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Short report

Prevalence of resistance to antiseptics and mupirocin among invasive coagulase-negative staphylococci from very preterm neonates in NICU: the creeping threat?

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SUMMARY

In neonatal intensive care units, topical agents represent an increasing part of the infection control armamentarium. Fifty-one coagulase-negative staphylococci (CNS) isolated from catheter-associated bloodstream infections in very preterm neonates were investigated in this study: 41.2% exhibited decreased susceptibility to at least one antiseptic (chlorhexidine 12%, benzalkonium 24%, acriflavine 33%) and 61% were resistant to mupirocin. *Qac*A/B, *mup*A and both genes were detected by polymerase chain reaction in 59%, 63% and 49% of CNS, respectively. Seventy-six percent of *Staphylococcus epidermidis* (5/5 pulsed-field-gel electrophoresis subgroups) and 11% of *Staphylococcus capitis* (1/3 subgroups) were multi-resistant. Skin antisepsis using low-concentration aqueous formulations and off-label mupirocin indications should benefit from a stewardship programme.

Introduction

In neonatal intensive care units (NICU), catheter-associated bloodstream infections (CABSI) due to coagulase-negative staphylococci (CNS) are the main healthcare-associated infections (HAI), occurring in 11% of patients. HAI increase morbidity, mortality and risk of long-term disabilities.[1] Preventive measures include the use of chlorhexidine (CHX) for skin antisepsis, gauze dressing and catheter injection hub-site disinfection.[1] Combination therapy with CHX skin disinfection and mupirocin nasal decolonization can be effective in managing outbreaks of *Staphylococcus aureus* infections.[2]

Unfortunately, the use of topical agents can select resistant strains of staphylococci. *Mup*A is the major resistance gene responsible for high-level mupirocin resistance.[3] Quaternary ammonium compound (QAC)-resistant determinants, encoded by *qac* family genes, are multi-drug efflux pumps that decrease bacterial susceptibility to cationic antiseptic agents.[4] Mainly located on mobile genetic elements as plasmids, these genes could be transferred between staphylococcal species.[4]

Beyond failure of CABSI preventive strategies, CNS that are resistant to topical agents could act as a reservoir of

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*mup*A and *qac* genes that could be transferred to *S. aureus*.[3,4] This paper reports a phenotypic and molecular study evaluating the prevalence of antiseptic- and mupirocin-resistant strains among CNS responsible for CABSI in a French referral NICU.

Methods

This study was performed in a level 3, 28-bedded NICU at the university-affiliated Antoine Béclère Hospital (Assistance Publique–Hôpitaux de Paris network). During the study period, 1799 neonates were admitted to the NICU (total 21,796 patient-days).

All non-duplicate CNS from clinically relevant CABSI diagnosed among preterm neonates (<32 completed weeks of gestation) between January 2009 and September 2011 were included. CABSI was defined according to international consensus criteria: one or more positive blood cultures associated with clinical and biological signs of infection with no other identified source and at least five days of intravenous vancomycin therapy.[5]

Staphylococcus epidermidis and *Staphylococcus capitis* were identified by specific *dnaJ* molecular probes, and other species were identified using conventional biochemical methods (IDStaph strip®, bioMérieux, Craponne, France). Antimicrobial susceptibility testing was performed by the disc diffusion method (meticillin, gentamicin, ofloxacin) or by determination of minimum inhibitory concentrations (MIC) (vancomycin, teicoplanin and mupirocin, Etest®, bioMérieux). Results were interpreted according to EUCAST recommendations (http://www.eucast.org/). The strains were classified as mupirocin susceptible (MIC ≤1 μg/mL), low-level resistant (2–256 μg/mL) or high-level resistant (≥512 μg/mL). Concerning the susceptibilities of antiseptics, MICs of CHX, benzalkonium chloride (BAC) and acriflavine (ACV) (Sigma-Aldrich, Saint-Quentin Fallavier, France) were determined using the Müeller-Hinton broth microdilution method in triplicate.[4] Although no longer considered an effective antiseptic, the cationic dye ACV constitutes a sensitive test to detect QAC resistance determinants.[6] Reduced antiseptic susceptibility was considered if the MIC was >2 μg/mL (CHX, BAC) or 16 μg/mL (ACV).[4,6] All isolates were tested for the presence of *mup*A and *qac*A/B genes by polymerase chain reaction (PCR) (Table I). Clonal distribution of *S. epidermidis* and *S. capitis* was evaluated using pulsed-field gel electrophoresis (GenePath *Sma*I kit and Fingerprinting II software®, Bio-Rad, Hercules, USA).

