate an infection, but the current data are not conclusive enough to implement in routine testing. Even if these elements are mainly found in hospital acquired CoNS strains, they can also be found less frequently in community acquired CoNS strains. *Ica* operon was found in community as well as hospital acquired strains but with a higher incidence among the latter [18]. Additionally, not all strains with these elements were accompanied by clinical signs of acute infection.

1.3.3 Virulence factors

Biofilm formation is the main virulence factor leading to CoNS foreign body-related infection (FBRI) [8]. The colonization of a foreign body happens mainly during insertion and not subsequently from a bacteremia [25]. The genes that promote biofilm formation are not present in all CoNS and vary not only among CoNS species but also among isolates within the same species. The process comprises of at least three phases: surface adhesion, biofilm accumulation and formation, and biofilm detachment Fig. 1 [25]. Biofilm formation can occur on both abiotic and biotic (host tissue) surfaces [20].

Attachment to surfaces involves physicochemical elements such as van der Waals forces and hydrophobic interactions with various proteinaceous and non-proteinaceous adhesins from the CoNS surface [20]. Adhesion is most likely not receptor-mediated, but a result of cell surface protein interaction with extracellular host products (e.g., fibrinogen).

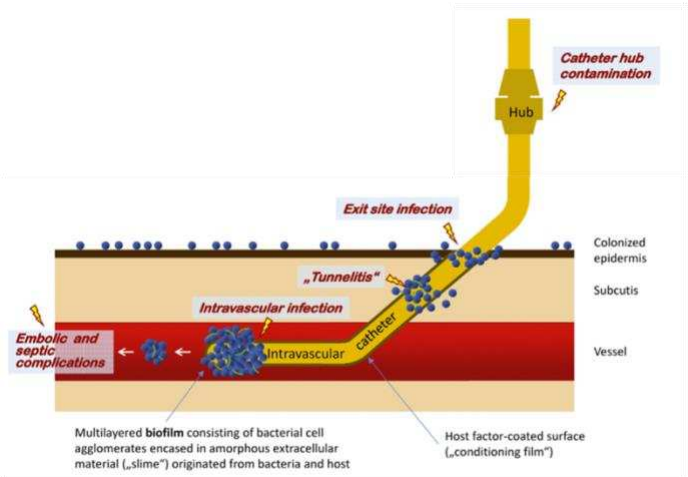

Fig. 1
The phases of the Biofilm formation: attachment, accumulation and detachment (retrieved with authors permission [25])

The AtlE surface autolysin not only facilitates attachment to the foreign material but also to the host cells. Moreover, it also has bacteriolytic activity [8, 20], best characterized for *S. epidermidis*, but was also reported in *S. saprophyticus* and other CoNS.

In the second phase, *Staphylococci* proliferate, resulting in the accumulation of multiple layers that are held together by polysaccharide intercellular adhesins, (PIAs) synthesized by the *ica*ADBC operon, and additional protein factors. In the detachment phase, enzymatic activity (protease, nuclease, hydrolase) prompts the dislocation of parts of the clusters, releasing bacterial emboli into the circulation. Thus, *Staphylococci*, on the one hand, can colonize other sites and, on the other hand, can cause emboli responsible for a complicated course of the disease.

Within the biofilm, the bacteria are no longer in a planktonic phase and the matrix forms a protective barrier against environmental factors, the host's immune response and, in particular, antibiotics. Biofilm-associated infections are therefore prone to therapy failure, complications and can eventually lead to chronic persistent infection [24].

1.3.4 Antibiotic susceptibility testing (AST)

Different AST methods are available, and their employment depends on the setting. Broth micro-dilution (BMD) is according to the European Committee on Antimicrobial Susceptibility Testing [26, 27] the gold standard method for rapidly growing aerobic bacteria (Fig. 2).

Shortly, within a well a known bacterial suspension will be incubated with the antibiotic(s) of interest for 18h to 24h and in the end the presence or the lack of turbidity will be recorded. Thus, the minimal inhibitory concentration (MIC) for each tested antibiotic is determined. MIC can be also determined by means of gradient test. Briefly, an antibiotic impregnated strip is applied on agar previously inoculated with a defined bacterial suspension and incubated together for certain amount of time and the MIC read where the ellipse meets the strip. (Fig. 2).

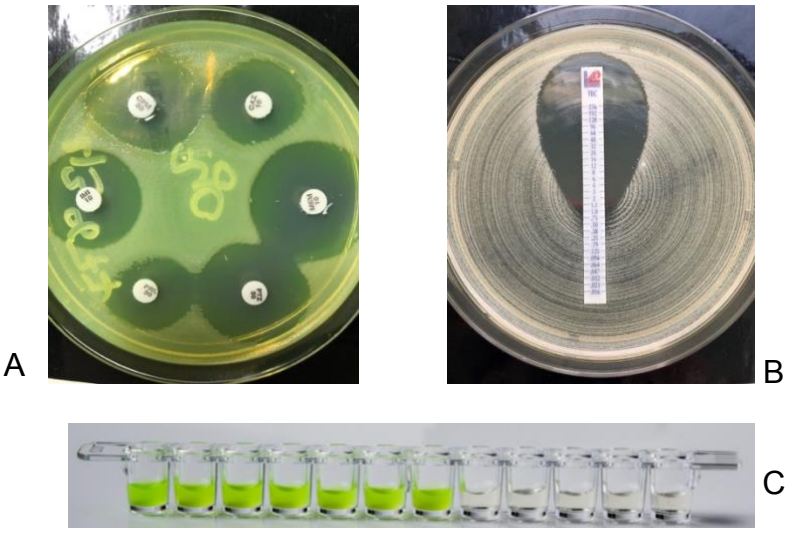

Fig. 2: A: disc diffusion, B. teicoplanin gradient test for CoNS (MIC of 1mg/L), C. microdilution (well 1-7 turbidity, 8-12 clear wells).

Agar dilution is similar to broth dilution but uses agar instead of broth. For disc diffusion, the method first described by Kirby and Bauer [28], the bacterial inoculum is spread evenly on agar, antibiotic-impregnated discs are then applied, and after incubation the zone of growth inhibition around each disc is measured [26]. The diameter of each inhibition zone, if present, determines whether the bacteria is susceptible to the antibiotic or not according to the existing EUCAST-defined breakpoints [29].

Some of these methods are multi-step and time-consuming and therefore not always suitable for a high sample throughput within a diagnostic setting. Therefore, automated methods are more convenient and suitable. Automated ASTs are generally speaking robust, with minimal handling time, cost-effective and require little technical effort.

1.4 Clinic

Among opportunistic Staphylococci species like *S. aureus, S. lugdunensis,* or the *S. epidermidis* group cause infection more often than the others. Some other saprophytic species are mainly found on plant or animals [30] and have not yet been associated with infections in humans. A challenging situation from a clinical perspective arises when CoNS cause an infection, thus requiring antibiotic

treatment and indwelling material removal. CoNS detected in a sample might be the result of a contamination during sampling or laboratory processing, or colonization of the surface or infection. Unfortunately, the clinical information is not very supportive because the onset is usually insidious, non-specific, and symptoms mild, therefore follow-up cultures are advisable. Bacteremia can be transient, intermittent, or continuous, thus CoNS identification in multiple samples taken at different time point can support the suspicion of an infection.

At first CoNS adhere to a surface, colonize it and only in certain circumstances (interference with skin health, ecology, and structure, or the immune system), may cause opportunistic local or systemic infections. Advances in modern medicine have led to an increased role of CoNS among patients who are immunocompromised, critically ill, long-term in-patients, or have implanted medical devices [8, 31-35]

CoNS strains have been reported to play a significant role not only among device-associated infections (intravascular catheters, cerebrospinal fluid shunts, prosthetic joint, vascular grafts, and peritoneal dialysis catheters) but also in osteomyelitis, infective endocarditis [33], surgical site infections [35], and infections in neonates [36]. Van Epps et al. showed [31] that 50–70% of healthcare-associated infections in the USA are a consequence of the utilization of a broad spectrum of available implantable medical devices, from the easily replaceable peripheral cannula to long-term devices, including extracorporeal life support, left ventricular assist devices, neurological devices, and joint prostheses.

CoNS strains cause 20–30%, and in some studies even up to 45% [32, 37] of central-line-associated bloodstream infections (CLABSIs) in intensive care units and 35–55% of cardiovascular implantable electronic device (CIED) infections [38]. Furthermore, the 2018 ECDC report [35], showed that overall, 50% of surgical site infections (SSIs) are due to Gram-positive cocci. CoNS strains were found in 26.4% of SSI after coronary artery bypass graft and 18.9% after hip prosthesis surgery. Amat-Santos et al. found that 24.5% of prosthetic valve endocarditis cases after transcatheter aortic valve replacement were caused by CoNS [33]. In addition, CoNS is a major cause of late-onset sepsis among neonates [36].

1.5 Treatment

Most CoNS strains display resistance to beta-lactam agents; therefore, glycopeptide antibiotics (GAs) are often the therapy of choice for these infections. Vancomycin and teicoplanin are naturally occurring actinomycete-derived first generation GA [39], whereas the recently clinically-approved second generation GA telavancin and dalbavancin are semi-synthetic lipoglycopeptides. GA fall into five structural sub-types sharing a backbone of seven aromatic amino acids (AA-1 to AA7) and five aromatics rings (A-E) [40]. All GAs share the same mechanism of action (inhibition of the cell synthesis) and have a similar structure and spectrum of activity (mainly aerobic Gram-positive bacteria). By binding to the amino terminal acyl-D-alanyl-D-alanine subunit of peptidoglycan transglycosylation and thus cell-wall biosynthesisis inhibited inducing cell death [39] [41].

Teicoplanin is a fermenting product of *Actinoplanes teichomyceticus*, which initially was found in a soil sample from Nimodi Village, Indore, India [42]. Teicoplanin complex consists of five related components (group A2) linked to the heptapeptide scaffold, a hydrolysis group and four other minor components [43]. The components ratio can vary with the strain and the growth media used in the fermentation process [44].

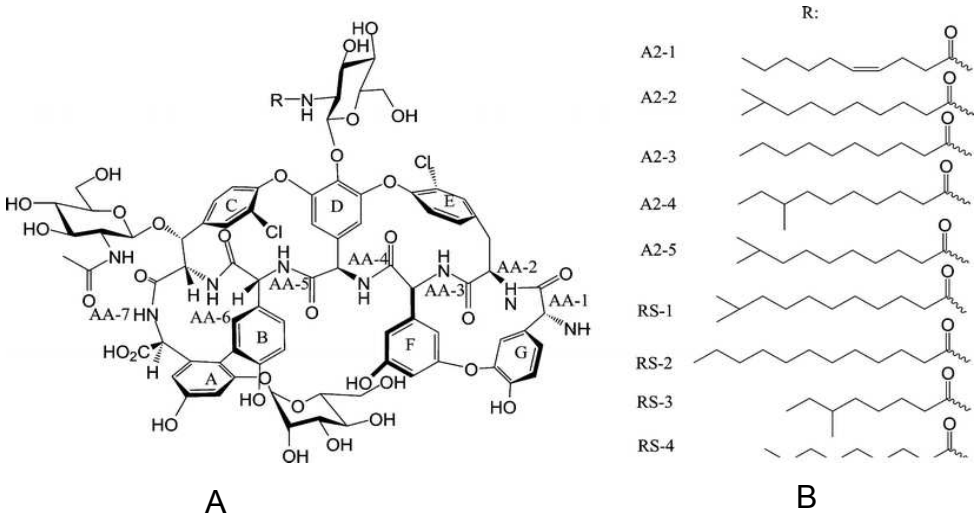

Fig 3. Structure of Teicoplanin: A. heptapeptide scaffold, B. five major groups (A2) and four minor groups (retrieved with authors permission [45]).

