Reduced expression of PBP-2A by neonatal meca-positive coagulase-negative staphylococci (CoNS) blood isolates: β-lactams are useful first-line agents for the treatment of neonatal CoNS sepsis, restricting the use of vancomycin

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Received 24 October 2011; returned 7 January 2012; revised 1 February 2012; accepted 27 February 2012

Objectives: Vancomycin use for neonatal coagulase-negative staphylococci (CoNS) sepsis is based on a high CoNS carriage rate of meca, encoding penicillin-binding protein (PBP)-2a, with low affinity for, and associated with resistance to, β-lactam antibiotics. The relationship between meca gene carriage, phenotypic expression of the gene by PBP-2a production and in vitro resistance to the β-lactam antibiotics oxacillin, cefazolin and amoxicillin/clavulanate was determined for 85 CoNS blood isolates randomly obtained from our collection of isolates from neonates with CoNS sepsis.

Methods: The relationship between meca gene carriage, phenotypic expression of the gene by PBP-2a production and in vitro resistance to the β-lactam antibiotics oxacillin, cefazolin and amoxicillin/clavulanate was determined for randomly obtained CoNS blood isolates from our collection of isolates from neonates with CoNS sepsis. The meca gene was detected using multiplex PCR, and PBP-2a expression was determined using a latex agglutination (LA) test (Oxoid). β-Lactam susceptibility was determined using the Phoenix automated system and, in addition, by Etest with interpretation of MIC values according to the reference MIC breakpoints adopted from the CLSI guidelines M100-S20, Infobase™.

Results: Among 85 CoNS blood isolates, 73 (86%) were meca positive and 12 (14%) were meca negative. None of the meca-negative isolates expressed PBP-2a and all were β-lactam susceptible. All meca-positive CoNS isolates were oxacillin resistant, although most oxacillin MICs were not very high, ranging from 2 to 8 mg/L for the majority of isolates. Only 8/73 (11%) meca-positive CoNS isolates had oxacillin MICs ≥ 32 mg/L (range 32 to >256 mg/L). meca-positive CoNS blood isolates, although fully resistant to oxacillin, were almost universally susceptible to cefazolin and amoxicillin/clavulanate, which was associated with a low expression rate of PBP-2a.

Conclusions: β-Lactam antibiotics are useful for the treatment of neonatal CoNS sepsis, reserving vancomycin for selected cases.

Keywords: neonatal sepsis, meca gene, β-lactam antibiotics, antibiotic susceptibility, cefazolin, amoxicillin/clavulanate

Introduction

Coagulase-negative staphylococci (CoNS) are the major causative agents of nosocomial sepsis in neonatal intensive care units (NICUs). Since almost all CoNS blood isolates carry the meca gene and are therefore reported to be resistant to β-lactam antibiotics, it is recommended to treat patients with CoNS sepsis with glycopeptides, most frequently vancomycin. The widespread use of vancomycin has raised concern, and resistance to glycopeptides in CoNS has already been reported. In the NICU of the University Medical Centre Utrecht, the Netherlands, first-generation cephalosporins are used as empirical agents in infants with CoNS sepsis. The great majority of CoNS blood isolates proved to be susceptible to cefazolin and amoxicillin/clavulanate, as determined by Etest. This is remarkable since most CoNS carry the meca gene, the gene coding for methicillin/oxacillin resistance. We postulated that the presence of the meca gene in CoNS blood isolates does not always result in production...
of penicillin-binding protein (PBP)-2a, the PBP with low affinity for β-lactam antibiotics, as it does in methicillin-resistant Staphylococcus aureus (MRSA).

**Methods**

The mecA gene was detected using multiplex PCR and PBP-2a expression was determined using a latex agglutination (LA) test (Oxoid). In 75 MRSA blood isolates collected from patients admitted to various departments in the hospital, expression of PBP-2a was determined to serve as a positive control and all 75 were strongly PBP-2a positive. β-Lactam susceptibility was determined using the Phoenix automated system and, in addition, by Etest with interpretation of MIC values according to the reference MIC breakpoints adopted from the CLSI guidelines, Infobase®M100-S20.

