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Performance of different commercial methods for determining minimum inhibitory concentrations of glycopeptides and linezolid against blood isolates of *Staphylococcus aureus*

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ABSTRACT

The aim of this study was to determine the accuracy of commercial systems (VITEK® 2, Etest and Sensititre®) in determining the minimum inhibitory concentrations of vancomycin, teicoplanin and linezolid of *Staphylococcus aureus* strains and to evaluate the reproducibility of each system in a clinical microbiology laboratory. In total, 115 strains of *S. aureus* isolated from blood cultures were tested with all three commercial methods as well as the broth microdilution method, which is designated as the standard for glycopeptides and linezolid. Fourteen different *S. aureus* strains were included in a reproducibility test for all methods and antibiotics. For these strains, antimicrobial susceptibility testing was repeated 10 times on different days with all four methods, each time using the same inoculum. All three commercial methods exhibited similar performance in categorisation of nearly all of the meticillin-susceptible *S. aureus* (MSSA) isolates. Discrepancies were registered for meticillin-resistant *S. aureus* (MRSA); 2.5% of the strains in the intermediate or resistant category with the VITEK 2 system were not recognised as resistant by Etest and Sensititre. Moreover, none of the three commercial methods provided accurate results compared with homemade broth microdilution. Reproducibility of vancomycin and teicoplanin was 100% with VITEK 2 and Sensititre and 98.75% with Etest. Microdilution showed a reproducibility of 95.6% with vancomycin and 83.1% with teicoplanin. In contrast to previous reports, the best agreement with microdilution was exhibited by VITEK 2 both for MSSA and MRSA. For the antibiotics tested, the best reproducibility was obtained with the VITEK 2 and Sensititre systems.

1. Introduction

Staphylococcus aureus is responsible for a wide range of diseases such as bacteraemia, pneumonia, endocarditis, osteomyelitis, septic arthritis, and infections of skin and soft tissue. The acquisition of antibiotic resistance, in particular development of resistance to meticillin, has become a problem for many hospitals around the world and recently also in the community [1].

Hospital infections involving meticillin-resistant *S. aureus* (MRSA) have become very common and tend to have a poor prognosis, with a worse outcome compared with meticillin-sensitive *S. aureus* (MSSA) [2,3].

Control of MRSA requires extensive knowledge of its role in infection, its epidemiology, its tendency to be clonal as well as its ability to accumulate resistance to many antimicrobials such as aminoglycosides, macrolides and fluoroquinolones [1,4]. In addition, the emergence of vancomycin-intermediate *S. aureus* (VISA) with minimum inhibitory concentrations (MICs) in the range of 4–8 mg/L, heterogeneous VISA (hVISA) containing subpopulations of cells for which the vancomycin MICs are 4–8 mg/L, and high-level vancomycin-resistant *S. aureus* (VRSA) with MICs $\geq$ 16 mg/L, although relatively uncommon, raises additional problems in the use of vancomycin for the treatment of infections caused by MRSA [5,6].

Recently, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/clinical_breakpoints/; clinical breakpoints v.3.1) modified the interpretive breakpoints for vancomycin susceptibility to $\leq$2 mg/L for susceptible strains and >2 mg/L for resistant strains. The same path was followed by the Clinical and Laboratory Standards Institute (CLSI), which in 2006 modified vancomycin breakpoints in the following way: from $\leq$4 mg/L to $\leq$2 mg/L to indicate a susceptible strain, whereas MICs of 4–8 mg/L and $\geq$16 mg/L are categorised as intermediate and resistant, respectively [7].

In vitro categorisation of an isolate as susceptible or resistant to an antibiotic is generally the parameter used by clinicians to select

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the appropriate therapy for bacterial infectious diseases. However, clinical studies on the use of vancomycin for the therapy of serious infections caused by *S. aureus* showed a lack of correlation between in vitro susceptibility and therapeutic outcome [5]. In these studies, the efficacy of vancomycin in the treatment of invasive *S. aureus* infection was higher if the isolate, categorised as susceptible, had an MIC of ≤0.5 mg/L, i.e. four times less than the susceptibility breakpoint (2 mg/L) [8,9]. This raised the possibility that MIC determination had a better predictive value than simple categorisation as susceptible for the outcome of serious *S. aureus* infections [9]. As a consequence, the clinical microbiology laboratory should assist the clinician by providing the MIC of the antibiotics used for treatment.

According to the CLSI and EUCAST, the most suitable method for determining the MIC of vancomycin is the broth microdilution method, but this method is very laborious and time consuming. However, several commercial methods that make MIC determination less cumbersome than homemade broth microdilution are now available for the clinical microbiology laboratory [10,11].

The aim of this study was to evaluate the performance of some of these commercial assays, such as Etest and VITEK® 2, also considering Sensititre® among the commercial methodologies available in a clinical microbiology laboratory, in determining the MIC of antistaphylococcal antibiotics largely used in the therapy of serious infections. We performed for the first time a complete performance analysis, not only on vancomycin but also on other last-resource antibiotics such as teicoplanin and linezolid, for *S. aureus* bloodstream infections (BSIs) in order to provide clinicians with important information about the susceptibility level of the isolate responsible for infections without burdening technicians with additional cumbersome and time-consuming assays.