GA resistance among CoNS has been reported but the underlying mechanism remains unclear.

Teicoplanin was approved in Europe for adults as well as for children in 1988 and according to the European Pharmacopoeia the six components should be available in a predetermined concentration in order to be eligible for release by the European Medicines Agency (EMA/194668/2013) [46]. Teicoplanin has similar efficacy to vancomycin but has been associated with fewer side effects and less nephrotoxicity than vancomycin [47, 48]. Therefore, teicoplanin has become a therapeutic alternative to vancomycin for certain patients (e.g., those with neutropenia [49], or renal dysfunction).

Teicoplanin can be administered intravenously or intramuscular, as a single daily dose after a loading phase. The teicoplanin dose, rate of administration and target serum trough level must be adjusted to the severity and infection site, age and renal function during the consolidation phase. Teicoplanin is neither absorbed orally nor metabolized [50], is up to 90% albumin-bound in serum and is excreted mainly renally. Teicoplanin has a long serum half-life (88 to 182 h) [50], with a good bioavailability in the lung, myocardium, and bone but little to no penetration in cerebral fluid or vitreous humour. Nevertheless, intraventricular administration has been described with satisfactory concentrations [50].

1.6 Ethics vote and animal experiment approval

In accordance with the Declaration of Helsinki (2013), ethical approval for the study was granted by the Ethics Committee of the Medical Faculty of the Heinrich-Heine University, Dusseldorf (Study-No.: 5694).

1.7 Aims of the study

Teicoplanin resistance has been reported increasingly over the years, but the published results are incongruous. In our laboratory we have made the same observation, but the questions as to whether teicoplanin resistance is rising and is it reliably detected, remain unanswered. Therefore, the aims of this study were to i.) assess the robustness of the routinely employed susceptibility testing by comparing it with other available methods; ii.) propose a diagnostic algorithm to

reliably detect the teicoplanin resistance and heteroresistance thereby also avoiding labour- intensive methods such as the population analysis method.

2 Publication

antibiotics

MDPI

Article

Teicoplanin-Resistant Coagulase-Negative Staphylococci: Do the Current Susceptibility Testing Methods Reliably Detect This Elusive Phenotype?

Adriana D. Balasiu * and Colin R. MacKenzie

Institute of Medical Microbiology and Hospital Hygiene, Heinrich-Heine University Hospital, 40225 Dusseldorf, Germany

* Correspondence: adriana.balasiu@med.uni-duesseldorf.de

Abstract: Coagulase-negative staphylococci (CoNS), members of the skin commensal microbiota, are increasingly associated with local or systemic infections due to a shift in patient populations in recent decades. Subsequently, more CoNS strains have been subjected to antibiotic susceptibility testing (AST), thus leading to the increased detection of teicoplanin resistance. However, data concerning teicoplanin resistance among CoNS strains remain limited, heterogeneous, and inconclusive. We collected 162 consecutive CoNS strains identified using Vitek-2 as teicoplanin-resistant and tested them with a range of AST methods. The results of standard and high inoculum broth microdilution (sBMD; hBMD), agar dilution (AD) after 24 h and 48 h incubation, standard and macrogradient diffusion strip (sGDT, MET), screening agar, and disc diffusion were compared to assess their robustness and to establish a diagnostic algorithm to detect teicoplanin resistance. sBMD was used as the reference method, and the lowest number of strains were teicoplanin-resistant using this method. sGDT and disc diffusion generated similar results to sBMD. Compared with sBMD, AD-24 h generated the lowest number of false teicoplanin-resistant strains, followed by hBMD, AD-48 h, and Vitek-2. sGDT, a fast, easy, affordable method in diagnostic settings, generated the highest rate of false teicoplanin-susceptible strains. Vitek-2 testing produced the highest number of teicoplanin-resistant strains. Only in two strains was the initial Vitek-2 teicoplanin resistance confirmed using five other AST methods. In conclusion, the different antibiotic susceptibility testing methods generated inconsistent, inconclusive, and discrepant results, thus making it difficult to establish a diagnostic algorithm for suspected teicoplanin resistance. Teicoplanin testing proved to be challenging and easily influenced by technical factors. This study aimed not only to raise awareness of teicoplanin resistance testing but also of the need for future studies focusing on the clinical efficacy of teicoplanin in relation to its susceptibility results.

Keywords: CoNS; teicoplanin; therapy; resistance; susceptibility testing

Citation: Balasiu, A.D.; MacKenzie, C.R. Teicoplanin-Resistant Coagulase-Negative Staphylococci: Do the Current Susceptibility Testing Methods Reliably Detect This Elusive Phenotype? *Antibiotics* **2023**, *12*, 611. https://doi.org/10.3390/antibiotics12030611

Academic Editor: Samantha Flores-Treviño

Received: 13 February 2023
Revised: 15 March 2023
Accepted: 17 March 2023
Published: 19 March 2023

1. Introduction

The coagulase-negative staphylococci (CoNS) include a large number of different *Staphylococcus* species and are part of the skin and mucous membrane commensal microbiota. In certain circumstances (interference with skin health, ecology, and structure, or the immune system), they may cause opportunistic local or systemic infections. Advances in modern medicine have led to an increased role of CoNS among patients who are immunocompromised, critically ill, long-term hospitalized, or have implanted medical devices [1–6].

CoNS strains have been reported to play a significant role not only among device-associated infections (intravascular catheters, cerebrospinal fluid shunts, prosthetic joint, vascular grafts, and peritoneal dialysis catheters) but also in osteomyelitis, infective endocarditis [3], surgical site infections [5], and infections in neonates [7]. Van Epps et al.

showed [1] that 50–70% of healthcare-associated infections in the USA are a consequence of a broad spectrum of available implantable medical devices, from the easily replaceable peripheral cannula to long-term devices, including extracorporeal life support, left ventricular assist devices, neurological devices, and joint prostheses.

CoNS strains cause 20–30%, and in some studies even up to 45% [2,8] of central-line-associated bloodstream infections (CLABSIs) in intensive care units and 35–55% of cardiovascular implantable electronic device (CIED) infections [9]. Furthermore, the 2018 ECDC report showed that [5], overall, 50% of surgical site infections (SSIs) are due to Gram-positive cocci. CoNS strains were found in 26.4% of SSI after coronary artery bypass graft and 18.9% after hip prosthesis surgery. Amat-Santos et al. found that 24.5% of prosthetic valve endocarditis cases after transcatheter aortic valve replacement were caused by CoNS [3]. In addition, CoNS is a major cause of late-onset sepsis among neonates [7].

Different AST methods, depending on the setting, can be performed: semi-automated or manually, using microdilution or agar dilution, disc diffusion, or gradient test. Most CoNS strains display resistance to beta-lactam agents; therefore, glycopeptide antibiotics (GAs) are often the therapy of choice for these infections. Vancomycin and teicoplanin are naturally occurring actinomycete-derived glycopeptide antibiotics [10]. GAs share the same mechanism of action (inhibition of the cell synthesis), structure, and spectrum of activity (mainly aerobic Gram-positive bacteria). GAs bind to the N-Acyl-D-Ala-D-Ala subunit of peptidoglycan, thus inhibiting cell-wall biosynthesis and inducing cell death [11]. Teicoplanin has similar efficacy to vancomycin but has been associated with fewer side effects and less nephrotoxicity than vancomycin [12,13]. Therefore, teicoplanin has become a therapeutic alternative to vancomycin for certain patients (e.g., those with neutropenia [14], or renal dysfunction).

Teicoplanin resistance has been increasingly reported over the years, but the published results are disparate. In our laboratory, we have made the same observation, and thus the main concern as to whether teicoplanin resistance is increasing remains unanswered. This leads to the question of which method is the most reliable to detect resistance to ensure that patients receive the appropriate therapy. Therefore, the aims of this study were to (i) assess the robustness of the routinely employed susceptibility testing by comparing it with other available methods and (ii) propose a diagnostic algorithm to detect the teicoplanin resistance and heteroresistance, thus avoiding labor-intensive population analysis methods.

2. Results

2.1. Patients and Included Isolates

Of the 162 tested isolates, 157 (96.9%) were *Staphylococcus epidermidis*, followed by *S. hominis* (3 isolates, 1.9%) and *S. haemolyticus* (2 isolates, 1.2%). In total, 96 (59.2%) strains were recovered from blood cultures, 76 (46.9%) of which were peripheral, and 20 (12.3%) were from central lines. The remaining 66 strains (40.8%) were isolated from tissue, intraoperative swabs, catheter tips, cerebrospinal fluid (CSF) from external ventricular drains (EVD), aspirates, respiratory samples, urine (from immunocompromised patients), and cell culture media (cell therapy products).

2.2. Vitek-2

The number of teicoplanin-resistant strains detected using Vitek-2 in our laboratory varied over 6 years between 20% and 32%, as shown by the annual resistance statistics listed in Table 1.

On retesting the 162 isolates using Vitek-2, 88 (54.3%) strains were susceptible to teicoplanin, and 74/162 (45.7%) were resistant. Most of the teicoplanin-resistant strains found with Vitek-2, i.e., 46/74 (62.2%), had a MIC of 8, while 27/74 (36.5%) had a MIC of 16, and 1 strain (1.3%) an MIC of 32. Moreover, Vitek-2 MIC distribution shows that the MICs are within close range of EUCAST defined teicoplanin breakpoint (Tables 2 and 3).

Vitek-2 found 63 teicoplanin-resistant strains not confirmed by sBMD, and in 14 strains, sBMD testing correlated with the Vitek-2 results (Table 2).

Table 1. Annual resistance statistics for CoNS for the entire clinic between 2015 and 2020, susceptible strains in percentage (%).

Year	Total	OXA	GEN	LEV	SXT	ERN	CLI	VAN	TEI	LIN	TIG	FOS	FUS	RIF	TET	DAP
2015	650	36	57	44	72	30	47	100	**68**	100	99	56	61	93	55	100
2016	669	31	54	42	72	28	44	100	**71**	100	100	57	-	92	-	99
2017	759	32	58	45	72	31	43	100	**74**	100	100	51	-	93	-	99
2018	619	39	64	49	73	33	50	100	**69**	100	100	59	-	92	-	100
2019	562	36	63	54	71	34	50	100	**84**	99	100	56	-	92	-	99
2020	497	37	66	54	70	36	52	100	**80**	99	100	61	-	94	-	98

OXA (oxacillin), GEN (gentamicin), LEV (levofloxacin), SXT (trimethoprim–sulfamethoxazole), ERN (erythromycin), CLI (clindamycin), VAN (vancomycin), TEI (teicoplanin), LIN (linezolid), TIG (tigecycline), FOS (fosfomycin), FUS (fusidic acid), RIF (rifampicin), TET (tetracycline), DAP (daptomycin).

