Original Article

An outbreak of methicillin resistant *Staphylococcus epidermidis* among neonates in a hospital in Saudi Arabia

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Abstract

Introduction: *Staphylococcus epidermidis* is a pathogen associated with nosocomial infection in neonatal intensive care units (NICU). This study investigates an outbreak of methicillin resistant *S. epidermidis* in an NICU in a hospital in Saudi Arabia.

Methodology: A total of 41 isolates identified as Gram-positive cocci were obtained from blood culture, umbilical wound swabs and endotracheal aspirate specimens of neonates, of which 29 were identified as *S. epidermidis*. Bacterial identification at the species level and determination of antibiotic resistance were performed by MicroScan (Dade Behring, USA). Genotyping was completed using randomly amplified polymorphic DNA (RAPD) and the *mecA* gene was detected by PCR.

Results: All 29 *S. epidermidis* isolates were found to be resistant to oxacillin and were positive for the *mecA* gene. The isolates showed several multidrug-resistance patterns; the resistance rates to gentamicin, erythromycin, clindamycin, and trimethoprim/sulfamethoxazole were 89.7%, 86.2%, 75.9% and 72.4%, respectively. All isolates were susceptible to vancomycin, teicoplanin, rifampin, synercid, and ciprofloxacin. Several genotypic and phenotypic patterns were detected among the *S. epidermidis* isolates: antibiogram typing showed seven different patterns, one of which was shared by 65% of the isolates, whereas the most prevalent RAPD genotype was shared by only five *S. epidermidis* isolates, and did not correlate with antibiotic resistance phenotype.

Conclusion: The diverse clonal origin of tested isolates indicates the presence of multiple *S. epidermidis* strains among neonates in the NICU setting

Key words: *Staphylococcus epidermidis*; NICU; methicillin resistance; Saudi Arabia

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Introduction

Staphylococcus epidermidis belongs to the coagulase negative staphylococci (CoNS), a group of Gram-positive cocci that causes a high incidence of bloodstream nosocomial infections [1]. *S. epidermidis* is a major nosocomial pathogen, frequently isolated from the normal skin microbiota of patients and healthy individuals [2,3].

Nosocomial infections caused by *S. epidermidis* have been reported in neonatal intensive care units (NICU) in hospitals in the Netherlands and in Italy [4,5]. Similar studies in Saudi hospitals reported that *S. epidermidis* caused 36-40% of infections in NICUs [6,7]. Moreover, cases of *S. epidermidis* pediatric bloodstream infections (55%) [8] in Saudi hospitals have been reported. Other studies have reported cases among adults with *S. epidermidis* bacteremia [9] and postoperative wound infections [10] in Saudi hospitals.

Staphylococcus epidermidis exhibits resistance to methicillin and consequently to all beta-lactams upon acquisition of the *mecA* gene. This gene encodes the PBP-2a, a penicillin binding protein that has low affinity for beta-lactams [11]. Methicillin resistant *S. epidermidis* isolates that circulate within hospital settings are mostly resistant to other classes of antibiotics [12] and can act as a reservoir of mobile genetic elements transferred to other *S. epidermidis* isolates as well as to other *Staphylococcus* species, such as *S. aureus* [11].

The geographical clonal dissemination of methicillin-resistant *S. epidermidis* has been demonstrated, as closely related pulsed-field gel electrophoresis (PFGE) types have been observed among isolates recovered in Iceland, Denmark, Mexico, Uruguay, Greece, and Cape Verde [12]. PFGE has also been used to detect the presence of endemic CoNS clones in NICUs in two hospitals in the Netherlands [13]. Raimundo *et al*. [14] identified one epidemic clone of *S. epidermidis* from an NICU in Australia using a combination of techniques including PFGE, randomly amplified polymorphic DNA (RAPD) and the antibiogram pattern. In a retrospective study between 2000 and 2002 of infants from an NICU in Canada with CoNS bacteremia, *S. epidermidis* was found to be the most common isolate. In this study, molecular typing was done with both PFGE and RAPD, with 14 RAPD patterns found but no predominant clone was identified [15]. Although PFGE is more discriminatory than RAPD [14], the latter technique has been used to type *S. epidermidis* isolates [14,16,17,18], as it provides results in a more timely manner and is more cost effective [4,14].

S. epidermidis has been found to be responsible for nosocomial infections in the Middle East region [9,17,18], but very few studies have attempted to determine the extent of clonal spread of these isolates in hospital settings using genotyping [17,18]. In this study, methicillin-resistant *S. epidermidis* (MRSE) was found to cause bacteremia in 70% of neonates during a 12-week outbreak in a 29-bed NICU at a tertiary hospital in Saudi Arabia. The possible clinical relatedness of these isolates was assessed using the RAPD technique. A correlation of RAPD and antibiotic resistance patterns was attempted.

