ORIGINAL ARTICLE

Comparison of Liofilchem and Etest gradient strips, and BD Phoenix, for the determination of vancomycin MIC in *Staphylococcus aureus*

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ABSTRACT

Introduction: Vancomcyin is the treatment of choice for serious infections caused by Gram-positive organisms, with therapy optimisation based on a calculation of the AUC_{24}/MIC_{BMD} ratio. Laboratories in New Zealand use a variety of methods to determine vancomycin MIC, including Vitek, Phoenix and Etest and Liofilchem MIC strips; however, there is a paucity of information on the performance of Liofilchem strips. This study compared Liofilchem and Etest gradient strips against Phoenix, for the determination of vancomycin MIC; also assessing variability between methods and operators to establish the reliability of reporting single dilution MIC values to clinicians.

Methods: A selection of 100 *Staphylococcus aureus* isolates, including 48 MRSA, were included in the study. Phoenix broth micro dilution (BMD), was performed using panel PMIC-84, and gradient strip MICs were performed using Etest and Liofilchem MIC Strips, recording single and double dilution MICs.

Results: All isolates were vancomycin susceptible, giving 100% categorical agreement. The essential agreement (EA) (MIC ±1 log_2) between all methods was 97%, with Phoenix and Etest showing the highest EA at 100% and modal values of 1.0 mg/L. Phoenix and Liofilchem had the lowest EA of 97%, due to the lower modal value of 0.5 mg/L produced by Liofilchem. Absolute agreement for single dilution values between Etest and Liofilchem was very low at 14%. Reader variability for the MIC strips ranged from 57% absolute agreement (Liofilchem at single dilution values) to 89% (Etest at double dilution values).

Conclusions: This study demonstrated high EA between methods, but considerable operator and method variation between MIC gradient strips. Liofilchem tended to produce MIC values one dilution lower than both Etest and Phoenix. These results have implications in terms of MIC method variability and the capacity of the laboratory to accurately report an absolute MIC result.

Key words: Liofilchem, Etest, Phoenix, MIC gradient strips, vancomycin, *Staphylococcus aureus.*

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INTRODUCTION

Vancomycin, a glycopeptide antibiotic which inhibits bacterial cell-wall synthesis, has been in use for the treatment of infections caused by Gram- positive organisms for over 60 years. Despite this extended period of use, decisions regarding optimisation of effective therapy, while avoiding toxicity, remain a problem for the treatment of serious infections such as bacteraemia.

The UK guidelines for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections have recently been updated (1). The review considered new evidence published over the last decade, new antimicrobials and changes in epidemiology in the UK. The use of vancomycin was still strongly recommended for the treatment of MRSA in serious infections such as bone and joint infections, severe cellulitis, bacteraemia and meningitis. While the guidelines discuss the use of trough serum concentrations to ensure non-toxic therapeutic levels of vancomycin, there is no mention of a laboratory input in terms of providing minimum inhibitory concentration (MIC) results to guide dosing.

In contrast, the Infectious Diseases Society of America (IDSA) therapeutic guidelines for serious MRSA infections, also published in 2020 (2), uses a different approach, looking to eliminate routine serum peak and trough concentration monitoring in favour of using a ratio of area-under-the-curve (AUC) over 24 hours to minimum inhibitory concentration (MIC) of ≥400. The AUC_{24}/MIC_{BMD} ratio ≥400 is based on the isolate MIC ≤ 1mg/L, as determined by reference broth microdilution (rBMD) method, in patients with normal renal function. However, the IDSA do not recommend waiting for the laboratory to complete vancomycin antimicrobial susceptibility testing (AST) before commencement of treatment because the AST report may take several days, laboratory testing methods vary and lack precision. Instead they advocate AUC-guided dosing, using limited serum sampling with Bayesian software interpretation, and assume a MIC_{BMD} of 1 mg/L (2).

So where does that leave the clinical laboratory in terms of testing and reporting vancomycin MICs, especially when several studies (3-5) have shown increased mortality or a worse clinical outcome in patients when the MIC is ≥1.5 mg/L? Can the laboratory determine an accurate and reliable MIC to the single or double dilution level when even the rBMD method has an acceptable variation of ±1 log_2 dilutions?