Furthermore, this study contributes to EUCAST criteria diffusion in order to help clinicians understand the different interpretation rules on glycopeptides with CLSI. This study also helps to evaluate the reproducibility of each commercial system, answering clinical questions about how reliable and useful the laboratory MIC data are for patient treatment.

2. Materials and methods

2.1. Bacterial strains

This study involved 115 *S. aureus* strains (75 MSSA and 40 MRSA) isolated from blood cultures during the period 2008–2010 from different patients and clinical settings at the University Hospital of Verona, Italy.

All of the strains were subcultured after removal from frozen storage. All of the methods and systems were tested on the same day using the same inoculum. Among the 115 strains, 40 were defined as MRSA by conventional methods [12].

Quality control strains tested were *S. aureus* ATCC 29213 (MSSA) and *Enterococcus faecalis* ATCC 29212.

2.2. Antimicrobial susceptibility testing (AST) methods

Broth microdilution arrays were prepared in the laboratory with ranges of two-fold dilutions from 0.12 mg/L to 128 mg/L for all the antibiotics tested, using cation-adjusted Muller–Hinton broth (Merck, Darmstadt, Germany).

ITSTAF panels were provided by Sensititre (TREK Diagnostic Systems, Cleveland, OH) and contained two-fold dilutions of vancomycin from 0.06 mg/L to 32 mg/L, teicoplanin from 0.03 mg/L to 32 mg/L and linezolid from 0.06 mg/L and 16 mg/L.

Etest strips (bioMérieux, Dalvägen, Sweden) were made by gradient from 0.016 mg/L to 256 mg/L of all three antibiotics and the test was performed on Mueller–Hinton plates following the manufacturer's instructions.

The AST-P580 panel of the VITEK 2 system (bioMérieux, Lyon, France) was used: vancomycin and teicoplanin had a range of ≤0.5 mg/L to ≥32 mg/L, whilst the linezolid range was between ≤0.5 mg/L and ≥8 mg/L. The results were interpreted with VITEK 2 system software v.5.01.

All of the AST results were interpreted according to the latest EUCAST (http://www.eucast.org/clinical_breakpoints/; clinical breakpoints v.3.1) and CLSI [13] documents. For EUCAST interpretation, the following breakpoints were used: vancomycin, susceptible (S) ≤2 mg/L, resistant (R) >2 mg/L; teicoplanin, S ≤2 mg/L, R, >2 mg/L; and linezolid, S ≤4 mg/L, R >4 mg/L. For CLSI interpretation, the following breakpoints were used: vancomycin, S ≤2 mg/L; intermediate (I) = 4–8 mg/L, R ≥16 mg/L; teicoplanin, S ≤8 mg/L, I = 16 mg/L, R ≥32 mg/L; and linezolid, S ≤4, R ≥8 mg/L. Results are reported separately for MSSA and MRSA.

Broth microdilution was considered the standard reference method for data analysis. Essential agreement (EA) was considered as results within an MIC ±1 $\log_2$ dilution. For Etest, non-$\log_2$ concentrations were rounded up to the next $\log_2$ concentration for data analysis. All MIC data were read by two independent readings, and discordant results were resolved by a third person blinded to the other results.

2.3. Reproducibility testing

Fourteen different *S. aureus* strains (four MSSA and ten MRSA), including the strains expressing resistant or intermediate category with one of the methods, as well as the *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 control strains were included in a reproducibility test for broth microdilution, VITEK 2, Sensititre and Etest.

For these strains, AST was repeated 10 times on different days with all four methods. Each time for the four methods, the same inoculum was used.

For evaluation of intralaboratory reproducibility, MIC data generated with broth microdilution, VITEK 2, Etest and Sensititre were first converted to $\log_2$ values. In the case of Etest readings, MIC data that ranked between two-fold dilutions were rounded up to the next two-fold dilution before conversion to the $\log_2$ scale. For each series of ten replicate MIC measurements for a specific strain–antimicrobial combination, the median of the $\log_2$ series and the $\log_2$ value of the median were determined. Finally, the mathematical differences between each of the ten $\log_2$ values in the replicate series and the $\log_2$ value of the median were summed. Analysis of these summed $\log_2$ distances (SLD) across all strain–antimicrobial combinations allowed classification of the variations in MIC data into different SLD categories. Intralaboratory reproducibility was considered acceptable for data sets classified into categories with an SLD of 0 or 1, corresponding to the range encompassing the median MIC ±1 dilution step. All MIC data were read by two independent readings, and discordant results were resolved by a third person blinded to the other results.

3. Results

MIC results and category interpretations for all methods are shown in Table 1 for vancomycin, Table 2 for teicoplanin and Table 3 for linezolid. For the microdilution method, which was designated as the standard for this study, 10% of the MRSA were in the intermediate category for vancomycin interpreted with CLSI criteria and in the resistant category interpreted with EUCAST criteria. The percentage of results in the intermediate or resistant category for the other methods ranged from 0% with Etest and Sensititre to 2.5% with the VITEK 2 system. Although almost of the strains remained in the range of sensitivity, it can be seen that the