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Short communication

Susceptibility patterns of coagulase-negative staphylococci to several newer antimicrobial agents in comparison with vancomycin and oxacillin

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ABSTRACT

Coagulase-negative staphylococci (CoNS) have emerged as important nosocomial pathogens. CoNS resistance to meticillin and other semisynthetic penicillins is now common. Elevated vancomycin minimal inhibitory concentrations (MICs) have been reported and are associated with worse treatment outcomes. Several newer antibiotics have recently become available for the treatment of Gram-positive infections. The purpose of this study was to assess the in vitro activity of telavancin, daptomycin, linezolid and tigecycline in comparison with oxacillin and vancomycin against 653 non-duplicate clinical isolates of CoNS by the agar dilution method. The greatest variability in MIC was observed for oxacillin. Presence of the *mecA* gene conferred higher MICs for oxacillin but did not influence MICs to all other antibiotics tested. Telavancin tended to have MICs that were 1–2 dilutions lower than vancomycin. Daptomycin had good activity against all isolates. *Staphylococcus haemolyticus*, *Staphylococcus hominis* subsp. *novobiosepticus*, *Staphylococcus saprophyticus*, *Staphylococcus schleiferi* and *Staphylococcus simulans* were the most daptomycin-susceptible CoNS species tested. The validity of the agar dilution method for daptomycin was confirmed, with >90% isolates having MICs that were within 1 dilution of parallel Etest results. Within-species MIC variation was most restricted for linezolid and tigecycline, with the exception of *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* that demonstrated higher overall MICs to tigecycline.

1. Introduction

Coagulase-negative staphylococci (CoNS) are common colonisers of human skin and are amongst the most commonly isolated organisms in the clinical microbiology laboratory. The ca. 30 species that constitute the CoNS have emerged as important clinical entities with varying pathogenic potential and antimicrobial resistance patterns. Data from the National Healthcare Safety Network indicate that CoNS are the most frequent cause of bloodstream infection, accounting for 36% of isolates [1]. Patients with prosthetic devices, intravascular catheters or other foreign bodies are especially at risk of CoNS infections. Resistance to meticillin and other semisynthetic penicillins is widespread amongst CoNS and there have also been several case reports of reduced vancomycin susceptibility [2].

This study was undertaken to assess the in vitro susceptibility patterns of various CoNS species (with and without the *mecA* gene) to daptomycin, telavancin, tigecycline and linezolid by the agar dilution method. For comparison, and to assess whether multiresistance patterns exist in this group of bacteria, all isolates were also tested by agar dilution against oxacillin and vancomycin. In addition, the daptomycin Etest (AB BIODISK, Solna, Sweden) was run in parallel to assess the validity of the agar dilution method.

2. Materials and methods

A total of 653 non-duplicate clinical isolates of CoNS, representing 15 different species and subspecies, were tested. Strains included in the study were from clinical specimens submitted to the Department of Microbiology, London Health Sciences Centre (London, Ontario, Canada). All strains were saved in glycerol phosphate buffer at −70 °C and were subcultured twice onto Columbia blood agar (Oxoid Inc., Nepean, ON, Canada) before testing. Isolates were identified by conventional biochemical tests, sensitivity to desferrioxamine and cellular fatty acid profiles, as outlined previously [3].

Minimal inhibitory concentrations (MICs) of daptomycin, telavancin, tigecycline, linezolid, oxacillin and vancomycin were determined by the agar dilution method as recommended by the Clinical and Laboratory Standard Institute (CLSI) using

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Mueller–Hinton agar (Oxoid Inc.) [4]. A 0.5 McFarland bacterial suspension in sterile distilled water was prepared directly from fresh cultures that had been plated on Columbia blood agar (Oxoid Inc.). The turbidity of the suspension was further adjusted so that 3-mm pins of a replicator delivered ca. 10^4 colony-forming units/spot onto Muller–Hinton agar plates. The concentrations of antibiotics tested ranged from 0.125 mg/L to 32 mg/L. Agar plates containing daptomycin were fortified with 50 mg/L calcium and were incubated at 35 °C for 18–20 h prior to reading. All other plates were incubated at 35 °C in air for 24 h before visual determination of MICs. CLSI susceptibility breakpoints are given in Table 1 [4].

Duplicate daptomycin agar dilution tests (as described above) were run in parallel with the daptomycin Etest performed as described by the manufacturer. Test strips were applied to the surface of 150-mm Mueller–Hinton agar plates (Oxoid Inc.) that had been inoculated with a swab dipped in a 0.5 McFarland suspension of the bacterial isolates. Plates were incubated for 18–20 h in ambient air at 35 °C prior to determination of MICs. The MIC was defined as the intersection of the bacterial growth margin with the Etest strip observed in reflected light. Etest MICs were rounded up to the next-higher log MIC for the purpose of comparison.

Staphylococcus aureus ATCC 29213, *S. aureus* ATCC 43300, *Enterococcus faecalis* ATCC 29212 and *E. faecalis* ATTCC 51299 were tested concurrently as quality control strains.

Using an in-house multiplex polymerase chain reaction (PCR) method, all strains were tested for the presence of the *mecA* and *nuc* genes as well as a conserved sequence of ribosomal DNA (internal control) as described previously [5].

3. Results

MICs for each strain/antibiotic combination as well as mean MIC_{50} and MIC_{90} values (MICs for 50% and 90% of the organisms, respectively) are shown in Table 1.

