Early Human Development 89 (2013) 661–665

Contents lists available at SciVerse ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Community-genotype strains of methicillin-resistant *Staphylococcus aureus* with high-level mupirocin resistance in a neonatal intensive care unit

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ARTICLE INFO

Article history:
Received 11 February 2013
Received in revised form 20 April 2013
Accepted 25 April 2013

Keywords:
Methicillin-resistant *Staphylococcus aureus*
Community-acquired
Mupirocin
Drug resistance
Newborn

ABSTRACT

Aim: The aim of this study was to investigate the genotypes of mupirocin-resistant methicillin-resistant *Staphylococcus aureus* (MR-MRSA) isolates in our neonatal intensive care unit (NICU) and their potential source.

Study design: One hundred one MRSA isolates obtained from 59 inborn and 42 outborn infants were identified and their antimicrobial susceptibility determined. Using pulse-field gel electrophoresis (PFGE) analysis, MR-MRSA isolates obtained from the neonatal patients in the NICU were compared with those from adult hospitalized in the same hospital and with community-associated MRSA (CA-MRSA) isolates recovered from different hospitals in Korea.

Results: Overall, 47% of CA-MRSA and 79% of healthcare-associated MRSA isolates exhibited high-level mupirocin resistance (HLMR). Forty-five percent of the outborn infants were considered to have CA-MRSA at the time of admission to our NICU. Most HLMR-MRSA isolates from neonates were grouped into a single cluster by PFGE analysis, and which included CA-MRSA isolates with HLMR recovered from outborn infants who were already colonized when they were transferred to our NICU. They belonged to the same PFGE group as the community-genotype strains isolated from different hospitals in Korea. HLMR-MRSA isolates from adults patients were classified as different clones. None of the attending staff in the NICU were nasal carriers.

Conclusion: Community-genotype strains of MRSA with HLMR may be imported to our NICU through obstetrics clinics and contribute to MRSA colonization or infection in facilities with a high rate of admission of outborn infants.

1. Introduction

Long-term use of mupirocin is generally considered to reduce its efficacy and induce resistance in *Staphylococcus aureus*, although some studies have demonstrated that mupirocin use decreases *S. aureus* colonization and infections without the development of mupirocin-resistant isolates, and others have shown that patients were colonized with mupirocin-resistant, methicillin-resistant *S. aureus* (MR-MRSA) in the absence of widespread mupirocin use [1–3].

Mupirocin resistance is classified into two phenotypes: low-level mupirocin resistance (LLMR) and high-level mupirocin resistance (HLMR). HLMR strains are associated with the failure of mupirocin to prevent colonization; fortunately, most *S. aureus* isolates are categorized as LLMR [4,5]. However, limitation in commercially available reagents makes it difficult in clinical practice to test mupirocin resistance and to monitor its prevalence.

In our neonatal intensive care unit (NICU), MRSA is the most common nosocomial pathogen and accounts for >95% of *S. aureus* isolates. We have implemented *S. aureus* surveillance cultures and universal precautions as part of routine infection control practices to identify colonized infants and to reduce the spread of *Staphylococcus*. However, we hesitated to use routine intranasal mupirocin for *S. aureus* nasal carriers for fear of inducing resistance or losing efficacy. Since new cards for antimicrobial susceptibility testing were introduced recently, allowing us to monitor mupirocin resistance practically, we have identified that the prevalence of mupirocin resistance among MRSA isolates is much higher in our NICU than in other facilities. This unequal distribution may be caused by the frequent use of mupirocin for superficial skin infection in the NICU or be associated with a susceptibility of the pediatric population including newborns to a specific mupirocin-resistant strains of *S. aureus*.

We studied the difference in the prevalence of mupirocin resistance between healthcare-associated (HA) and community-associated (CA)-MRSA in our NICU and the number of prescriptions for mupirocin dispensed from our NICU in recent years compared with those from

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http://dx.doi.org/10.1016/j.earlhumdev.2013.04.014

other departments. In addition, we used pulsed-field gel electrophoresis (PFGE) to compare the genotypes of MR-MRSA isolates from neonates in our NICU with those of isolates obtained from adult patients in the same hospital and from patients from other institutions in Korea.

2. Materials and methods

2.1. Case selection

From April 2011 to March 2012, 223 newborns were admitted to the NICU of our medical institution. To identify the MRSA colonization status of all patients, we initially obtained specimens from anterior nasal cavities of all newborns within 48 h after admission and then collected weekly until discharge. Only the first MRSA isolate from each patient was included in the study. In addition, cultures of the tips of the central venous catheter and the endotracheal tube and MRSA-positive body fluid samples (blood and pus) obtained from the infants with symptoms and signs suggestive of bacterial infection during the study period were included. Based on the interval of 48 h from admission to the initial collection of clinical samples, patients born outside the hospital (outborns) in whom the culture-positive sample was obtained more than 48 h after admission were considered to be HA-MRSA, whereas MRSA isolates from outborns that were obtained within 48 h after admission were considered to be CA-MRSA. All MRSA isolates obtained from infants born within the hospital (inborns) and admitted to NICU soon after birth were categorized as HA-MRSA. Readmission to the NICU from home after discharge is discouraged in principle.