As recommended by the French Society of Infection Control, skin antisepsis of preterm neonates was performed using an aqueous combination of CHX (0.25%), BAC (0.025%) and benzyl alcohol (4%) (Biseptine®, Bayer Healthcare, Loos, France; total consumption 505 L).[8] Although contraindicated in this high-risk population, alcoholic CHX 0.5% solution was introduced in 2011 instead of alcoholic povidone-iodine to disinfect catheter hubs (total consumption 412 L). Neither devices nor dressings impregnated with antiseptic were used. Nasal mupirocin (Bactroban®, GlaxoSmithKline, Marly-le-Roi, France; total consumption 456 g, 0.25 g per admission) was applied three times per day for five consecutive days, and continued once daily until removal of the intranasal tube in the following circumstances: (i) proven or suspected meticillin-susceptible *S. aureus* (MSSA) colonization in neonates with an intranasal tube; (ii) during outbreaks of MSSA colonization; (iii) in neonates with nasal skin lesions; and (iv) in neonates with an intranasal tube who had a high burden of CNS in the oropharynx.

Results

Over the study period, 51 strains were collected from extremely preterm (67.4%) or very preterm (32.6%) neonates (all weighing <1500 g). The species distribution was: *S. epidermidis* ($N = 25$), *S. capitis* ($N = 18$), *Staphylococcus haemolyticus* ($N = 2$) and other species ($N = 6$).

All but one strain was resistant to meticillin, and 88% and 30% were resistant to gentamicin and ofloxacin, respectively. All strains were susceptible to vancomycin, and all but two strains were susceptible to teicoplanin. Sixty-one percent (31/51) of the strains were resistant to mupirocin (MIC_{50} and MIC_{90} 2048 μg/mL, range 0.125–2048 μg/mL). All but one of these strains were highly resistant to mupirocin and harboured *mup*A (MIC of the low-level-resistant strain 128 μg/mL). At species level, 80% (20/25) of *S. epidermidis* and 39% (7/18) of *S. capitis* were resistant to mupirocin.

The percentage of strains with reduced susceptibility to antiseptics was 12% for CHX (6/51), 24% for BAC (12/51) and 33% for ACV (17/51); 41% of CNS (21/51) exhibited reduced susceptibility to at least one antiseptic. *Qac*A/B was identified in 63% (32/51) of the strains, including 92% (23/25) of *S. epidermidis* and 17% (3/18) of *S. capitis*. This gene was systematically identified in strains with reduced susceptibility to at least one antiseptic. *QacA/B* was identified in four of the six strains that showed reduced susceptibility to CHX; this ratio reached 12/12 for BAC and 17/17 for ACV. Among the *qacA/B*-positive CNS, 13 were fully susceptible to antiseptics. MIC_{50},

Table I
Sequences of primers and polymerase chain reaction (PCR) conditions used for the detection of *mup*A and *qac*A/B genes

Targeted gene	Primers	Size	Hybridisation temperature	PCR conditions	Reference
*qac*A/B	FW: 5′-GCA-GAA-AGT-GCA-GAG-TTC-G	361 bp	58 °C	30 cycles: 20 s at 94 °C, 20 s at 53 °C, 20 s at 72 °C	6
	REV: 5′-CCA-GTC-CAA-TCA-TGC-CTG		56 °C		
*mup*A	FW: 5′-TAT-ATT-ATG-GCA-TGG-AAG-GTT-GG	458 bp	46.6 °C	30 cycles: 30 s at 95 °C, 50 s at 45 °C, 50 s at 72 °C	7
	REV: 5′-AAT-AAA-ATC-AGC-TGG-AAA-GTG-TTG		45.4 °C		