Table 2. Antimicrobial resistance routinely performed using Vitek-2 *.

Vitek-2	EUCAST							
	Susceptible (S) ≤ 4 mg/L (%)				Resistant > 4 mg/L (%)			Total
MIC Teicoplanin mg/L	≤0.5	1	2	4	8	16 *	32 *	
S. epidermidis	15 (9.3)	3 (1.8)	24 (14.8)	46 (28.4)	44 (27.2)	24 (14.8)	1 (0.6)	157
S. haemolyticus	-	-	-	-	1 (0.6)	1 (0.6)	-	2
S. hominis	-	-	-	-	1 (0.6)	2 (1.2)	-	3
Total %	88 (54.3)				74 (45.7)			162

* According to CLSI: strains with a MIC of 16 mg/L would be assigned to intermediate strains and the strain with a MIC of 32 mg/L would be resistant.

Table 3. Teicoplanin MIC distribution with Vitek-2.

Tei	AB	OXA	GEN	LEV	SXT	ERN	CLI	VAN	LIN	TIG	FOS	FUS	RIF	TET	DAP
S (88)	R	62	37	42	23	59	41	-	-	-	17	42	5	57	1
	S	26	51	46	65	29	57	88	88	88	71	46	83	31	87
R (74)	R	66	38	58	25	57	53	-	-	-	17	35	3	29	-
	S	8	36	16	49	17	21	74	74	73 **	56 **	28 **	71	45	73 **
Total															
	R	128	75	100	48	116	94	-	-	-	34	77	8	86	1
	%	79	46.3	61.7	29.6	71.6	58.0	-	-	-	21.0	47.5	4.9	53.1	0.6
	S	34	87	62	114	46	68	162	161	161	127	84	154	76	160
	%	21	53.7	38.3	70.4	28.4	42.0	100	99.4	99.4	78.4	51.8	95.1	46.9	98.7

AB (antibiotic), OXA (oxacillin), GEN (gentamicin), LEV (levofloxacin), SXT (trimethoprim–sulfamethoxazole), ERN (erythromycin), CLI (clindamycin), VAN (vancomycin), TEI (teicoplanin), LIN (linezolid), TIG (tigecycline), FOS (fosfomycin), FUS (fusidic acid), RIF (rifampicin), TET (tetracycline), DAP (daptomycin). ** For one strain, TIG, FOS, FUS, and DAP were not tested.

Using Vitek-2, 79% of the strains were oxacillin-resistant, and none of the strains displayed resistance to vancomycin or linezolid. All the antibiotics tested using Vitek-2 are listed in Table 3.

2.3. Standard and High-Broth Microdilution (sBMD and hBMD)

Using sBMD, 146/162 (90.1%) were teicoplanin-susceptible, and 16/162 (9.9%) were resistant. With hBMD, only 109/162 strains (67.3%) were susceptible, and 53/162 (32.7%) were resistant. With sBMD, most of the strains (62.9%) had a MIC of 2 or 4, whereas using hBMD, the majority (58.0%) had a MIC of 4 or 8. The 39 (24.1%) teicoplanin-resistant strains in the hBMD assay had a MIC of 4 (28 strains), 2 (10 strains), and 0.5 (1 strain) in sBMD. These results are summarized in Tables 4–6.

Table 4. sBMD MICs.

sBMD	EUCAST							
	Susceptible (S) ≤ 4 mg/L (%)				Resistant > 4 mg/L (%)			Total
MIC Teicoplanin mg/L	≤0.5	1	2	4	8	16	>16	
S. epidermidis	15	29	48	53	12	-	-	157
S. haemolyticus	-	-	-	-	-	1 *	1 *	2
S. hominis	-	-	-	-	2	-	-	3
	9.3	17.9	29.6	33.3	8.6	0.6	0.6	162

* According to CLSI: the strain with a MIC of 16 mg/L would be assigned to intermediate and the strain with a MIC of >16 would be resistant.

Table 5. The minimum inhibitory concentration of the staphylococci strains using hBMD.

hBMD	EUCAST							
	Susceptible (S) ≤ 4 mg/L (%)				Resistant > 4 mg/L (%)			Total
MIC Teicoplanin mg/L	≤0.5	1	2	4	8	16	>16	
S. epidermidis	11	16	35	45	44	5 *	1 *	157
S. haemolyticus	-	-	-	-	2	-	-	2
S. hominis	-	-	-	-	1	-	-	3
	6.8	9.9	21.6	29.0	29.0	3.1	0.6	162

* According to CLSI: the strain with a MIC of 16 mg/L would be assigned to intermediate and the strain with a MIC of > 16 would be resistant.

Table 6. MIC distribution using sBMD vs. Vitek-2 and the respective EA, CA, and ME.

EUCAST Category	sBMD Teicoplanin mg/L	sBMD No.	MIC by Vitek-2, (No.)							
			Susceptible				Resistant			
			0.5	1	2	4	8	16	32	
Resistant (R)	32	-	-	-	-	-	-	-	-	EA 81 (50) CA 106 (65.4) ME 59 (36.4)
	16	2	-	-	-	-	2	-	-	
	8	11	-	-	-	-	3	7	1	
Total R		13 (8%)	-				13			
Susceptible (S)	4	40	-	-	4	6	18	12	-	
	2	49	2	2	4	20	18	3	-	
	1	22	2	1	6	7	3	3	-	
	0.5	25	5	-	8	11	-	1	-	
	<0.5	11	6	-	3	1	1	-	-	
Total S		147 (90.7%)	88 (54.3)				59 (36.4)			

Notably, 2 strains of 162 were not tested due to lack of growth. EA, essential agreement; CA, categorical agreement; ME, major error.

Vancomycin MIC was measured by means of sBMD and Vitek-2. All the samples were vancomycin-susceptible using both methods. While with sBMD, the majority 144/162 (88.9%) of the strains had a MIC of 2 mg/L, with Vitek-2, 70 (43.2%) had a MIC of 1 mg/L and 80 (49.4%) had a MIC of 2 mg/L.

2.4. Agar-Diffusion 24 h and 48 h Incubation (AD-24 h and AD-48 h)

In the AD-24 h assay, 128 (79%) strains were teicoplanin-susceptible, 33 (20.4%) were resistant, and 1 strain displayed no growth after 20–24 h incubation. Among the 33 teicoplanin-resistant strains in hBMD, 20 (12.3%) strains were susceptible, and 13 were resistant using sBMD, while with Vitek-2, 7 were susceptible, and 26 were resistant. AD-24 h, on the one hand, failed to recognize accurately 3 teicoplanin-resistant strains from sBMD, but on the other hand, generated 20 more resistant strains than sBMD. AD-24 h and the other AST results are summarized in Tables 7 and 8.

Table 7. MICs using agar dilution after 24 h and 48 h incubation.

Agar Dilution (AD) *	**EUCAST**									
	Susceptible (S) ≤ 4 mg/L								**Resistant > 4 mg/L**	
Incubation	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Teicoplanin (mg/L)	0.5		1		2		4		≥8	≥8
S. epidermidis	1	1	16	6	61	46	48	44	31	59
S. haemolyticus	-	-	-	-	-	-	-	-	-	2
S. hominis	-	-	-	-	-	-	-	-	2	3

* One strain remained without growth.

Table 8. Results of AD-24 h vs. other AST assay methods.

AD-24 h	**sBMD**	**AD-48 h**	**Vitek-2**	**sGDT**	**Screening McF 0.5**	**Screening McF 2**	**Disc Diffusion (CLSI)**
128 S	125 S	97 S	80 S	125 S	80 pos	114 pos	126 S
	3 R	31 R	48 R	1 R	44 neg	12 neg	-
	-	-	-	2 NE	4 NE	2 NE	2 NE
33 R	20 S	-	7 S	31 S	33 pos	33 pos	31 S
	13 R	33 R	26 R	2 R	-	-	2 I
	-	-	-	-	-	-	
1 NG	1 S	NG	1 S	NE	-	-	NG

S, susceptible; R, resistant; NE, not evaluable; NG, no growth; pos, positive; neg, negative; I, intermediate according to CLSI.

In AD after 48 h incubation, only 98 (60.5%) strains remained susceptible, 63 (38.9%) were resistant, and 1 strain displayed no growth. Notably, 31 strains, initially tested in AD-24 h as susceptible with a MIC of 4 (30) and 2 (1), were resistant after 48 h incubation. Only 14 of the 16 teicoplanin-resistant strains in sBMD were among the 63 teicoplanin-resistant strains in AD-48 h. Testing with sBMD and AD-48 h found the highest number of susceptible strains, whereas using Vitek-2 and AD-48 h, most strains were teicoplanin-resistant (51). Further results are depicted in Table 9.

Table 9. Results of AD-48 h vs. other AST methods.

AD-24 h	sBMD	AD-48 h	Vitek-2	sGDT	Screening McF 0.5	Screening McF 2	Disc Diffusion (CLSI)
97 S	95 S	97 S	74 S	96 S	50 pos	84 pos	96 S
	2 R	-	23 R		44 neg	12 neg	-
	-	-	-	1 NE *	3 NE	1 NE	1 NE
64 R	50 S	31 S	13 S	60 S	63 pos	63 pos	60 S
	14 R	33 R	51 R	3 R	-	-	2 I
	-	-	-	1 NG	1 NE-	NE	2 NE
1 NG	1 S	NG	1 S	NE	-	-	NG

* S, susceptible; R, resistant; NE, not evaluable; NG, no growth; pos, positive; neg, negative; I, intermediate according to CLSI.

2.5. Standard Gradient Diffusion Test (sGDT) and Macrodilution Gradient Test (MET)

All but three strains tested teicoplanin-susceptible by means of sGDT. Most of the strains displayed a MIC of 1 mg/L (81/162) or 2 mg/L (44/162). The assay recognized only 3 of the 16 teicoplanin-resistant strains from sBMD, thus generating the highest rate not only of CA but also of vME (Table 10).

Table 10. Susceptibility results using sGDT.

sGDT	EUCAST					
	Susceptible (S) ≤ 4 mg/L (%)				Resistant > 4 mg/L (%)	
MIC Teicoplanin mg/L	≤0.5	1	2	4	8	16
S. epidermidis	23	81	42	8	-	-
S. haemolyticus	-	-	-	-	2	-
S. hominis	-	-	2	-	-	1
% *	14.2	50	27.2	4.9	1.2	0.6

* Two strains display no growth, and for one strain, the MIC could not be read (1.8%).

The values obtained using MET are not strictly speaking MICs. After 48 h incubation, 157/162 (96.9%) strains displayed growth at a MIC lower than 8 mg/L, 1 strain at 8 mg/L, 1 strain at 12 mg/L, and 2 (1.2%) (both *S. epidermidis*) strains failed to grow. The strains displaying growth at 8 mg/L were also tested for vancomycin resistance because according to the EUCAST criteria, the reading of teicoplanin at 8 mg/L is not enough in itself to assign a strain as vancomycin-resistant or as a heteroresistant strain. The two strains with high MET readings were confirmed using all other AST assays, except via AD-24 h and disc diffusion. The AST results are collated in Table 11.

Table 11. Comparison of strains with high MET values (≥8 mg/L) with other AST assays.