Oxacillin demonstrated the greatest variability of all antibiotics tested. The MIC_{90} of *mecA*-negative isolates demonstrated a three-fold dilution difference (0.25 mg/L vs. 2.0 mg/L), whilst that of *mecA*-positive strains was consistently ≥8 mg/L. Presence or absence of the *mecA* gene did not appear to influence MICs to all other antibiotics tested, as judged by a greater than two-fold dilution difference.

The overall MIC_{50} and MIC_{90} values of telavancin for all isolates (irrespective of species) were 0.25 mg/L and 0.5 mg/L, respectively, whilst those for vancomycin were 1.0 mg/L and 2.0 mg/L. This represents a 2–4 dilution difference between the two agents.

Daptomycin demonstrated very good activity against all species of CoNS. Only 7/653 strains (1.1%) were categorised as non-susceptible with a MIC ≥2 mg/L. *Staphylococcus haemolyticus*, *Staphylococcus hominis* subsp. *novobiosepticus*, *Staphylococcus saprophyticus*, *Staphylococcus schleiferi* and *Staphylococcus simulans* were most susceptible, all having MIC_{90} values ≤0.25 mg/L.

A correlation between the degree of vancomycin and daptomycin susceptibility was observed. Of the strains with a vancomycin MIC ≤ 0.5 mg/L, 49% had a daptomycin MIC ≤ 0.25 mg/L, but only 14% of strains with a vancomycin MIC of 2.0 mg/L had a similar degree of susceptibility to daptomycin.

Good agreement was observed between the daptomycin Etest and agar dilution methods. Identical results were obtained for 38% of isolates, and incongruent isolates only differed by 1 dilution in 56% of isolates. Discordant agar dilution and Etest results demonstrated higher MICs for the agar dilution subset. Despite this tendency, 95.4% and 93.4% of results of agar dilution were within 1 dilution of the Etest MIC. Reproducibility of the agar dilution method was excellent, with 525 (80.4%) MIC values being identical and another 20.3% of MICs being within 1 dilution of each other.

Within-species variation of MICs to linezolid was restricted to 1–2 dilutions. *Staphylococcus saprophyticus* and *Staphylococcus xylosus* showed the highest overall MIC_{90} to linezolid (4.0 mg/L and 2.0 mg/L, respectively). Tigecycline also tended to have minimal within-species variation, with the exception of *Staphylococcus epidermidis* and *S. haemolyticus*. These two species also demonstrated higher overall tigecycline MIC_{90} value (1.0 mg/L) than the other CoNS species tested (≤0.5 mg/L). No correlation between linezolid and tigecycline susceptibility was evident.

4. Discussion

Resistance to meticillin and other semisynthetic penicillins is widespread amongst CoNS species that are commonly associated with human infections [5]. Although glycopeptide resistance was first observed in a strain of *S. haemolyticus*, there are only isolated reports of infections with CoNS with reduced susceptibility to vancomycin [2,6].

Daptomycin is a cyclic lipopeptide antibiotic naturally produced by *Streptomyces roseosporus* with a novel mode of bactericidal action against Gram-positive organisms. Studies have shown that daptomycin disrupts the functional integrity of the cytoplasmic membrane in the presence of physiological concentrations of calcium [7].

Excellent daptomycin activity against CoNS has previously been demonstrated [8], however the isolates in those studies were not speciated. Like the current investigation, previous papers report equal daptomycin activity against oxacillin-susceptible and -resistant strains of staphylococci [8]. Johnson et al. [9] evaluated calcium-supplemented Etest strips against an agar dilution method and found the results obtained by both methods to be comparable. Interestingly, they also noted that the MICs of *S. aureus* were higher when measured by the agar dilution method.

Daptomycin has excellent activity against all species of CoNS. The CLSI and US Food and Drug Administration (FDA) have defined only 'susceptible' criteria for daptomycin since resistance is rare and an interpretive breakpoint cannot be established. Results of this and previous in vitro studies, combined with the clinical efficacy and safety of daptomycin, indicate that it is an acceptable alternative for the treatment of serious CoNS infections, especially when bactericidal activity is desirable. The present experiments demonstrate that the agar dilution method is a convenient alternative in vitro technique that produces satisfactory susceptibility results.

Telavancin is a semisynthetic derivative of vancomycin with an additional hydrophobic and hydrophilic moiety. In contrast to vancomycin, telavancin is more rapidly bactericidal against Gram-positive organisms, including staphylococci, owing to its dual mechanism of action. The first mechanism is similar to the action of vancomycin, where the drug binds non-covalently to the terminal D-Ala-D-Ala both of lipid II and immature non-cross-linked glycan strands. This binding prevents peptidoglycan polymerisation and cross-linking steps. The second mechanism involves non-covalent binding of the telavancin molecule to membrane-bound lipid II. The lipophilic moiety of telavancin interacts directly with the bacterial cell membrane, resulting in depolarisation and increased permeability of the cell membrane. As shown in this study, telavancin MICs have previously been reported to be two to eight times lower than corresponding vancomycin MICs [10].

None of the strains used in this study had decreased susceptibility to vancomycin. However, increased telavancin MICs have been observed in enterococci and in two strains of *S. epidermidis* with reduced susceptibility to glycopeptides [11]. Previous studies have demonstrated slightly greater telavancin activity amongst *Staphylococcus capitis*, *S. epidermidis*, *S. hominis*, *Staphylococcus lugdunensis*