One hundred one newborn infants were positive for MRSA colonization or infection. Of these, 74 isolates showed mupirocin resistance. We aimed to analyze the genotypes of the MR-MRSA strains. We randomly selected two MR-MRSA isolates each month, so 25 isolates were selected during the study period of 1 year. For molecular typing of MRSA isolates by PFGE, HA-MRSA recovered from three adults hospitalized in separate wards in the same hospital, one strain of mupirocin-resistant, methicillin-susceptible *S. aureus* (MR-MSSA) obtained from an outpatient child and a mupirocin-susceptible MRSA (MS-MRSA) obtained from another newborn in the same NICU were included. We added to the analysis MRSA strains collected from pediatric and adult patients from different areas in Korea, for which the multilocus sequence types (MLST) and staphylococcal cassette chromosome *mec* (SCC*mec*) types were already defined.

We compared mupirocin prescriptions dispensed from our NICU over a 3-year period with those prescribed by other departments. All attending staffs in our NICU were interviewed about symptoms of skin infection and without prior notice were screened for MRSA carriage by swabs of the anterior nasal cavity and cultures of nurse's hands. Environmental screening swabs were taken from baby warmers, incubators, blood pressure cuffs, stethoscopes, bathtubs, thermometers and body weight scales.

2.2. Antimicrobial susceptibility and molecular typing

Identification of bacteria and antimicrobial susceptibility were determined using a Vitek II (bioMérieux, Hazelwood, MO, USA). Additionally antimicrobial susceptibility tests, if needed, were performed using the disc diffusion method in accordance with Clinical and Laboratory Standards Institute protocols [6]. The phenotypes of mupirocin resistance were determined according to the manufacturer's instructions. The minimum inhibitory concentration (MIC) breakpoints were as follows: mupirocin susceptibility was defined as an MIC of <8 mg/L, LLMR as an MIC of 8–256 mg/L, and HLMR as an MIC of ≥512 mg/L [7].

Molecular typing of *S. aureus* isolates was performed by the harmonized PFGE protocol described by Murchan et al. [8]. The recommended reference standard, NCTC 8325, was positioned in every fifth or sixth lane to allow later normalization of electrophoretic patterns across the gel. Digital images were stored electronically as .tiff files and analyzed visually and with GelCompar (Applied Maths, Kortrijk, Belgium), using the Dice coefficient, and represented by unweighted pair group method analysis with 1% tolerance and 0.5% optimization settings. A cluster was defined by a similarity cutoff of 80% and a difference of six or fewer bands, as described by Tenover et al. [9].

2.3. Statistical analysis

Statistical analysis was performed with SPSS software, version 13.0 (SPSS Inc., Chicago, IL). Categorical variables were analyzed using the chi-Square test or Fisher's exact test and continuous variables were analyzed with the Student *t*-test or the Mann–Whitney *U* test.

3. Results

3.1. Clinical characteristics of patients and antimicrobial susceptibilities

During study period, 101 of 223 newborn infants admitted to our NICU were positive for MRSA (45.3%). All were considered to be only colonized patients with MRSA, except two patients with bacteremia and four with skin and soft tissue infections. The infections were caused by MR-MRSA and occurred after 48 h from admission. A total of 101 isolates were obtained from 59 inborn and 42 outborn infants. Nineteen of the 42 outborns were already colonized at the time of admission and were considered to have CA-MRSA. Eighty-two (81.2%) isolates were considered HA-MRSA (Table 1). Twenty-seven of the *S. aureus* isolates were MS-MRSA and 74 (73.3%) were MR-MRSA. All MR-MRSA isolates exhibited HLMR. A comparison of the detailed demographic data between patients with CA- and HA-MRSA is illustrated in Table 1. The newborns infected by or colonized with HA-MRSA had younger gestational age and lower birth weight than the infants colonized with CA-MRSA, because most preterm deliveries occurred in hospital rather than in primary care clinics. The rates of Cesarean section were also high because of the high rate of preterm birth. However, the mean age at MRSA acquisition did not differ significantly between the two groups. All isolates from neonates were susceptible to ciprofloxacin, trimethoprim–sulfamethoxazole (TMP-SMX) and vancomycin. HA-MRSA isolates showed a higher prevalence than CA-MRSA of resistance to mupirocin, gentamicin and tetracycline ($P < 0.05$) (Table 1).

When staff and environmental screening was conducted without notice, no MRSA-positive samples were obtained from the nares of

Table 1
Comparison of clinical characteristics of culture-positive patients and antimicrobial susceptibility for HA- and CA-MRSA based on the interval of 48 h from admission to the initial collection of clinical samples.

	HA-MRSA (n = 82)	CA-MRSA (n = 19)	*P*
Patient characteristics			
Gestational age, weeks	33.5 ± 3.9	39.3 ± 1.0	<0.05
Birth weight, g	2084 ± 885	3278 ± 385	<0.05
Age at first detection of MRSA, days	11.7 ± 10.8	7.3 ± 4.9	<0.05
Male to female ratio	47:35	12:7	NS
Cesarean section, n (%)	60 (73.2)	5 (26.3)	<0.05
Outborn infants, n (%)	23 (28.0)	19 (100)	<0.05
Antimicrobial resistance, n (%)			
Mupirocin	65 (79.3)	9 (47.4)	<0.05
Gentamicin	63 (76.8)	6 (31.6)	<0.05
Tetracycline	66 (80.5)	11 (57.9)	<0.05
Erythromycin	62 (78.5)	13 (72.2)	NS
Clindamycin	61 (77.2)	12 (63.2)	NS
Fusidic acid	1 (1.2)	0	NS
Ciprofloxacin	82 (100)	19 (100)	NS
TMP-SMX	82 (100)	19 (100)	NS

Abbreviations: HA-MRSA, healthcare-associated methicillin-resistant *Staphylococcus aureus*; CA-MRSA, community-associated methicillin-resistant *Staphylococcus aureus*; TMP-SMX, trimethoprim–sulfamethoxazole; NS, not significant.