Emergence of High-Level Mupirocin Resistance in Coagulase-Negative Staphylococci Associated with Increased Short-Term Mupirocin Use

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In our hospital, mupirocin has increasingly been used for peri-operative decolonization of *Staphylococcus aureus*. The target for mupirocin is isoleucyl tRNA synthetase (*ileS*). High-level resistance to mupirocin is conferred by acquisition of plasmids expressing a distinct *ileS* gene (*ileS2*). Here we evaluated the longitudinal trends in high-level mupirocin resistance in coagulase-negative staphylococci (CoNS) and linked this to the presence of *ileS2* genes and mupirocin use. We assessed mupirocin resistance in CoNS bloodstream isolates from 2006 to 2011 tested by Phoenix automated testing (PAT). We evaluated the reliability of PAT results using Etest. PAT species determination was confirmed by MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) mass spectrometry. We investigated the presence of *ileS2* in the first 100 consecutive CoNS bloodstream isolates of each year using RT-PCR. Mupirocin use increased from 3.6 kg/year in 2006 to 13.3 kg/year in 2010 and correlated with the increase in the percentage of CoNS isolates carrying *ileS2* (8% in 2006 to 22% in 2011; Spearman’s rho, 0.137; *P* = 0.01). The sensitivity and specificity of PAT for detecting high-level mupirocin resistance were 0.97 and 0.97, respectively. *ileS2* was detected in 81 of 82 phenotypically highly mupirocin-resistant strains and associated with resistance to ciprofloxacin, erythromycin, and clindamycin. In conclusion, we found a rapid increase in high-level resistance to mupirocin and resistance to other antibiotics in CoNS associated with an increase in mupirocin use. The associated resistance to other antibiotics may result in a reduction of oral antibiotic options for prolonged treatment of prosthetic infections with CoNS.

**MATERIALS AND METHODS**

Mupirocin susceptibility was tested in all staphylococcal bloodstream isolates obtained between 2006 and 2011. Susceptibility had been tested routinely by Phoenix automated testing (PAT) (Becton, Dickinson and Company, Breda, the Netherlands). All isolates had been stored at −70°C. CoNS were distinguished from *S. aureus* by tube coagulase, DNase, and slide testing. Species determination was performed by MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) mass spectrometry (Bruker Daltonics, Bremen, Germany). Mupirocin susceptibility of the first 40 consecutive CoNS isolates of each year was also determined by Etest according to the manufacturer’s guidelines on Mueller-Hinton agar (AB Biodisk, Mannheim, Germany). Susceptibility to ciprofloxacin, trimethoprim-sulfamethoxazole (TMP-SMX), erythromycin, clindamycin, tetracycline, and oxacillin was tested by PAT and interpreted according to CLSI guidelines (3a).

The presence of the *ileS2* gene was determined in the first 100 consecutive blood culture CoNS isolates of each year (from 2006 to 2011) using a Lightcycler 480 real-time PCR system (Roche Diagnostics, Mannheim, Germany). For this, isolates were grown overnight at 37°C on sheep blood agar (Oxoid Deutschland GmbH, Wesel, Germany), and three to five colonies were suspended in 1 ml lysis buffer (Roche Diagnostics, Mannheim, Germany). The primers Fw (5’-CTAAAGATTTAGGATACGGTGAC) and Rev (5’-GGATGTAATAATATCCGACGTTC) (Invitrogen, Breda, the Netherlands) were designed to amplify the *ileS2* gene based on the previously described sequence (10). Samples were heated to 95°C for 10 min. Forty-five cycles were run at 95°C for 15 s and at 60°C for 1 min.

Received 6 February 2012 Returned for modification 14 March 2012 Accepted 17 June 2012 Published ahead of print 3 July 2012 Address correspondence to Erik Bathoorn, d.bathoorn@umcutrecht.nl.

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doi:10.1128/JCM.00302-12
Samples were cooled to 40°C for 40 s. PCR tests were performed in duplicate. A mupirocin-susceptible S. aureus strain and a strain carrying the ileS2 gene (donated by A. J. de Neeling, Laboratory for Infectious Diseases and Perinatal Screening, National Institute for Public Health and the Environment) were used as controls in every run; phocine herpesvirus was used as an internal control. In case of discrepancy between PCR ileS2 gene detection and mupirocin susceptibility, PCR was repeated with susceptibility testing by Etest and species determination by MALDI-TOF mass spectrometry. We analyzed the mupirocin susceptibility of all S. aureus bloodstream isolates from 2006 to 2011 tested by PAT.

Mupirocin use data were provided by the Department of Clinical Pharmacy of our hospital. For peri-operative decolonization, mupirocin was used twice daily from the day of surgery until 5 days after surgery. Pharmacy of our hospital. For peri-operative decolonization, mupirocin was used twice daily from the day of surgery until 5 days after surgery. Pharmacy of our hospital. For peri-operative decolonization, mupirocin was used twice daily from the day of surgery until 5 days after surgery. Pharmacy of our hospital. For peri-operative decolonization, mupirocin was used twice daily from the day of surgery until 5 days after surgery. Pharmacy of our hospital. For peri-operative decolonization, mupirocin was used twice daily from the day of surgery until 5 days after surgery. Pharmacy of our hospital. For peri-operative decolonization, mupirocin was used twice daily from the day of surgery until 5 days after surgery. Pharmacy of our hospital. For peri-operative decolonization, mupirocin was used twice daily from the day of surgery until 5 days after surgery. Pharmacy of our hospital. For peri-operative decolonization, mupirocin was used twice daily from the day of surgery until 5 days after surgery.