Methodology

Bacterial strains

Samples of blood culture, cerebrospinal fluid (CSF), umbilical wound swabs and endotracheal aspirates were taken from 41 neonates. Blood culture and CSF were collected by the authorized neonatologist physician. Endotracheal aspirates and umbilical swabs were collected using sterile swab collection tubes. Blood culture samples were collected in blood culture bottles (BD BACTEC Peds Plus/F)) and assayed in the BACTEC 9120 system (Becton Dickinson, Sparks, MD, USA). All other samples were plated without delay on the routine culture media plates, i.e., blood agar, MacConkey agar, chocolate agar and Sabouraud's dextrose agar (BD BBL, Franklin Lakes, NJ, USA) and incubated at 37°C for 24 hours. Forty-one Gram-positive cocci were recovered. The coagulase-negative staphylococci (CoNS) were identified by colony morphology, Gram stain reactions, positive catalase test, and negative coagulase test using the Staphauruex Plus system (Murex Biotech Ltd, Dartford, United Kingdom). Further biochemical identification of CoNS to the species level was performed using MicroScan POS Combo Panel Type PC 1A (Dade Behring, MicroScan, Sacramento, California, USA), and 29 *S. epidermidis* isolates were identified, of which 24 were isolated from blood culture. The patients were sampled daily for three consecutive days, where the same organism, *S. epidermidis* was isolated repeatedly. Isolates were stored in glycerol-containing medium at -70°C until further analysis and were subcultured on Tryptic Soy Agar (Difco, Detroit, USA).

Determination of MICs

The minimal inhibitory concentrations (MICs) of antibiotics were determined using MicroScan POS Combo Panel Type PC 1A (Dade Behring, MicroScan, Sacramento, California, USA) at the hospital laboratory. The antibiotic panel included beta-lactams such as oxacillin, penicillin, ampicillin, cefazolin, cefepime, cefotaxime, cefuroxime, cephalothin, imipenem, meropenem, ticarcillin, and the beta-lactam/inhibitor combination, amoxicillin/clavulanic acid. Other antibiotic classes included trimethoprim/sulfamethoxazole, erythromycin, clindamycin, gentamicin, chloramphenicol, and tetracycline. Results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines [19]. All isolates were found to be resistant to oxacillin (MIC $\geq$ 0.5µg/ml) and all other tested beta-lactams. The majority of the isolates (89.7%) were resistant to gentamicin (MIC > 8µg/ml), 86.2% were resistant to erythromycin (MIC > 4µg/ml), 75.9% were resistant to clindamycin (MIC > 2µg/ml), and 72.4% were resistant to trimethoprim/sulfamethoxazole (MIC > 2/38µg/ml). Low resistance rates (6.9%) to tetracycline (MIC $\geq$ 16 µg/ml) and chloramphenicol (10.3% with MIC $\geq$ 32 µg/ml) were found among the clinical isolates. All isolates were susceptible to vancomycin (MIC $\leq$ 2µg/ml), teicoplanin (MIC $\leq$ 4µg/ml), rifampin (MIC $\leq$ 1µg/ml), synercid (MIC $\leq$ 1µg/ml), and ciprofloxacin (MIC $\leq$ 2µg/ml).

RAPD analysis

The DNA template for RAPD analysis was obtained as previously described [20] using Triton X-

100 lysis buffer. RAPD PCR was done as previously described [16] using two different primers: ERIC-2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') or primer3 (5'-TTATGTAAAAGGACGGCCAGT-3') [16]. RAPD amplified products from the 29 *S. epidermidis* clinical isolates were analyzed on a 1.3% agarose gel by horizontal electrophoresis, in a Tris-acetate-EDTA buffer. Gels were visualized under UV light and photographed with a computer-controlled image analyzer (Whatman, Biometra, USA). The resulting banding patterns were counted, compared, and assigned type names. Patterns differing by one band were considered different types. Differences in band intensity were not taken into consideration [21]. The PCR assay was repeated from fresh cultures for each isolate in order to obtain reproducible results.

Detection of mecA *gene*

Chromosomal DNA of *S. epidermidis* isolates was extracted using a commercially available QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR was performed as described previously [20] with the following specific primers: MRS1 (5'-TAGAAATGACTGAACGTCCG-3') and MRS2 (5'-TTGC GATCAATGTTACCGTAG-3'). A 154-bp amplified DNA fragment was detected on a 2% agarose gel (Roche, Spain) using ΦX174 DNA / BSU RI (HaeIII) as a DNA marker at 100 V for one hour and 30 minutes.