No.	MET	sBMD	hBMD	Vitek-2	sGDT	AD-24 h	AD-48 h	Screening McF 0.5	Disc Diffusion	Material	Strain ID
71	8	R	R	R	R	S	R	pos	S	BC	*S. haemolyticus*
72	12	R	R	R	R	R	R	pos	I	BC	*S. hominis*

sBMD, standard broth microdilution; hBMD, high-broth microdilution; sGDT, standard gradient strip; BC, blood culture; AD, agar dilution; S, susceptible; R, resistant; I, intermediate according to CLSI; pos, positive.

2.6. Disc Diffusion and Screening Agar

By means of disc diffusion, all the samples except two were susceptible, according to the CLSI criteria, thus confirming that this method does not reliably detect teicoplanin resistance.

By means of screening agar (5 mg/L teicoplanin) using a standard 0.5 McF inoculum, 113/162 (69.8%) strains were positive, suggesting a teicoplanin MIC of over 5 mg/L and thus resistant. The remaining 44 (27.2%) were negative, 4 could not be evaluated, and 1 was not performed. Notably, 147/162 (90.7%) strains were positive when an McF 2 inoculum was used, 12 were negative, 2 could not be evaluated, and in 1, this was not performed. The most positive strains in a screening method, 99 (61.1%) strains with McF 0.5 and 131 (80.9%) using McF 2, were among the strains tested susceptible with sBMD and therefore would be falsely assigned as teicoplanin-resistant, which would correspond to the highest ME among all the employed AST methods. A summary of the results comparing the AST methods is depicted in Table 12.

Table 12. Teicoplanin susceptibility tested via AST and the EA, CA, vME, and ME yielded when compared with sBMD.

Method	Strain	No. % Isolates		EA	CA	vME	ME
		Susceptible	Resistant				
		≤4	>4				
sBMD	All strains	146 (90.1)	16 (9.9)				
	S. epidermidis	145 (89.5)	12 (7.4)				
	S. haemolyticus	-	2 (1.2)				
	S. hominis	1 (0.6)	2 (1.2)				
hBMD	All strains	109 (67.3)	53 (32.7)	137 (84.6)	121 (74.7)	2 (1.2)	39 (24.1)
	S. epidermidis	107 (66)	50 (30.9)	132 (81.5)	117 (72.2)	1 (0.6)	39 (24.1)
	S. haemolyticus	-	2 (1.2)	2 (1.2)	2 (1.2)	-	-
	S. hominis	2 (1.2)	1 (0.6)	3 (1.8)	2 (1.2)	1 (0.6)	-
Vitek-2	All strains	88 (54.3)	74 (45.7)	103 (63.6)	94 (58.0)	5 (3.1)	63 (38.9)
	S. epidermidis	88 (54.3)	69 (42.6)	99 (61.1)	90 (55.6)	5 (3.1)	62 (38.2)
	S. haemolyticus	-	2 (1.2)	2 (1.2)	2 (1.2)	-	-
	S. hominis	-	3 (1.8)	2 (1.2)	2 (1.2)	-	1 (0.6)
AD-24 h [1]	All strains	128 (79)	33 (20.4)	146 (90.1)	138 (85.2)	3 (1.8)	20 (12.4)
	S. epidermidis	125 (77.2)	31 (19.1)	142 (87.7)	134 (82.7)	2 (1.2)	20 (12.4)
	S. haemolyticus	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	-
	S. hominis	1 (0.6)	2 (1.2)	3 (1.8)	3 (1.8)	-	-
AD-48 h [1]	All strains	97 (59.9)	64 (39.5)	132 (81.5)	109 (67.3)	2 (1.2)	50 (30.9)
	S. epidermidis	97 (59.9)	59 (36.4)	127 (78.4)	105 (64.8)	2 (1.2)	49 (30.2)
	S. haemolyticus	-	2 (1.2)	2 (1.2)	2 (1.2)	-	-
	S. hominis	-	3 (1.8)	3 (1.8)	2 (1.2)	-	1 (0.6)
sGDT [2]	All strains	156 (96.3)	3 (1.8)	118 (72.8)	146 (90.1)	13 (8.0)	-
	S. epidermidis	154 (95.1)	-	114 (70.4)	142 (87.7)	12 (7.4)	-
	S. haemolyticus	-	2 (1.2)	2 (1.2)	2 (1.2)	-	-
	S. hominis	2 (1.2)	1 (0.6)	2 (1.2)	2 (1.2)	1 (0.6)	-

Strains without growth: [1] one strain and [2] three strains.

3. Discussion

The AST results and the institutional yearly resistance statistics confirmed the previously published data [4,15–17] that CoNS strains are highly resistant to most commonly used beta-lactam antibiotic agents, leaving glycopeptides, linezolid, and daptomycin as the most important therapeutic options. A number of aspects should be considered when

choosing the appropriate treatment, including side effects, risk of developing resistance during therapy, therapeutic drug monitoring, cost, and availability. Teicoplanin has been considered an alternative to vancomycin due to its lower nephrotoxicity, reduced drug interactions, and once-daily administration.

Teicoplanin resistance has been reported in the USA and the UK since the early 1980s, but the published data since then [18] do not reflect the actual incidence and its impact on therapeutical use. Teicoplanin resistance is an increasing and emerging challenge, but published data are inconclusive due to a number of factors. These include the different methods employed (e.g., broth microdilution vs. disk diffusion [4]); settings, diagnostic vs. research (e.g., broth microdilution vs. population analysis); the standards employed (e.g., CLSI vs. EUCAST defined breakpoints); the inclusion of diverse cohorts (e.g., catheter-related bacteremia vs. healthy volunteers [17,19]; the bacterial species studied (most studies have focused on *S. aureus* and fewer on CoNS [20]); clonal dissemination [21]; data generated at different time points [22]; or that teicoplanin was not tested. Thus, to date, reports have probably underestimated the true incidence of teicoplanin resistance and are still insufficient to identify its underlying mechanisms with certainty.

It is still unclear if increasing teicoplanin resistance should be attributed to one or several possible underlying mechanisms. The mechanism is neither well defined nor adequately studied. Several mechanisms have been proposed such as cellular aggregates and antibiotic retention [23] or cell-wall alteration through reorganization or thickening [24,25]. Perhaps even more worrying is that teicoplanin resistance has been shown to develop under therapy [26,27]. Biavasco et al. pointed out that the AST employed for teicoplanin can be easily influenced by technical factors such as methods, media, inoculum, and incubation time [28]. Furthermore, it has been shown that the physical properties of teicoplanin—a large, lipophilic, and negatively charged molecule—have an impact upon testing by generating a lower diffusion coefficient on agar compared with vancomycin [29].

Broth microdilution is generally regarded as the gold standard method for antibiotic susceptibility testing; however, few laboratories use it for routine purposes. To optimize laboratory workflow with a high sample throughput, semi-automated devices such as Vitek-2 are employed routinely for the AST of fast-growing bacteria. Generally speaking, Vitek-2 performs well: It is fast and robust, with minimum hands-on time, is cost-effective, and requires little technical expertise. In our laboratory, using Vitek-2, a rapid rise in teicoplanin-resistant CoNS strains was observed in 2015. Baris et al. also reported an increased number of teicoplanin-resistant strains with BD Phoenix [16]. As in our study, most of the samples tested as teicoplanin-resistant using Vitek-2 were not confirmed via sBMD, leading to the highest rate of ME among the AST methods. The majority of teicoplanin MIC, either 4 or 8 mg/L (56.8%), determined using Vitek-2 were close to the EUCAST epidemiological cut-off (ECOFF) value for CoNS (MIC 4 mg/L), thus having an impact upon the generated EA and CA. Meanwhile, most of the MIC in sBMD (54.7%) were concentrated at the upper limit of the range (2 or 4 mg/L), thus conforming to the published EUCAST MIC distribution determining the teicoplanin breakpoints for CoNS. Vaudaux et al. found a similar MIC distribution using macrodilution but not microdilution. Moreover, MIC distribution was different when performed using macrodilution or microdilution [30].

According to these results, the AST performance for teicoplanin does not fulfill the CLSI criteria of 90% agreement for both EA and CA [31]. It is difficult to establish a diagnostic workflow that reliably confirms teicoplanin resistance among routinely tested strains. Firstly, EA and CA differ in test, antibiotic, and methodology, confirming the results of Campana et al. Moreover, their results showed that EA and CA vary with species (e.g., CA for strip test for *S. aureus* (100%) vs. 75% for CoNS according to CLSI) [32]. Secondly, most of the routinely employed AST assays use a low bacterial inoculum and are fast, whereas the strains that might bear heteroresistance are first detected at CFU above 10^6 CFU/mL and after a longer incubation time (48 h). With a final inoculum of 5×10^5 CFU/well, the microdilution assay, the current gold standard method, is unable to

reliably detect heteroresistance [30]. Routinely employed methods probably do not detect heteroresistant strains, which may have a negative impact on therapeutic outcomes.

Teicoplanin AST is easily influenced by inoculum, incubation time, media, and method and is more variable than vancomycin AST. All these suggest that a re-evaluation of diagnostic methods, breakpoints, and their capacity to accurately identify teicoplanin resistance and heteroresistance among clinically relevant CoNS strains is needed. A possible diagnostic algorithm should encompass different steps that can be carried out in a routine setting: a rapid automated AST to identify possible resistance, followed by a high inoculum and a longer incubation period method to confirm resistance or susceptibility. The second method should preferably be fast, commercially available for routine settings, have a low cost, and be reliable and reproducible. A possible option would be MET. MET is a method with low hands-on time, but adjustments are needed for it to be as reliable for use with CoNS as it is for *S. aureus*. The strains with suspected teicoplanin resistance could be further tested in reference laboratories by means of population analysis profiles (PAPs). PAP is the gold standard method to detect heteroresistance. This is a demanding time-consuming method, difficult to implement in a routine setting, and poses the risk of selecting resistance instead of finding it [33]. Using a different method in the second step is challenging because not all laboratories have the option to produce the necessary in-house plates.

These results do not confirm an increased vancomycin resistance as previously thought or predicted. This may be due to an underlying mechanism that involves only teicoplanin or that the teicoplanin molecule presents technical difficulties causing an unreliable result [3]. A similar situation applies to colistin [34].

In conclusion, extensive teicoplanin susceptibility testing showed that the results obtained using a single method could not be fully confirmed by employing various other methods. Due to a high discrepancy among the methods tested, no algorithm can be proposed to reliably detect teicoplanin resistance. The fact that the results were so diverse suggests that all the aspects involved in teicoplanin testing should be re-evaluated so that improvements can be made not only in the laboratory but also in establishing reliable breakpoints. Given the relevance that these results pose for antibiotic therapy, further clinical studies looking into the clinical efficacy of teicoplanin and in vitro teicoplanin testing are of great importance.

4. Materials and Methods

In accordance with the Declaration of Helsinki (2013), ethical approval for this study was granted by the Ethics Committee of the Medical Faculty of Heinrich Heine University, Dusseldorf (Study No. 5694/26.9.2016).

4.1. Bacterial Strains

For this study, 162 consecutive CoNS strains were collected from August 2015 to August 2016 at the Institute of Medical Microbiology and Hospital Hygiene, Heinrich Heine University Hospital, Düsseldorf. The strains were selected based on non-susceptibility against teicoplanin and were recovered from different samples such as blood culture, soft-tissue infections, or central lines. Routinely, putative clinically relevant isolates were subjected to identification and susceptibility testing. Identification was performed with Vitek® MS (bioMérieux, Marcy l'Etoile, France), a matrix-assisted laser desorption ionization–time of flight mass spectrometry method (MALDI-TOF MS). Antibiotic susceptibility testing was performed with Vitek 2 (bioMérieux, Marcy l'Etoile, France) AST- P654 cards.

