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Risk factors for extended-spectrum β-lactamase-producing *Serratia marcescens* and *Klebsiella pneumoniae* acquisition in a neonatal intensive care unit

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Summary We investigated the molecular epidemiology of gentamicin-resistant, extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* and *Serratia marcescens*, and risk factors associated with their acquisition in a neonatal intensive care unit (NICU) of a university hospital in Italy. During the study period (April–November 2004), *S. marcescens* was responsible for six infections and 31 colonisations, while *K. pneumoniae* was responsible for six infections and 103 colonisations. Concurrent isolation of both organisms occurred in 24 neonates. Molecular typing identified one major pulsed-field gel electrophoresis pattern each for *S. marcescens* and *K. pneumoniae* strains isolated during the study period. An 80 kb plasmid containing bla_{SHV-12}, bla_{TEM-1} and *aac(6′)-Ib* genes, isolated from both *S. marcescens* and *K. pneumoniae* strains, and showing identical restriction profiles, transferred resistance to third-generation cephalosporins to a previously susceptible *Escherichia coli* host. Birthweight, gestational age and use of invasive devices were significantly associated with *S. marcescens* and *K. pneumoniae* acquisition on univariate analysis, while empiric antimicrobial treatment with ampicillin and gentamicin, and duration of hospital stay, proved to

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be the only independent risk factors. In conclusion, conjugal plasmid transfer and empiric antimicrobial therapy with ampicillin and gentamicin might have contributed to the selection and spread of gentamicin-resistant ESBL-producing Enterobacteriaceae in the NICU.

Introduction

Extended-spectrum β-lactamases (ESBLs) are widespread, particularly in the hospital environment.[1,2] They are usually encoded by genes located on transferable plasmids, they confer resistance to penicillins, third-generation cephalosporins and monobactams, and are commonly found in Gram-negative bacteria, particularly Enterobacteriaceae.[1–9]

Outbreaks by ESBL-producing organisms have been described frequently in neonatal intensive care unit (NICU) settings.[6–13] Clinical conditions and treatments predisposing to infection and/or colonisation by such pathogens have been investigated but very few studies were able to identify independent risk factors.[2,10–15] Moreover, variables associated with colonisation and/or infection vary considerably between the different studies.[2,10–13]

We recently described an increase in the number of ESBL-producing *Klebsiella pneumoniae* isolates in the NICU of our university hospital from September 2002 to December 2004.[9] Aims of the present study were: (i) to analyse the molecular epidemiology of concurrent outbreaks by ESBL-producing *Serratia marcescens* and *K. pneumoniae* occurring in the same ward; (ii) to study the microbial factors predisposing to selection and spread of ESBL-producing bacteria; and (iii) to identify underlying clinical conditions associated with acquisition.

Methods

The tertiary-level NICU of the University 'Federico II' hospital of Naples, Italy serves approximately 300 admissions per year and consists of three rooms with a maximum capacity of eight neonates per room. Sinks, chlorhexidine/alcohol hand disinfectants and gloves are available in each room. Handwashing before and between each patient contact is routinely performed by staff members and visiting parents.

Surveillance of nosocomial infection was prospectively carried out as previously described.[15] Surveillance swabs from the nose, pharynx and rectum of each neonate admitted to the ward were analysed weekly. Informed consent to participate in this study was obtained from patients' parents. The study protocol was reviewed and approved by the local ethics committee.

ESBL-producing Enterobacteriaceae isolated from surveillance swabs and from clinical samples of babies in the NICU were included in the study. Isolates were identified using the API 20E manual identification system (bioMérieux, Marcy-L'Etoile, France) or the Phoenix automatic system (Becton Dickinson Bioscience, Spark, MD, USA). Susceptibility tests were performed using the Phoenix NMIC/ID panel, according to the manufacturer's instructions. ESBL activity was evaluated as previously described.[9] Mating experiments were performed as previously described.[9] DNA macrorestriction and pulsed-field gel electrophoresis (PFGE) of *S. marcescens* and *K. pneumoniae* isolates was performed as previously described.[6,15] Genomic and plasmid DNA preparations and PCR amplifications for bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ genes were performed as previously described.[9] Amplification of *ampC* was performed with primers 5′-GCCGATACCCTGCAACCT-3′ and 5′-TGGCCGTCAGCGCTTCTC-3′ specific for *S. marcescens ampC*. PCR amplifications for *aac(6′)* genes were performed as previously described.[16] DNA sequencing was performed as previously described.[9]

Patients' charts and bacterial isolates from surveillance swabs and clinical specimens of all neonates admitted to the ward during the outbreak period (April—November 2004) were reviewed to assess risk factors. Patients enrolled in the analysis were those included in the active surveillance system (NICU stay >48 h and weekly surveillance swabs taken at least once). Neonates admitted to the ward before April 2004 and/or discharged after November 2004 were excluded. The following risk factors, known to be associated with nosocomial NICU infections,[6,7,10–15] were analysed: birthweight, gestational age, total days of hospital stay, use of invasive devices (percentage and days of both central venous catheters and/or endotracheal tube), and first-line antimicrobial