Results and discussion

Medical data of patients

A total of 49 newborn patients were admitted into the NICU of North West Armed Forces hospital in Tabuk, Saudi Arabia, between 5 December 2004 and 26 February 2005. These neonates were born in the hospital and admitted into the NICU due to risk factors associated with the mothers or the babies (Table 1). Of the 49 neonates, 41 were found to have Gram-positive coccal infections. *S. epidermidis* accounted for 29 of these cases (70%), with other Gram-positive isolates identified as streptococci and *S. hemolyticus*. Of the 29 neonates infected with *S. epidermidis*, 18 were male and 11 were female, all but one had low birth weight, and most had risk factors such as congenital heart diseases (Table 1). Mortality was reported for 10% of patients (Table 1).

Antimicrobial susceptibility

The 29 *S. epidermidis* clinical isolates were tested to determine the MICs of eighteen antibiotics. The isolates showed several multidrug-resistance patterns (Table 2). The high resistance rates to beta-lactams (100%) and gentamicin (89.7%) among the isolates could be due to the increased pressure on ampicillin and gentamicin in the NICU (Table 1).These results are in agreement with those of Villari *et al*. [5], who reported a 94.6% resistance to both these antibiotics among their *S. epidermidis* isolates. The authors in that study suggested that increased pressure through use of gentamicin in the NICU might have selected for gentamicin-resistant strains. Antibiotic resistance has been found to be a selective force for certain CoNS strains that circulate within a hospital environment [12]. It has been reported that the possession of *mecA* by CoNS strains also helps such isolates to spread in the NICU [4,21], which may have been the case in this study, as all 29 clinical isolates possessed *mecA*.

In this study, vancomycin was the drug of choice, prescribed to 21 of 29 cases (Table 1). The results of susceptibility testing demonstrated that all *S. epidermidis* isolates were not only resistant to oxacillin, but also exhibited a range of multidrug-resistant patterns. In an attempt to use the antibiotic resistance profile as an epidemiological marker, seven different patterns were found (Tables 1 and 2). Pattern V was shared by 19 of the isolates (65%) (Table 2). Thus antibiotyping was not a helpful method to discriminate between 65% of *S. epidermidis* isolates.

Genotyping

To take preventive measures and implement infection control, identification of the source of infection is required. In this study, the use of RAPD was useful in discriminating between *S. epidermidis* strains tested, confirming what was found in previous studies [17,18,21]. Moreover, Khashu *et al*. [15] found that RAPD was sufficiently discriminatory to detect differences between strains of *S. epidermidis*. In fact, the comparison between PFGE and RAPD results obtained in their study indicated that the RAPD method concurred with results from PFGE analysis [15].

In this study, RAPD analysis revealed 17 distinct patterns with primer ERIC-2 (Table 1), showing between three and five bands in each pattern of relative size ≤ 1 kb (data not shown). Pattern 1 one was common to six *S. epidermidis* isolates designated 31, 47, 29, 41, 90, and 85 (Table 1). Each of the RAPD patterns 2 to 7 was found to be shared by two or three isolates (Table 1). A total of 10 isolates had unique RAPD patterns. RAPD analysis using primer3 was less discriminatory, amplifying only two bands of

Table 1. Medical data for 29 patients admitted to NICU during period between 5-12-04 and 26-2-05. The table also shows different patterns obtained by phenotyping and genotyping of the 29 isolates

Isolate code[a]	Sex of neonate	Type of delivery	Weight at birth (Kg)	Risk Factor if any	Hospital stay (days)	Discharge or Death	Diagnosis	Ventilator	Site of isolation	Antibiotics used	Antibiogram[b]	RAPD analysis	
												Primers	
												ERIC2	3
4	M	SVD	1.276	Preterm Rh incompatibility	36	Discharged	Preterm NEC Hyperbilirubinemia Sepsis	No	Blood	AMP, GEN VAN, CAZ	V	5	1
31	M	C/S	N/A	N/A	7	Discharged	Preterm, RDS	yes	Blood	AMP/SUL	V	1	1
85	M	SVD	1.027	N/A	35	Discharged	Preterm G6PD deficiency MRSE sepsis	No	Blood	VAN, CAZ	V	1	1
41	F	Breech birth	0.746	Triplet	75	Discharged	Preterm, RDS Sepsis, Umbilical hernia	Yes	Blood	AMP, GEN VAN, CAZ	V	1	1
47	M	C/S	1.096	Triplet Preterm	85	Discharged	Preterm, RDS NEC, Sepsis	Yes	Blood CSF, ETT	VAN	III	1	1
29	F	Emergency C/S	1.084	Triplet	50	Discharged	Preterm, RDS Lung collapse MRSE sepsis Urea plasma pneumonia Nasal septal defects	yes	Blood	VAN	III	1	1
6	M	Emergency C/S	1.259	LBW Preterm	29	Discharged	Abruption placenta	Yes	Blood	AMP, GEN	V	7	1
63	M	Emergency C/S	0.8	LBW Preterm	133	Discharged	Sepsis, NEC	Yes	Blood Abscess	VAN, TEC, CLI	V	DP	1
17	F	Emergency C/S	1.588		31	Discharged	Preterm Mild RDS	yes	Blood	AMP, GEN	I	DP	1
16	F	Emergency C/S Pre-eclampsia	0.828	Preterm VLBW	64	Discharged	Preterm, LBW RDS, Sepsis, PDA	Yes	Blood	AMP, GEN VAN	V	6	1
34	M	Breech birth	1.061	N/A	100	Discharged	Preterm, RDS IVH Seizure, Jaundice, Umbilical Hernia , Post hemorrhagic hydrocephalus, MRSE sepsis	yes	Blood	AMP, GEN VAN, CAZ	V	7	1
32	M	SVD	1.535	HBV reactive	32	Discharged	Preterm	no	Blood	VAN	VII	DP	1
35	M	Emergency	N/A	Brother died	68	Death	Preterm	Yes	Blood	VAN, CAZ	V	3	1