**Results**

Figure 1 shows that among 85 CoNS blood isolates, 73 (86%) were mecA positive and 12 (14%) were mecA negative. None of the mecA-negative isolates expressed PBP-2a and all were β-lactam susceptible. All mecA-positive CoNS isolates were oxacillin-resistant, although most oxacillin MICs were not very high, ranging from 2 to 8 mg/L for the majority of isolates. Only 8/73 (11%) mecA-positive CoNS isolates had oxacillin MICs ≥32 mg/L (range 32 to >256 mg/L). Of the mecA-positive isolates, 41/73 (56%) expressed PBP-2a and 32/73 (44%) were negative in the PBP-2a LA test. There was no clear relationship between oxacillin MIC and PBP-2a expression, although the CoNS isolate with the highest oxacillin MIC (>256 mg/L) strongly expressed PBP-2a. In contrast to the universal resistance to oxacillin among the mecA-positive CoNS isolates, the great majority of these isolates appeared susceptible to cefazolin and amoxicillin/clavulanate. Susceptibility was highest among the 32 mecA-positive but PBP-2a-negative isolates, with 30/32 (94%) susceptible to cefazolin and amoxicillin/clavulanate, but even the majority of the mecA-positive and PBP-2a-expressing CoNS isolates appeared to be susceptible to cefazolin (32/41, 78%) and amoxicillin/clavulanate (31/41, 75%). Differentiation of the 41 PBP-2a-positive CoNS blood isolates revealed that 25/41 (61%) isolates only weakly expressed the PBP-2a protein (LA test positive between 3 and 10 min), as opposed to the 16/41 (39%) isolates in which the LA test was positive within 3 min. The Etest showed that 23/25 (92%) of the weakly PBP-2a-positive CoNS blood isolates were susceptible to cefazolin and 22/25 (88%) to amoxicillin/clavulanate. Remarkably, 9/16 (56%) of the CoNS blood isolates with a strongly positive PBP-2a LA test still appeared to be cefazolin and amoxicillin/clavulanate susceptible.

**Discussion**

The most striking finding of this study is the discrepancy among mecA-positive CoNS blood isolates between universal resistance to oxacillin and apparent susceptibility to cefazolin and amoxicillin/clavulanate. In contrast to MRSA, in CoNS mecA gene carriage may not necessarily be synonymous with detectable β-lactam resistance. This is most clearly demonstrated by Staphylococcus sciuri, in which universal mecA carriage is rarely accompanied by β-lactam resistance. In addition, in mecA-positive strains from various CoNS species phenotypic expression of oxacillin resistance was often difficult to detect. In most mecA-positive CoNS this gene is under strong repression, presumably by the mecR1-mecI and blaR1-blaI operons.

The discrepancy between mecA gene carriage and PBP-2a expression may be explained by the way in which binding of β-lactams to the active site of the PBP-2a molecule is arranged. Crystallographic studies have indicated that older penicillins and cephalosporins bind relatively well to the active site of the PBP-2a protein. In contrast to methicillin, which shows a poorer fit, retarding the formation of the acyl-PBP intermediate, which is essential for the inhibitory action of β-lactams on the function of PBPs. In addition, we also found that an appreciable number (nearly 80%) of mecA-positive CoNS strains do not express or only weakly express PBP-2a. This discordance between carriage of the mecA gene and expression of PBP-2a has been noted for a number of CoNS species, most notably S. sciuri. It is evident that relatively low expression of PBP-2a may add to the
discrepancy between oxacillin resistance and susceptibility to older β-lactams, because such low amounts are apparently sufficient for oxacillin resistance as a result of the poor fit of oxacillin/methicillin for the active site of PBP-2a. At the same time older β-lactams may be even more active due to their higher binding affinity for PBP-2a. This relatively good activity of older β-lactams such as cefazolin and amoxicillin/clavulanate against oxacillin-resistant staphylococci is not a unique finding. More than a decade ago it was shown that amoxicillin/clavulanate and ampicillin/subactam are effective in the treatment of endocarditis due to MRSA and methicillin-resistant CoNS in animal models.8,9 Despite these findings, these agents have rarely been tried in humans. In the NICU, vancomycin is prescribed most often for the treatment of CoNS sepsis, but occasionally other agents have been used successfully, notably cloxacillin and ampicillin/sublactam, while our own experience with cefazolin/fcefotin has been favourable for more than a decade.5 Previous studies have indicated that, despite the in vitro resistance of CoNS to cloxacillin, infants with CoNS sepsis clinically improved on treatment with cloxacillin.10,11 The apparent efficacy of cefazolin and ampicillin/sublactam (which is comparable to that of amoxicillin/clavulanate) is explained by the in vitro susceptibility results of the present study, while the successful treatment results with cloxacillin may be explained by the relatively low MICs of this agent for mecA-positive CoNS found in the present study and by others.5 Peak free plasma concentrations of this antibiotic in neonates exceed these MICs multifold with current dosing schedules and a level >2 mg/L is maintained for a considerable part of the dosing interval.12 These peak and sustained levels may be sufficient to inhibit further growth of CoNS in the bloodstream and, in the meantime, permit the neonatal host to boost its defences to clear bacteria by opsonophagocytosis.

In conclusion, β-lactam antibiotics may be useful alternatives for the treatment of neonatal CoNS sepsis, with vancomycin reserved for selected cases.

Funding
This study was supported by internal funding.

Transparency declarations
None to declare.

References