Using a rBMD method is time consuming and expensive and there are no breakpoints for disc diffusion testing, so most clinical laboratories in New Zealand use automated methods such as Phoenix or Vitek, or gradient MIC strips such as Etest or Liofilchem. Previous studies evaluating methods for the determination of vancomycin MIC have compared Vitek, Phoenix and Etest (3-5); however, there is a paucity of information on the performance of Liofilchem MIC strips. This study compared the performance of Liofilchem and Etest gradient strips, and $Phoenix_{BMD}$, for the determination of vancomycin MIC, against 100 clinical *Staphylococcus aureus* isolates, including 48 MRSA. The study also assessed the variability between operators and methods to determine the reliability of reporting single dilution MIC values to clinicians.

METHODS

The study consisted of a retrospective selection of 100 non-duplicate *Staphylococcus aureus* isolates, including 48 MRSA, collected between 2011 and 2021. All isolates were obtained from clinical samples, predominantly blood cultures and other sterile-site samples, which had been processed at Canterbury Health Laboratories. All isolates had a previous vancomycin susceptibility result determined by Phoenix (BD Diagnostics, USA) broth micro dilution (BMD), using panel PMIC-84. The BD -EpiCenter database was searched to selectively find isolates with an MIC of 2 mg/L, revealing 9 isolates which were included in the study. Isolates stored at -70°C were sub-cultured twice onto Colombia Blood Agar. *S. aureus* ATCC 29213 was used as a control strain.

Confirmation of isolate identification was performed by MALDI -TOF (Bruker Daltonics, USA). Gradient strip MICs were prospectively performed using Etest (bioMèrieux, France) and Liofilchem MIC Strip Test (Liofilchem, Italy), following the manufacturer's instructions, with interpretation according to EUCAST breakpoints. Gradient strips were independently read by three scientists, and the modal value was used as the consensus MIC. For the purposes of this evaluation, single dilution (0 $\log_2$) values were recorded as well as doubling dilution values.

RESULTS

One hundred non-duplicate *S. aureus* isolates were tested for vancomycin susceptibility using three different methods: Phoenix BMD, Etest gradient strips and Liofilchem MIC strip tests. The Phoenix provides MICs as doubling dilution concentrations (range ≤0.5 to >16 mg/L). MIC gradient strips are normally rounded up to the next doubling dilution value; however, for the purposes of this evaluation, MIC strip results were recorded at both the single and double dilution value.

Operator variability

The MIC strips were independently read by three scientists, with the modal value used as the consensus MIC for further method comparisons. Since interpretation of the MIC endpoint can be subjective and operator-dependent, the results were first assessed for variation between operators, at both the single and double dilution endpoints. For the Etest, at the single dilution reading, 77/100 (77%) of isolates had the same MIC value (absolute agreement) recorded by each of the three readers, with the remaining 23 isolates having matching MICs for two of the three readings (Table 1). When considering the doubling dilution results, 89% of the isolates had matching MICs, recorded by each of the three readers. Endpoint interpretation for the Liofilchem strip was less consistent, with only 57% of isolates recording the same MIC value for the single dilution results, by all three readers, increasing to 79% agreement for the double dilution values (Table 1). For both gradient strips, there were no isolates for which three different MIC endpoints were recorded. These results show good inter-operator concordance, confirming the use of the modal MIC for further analysis.

Table 1. Inter-operator consensus of three readers for vancomycin MIC of 100 *S. aureus*, determined by Etest and Liofilchem gradient strips.

AST method	Reader absolute agreement (%)	Two reader absolute agreement (%)
Etest		
single dilution	77	23
double dilution	89	11
Liofilchem		
single dilution	57	43
double dilution	79	21

Categorical and essential agreement

All isolates were categorised as vancomycin susceptible (MIC ≤ 2 mg/L) by all methods, giving 100% categorical agreement (CA). The essential agreement (EA) between methods, at the doubling dilution MIC value, was based on having no more than one concentration different from that reported by the other method (MIC ±1 $\log_2$). Overall, the EA between all methods was high at 97% (Table 2). Phoenix and Etest showed the highest EA at 100%, while Etest and Liofilchem showed 99% EA at the ±1 $\log_2$ level and 97% EA at the ±0 $\log_2$ level. Phoenix and Liofilchem had the lowest EA of 97%.