The strains were stored in 80% glycerol in a Mueller–Hinton Broth (MHB) (commercially dehydrated base from Oxoid, Thermo Scientific, Basingstoke, United Kingdom) (v/v) at −80 °C until additional testing was performed. To perform further testing, the strains were subcultured on Columbia agar supplemented with 5% sheep blood (COS Agar) (bioMérieux, Marcy l'Etoile, France), incubated at 36 ± 1 °C in an atmosphere enriched with 5–10% CO_2 for 18–24 h. Subsequently, a single colony was picked, subcultured on COS Agar, and incubated for another 18–24 h under the same conditions.

4.2. *Antimicrobial Susceptibility Testing (AST)*

The minimal inhibitory concentration (MIC) was determined on a standard 0.5 McFarland bacterial suspension in a 0.85% saline solution using different susceptibility testing methods. The MIC is reported either in mg/L or µg/mL, and strains were classified as susceptible or resistant according to EUCAST breakpoints.

4.2.1. Broth Microdilution

Broth microdilution (BMD) was performed according to the method recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (ISO 20776-1) and used as the reference method for antimicrobial susceptibility testing (AST) of rapidly growing aerobic bacteria. Both antibiotics used in this assay, vancomycin (V2002-100MG) and teicoplanin (T0578-100MG) (Sigma-Aldrich, Darmstadt, Germany), were resuspended in water at a concentration of 5120 mg/L (stock solution) and kept in aliquots at −80 °C until use. Ready-to-use antimicrobial solutions were freshly prepared from the stock solutions on the day of the assay using the Mueller–Hinton Broth (MHB). For the assay, 100 zµL MHB was added in each well of a 96-well flat bottom plate. Then, 100 µL antibiotic with the highest concentration (32 mg/L) was added to the wells of the first column using a multichannel pipette, and mixed (pipetted 5 times), thus achieving a final concentration of 16 mg/L in the first dilution (wells A1-H1). Afterward, 100 µL suspension was transferred to the corresponding well in the second column. This process was repeated up to the 10th column, from which 100 µL were discarded. As a result, a serial twofold dilution was generated to a final concentration of 0.03 mg/L. In addition to the ten antibiotic concentrations columns, growth/positive control (column 11—MHB and bacterial inoculum without antibiotic) and negative control (column 12—only MHB) were tested. To all the wells other than the negative control column, 10 µL of the standard bacterial inoculum (5×10^5 colony forming units/mL (CFU/mL)) was added. To obtain a standard inoculum, each strain was resuspended in 0.85% saline to a 0.5 on the McFarland scale (McF) (1–2×10^8 CFU/mL), followed by a 1:20 dilution (5×10^6 CFU/mL). The 96-well-plate was sealed and incubated for 24 h at 36 ± 1 °C air (according to ISO 20776-1), and the OD was then measured at 620 nm with a Sunrise TW absorbance reader (Tecan Trading AG, Männedorf, Switzerland). An absorbance of >0.5 was considered positive for bacterial growth.

A second BMD assay was performed under similar conditions but with a higher bacterial inoculum (hBMD). For the bacterial inoculum, the strains were resuspended in 0.85% saline to a 0.5 McF, diluted 1:2 (5×10^7 CFU/mL), and 10 µL added to the well to a final concentration of 5×10^6 CFU/mL. The plates were sealed and incubated for 18 ± 2 h, and OD was measured.

4.2.2. Agar Dilution

For agar dilution (AD) assay, the Mueller–Hinton agar (dehydrated base from Oxoid, Thermo Scientific, Basingstoke, United Kingdom) was autoclaved and cooled to 45–50 °C and adjusted to a 7.3 pH, and teicoplanin from the stock solution was added to final concentrations of 0.25, 0.5, 1, 2, 4, and 8 mg/L. Additionally, drug-free plates were prepared and used for growth control. The prepared plates were kept wrapped at 4 °C and brought to room temperature before being subjected to previously described procedures [33,35,36]. Briefly, a 0.5 McF (1–2×10^8 CFU/mL) standard bacterial suspension was serially diluted 1:10 to 10^3 CFU/mL, and 10 µL from each dilution was transferred to the plates and incubated for 20–24 h and 48 h, after which the colonies were counted.

4.2.3. Glycopeptide Antibiotic Susceptibility Testing (EUCAST)

EUCAST endorses the use of standard gradient diffusion test (sGDT), macrodilution gradient test (MET), and screening agar as detection methods of glycopeptide non-susceptible *S. aureus* strains [37]. These assays have been recommended by EUCAST for *S. aureus* for research use only but have neither been suggested nor validated for CoNS. The obtained results are therefore not suitable for clinical interpretation.

The teicoplanin standard gradient diffusion strip test (sGDT) was performed according to the manufacturer's instruction using teicoplanin MIC test strips (range 0.016–256 µg/mL) (MTS; Liofilchem, Italy) [38] on a 0.5 McF standard bacterial inoculum on Mueller–Hinton agar (MHE) plates (BioMérieux, France). The MIC in mg/L was read after 16–20 h incubation, representing the point where the formed symmetrical ellipse met the strip.

MET was performed according to EUCAST and the manufacturer's instructions. Briefly, colonies from a 24 h old culture were resuspended in 2 mL 0.85% saline to McF 2 (heavier inoculum), streaked evenly on a brain–heart infusion (BHI) agar (Graso Biotech, Poland), and left to dry. Teicoplanin gradient strips were applied to the surface, incubated at 37 °C air, and read after 24 and 48 h. Not only was the value documented but also the presence of hazes, microcolonies, and isolated colonies.

4.2.4. Screening Agar

For the agar screening method, in-house Mueller–Hinton agar plates with and without 5 mg/L teicoplanin were produced and used based on the previously described protocol [39]. Briefly, colonies were suspended in 0.85% saline to an McF 0.5 and McF 2.0, and 10 µL of each inoculum were evenly distributed on the surface of the agar, incubated at 37 °C in air, and the growth was assessed after 24 and 48 h.

4.2.5. Disc Diffusion

Disc diffusion was performed, even though this approach is no longer EUCAST-recommended. CLSI version 2012 released breakpoints for disc diffusion warning indicating that it is unknown if the method can discriminate between susceptible and resistant strains to teicoplanin. For disc diffusion, the bacterial inoculum was evenly distributed on MHE plates (bioMérieux, Marcy l'Etoile, France), teicoplanin 30 mg discs (Liofilchem, Italy) placed on the surface, and incubated at 36 ± 1 °C in air. The inhibition zone was read after 24 h and interpreted according to the Clinical and Laboratory Standards Institute (CLSI).

4.2.6. Quality Controls

All the performed tests included negative and positive controls. *S. aureus* ATCC 29213 (teicoplanin reference range 0.25–1 mg/L, vancomycin reference range 0.5–2 mg/L) was included as a positive control (quality controls; QC strains) in all the assays under the same conditions as the CoNS strains [40]. The test results were considered valid only when the QC strain was tested within the EUCAST-given ranges. The AD assay included three additional strains as controls: *Enterococcus faecalis* ATCC 29212 (teicoplanin reference range 0.25–1 mg/L, vancomycin reference range 1–4 mg/L); the vancomycin-resistant *S. aureus* (VRSA) strain Mu50 (ATCC 700699); and Mu3 (ATCC 700698), a methicillin-resistant *S. aureus* (MRSA) strain with heterogeneous resistance to vancomycin.

4.3. EUCAST Rules, Results Interpretation, and Data Analysis

All the AST results, except disc diffusion, were interpreted according to EUCAST breakpoints [41] and assigned to susceptible (MIC $\leq$ 4 mg/L) or resistant (MIC > 4 mg/L). The MIC values were reported in serial 1:2 dilutions and intermediate values as the next higher MIC. CLSI criteria were used to assess the results of disc diffusion and sBMD. According to CLSI, the strains were susceptible at MIC $\leq$ 8 mg/L, with zone diameter $\geq$14 mm; intermediate at MIC 16 mg/L, with zone diameter 11–13 mm; or resistant at MIC $\geq$ 32 mg/L, with zone diameter $\leq$ 10 mm [42].

Data were analyzed by comparing the measured MIC values and the corresponding interpretation generated using Vitek-2, hBMD, AD-24 h, AD-48 h, sGDT, MET, and screening agar with those from sBMD, the EUCAST recommended reference method. A very major error (vME) was defined as a false-susceptible result, whereas a major error (ME) was considered a false-resistant result compared with the results of sBMD. An essential agreement (EA) was considered when the MICs fell within the 1 $\log_2$ dilution of the MIC determined using sBMD, while categorical agreement (CA) was assigned to the isolate

rated with the same interpretation category results (S/R) as sBMD. Acceptable performance for a method was defined as a percentage ≥90% for EA, CA, and ≤3% for vME or ME [31].

Author Contributions: Conceptualization, C.R.M. and A.D.B.; methodology, A.D.B.; writing—original draft preparation, A.D.B.; writing—review and editing, C.R.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee) of The Medical Faculty of Heinrich Heine University, Dusseldorf (Protocol Code 5694, on 26.09.2016).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are presented in the tables, no additional data was generated.

