A Comparison of MICs Determined by the CDS, CLSI and EUCAST Reference Techniques

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Introduction

In 1971, Ericsson and Sherris published their report of an international collaborative study on antibiotic sensitivity testing[1]. Ericsson and his colleagues demonstrated that with a carefully standardised technique the diameter of the zone of inhibition around a disc containing an antibiotic could be correlated to the quantitative susceptibility of an organism. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism under defined *in vitro* conditions[2]. It is the accepted way of determining the quantitative susceptibility of an organism. It is these values to which disc diffusion tests such as CDS, CLSI and EUCAST are calibrated.

Using a standard method allows laboratories to provide clinicians with a confident scientific prediction of the likely response of a patient to antibiotic therapy. We assess the level of agreement of MICs determined by agar dilution using two types of agar and broth microdilution.

Method

Two-fold dilutions of antibiotic in water, buffer or broth were prepared and incorporated into agar or distributed into a micro titre plate.

An inoculum of 0.75 OD at 640 nm was prepared on a spectrophotometer from an overnight culture of ATCC and NCTC standard reference strains. For agar dilution the suspension was delivered in thirty two 4µL spots using a Steer's replicator, giving a final concentration of 10^4 cfu. For broth microdilution the inoculum was diluted in Mueller-Hinton broth to give a final concentration of approximately 10^5 cfu.

The plates were incubated for 16-20 hours at 35^{o}C.

Reference methods have MICs within ± 1 two-fold dilution of the 'true' end-point. When new or existing methods are compared to reference standards results that fall within one doubling dilution are defined as having "essential agreement"[3].

Results

Essential agreement was calculated for MIC results that fell within ± 1 doubling dilution. The results are summarised in the graphs below.

Overall the three references techniques showed a level of agreement greater than 85% for the majority of antibiotic families, as seen in Figure 1. No differences greater than ± 2 doubling dilutions were observed between any of the methods.

Results cont.

Figure 2 demonstrates the MICs generated on Sensitest and Mueller-Hinton agar. There was complete consensus with the two agar types. Agar dilution on Sensitest agar, when compared with broth microdilution also had a high level of agreement as shown in figure 3. There was greater than 90% agreement between the two methods, except with the aminoglycosides. The majority of the discordant aminoglycoside results were observed with enterococci and the broth microdilution technique.

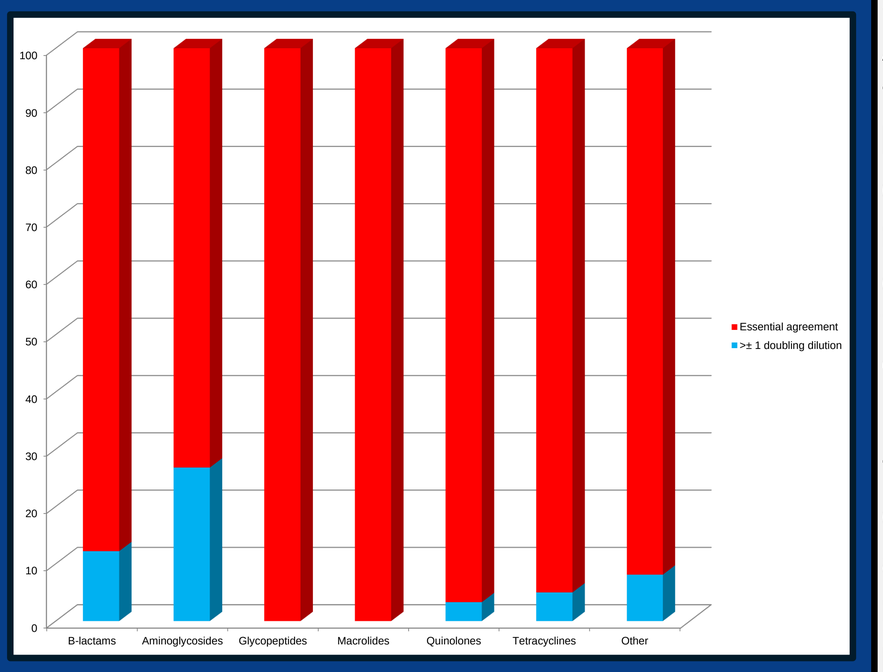

Figure 1: CDS v CLSI v EUCAST
Greater than 85% consensus is seen in 33/34 antimicrobial agents from all major drug classes.