Table 2. Essential and absolute agreement between MIC methods for the determination of vancomycin MIC in 100 *S. aureus* isolates.

AST method	Essential agreement (%)	Absolute agreement (%)
All methods	97	42
Phoenix V Etest	100	89
Phoenix V Liofilchem	97	47
Liofilchem V Etest (1± dilution)	99	46
Liofilchem V Etest (0± dilution)	97	14

Absolute agreement

Considering absolute agreement (matching MIC values) between methods, at the doubling-dilution concentrations, there is greater variation, with an all-method agreement of only 42% (Table 2). The Phoenix and Etest showed the highest absolute agreement at 89%, while MIC values for Phoenix and Liofilchem agreed only 47% of the time. Comparing gradient strips Etest and Liofilchem, the absolute agreement was low at 46%. Strikingly only 11% of MICs were ≥1.0 mg/L by Liofilchem, whereas 76% of MICs were ≥1.0 mg/L by Etest. Comparing Etest with Liofilchem at the single dilution level, the MICs were in absolute agreement for only 14% of the isolates. Furthermore for three isolates the results differed by three one-fold dilution values: all with Etest MICs higher than Liofilchem.

Modal values and MIC scattergrams

The low level of agreement between Liofilchem MICs and those produced by Phoenix and Etest, can also be demonstrated by the different modal values and shown in the scattergrams of MIC results (Figure 1). The modal value for Phoenix was 1.0 mg/L, with 82/100 (82%) of isolates having this MIC, with an additional 9 isolates having an MIC of 0.5 mg/L and the remaining 9 isolates having an MIC of 2 mg/L. At the doubling dilution concentrations, the Etest MICs aligned closely with the Phoenix results, producing a modal value of 1.0 mg/L, with 85% of isolates having this MIC, 6 isolates with an MIC of 0.5 mg/L and the remaining 9 isolates having an MIC of 2 mg/L. In contrast, Liofilchem had a modal value of 0.5 mg/L, although that comprised only 52% of isolates, with the remaining 48% producing an MIC of 1.0 mg/L.

There were 11 isolates which produced an MIC >1 mg/L by any method, only one of which was a MRSA. Seven of these isolates were detected by both Phoenix and Etest, with an additional two isolates detected by each system; however, for Liofilchem there were no isolates which had an MIC >1 mg/L (Figure 1).