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

References

1. Van Epps, J.S.; Younger, J.G. Implantable Device-Related Infection. *Shock* **2016**, *46*, 597–608. [CrossRef] [PubMed]
2. Plachouras, D.; Savey, A.; Palomar, M.; Moro, M.; Lebre, A.; McCoubrey, J. Incidence and microbiology of central line-associated bloodstream infections in European intensive care units: Results from the European Healthcare-Associated Infections surveillance Network (HAI-Net). In Proceedings of the European Conference of Clinical Microbiology and Infectious Diseases (ECCMID), Madrid, Spain, 24 April 2018.
3. Amat-Santos, I.J.; Messika-Zeitoun, D.; Eltchaninoff, H.; Kapadia, S.; Lerakis, S.; Cheema, A.N.; Gutiérrez-Ibanes, E.; Munoz-Garcia, A.J.; Pan, M.; Webb, J.G.; et al. Infective endocarditis after transcatheter aortic valve implantation: Results from a large multicenter registry. *Circulation* **2015**, *131*, 1566–1574. [CrossRef]
4. Bora, P.; Datta, P.; Gupta, V.; Singhal, L.; Chander, J. Characterization and antimicrobial susceptibility of coagulase-negative staphylococci isolated from clinical samples. *J. Lab. Physicians* **2018**, *10*, 414–419. [CrossRef]
5. ECDC. Healthcare-associated infections: Surgical site infections. In *Annual Epidemiological Report for 2017*; ECDC: Solna, Sweden, 2019.
6. Kloos, W.E.; Bannerman, T.L. Update on clinical significance of coagulase-negative staphylococci. *Clin. Microbiol. Rev.* **1994**, *7*, 117–140. [CrossRef]
7. Klingenberg, C.; Aarag, E.; Rønnestad, A.; Sollid, J.E.; Abrahamsen, T.G.; Kjeldsen, G.; Flaegstad, T. Coagulase-negative staphylococcal sepsis in neonates. Association between antibiotic resistance, biofilm formation and the host inflammatory response. *Pediatr. Infect. Dis. J.* **2005**, *24*, 817–822. [CrossRef]
8. ECDC. Healthcare-Associated Infections Acquired in Intensive Care Units. 2019. Available online: https://www.ecdc.europa.eu/sites/default/files/documents/AER_for_2017-HAI.pdf (accessed on 6 December 2021).
9. Kusumoto, F.M.; Schoenfeld, M.H.; Wilkoff, B.L.; Berul, C.I.; Birgersdotter-Green, U.M.; Carrillo, R.; Cha, Y.-M.; Clancy, J.; Deharo, J.-C.; Ellenbogen, K.A.; et al. 2017 HRS expert consensus statement on cardiovascular implantable electronic device lead management and extraction. *Heart Rhythm* **2017**, *14*, e503–e551. [CrossRef] [PubMed]
10. Butler, M.S.; Hansford, K.A.; Blaskovich, M.A.; Halai, R.; Cooper, M.A. Glycopeptide antibiotics: Back to the future. *J. Antibiot.* **2014**, *67*, 631–644. [CrossRef] [PubMed]
11. Yim, G.; Thaker, M.N.; Koteva, K.; Wright, G. Glycopeptide antibiotic biosynthesis. *J. Antibiot.* **2014**, *67*, 31–41. [CrossRef] [PubMed]
12. Svetitsky, S.; Leibovici, L.; Paul, M. Comparative efficacy and safety of vancomycin versus teicoplanin: Systematic review and meta-analysis. *Antimicrob. Agents Chemother.* **2009**, *53*, 4069–4079. [CrossRef]
13. Wood, M.J. The comparative efficacy and safety of teicoplanin and vancomycin. *J. Antimicrob. Chemother.* **1996**, *37*, 209–222. [CrossRef]
14. Kato-Hayashi, H.; Niwa, T.; Ohata, K.; Harada, S.; Matsumoto, T.; Kitagawa, J.; Tsurumi, H.; Suzuki, A. Comparative efficacy and safety of vancomycin versus teicoplanin in febrile neutropenic patients receiving hematopoietic stem cell transplantation. *J. Clin. Pharm. Ther.* **2019**, *44*, 888–894. [CrossRef]
15. Lee, J.Y.H.; Monk, I.R.; Gonçalves da Silva, A.; Seemann, T.; Chua, K.Y.L.; Kearns, A.; Hill, R.; Woodford, N.; Bartels, M.D.; Strommenger, B.; et al. Global spread of three multidrug-resistant lineages of Staphylococcus epidermidis. *Nat. Microbiol.* **2018**, *3*, 1175–1185. [CrossRef] [PubMed]
16. Baris, A.; Malkocoglu, G.; Buyukyanbolu, E.; Aslan, F.M.; Bayraktar, B.; Aktas, E. Evaluation of Teicoplanin Resistance Detected by Automated System in Coagulase Negative Staphylococci: A Comparison with Gradient Test and Broth Microdilution Methods. *Curr. Microbiol.* **2020**, *77*, 3355–3360. [CrossRef] [PubMed]

17. Marincola, G.; Liong, O.; Schoen, C.; Abouelfetouh, A.; Hamdy, A.; Wencker, F.D.R.; Marciniak, T.; Becker, K.; Köck, R.; Ziebuhr, W. Antimicrobial Resistance Profiles of Coagulase-Negative Staphylococci in Community-Based Healthy Individuals in Germany. *Front. Public Health.* **2021**, *9*, 684456. [CrossRef] [PubMed]
18. Del Bene, V.E.; John, J.F.; Twitty, J.A., Jr.; Lewis, J.W. Anti-staphylococcal activity of teicoplanin, vancomycin, and other antimicrobial agents: The significance of methicillin resistance. *J. Infect. Dis.* **1986**, *154*, 349–352. [CrossRef] [PubMed]
19. Cherifi, S.; Byl, B.; Deplano, A.; Nonhoff, C.; Denis, O.; Hallin, M. Comparative epidemiology of Staphylococcus epidermidis isolates from patients with catheter-related bacteremia and from healthy volunteers. *J. Clin. Microbiol.* **2013**, *51*, 1541–1547. [CrossRef] [PubMed]
20. Satola, S.W.; Farley, M.M.; Anderson, K.F.; Patel, J.B. Comparison of detection methods for heteroresistant vancomycin-intermediate Staphylococcus aureus, with the population analysis profile method as the reference method. *J. Clin. Microbiol.* **2011**, *49*, 177–183. [CrossRef] [PubMed]
21. Miragaia, M.; Couto, I.; Pereira, S.F.F.; Kristinsson, K.G.; Westh, H.; Jarløv, J.O.; Carriço, J.; Almeida, J.; Santos-Sanches, I.; de Lencastre, H. Molecular characterization of methicillin-resistant Staphylococcus epidermidis clones: Evidence of geographic dissemination. *J. Clin. Microbiol.* **2002**, *40*, 430–438. [CrossRef]
22. Camargo, C.H.; Mondelli, A.L.; Boas, P.J. Comparison of teicoplanin disk diffusion and broth microdilution methods against clinical isolates of Staphylococcus aureus and S. epidermidis. *Braz. J. Microbiol.* **2011**, *42*, 1265–1268. [CrossRef]
23. Sieradzki, K.; Villari, P.; Tomasz, A. Decreased susceptibilities to teicoplanin and vancomycin among coagulase-negative methicillin-resistant clinical isolates of staphylococci. *Antimicrob. Agents Chemother.* **1998**, *42*, 100–107. [CrossRef]
24. Howden, B.P.; Davies, J.K.; Johnson, P.D.; Stinear, T.P.; Grayson, M.L. Reduced vancomycin susceptibility in Staphylococcus aureus, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: Resistance mechanisms, laboratory detection, and clinical implications. *Clin. Microbiol. Rev.* **2010**, *23*, 99–139. [CrossRef]
25. Becker, K.; Heilmann, C.; Peters, G. Coagulase-negative staphylococci. *Clin. Microbiol. Rev.* **2014**, *27*, 870–926. [CrossRef]
26. Schwalbe, R.S.; Stapleton, J.T.; Gilligan, P.H. Emergence of vancomycin resistance in coagulase-negative staphylococci. *N. Engl. J. Med.* **1987**, *316*, 927–931. [CrossRef] [PubMed]
27. Arioli, V.; Pallanza, R. Teicoplanin-resistant coagulase-negative staphylococci. *Lancet* **1987**, *1*, 39. [CrossRef] [PubMed]
28. Biavasco, F.; Vignaroli, C.; Varaldo, P.E. Glycopeptide resistance in coagulase-negative staphylococci. *Eur. J. Clin. Microbiol. Infect. Dis.* **2000**, *19*, 403–417. [CrossRef]
29. Cavenaghi, L.A.; Biganzoli, E.; Danese, A.; Parenti, F. Diffusion of teicoplanin and vancomycin in agar. *Diagn. Microbiol. Infect. Dis.* **1992**, *15*, 253–258. [CrossRef] [PubMed]
30. Vaudaux, P.; Huggler, E.; Bernard, L.; Ferry, T.; Renzoni, A.; Lew, D.P. Underestimation of vancomycin and teicoplanin MICs by broth microdilution leads to underdetection of glycopeptide-intermediate isolates of Staphylococcus aureus. *Antimicrob. Agents Chemother.* **2010**, *54*, 3861–3870. [CrossRef]
31. Humphries, R.M.; Ambler, J.; Mitchell, S.L.; Castanheira, M.; Dingle, T.; Hindler, J.A.; Koeth, L.; Sei, K. CLSI Methods Development and Standardization Working Group Best Practices for Evaluation of Antimicrobial Susceptibility Tests. *J. Clin. Microbiol.* **2018**, *56*, e01934-17. [CrossRef]
32. Campana, E.H.; Carvalhaes, C.G.; Nonato, B.; Machado, A.M.; Gales, A.C. Comparison of M.I.C.E. and Etest with CLSI agar dilution for antimicrobial susceptibility testing against oxacillin-resistant *Staphylococcus* spp. *PLoS ONE* **2014**, *9*, e94627. [CrossRef]
33. Wootton, M.; Howe, R.A.; Hillman, R.; Walsh, T.R.; Bennett, P.M.; MacGowan, A.P. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in Staphylococcus aureus in a UK hospital. *J. Antimicrob. Chemother.* **2001**, *47*, 399–403. [CrossRef]
34. Matuschek, E.; Åhman, J.; Webster, C.; Kahlmeter, G. Antimicrobial susceptibility testing of colistin—Evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. *Clin. Microbiol. Infect.* **2018**, *24*, 865–870. [CrossRef] [PubMed]
35. EUCAST. Media Preparation for EUCAST Disk Diffusion Testing and for Determination of MIC Values by the Broth Microdilution Method. 2020. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2020_manuals/Media_preparation_v_6.0_EUCAST_AST.pdf (accessed on 6 December 2021).
36. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. 2012. Available online: https://clsi.org/standards/products/microbiology/documents/m07/ (accessed on 6 December 2021).
37. EUCAST. EUCAST Guidelines for Detection of Resistance Mechanisms and Specific Resistances of Clinical and/or Epidemiological Importance, Version 2.0. 2017. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf (accessed on 6 December 2021).
38. Liofilchem. MTSTM Technical Sheet Staphylococci-Rev.5/ 31 May 2021. 2021. Available online: https://www.liofilchem.com/images/brochure/mic_test_strip_patent/MTS20.pdf (accessed on 10 June 2022).
39. Hiramatsu, K.; Aritaka, N.; Hanaki, H.; Kawasaki, S.; Hosoda, Y.; Hori, S.; Fukuchi, Y.; Kobayashi, I. Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to vancomycin. *Lancet* **1997**, *350*, 1670–1673. [CrossRef] [PubMed]

40. EUCAST. Routine and Extended Internal Quality Control for MIC Determination and Disk Diffusion as Recommended by EUCAST. 2016. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/QC/v_6.1_EUCAST_QC_tables_routine_and_extended_QC.pdf (accessed on 10 June 2022).
41. EUCAST. Breakpoint Tables for Interpretation of MICs and Zone Diameters Version 7.1, valid from 10 March 2017. 2017. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf (accessed on 10 June 2022).
42. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*; Twenty-Second Informational Supplement 2012; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2012.

3 Discussion

The AST results and the institutional yearly resistance statistics confirmed previously published data [13, 34, 51, 52] that CoNS strains are highly resistant to most commonly used beta-lactam antibiotic agents, leaving glycopeptides, linezolid, and daptomycin as the most important therapeutic options. A number of aspects should be considered when choosing the appropriate treatment including side effects, risk of developing resistance during therapy, therapeutic drug monitoring, cost, and availability. Teicoplanin has been considered an alternative to vancomycin due to its lower nephrotoxicity, reduced drug interactions, and once-daily administration.

