Detection of methicillin–resistant *Staphylococcus aureus* with cefoxitin 10 µg discs using the CDS method

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Introduction

An alternative antibiotic will be required to test methicillin susceptibility of *Staphylococcus aureus* when methicillin discs are no longer manufactured. Although oxacillin 1 µg discs are recommended by the National Committee for Clinical Laboratory Standards (NCCLS) they are unsatisfactory. Some strains of susceptible *Staph. aureus* that lack the *mecA* gene appear resistant whilst others with the *mecA* gene appear susceptible (Figure 1 & 2).

Aim

Cefoxitin 10 µg discs were evaluated for the detection of *Staph. aureus* possessing the *mecA* gene. The agar dilution MIC of cefoxitin was correlated with the inhibitory zone sizes around cefoxitin 10 µg discs according to the Calibrated Dichotomous Sensitivity (CDS) method and with the presence or absence of the *mecA* gene.

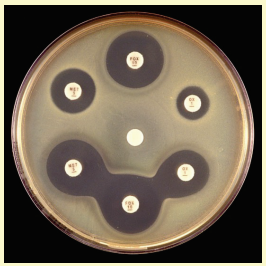

Figure 1: A *mecA* gene-negative *Staph. aureus* resistant to oxacillin (OX 1) but susceptible to methicillin (MET 5) and cefoxitin (FOX 10). Clavulanate 2 µg was added to the same 3 antibiotic discs in the lower half of the plate. The enhancement of the inhibitory zones around oxacillin and methicillin by clavulanate demonstrated that the isolate was a borderline oxacillin-resistant (BORSA) strain with high β-lactamase activity. The blank disc in the centre is a control and contains 2 µg clavulanate.

Method

Organisms: Eighty clinical isolates of *Staph. aureus* were used in the study. Identification was based on a positive tube coagulase test and the presence of the *nuc* gene.

Antibiotic discs: Cefoxitin 10 µg discs (Oxoid) were used for agar discs diffusion tests that were carried out according to the CDS method[1].

Materials: Sensitest Agar (Oxoid CM 409) was used for agar disc diffusion tests with cefoxitin 10 µg discs and for determining the MIC of cefoxitin using an inoculum of 10^4 cfu. Inoculated plates were incubated in air for 18 hours at 35ºC.

Detection of the mecA gene: A PCR assay was used to detect the *mecA* gene[2].

Inhibitory zones around cefoxitin 10 µg discs: The inhibitory zone sizes around cefoxitin 10 µg discs were correlated with the MIC of cefoxitin and the presence or absence of the *mecA* gene.

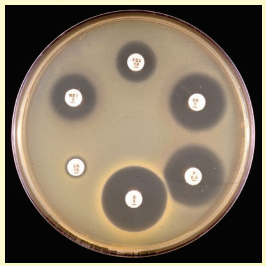

Figure 2: A *mecA* gene-positive *Staph. aureus* (MRSA) falsely susceptible to oxacillin (OX 1) but resistant to methicillin (MET 5) and cefoxitin (FOX 10). The isolate is β-lactamase-negative.

Results

Forty-four isolates of *Staph. aureus* possessed the *mecA* gene. The MIC of cefoxitin was 8-16 mg/L for 34 of these and > 64 mg/L for the other 10.

The MIC of cefoxitin for 36 isolates that lacked the *mecA* gene ranged from 2 to 4 mg/L.

The annular radius of the inhibitory zone around cefoxitin 10 µg discs was < 6 mm for all strains that possessed the *mecA* gene (Fig 3). Conversely, the inhibitory zone size around cefoxitin 10 µg discs was ≥ 6 mm for all *mecA* gene-negative strains.

The inhibitory zone sizes around cefoxitin 10 µg discs related to the MIC of cefoxitin and the presence or absence of the *mecA* gene are shown in Fig. 4.

Two *mecA* gene-positive (MRSA) strains that lacked β-lactamase were resistant to cefoxitin but appeared susceptible to oxacillin (Fig. 2) and two *mecA* gene-negative strains that were resistant to oxacillin (BORSA strains) were susceptible to cefoxitin (Fig. 1).

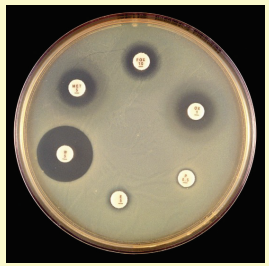

Figure 3: *Staph. aureus* resistant to cefoxitin (FOX 10), methicillin (MET 5) and oxacillin (OX 1). The *mecA* gene was present.

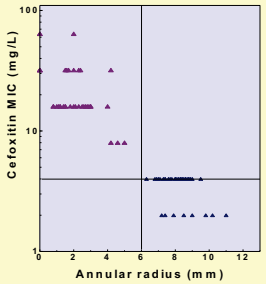

Figure 4: The annular radius (mm) of the inhibitory zone sizes around cefoxitin 10 µg discs related to the MIC (mg/L) of cefoxitin for 80 isolates of *Staph. aureus*. Points in purple ( ) are strains with the *mecA* gene. Points in blue ( ) are strains lacking the *mecA* gene.

Conclusion

Cefoxitin 10 µg discs are a suitable alternative to methicillin 5 µg discs for detecting *mecA* gene-positive *Staph. aureus* when using the CDS method. The annular radius of the inhibitory zone size around cefoxitin 10 µg discs for methicillin-susceptible strains (including BORSA strains) that lacked the *mecA* gene was **≥** 6 mm with the corresponding MIC of cefoxitin **≤** 4 mg/L. The inhibitory zone size around cefoxitin 10 µg discs for strains possessing the *mecA* gene was < 6 mm with the corresponding MIC of cefoxitin ≥ 8 mg/L.

References

1. Bell, S.M., Gatus, B.J., Pham, J.N. & Rafferty, D.L. (2002). Antibiotic susceptibility testing by the CDS method. A manual for Medical and Veterinary Laboratories. South Eastern Area Laboratory Services. Sydney, New South Wales, Australia. ISBN 0-9581853-0-1.

2. Maes, N., Magdalena, J., Rottiers, S., De Gheldre, Y. & Strulens, M. J. (2002). Evaluation of a of a triplex PCR assay to discriminate *Staphylococcus aureus* from coagulase-negative staphylococci and determine methicillin resistance from blood cultures. *Journal of Clinical Microbiology*. **40**: 1514-7.