RESULTS

The University Medical Center Utrecht (UMC Utrecht) is a 1,042-bed academic teaching hospital in the center of the Netherlands, with about 28,000 clinical and 15,000 day care hospitalizations and 334,000 outpatient visits annually. In the 5-year study period, there were 595 CoNS blood culture isolates, and the prevalence of high-level mupirocin resistance due to ileS2 increased from 8% in 2006 to 22% in 2011 (Fig. 1). The annual volume of mupirocin use increased from 3.6 kg in 2006 to 13.3 kg in 2010, which correlates with the trend in high-level resistance among CoNS (Spearman’s rho, 0.137; P = 0.01). The median duration of mupirocin use per patient was 4.3 days (interquartile range, 2.5 to 5.0 days).

Only 2 of 362 S. aureus blood isolates collected between 2006 and 2011 were highly resistant to mupirocin.

Among 238 CoNS bloodstream isolates that were further investigated (2 isolates did not grow), S. epidermidis was most prevalent (n = 150, 63%), and was also the most common species with high-level resistance to mupirocin (n = 25; 78% of all isolates with high-level resistance) (Table 1). The median time from start of hospitalization to the day on which the positive blood culture was taken was 9 days.

Among 237 isolates tested by Etesting and PAT (two isolates did not grow, and PAT testing could not provide a result for 1 isolate), there was agreement at the level of 512 mg/liter for 230 isolates (199 with MICs of <512 mg/liter and 31 with MICs of ≥512 mg/liter). Six isolates had MICs of <512 mg/liter by Etest but MICs of ≥512 mg/liter by PAT, and one isolate had results the other way around. When the Etest was used as a reference, both sensitivity and specificity of PAT were 0.97.

The correlation between ileS2 gene detection with RT-PCR and MIC (cutoff, 512 mg/liter) was determined for 595 isolates (3 were reidentified as Rothia mucilaginosa, Kocuria sp., and Micrococcus sp., which are naturally resistant to mupirocin, and were therefore excluded from analysis; 2 isolates did not grow). In isolates with MICs of ≥512 mg/liter (n = 85), ileS2 RT-PCR was negative for 1. ileS2 PCR cycle threshold values for phenotypically highly resistant isolates ranged from 19.5 to 37.8. In 3 of 513 isolates with MICs of <512 mg/liter, ileS2 was detected, with cycle...
of the ileS2 gene and could change to highly resistant phenotypes under mupirocin pressure (7). We found two isolates with a clearly positive PCR signal which did not display phenotypic resistance to mupirocin. A mutation in the ileS2 gene might be an explanation for this discrepancy between the genotype and phenotype of these four isolates, but this has not been investigated.

We and others have found correlations between resistance to ciprofloxacin, TMP-SMX, doxycycline, and clindamycin and high-level mupirocin resistance (9). This finding has important clinical consequences, as CoNS are frequent causes of prosthetic infections. Treatment of these infections consists of intravenous antibiotics, usually for 2 weeks, followed by prolonged treatment with oral antibiotics. In case of nonsusceptibility to these antibiotics, long-term intravenous treatment is necessary (25).

Several mechanisms have been described for coresistance to mupirocin and other antibiotic classes. Plasmids carrying the ileS2 gene are diverse in size and antibiotic resistance phenotype (13, 20). The co-occurrence of genes encoding resistance to mupirocin and other antibiotic classes on the same plasmid has been described (5, 17). Sequencing of a plasmid from the epidemic strain USA300 (pUSA03) has shown the co-occurrence of genes encoding lower susceptibility to macrolides and lincosamides (6). Clonal spread of the strain with this plasmid could explain coresistance (5). The majority of high-level mupirocin-resistant strains (25 of 30) we have found were S. epidermidis. We typed these isolates by the method described by Johansson et al. (14). We observed some small clusters of multiresistant isolates of the same type. However, there was no evidence for extensive clonal spread of a particular S. epidermidis type among the resistant strains. Both the detection of the small clusters and the median time of hospitalization to the day on which the positive blood culture was taken of 9 days suggests that at least to some extent, ileS2-carrying isolates were acquired in the hospital. Future prospective trials may provide more conclusive answers on this. The ability of a plasmid encoding high-level mupirocin resistance to mobilize nonconjugated plasmids has also been reported (23). Another mechanism might be selection of restriction-deficient strains. A deficiency in the restriction system results in strains hypersusceptible to horizontal transfer of plasmids (4). Such strains may be selected during mupirocin use and are more likely to possess DNA of plasmids encoding resistance to other antibiotic classes in addition to mupirocin resistance.

We conclude that an increase in hospital mupirocin use is associated with a rapid increase in high-level resistance to mupirocin and resistance to other antibiotics in CoNS. This may have direct clinical consequences in the treatment of prosthetic infections and may, in the long term, increase the risk of high-level resistance to mupirocin in S. aureus.

**REFERENCES**