Teicoplanin resistance has been reported in the USA and the UK since the early 1980s, but the published data since then [53] do not reflect the actual incidence and its impact on therapeutical use. Teicoplanin resistance is an increasing and emerging challenge, but published data are inconclusive due to a number of factors. These include the different methods employed (e.g., broth microdilution vs. disk diffusion [34]); settings (diagnostic vs. research e.g., broth microdilution vs. population analysis); the standards employed (e.g., Clinical & Laboratory Standards Institute- CLSI vs. EUCAST defined breakpoints); the inclusion of diverse cohorts (e.g., catheter-related bacteremia vs. healthy volunteers [18, 52]; the bacterial species studied (most studies have focused on S. aureus and fewer on CoNS [54]); clonal dissemination [55]; data generated at different time points [56]; or that teicoplanin was not tested. Thus, to date, reports have probably underestimated the true incidence of teicoplanin resistance and are still insufficient to identify its underlying mechanisms with certainty.

It is still unclear if increasing teicoplanin resistance should be attributed to one or several possible underlying mechanisms. The mechanism is neither well defined nor adequately studied. Several mechanisms have been proposed such as cellular aggregates and antibiotic retention [57] or cell-wall alteration through reorganization or thickening [20, 58]. Perhaps even more worrying is that teicoplanin resistance has been shown to develop under therapy [59, 60]. Biavasco et al. pointed out that the testing methods employed for teicoplanin can be easily influenced by technical factors such as the methodology, media,

inoculum, and incubation time [61]. Furthermore, it has been shown that the physical properties of teicoplanin—a large, lipophilic, and negatively charged molecule have an impact upon testing by generating a lower diffusion coefficient on agar compared with vancomycin [62].

Broth microdilution is generally regarded as the gold standard method for antibiotic susceptibility testing; however, few laboratories use it for routine purposes. To optimize laboratory workflow with a high sample throughput, semi-automated devices such as Vitek-2 are employed routinely for the AST of fast-growing bacteria. Generally speaking, Vitek-2 performs well; It is fast and robust, with minimum hands-on time, is cost-effective, and requires little technical expertise. In our laboratory, using Vitek-2, a rapid rise in teicoplanin-resistant CoNS strains was observed in 2015. Baris et al. also reported an increased number of teicoplanin-resistant strains with a similar semi-automated system, BD Phoenix [51]. In this study, as in ours, most of the samples tested as teicoplanin-resistant using Vitek-2 were not confirmed via sBMD, resulting in the highest rate of ME among the AST methods. The majority of teicoplanin MIC, determined using Vitek-2 were either 4 or 8 mg/L (56.8%) – at or close to the EUCAST epidemiological cut-off (ECOFF) value for CoNS (MIC 4 mg/L), thus having an impact upon the generated EA and CA. Meanwhile, most of the MIC in sBMD (54.7%) were concentrated at the upper limit of the range (2 or 4 mg/L), thus conforming to the published EUCAST MIC distribution determining the teicoplanin breakpoints for CoNS. Vaudaux et al. found a similar MIC distribution using macrodilution but not microdilution. Moreover, MIC distribution was different when performed using macrodilution or microdilution [63].

According to these results, the AST performance for teicoplanin does not fulfill the CLSI criteria of 90% agreement for both EA and CA [64]. It is difficult to establish a diagnostic workflow that reliably confirms teicoplanin resistance among routinely tested strains. Firstly, EA and CA differ in test, antibiotic, and methodology, confirming the results of Campana et al. Moreover, their results showed that EA and CA vary with species (e.g., CA for strip test for *S. aureus* (100%) vs. 75% for CoNS according to CLSI) [65]. Secondly, most of the routinely employed AST assays use a low bacterial inoculum and are fast, whereas the strains that might bear heteroresistance are first detected at CFU above 10^6

CFU/mL and after a longer incubation time (48 h). With a final inoculum of 5 × 10^5 CFU/well, the microdilution assay, the current gold standard method, is unable to reliably detect heteroresistance [63]. Routinely employed methods probably do not detect heteroresistant strains, which may have a negative impact on therapeutic outcomes.

Due to the discussed difficulty in obtaining reliable, consistent susceptibility data for teicoplanin in CoNS it would be useful to generate a diagnostic algorithm, in which screening for resistance in a first simple test would detect possible resistance, e.g., a rapid automated AST and lead to further, more labor-intensive testing such as broth. Or agar-dilution, which involve a high inoculum and a longer incubation. The second method should preferably be fast, commercially available for routine settings, have a low cost, and be reliable and reproducible. A possible option would be MET. MET is a method with low hands-on time, but adjustments are needed for it to be as reliable for use with CoNS as it is for *S. aureus*. The strains with suspected teicoplanin resistance could be further tested in reference laboratories by means of population analysis profiles (PAPs), whichis the gold standard method to detect heteroresistance. This is a demanding time-consuming method, difficult to implement in a routine setting, and poses the risk of selecting in vitro resistance instead of finding it [66]. Using a different method in the second step is challenging because not all laboratories have the option to produce the necessary in-house plates.

These results do not detect an increased vancomycin resistance associated with teicoplanin resistance as previously predicted. This may be due to an underlying mechanism that involves only teicoplanin or that the teicoplanin molecule presents technical difficulties causing an unreliable result [33]. In this respect similar results are seen with colistin resistance testing [67].

The study has drawbacks. It is a retrospective, descriptive and focuses mainly on the microbiology aspects. For a complete overview of the situation further studies are needed which will include clinical aspects and more importantly the response to therapy.

In conclusion, extensive teicoplanin susceptibility testing showed that the results obtained using a single method could not be fully confirmed by employing various other methods. Due to a high discrepancy among the methods tested, no suitable algorithm can be proposed to reliably detect teicoplanin resistance. The fact that the results were so diverse suggests that all the aspects involved in teicoplanin testing should be re-evaluated so that improvements can be made not only in the laboratory but also in establishing reliable breakpoints. Given the relevance that these results pose for antibiotic therapy, further clinical studies looking into the clinical efficacy of teicoplanin and in vitro teicoplanin testing are of great importance.

4 Bibliography and list of sources

1. Balasiu AD, MacKenzie CR. Teicoplanin-Resistant Coagulase-Negative Staphylococci: Do the Current Susceptibility Testing Methods Reliably Detect This Elusive Phenotype? Antibiotics (Basel). 2023;12(3). 10.3390/antibiotics12030611.

2. Ogston A. Micrococcus Poisoning. J Anat Physiol. 1882;17(Pt 1):24-58.

3. Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. International Journal of Systematic and Evolutionary Microbiology. 2020;70(11):5607-12. https://doi.org/10.1099/ijsem.0.004332.

4. FJ. R. Micro-Organismen bei den Wund-Infections Krankeiten des Menschens.: J F Bergmann; 1884.

5. Fairbrother R. Coagulase production as a criterion for the classification of the staphylococci. The Journal of Pathology and Bacteriology. 2005;50:83-8. 10.1002/path.1700500112.

6. Garrity G, De Vos P, Jones D, Kreig N, Ludwig W, Rainey F, et al. Bergey's Manual of Systematic Bacteriology. Volume 3. The Firmicutes2010.

7. Lamers RP, Muthukrishnan G, Castoe TA, Tafur S, Cole AM, Parkinson CL. Phylogenetic relationships among Staphylococcus species and refinement of cluster groups based on multilocus data. BMC Evolutionary Biology. 2012;12(1):171. 10.1186/1471-2148-12-171.

8. Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. Clin Microbiol Rev. 1994;7(1):117-40. 10.1128/CMR.7.1.117.

9. Schleifer KH, Kloos WE. Isolation and Characterization of Staphylococci from Human Skin I. Amended Descriptions of Staphylococcus epidermidis and Staphylococcus saprophyticus and Descriptions of Three New Species: Staphylococcus cohnii, Staphylococcus haemolyticus, and Staphylococcus xylosus. International Journal of Systematic and Evolutionary Microbiology. 1975;25(1):50-61. https://doi.org/10.1099/00207713-25-1-50.

10. Kloos WE, Schleifer KH. Isolation and Characterization of Staphylococci from Human Skin II. Descriptions of Four New Species: Staphylococcus warneri, Staphylococcus capitis, Staphylococcus hominis, and Staphylococcus simulans1. International Journal of Systematic and Evolutionary Microbiology. 1975;25(1):62-79. https://doi.org/10.1099/00207713-25-1-62.

11. Colleen S, Hovelius B, Wieslander A, Mårdh PA. Surface properties of Staphylococcus saprophyticus and Staphylococcus epidermidis as studied by adherence tests and two-polymer, aqueous phase systems. Acta Pathol Microbiol Scand B. 1979;87(6):321-8. 10.1111/j.1699-0463.1979.tb02446.x.

12. Tacconelli E, Tumbarello M, Donati KG, Bettio M, Spanu T, Leone F, et al. Glycopeptide resistance among coagulase-negative staphylococci that cause bacteremia: epidemiological and clinical findings from a case-control study. Clin Infect Dis. 2001;33(10):1628-35. 10.1086/323676.

13. Lee JYH, Monk IR, Goncalves da Silva A, Seemann T, Chua KYL, Kearns A, et al. Global spread of three multidrug-resistant lineages of Staphylococcus epidermidis. Nat Microbiol. 2018;3(10):1175-85. 10.1038/s41564-018-0230-7.

14. Begović J, Jovčić B, Papić-Obradović M, Veljović K, Lukić J, Kojić M, et al. Genotypic diversity and virulent factors of Staphylococcus epidermidis isolated from human breast milk. Microbiological Research. 2013;168(2):77-83. https://doi.org/10.1016/j.micres.2012.09.004.

15. Lina B, Vandenesch F, Etienne J, Kreiswirth B, Fleurette J. Comparison of coagulase-negative staphylococci by pulsed-field electrophoresis. FEMS Microbiol Lett. 1992;71(2):133-38. 10.1016/0378-1097(92)90501-e.

16. Lang S, Livesley MA, Lambert PA, Elliott J, Elliott TS. The genomic diversity of coagulase-negative staphylococci associated with nosocomial infections. J Hosp Infect. 1999;43(3):187-93. 10.1053/jhin.1999.0245.

17. Wisplinghoff H, Rosato AE, Enright MC, Noto M, Craig W, Archer GL. Related clones containing SCCmec type IV predominate among clinically significant Staphylococcus epidermidis isolates. Antimicrob Agents Chemother. 2003;47(11):3574-9. 10.1128/aac.47.11.3574-3579.2003.

18. Cherifi S, Byl B, Deplano A, Nonhoff C, Denis O, Hallin M. Comparative epidemiology of Staphylococcus epidermidis isolates from patients with catheter-related bacteremia and from healthy volunteers. J Clin Microbiol. 2013;51(5):1541-7. 10.1128/JCM.03378-12.

19. Miragaia M, Thomas JC, Couto I, Enright MC, de Lencastre H. Inferring a population structure for Staphylococcus epidermidis from multilocus sequence typing data. J Bacteriol. 2007;189(6):2540-52. 10.1128/jb.01484-06.

20. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin Microbiol Rev. 2014;27(4):870-926. 10.1128/cmr.00109-13.

21. Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, et al. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol. 2006;4(4):295-305. 10.1038/nrmicro1384.

22. Besier S, Ludwig A, Ohlsen K, Brade V, Wichelhaus TA. Molecular analysis of the thymidine-auxotrophic small colony variant phenotype of Staphylococcus aureus. Int J Med Microbiol. 2007;297(4):217-25. 10.1016/j.ijmm.2007.02.003.