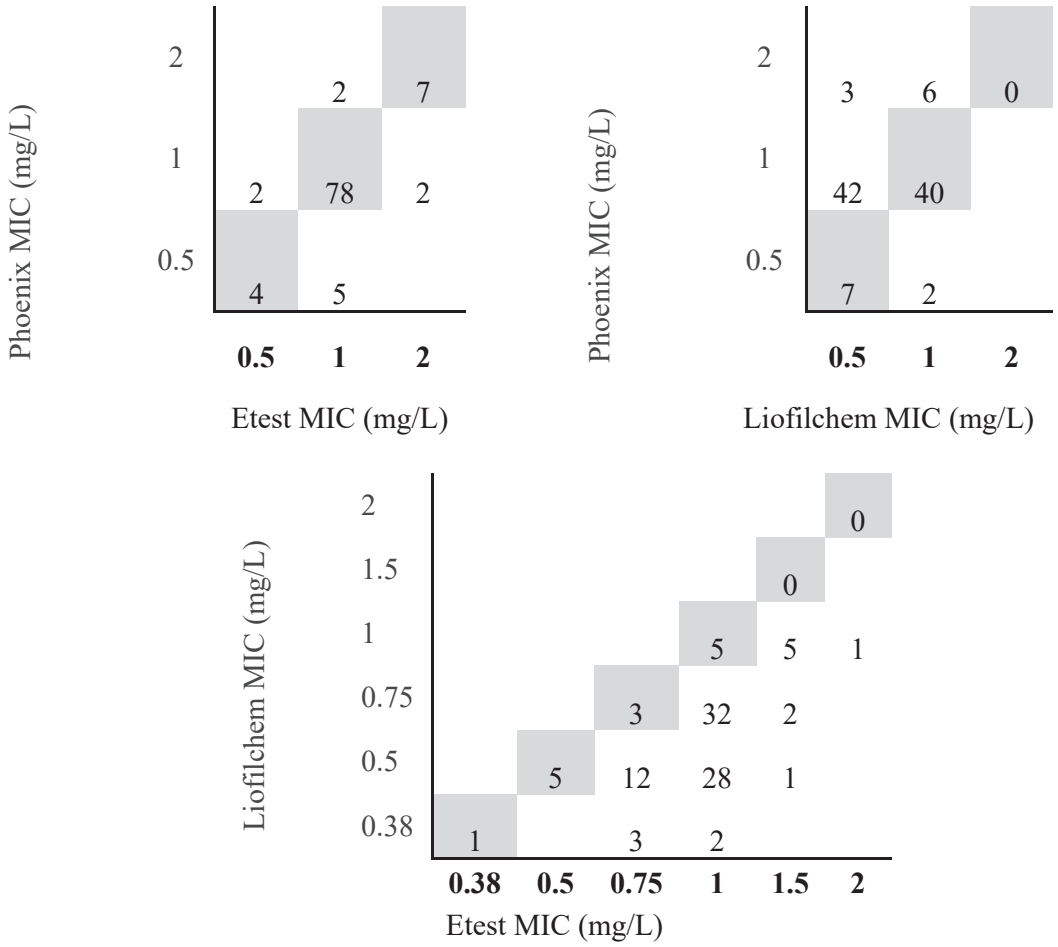

Figure 1. Scattergrams of MIC results (mg/L) for 100 *S. aureus*, comparing Phoenix with Etest, Phoenix with Liofilchem and Liofilchem with Etest.

DISCUSSION

This study was performed to evaluate vancomycin Liofilchem MIC strips and to assess the reliability of reporting single dilution MIC values. We found 100% CA and high EA, at doubling dilution MICs, between all methods for the determination of vancomycin MIC against 100 *S. aureus* (48 MRSA) isolates. However, if a laboratory is required to produce a vancomycin MIC result for a clinician's AUC_{24}/MIC_{BMD} calculation, the MIC level should be accurate and reproducible, so that small changes in the MIC do not have a dramatic effect on the dose given, nor initiate an unnecessary switch in treatment options.

We have demonstrated considerable variation between methods, ranging as low as 14% absolute agreement between Etest and Liofilchem at the single-dilution concentration. When MIC values were rounded up to the next doubling dilution, the absolute agreement between all methods was still poor. Phoenix and Etest results aligned most closely for absolute agreement and essential agreement. Furthermore, both produced a modal value of 1 mg/L and had similar MIC distributions for the study isolates. On the other hand, Liofilchem showed discordant absolute agreement between both Phoenix and Etest, frequently producing MICs 1 $\log_2$ concentration lower, with a modal value of 0.5 mg/L.

It is interesting that our study has shown such a high level of concordance between Phoenix and Etest. This finding conflicts with previous studies which have found that Etest tends to produce MIC values 1 to 2 dilutions higher than reference BMD (rBMD) and that Phoenix produces MICs 1 dilution lower (4,6). In a study by Rybak *et al*. evaluating the ability of Etest, Microscan, Vitek 2 and Phoenix to determine the vancomycin MIC of 200 MRSA isolates, the authors found that the Phoenix achieved 66.2% absolute agreement with rBMD. On the other hand, Etest achieved only 36.7% agreement, with the low rate attributed to Etest producing MIC values 1 to 2 dilutions higher than rBMD. Rybak suggested that Etest might be a conservative approach to determining vancomycin MIC, especially in patients with serious infections (4). An investigation by Riedel *et al*. of 150 *S. aureus* isolates (100 MRSA), comparing Microscan, Phoenix and Etest with rBMD (6), showed similar findings in that the modal MIC for rBMD, Microscan and Etest was 1.0 mg/L, with Etest commonly producing MICs 1 $\log_2$ concentration higher than rBMD; while Phoenix produced MICs 1 $\log_2$ lower and a modal MIC of 0.5 mg/L (6).