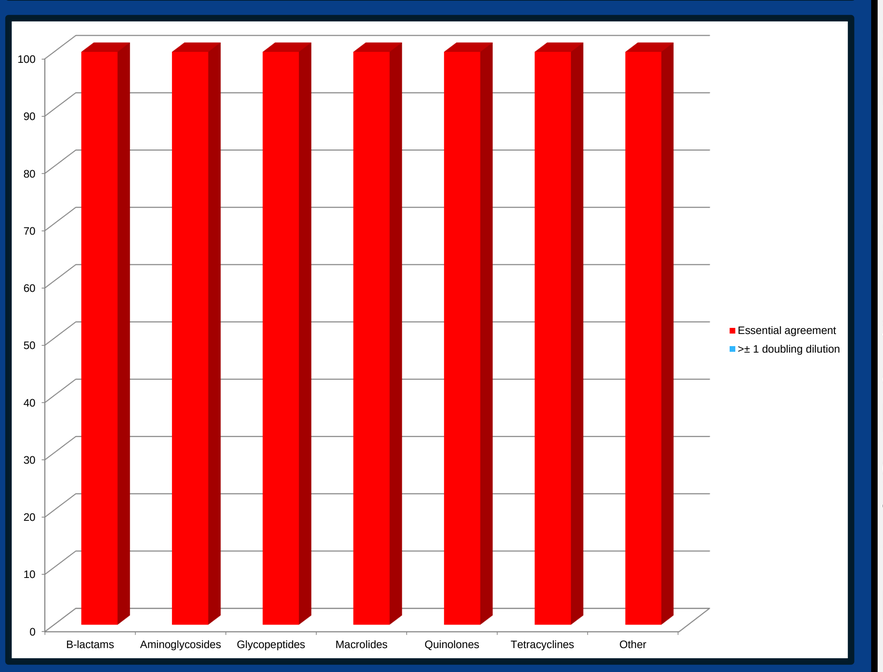

Figure 2: CDS v CLSI
MICs determined by agar dilution on Sensitest agar (CDS) and Mueller-Hinton agar (CLSI) had 100% agreement across all antibiotic agents and organisms.

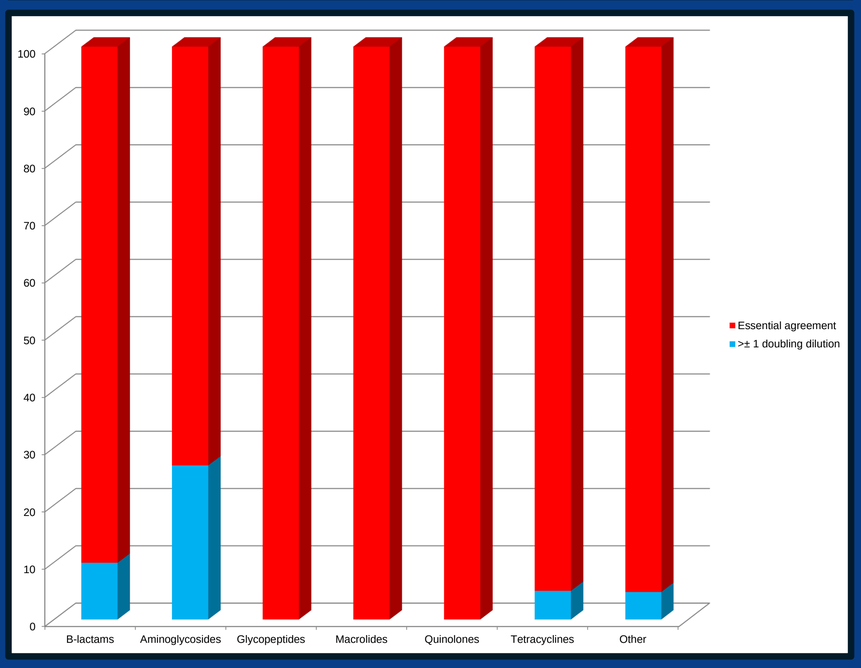

Figure 3: CDS v Broth
A comparison of results determined by agar dilution on Sensitest agar (CDS) and by broth microdilution using Mueller-Hinton broth (EUCAST). With the exception of the aminoglycoside group there was greater than 90% agreement between the two methods.

Discussion

The agar dilution methods described by Ericsson and Sherris[1] formed the basis of the original calibrations of the CDS, and CLSI methods. Later EUCAST and most automated susceptibility testing techniques were based on their broth dilution method. In their report, Ericsson and Sherris found that the differences in the (geometric mean) MICs by broth and agar dilution methods was within one doubling dilution for 80% of the observations made. Only one strain differed by more than two dilution steps[1]. Similar concordance was found in our study. Among the agar dilution method using two agar types, Sensitest and Mueller-Hinton, and the broth microdilution test, no result fell more than 2 dilution steps from any other. In fact, the vast majority were either equivalent or no more than one doubling dilution from either of the other two. These latter results are classified as having "essential agreement".

MIC values are influenced by choice of technique, medium, inoculum size, incubation conditions and the precision with which the antibiotic concentrations are prepared. Low level resistance to aminoglycosides is an inherent property of enterococci due to low uptake of these agents[4]. Aminoglycosides act primarily by impairing bacterial protein synthesis. Transport of these agents across the cytoplasmic membrane is dependent upon electron transport. It is blocked or inhibited by divalent cations, hyperosmolarity, low pH and anaerobiosis[5]. Due to the nature of the testing medium, broth microdilution is more likely to transition to an acid pH as the sugars are fermented. Although no specific investigation has been undertaken to account for the 2 fold difference observed between broth and agar determination of gentamicin MICs for *E.faecalis* strains, it is likely that these factors are partly responsible.

The results of the present study show a high level of agreement between the CDS, CLSI and EUCAST techniques of MIC determination. It would be valid to compare the breakpoints of the three methods because of the degree of relatedness of the techniques.

References

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