23. Sadovskaya I, Vinogradov E, Li J, Jabbouri S. Structural elucidation of the extracellular and cell-wall teichoic acids of Staphylococcus epidermidis RP62A,

a reference biofilm-positive strain. Carbohydr Res. 2004;339(8):1467-73. 10.1016/j.carres.2004.03.017.

24. Büttner H, Mack D, Rohde H. Structural basis of Staphylococcus epidermidis biofilm formation: mechanisms and molecular interactions. Frontiers in Cellular and Infection Microbiology. 2015;5. 10.3389/fcimb.2015.00014.

25. Heilmann C, Ziebuhr W, Becker K. Are coagulase-negative staphylococci virulent? Clin Microbiol Infect. 2019;25(9):1071-80. 10.1016/j.cmi.2018.11.012.

26. EUCAST. Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method 2020 [Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2020_manuals/Media_preparation_v_6.0_EUCAST_AST.pdf (accessed on 6 of December 2021).

27. 20776-1:2019 DEI. Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices- Part 1: Broth micro-dilution refence method for testing antmicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases 2020 (accessed on 6 of December 2021)

28. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6.

29. EUCAST. Breakpoint tables for interpretation of MICs and zone diameters Version 7.1, valid from 2017-03-10 2017 [Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf (accessed on 6 of December 2021).

30. Schmidt VM, Williams NJ, Pinchbeck G, Corless CE, Shaw S, McEwan N, et al. Antimicrobial resistance and characterisation of staphylococci isolated from healthy Labrador retrievers in the United Kingdom. BMC Veterinary Research. 2014;10(1):17. 10.1186/1746-6148-10-17.

31. VanEpps JS, Younger JG. Implantable Device-Related Infection. Shock. 2016;46(6):597-608. 10.1097/shk.0000000000000692.

32. Plachouras D, Savey A, Palomar M, Moro M, Lebre A, McCoubrey J. Incidence and microbiology of central line-associated bloodstream infections in European intensive care units: Results from the European Healthcare-Associated Infections surveillance Network (HAI-Net). ePoster presented at: European Conference of Clinical Microbiology and Infectious Diseases (ECCMID); 24 April 2018. Madrid, Spain.

33. Amat-Santos IJ, Messika-Zeitoun D, Eltchaninoff H, Kapadia S, Lerakis S, Cheema AN, et al. Infective endocarditis after transcatheter aortic valve implantation: results from a large multicenter registry. Circulation. 2015;131(18):1566-74. 10.1161/circulationaha.114.014089.

34. Bora P, Datta P, Gupta V, Singhal L, Chander J. Characterization and antimicrobial susceptibility of coagulase-negative staphylococci isolated from clinical samples. J Lab Physicians. 2018;10(4):414-9. 10.4103/JLP.JLP_55_18.

35. ECDC. Healthcare-associated infections: surgical site infections

Annual Epidemiological Report for 2017. 2019.

36. Klingenberg C, Aarag E, Rønnestad A, Sollid JE, Abrahamsen TG, Kjeldsen G, et al. Coagulase-negative staphylococcal sepsis in neonates. Association between antibiotic resistance, biofilm formation and the host inflammatory response. Pediatr Infect Dis J. 2005;24(9):817-22. 10.1097/01.inf.0000176735.20008.cd.

37. ECDC. Healthcare-associated infections acquired in intensive care units 2019 [Available from: https://www.ecdc.europa.eu/sites/default/files/documents/AER_for_2017-HAI.pdf (accessed on 6 of December 2021).

38. Kusumoto FM, Schoenfeld MH, Wilkoff BL, Berul CI, Birgersdotter-Green UM, Carrillo R, et al. 2017 HRS expert consensus statement on cardiovascular implantable electronic device lead management and extraction. Heart Rhythm. 2017;14(12):e503-e51. 10.1016/j.hrthm.2017.09.001.

39. Butler MS, Hansford KA, Blaskovich MA, Halai R, Cooper MA. Glycopeptide antibiotics: back to the future. J Antibiot (Tokyo). 2014;67(9):631-44. 10.1038/ja.2014.111.

40. Nicolaou KC, Boddy CN, Bräse S, Winssinger N. Chemistry, Biology, and Medicine of the Glycopeptide Antibiotics. Angew Chem Int Ed Engl. 1999;38(15):2096-152. 10.1002/(sici)1521-3773(19990802)38:15<2096::aid-anie2096>3.0.co;2-f.

41. Van Bambeke F, Van Laethem Y, Courvalin P, Tulkens PM. Glycopeptide antibiotics: from conventional molecules to new derivatives. Drugs. 2004;64(9):913-36. 10.2165/00003495-200464090-00001.

42. Parenti F, Beretta G, Berti M, Arioli V. Teichomycins, new antibiotics from Actinoplanes teichomyceticus Nov. Sp. I. Description of the producer strain, fermentation studies and biological properties. J Antibiot (Tokyo). 1978;31(4):276-83. 10.7164/antibiotics.31.276.

43. Borghi A, Antonini P, Zanol M, Ferrari P, Zerilli LF, Lancini GC. Isolation and structure determination of two new analogs of teicoplanin, a glycopeptide antibiotic. The Journal of Antibiotics. 1989;42(3):361-6.

44. Malabarba A, Strazzolini P, Depaoli A, Landi M, Berti M, Cavalleri B. Teicoplanin, antibiotics from Actinoplanes teichomyceticus nov. sp. VI. Chemical degradation: physico-chemical and biological properties of acid hydrolysis products. J Antibiot (Tokyo). 1984;37(9):988-99. 10.7164/antibiotics.37.988.

45. Jung H-M, Jeya M, Kim S-Y, Moon H-J, Kumar Singh R, Zhang Y-W, et al. Biosynthesis, biotechnological production, and application of teicoplanin: current

state and perspectives. Applied Microbiology and Biotechnology. 2009;84(3):417-28. 10.1007/s00253-009-2107-4.

46. Agency EEM. Teicoplanin

Assessment report. 2013.

47. Svetitsky S, Leibovici L, Paul M. Comparative efficacy and safety of vancomycin versus teicoplanin: systematic review and meta-analysis. Antimicrob Agents Chemother. 2009;53(10):4069-79. 10.1128/AAC.00341-09.

48. Wood MJ. The comparative efficacy and safety of teicoplanin and vancomycin. J Antimicrob Chemother. 1996;37(2):209-22. 10.1093/jac/37.2.209.

49. Kato-Hayashi H, Niwa T, Ohata K, Harada S, Matsumoto T, Kitagawa J, et al. Comparative efficacy and safety of vancomycin versus teicoplanin in febrile neutropenic patients receiving hematopoietic stem cell transplantation. J Clin Pharm Ther. 2019;44(6):888-94. 10.1111/jcpt.13011.

50. Wilson AP. Clinical pharmacokinetics of teicoplanin. Clin Pharmacokinet. 2000;39(3):167-83. 10.2165/00003088-200039030-00001.

51. Baris A, Malkocoglu G, Buyukyanbolu E, Aslan FM, Bayraktar B, Aktas E. Evaluation of Teicoplanin Resistance Detected by Automated System in Coagulase Negative Staphylococci: A Comparison with Gradient Test and Broth Microdilution Methods. Curr Microbiol. 2020;77(11):3355-60. 10.1007/s00284-020-02144-7.

52. Marincola G, Liong O, Schoen C, Abouelfetouh A, Hamdy A, Wencker FDR, et al. Antimicrobial Resistance Profiles of Coagulase-Negative Staphylococci in Community-Based Healthy Individuals in Germany. Front Public Health. 2021;9:684456. 10.3389/fpubh.2021.684456.

53. Del Bene VE, John JF, Jr., Twitty JA, Lewis JW. Anti-staphylococcal activity of teicoplanin, vancomycin, and other antimicrobial agents: the significance of methicillin resistance. J Infect Dis. 1986;154(2):349-52. 10.1093/infdis/154.2.349.

54. Satola SW, Farley MM, Anderson KF, Patel JB. Comparison of detection methods for heteroresistant vancomycin-intermediate Staphylococcus aureus, with the population analysis profile method as the reference method. J Clin Microbiol. 2011;49(1):177-83. 10.1128/JCM.01128-10.

55. Miragaia M, Couto I, Pereira SF, Kristinsson KG, Westh H, Jarlov JO, et al. Molecular characterization of methicillin-resistant Staphylococcus epidermidis clones: evidence of geographic dissemination. J Clin Microbiol. 2002;40(2):430-8. 10.1128/JCM.40.2.430-438.2002.

56. Camargo CH, Mondelli AL, Boas PJ. Comparison of teicoplanin disk diffusion and broth microdilution methods against clinical isolates of Staphylococcus aureus and S. epidermidis. Braz J Microbiol. 2011;42(4):1265-8. 10.1590/S1517-83822011000400003.

57. Sieradzki K, Villari P, Tomasz A. Decreased susceptibilities to teicoplanin and vancomycin among coagulase-negative methicillin-resistant clinical isolates of staphylococci. Antimicrob Agents Chemother. 1998;42(1):100-7. 10.1128/aac.42.1.100.

58. Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in Staphylococcus aureus, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev. 2010;23(1):99-139. 10.1128/cmr.00042-09.

59. Schwalbe RS, Stapleton JT, Gilligan PH. Emergence of vancomycin resistance in coagulase-negative staphylococci. N Engl J Med. 1987;316(15):927-31. 10.1056/nejm198704093161507.

60. Arioli V, Pallanza R. Teicoplanin-resistant coagulase-negative staphylococci. Lancet. 1987;1(8523):39. 10.1016/s0140-6736(87)90724-0.

61. Biavasco F, Vignaroli C, Varaldo PE. Glycopeptide resistance in coagulase-negative staphylococci. Eur J Clin Microbiol Infect Dis. 2000;19(6):403-17. 10.1007/s100960000299.

62. Cavenaghi LA, Biganzoli E, Danese A, Parenti F. Diffusion of teicoplanin and vancomycin in agar. Diagnostic Microbiology and Infectious Disease. 1992;15(3):253-8. https://doi.org/10.1016/0732-8893(92)90121-9.

63. Vaudaux P, Huggler E, Bernard L, Ferry T, Renzoni A, Lew DP. Underestimation of vancomycin and teicoplanin MICs by broth microdilution leads to underdetection of glycopeptide-intermediate isolates of Staphylococcus aureus. Antimicrob Agents Chemother. 2010;54(9):3861-70. 10.1128/AAC.00269-10.

64. Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, et al. CLSI Methods Development and Standardization Working Group Best Practices for Evaluation of Antimicrobial Susceptibility Tests. J Clin Microbiol. 2018;56(4). 10.1128/JCM.01934-17.

65. Campana EH, Carvalhaes CG, Nonato B, Machado AM, Gales AC. Comparison of M.I.C.E. and Etest with CLSI agar dilution for antimicrobial susceptibility testing against oxacillin-resistant Staphylococcus spp. PLoS One. 2014;9(4):e94627. 10.1371/journal.pone.0094627.

66. Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in Staphylococcus aureus in a UK hospital. J Antimicrob Chemoth. 2001;47(4):399-403. DOI 10.1093/jac/47.4.399.

67. Matuschek E, Åhman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin - evaluation of seven commercial MIC products against standard broth microdilution for Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter spp. Clin Microbiol Infect. 2018;24(8):865-70. 10.1016/j.cmi.2017.11.020.