Contrary to these studies, a recent review by Brusamarello *et al*. (7) found a high rate of variability between rBMD and Etest, ranging from 0% to 89%. Moreover, in five of six studies vancomycin MICs determined by Etest were typically either concordant with, or lower than, rBMD. Collectively, these conflicting findings between methods would suggest that establishing a true vancomycin MIC is highly method and user dependent.

The difference between our study and others might be explained by different Phoenix panel types; however, our institution has been using the Phoenix for over 10 years, with several different panel types, testing over 54,000 *S. aureus* isolates. During this period 85% of isolates had an MIC of 1.0 mg/L, 16.6% had an MIC of 0.5 mg/L and 0.6% had an MIC of 2 mg/L, demonstrating a consistency of results. Differences in MIC gradient strip values might be attributed to reader subjectivity or institutional interpretation of endpoint cut-off (8).

MIC endpoint interpretation is important as studies investigating treatment outcomes in relation to MICs have found an association between a high vancomycin MIC and treatment failure (3,5). Van Hal *et al*. conducted a meta-analysis of 22 studies which reported on vancomycin treatment outcomes for MRSA infections. The authors concluded that a high vancomycin MIC of ≥2 mg/L was a predictor of treatment failure, and a higher mortality was associated with MRSA blood stream infections if the vancomycin Etest was ≥1.5 mg/L. Their analysis found Etest results to be 0.5 to 1 dilution higher than rBMD and recommended its use to detect patients potentially at risk for treatment failure (3). Similarly, Chen *et al*. retrospectively reviewed over 300 patients with MRSA bacteraemia who were treated with vancomycin, finding that high MICs by Etest was an independent predictor of patient mortality (5).

However, there are many opposing voices arguing against attributing treatment failures based on a single MIC value. A robust meta-analysis by Dalton *et al*., emphasising treatment failure and mortality outcomes, showed that differences in AUC and MIC methodology, combined with varying patient co-morbidities and sites of infection, made it difficult to compare studies (9). The authors failed to find a meaningful relationship between AUC/MIC and predication of clinical outcome.

More importantly is the premise that routine laboratories can provide an accurate and reproducible MIC value that can be reliably used in the AUC_{24}/MIC_{BMD} calculation to guide dosing (8). Indeed, laboratory MIC determination is fraught with difficulties including method variations, strain heterogenicity, operator interpretation and inter-laboratory differences (8). Even within the accepted MIC variation of ±1 $\log_2$ dilutions, a single MIC value could be 0.5 mg/L or 2 mg/L on any subsequent repeat test. Therefore, even a small over estimation of MIC could initiate a change in therapy or potentially result in patient toxicity if the dosage is adjusted accordingly upwards (7). It might be that other variables, such as the site or underlying cause of infection together with patient co-morbidities, play a greater role in determining patient outcomes rather than isolate MIC. Further research into the treatment of MRSA bacteraemia, using alternative options such as daptomycin combined with beta-lactams, may offer a better pathway for clinicians (10).

Limitations

This study has some limitations including no comparison against the reference BMD method, the isolates were obtained from only one institution and clinical outcomes in relation to MIC values were not investigated.

In summary, this study has found total categorical agreement, and a high level of essential agreement at doubling dilution concentrations, for vancomycin susceptibility testing between Phoenix, Etest and Liofilchem. In addition, there was high absolute concordance between Etest and Phoenix, with both yielding a modal value of 1.0 mg/L and having a similar distribution of MIC results. However, there was considerable variation between Liofilchem and Etest, with very low absolute agreement. This study observed that the Liofilchem MIC gradient strips produced MIC values one dilution lower than both Etest and Phoenix. This result has implications in New Zealand as many laboratories use the Liofilchem vancomycin MIC strips and they may be unaware that this product may undercall MIC values.

The results of this study call into doubt the ability of the laboratory to accurately produce an absolute MIC at any level better than a categorical result. Reporting of MICs at the single dilution level is highly discouraged. In general, clinicians should assume a vancomycin MIC of 1 mg/L ±1 $\log_2$ dilutions. However, as part of individualised therapy in patients who have serious MRSA infections, the laboratory MIC result could be reported as part of the clinical decision bundle, contingent with the clinician's understanding of MIC test variability and the laboratory method used.

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