Table 1. *(continued)*

		C/S Breech		with the same condition			Pena shokier syndrome						
45	M	SVD	0.710	*Morganella morganii* In cervical suture Candida spp.	12	Death	Preterm, RDS VLBW	Yes	Blood	AMP, GEN AMB	V	6	2
50	F	SVD	1.422	N/A	54	Discharged	RDS Hyperbilirubinemia *Klebsiella* spp. sepsis Vit E deficiency. Upper airway obstruction	yes	Blood ETT	VAN, CAZ	V	DP	1
43	M	Elective C/S	3.35	N/A	15	Discharged	Respiratory distress Right upper lobe collapse	Yes	Umbilical swab,Groin	VAN, CAZ	II	DP	1
49	F	SVD	0.835	N/A	61	Discharged	Preterm RDS	Yes	Blood	AMP, GEN VAN, CAZ	V	DP	3
72	F	SVD	0.812	Triplet	83	Discharged	Preterm Severe RDS ABO incompatibility PDA post legation Aspiration pneumonia	Yes	Blood	AMP, GEN	IV	4	1
39	M	SVD	1.279	N/A	10	Death	VATER association Imperforated anus	No	Blood	AMP, GEN VAN, CAZ	V	DP	1
66	M	SVD	2.257	N/A	24	Discharged	Preterm Cong Pneuom. Sepsis Neonatal anemia	No	Blood	GEN VAN, CAZ	VI	4	1
30	F	Breech birth	1.191	Twin Concentric LVH	48	Discharged	Preterm Mild RDS MRSE-resolved NEC - resolved	Yes	Blood Urine	AMP, GEN AMB	V	5	1
67	F	Elective C/S	1.621	Twins	31	Discharged	Preterm, LBW Large ASD, Anterior displaced anus	Yes	Blood	VAN	V	2	1
76	M	SVD	1.731	Triplet	22	Discharged	Preterm Small for gest. Age Hyperbilirubinemia	No	Blood	VAN	V	4	4
70	F	Emergency C/S	0.933	IUGR	51	Discharged	Preterm, RDS, IVH, Feed intolerance	No	Blood	AMP, GEN VAN, CLI TEC	I	2	1
88	M	EmergencyC/S	1.0909	Triplet	25	Discharged	Preterm	Yes	Blood	VAN	VI	3	1
84	M	SVD	0.965	Congenital heart disease	52	Discharged	Preterm, VLBW IVH, PDA closed medically, ASD Anemia	Yes	Blood CSF	AMP, GEN	IV	DP	1
61	M	SVD	1.024	Triplet	49	Discharged	Preterm, VLBW	No	Blood	TEC	V	DP	1

Table 1. *(continued)*

				Preterm			MRSE sepsis Jaundice, Anemia						
90	M	Emergency C/S	1.128	Preterm	42	Discharged	Neonatal jaundice MRSE sepsis Symmetrical IUGR,RDS	Yes	Blood	VAN, TEC	V	1	2
62	F	SVD	1.279	Twins G6PDdeficiency	41	Discharged	Preterm RDS, Sepsis	No	Blood	AMP,GEN VAN	V	DP	1

SVD: Spontaneous vaginal delivery, Emergency C/S: caesarian section, CSF: Cerebrospinal fluid, N/A: not available, ETT: Endotracheal aspirate, NEC: necrotizing enterocolitis, Preterm RDS: Preterm respiratory distress syndrome, Symmetrical IUGR: symmetrical intrauterine growth retardation, VLBW: very low birth weight, ASD: Atrial septal defect, LBW: low birth weight
DP: each isolate showed a unique pattern.
Antibiotic abbreviations: AMP: ampicillin, GEN: gentamicin, VAN: vancomycin, CAZ: ceftazidime, AMB: Amphotericin B, TEC: Teicoplanin, CLI: clindamycin
a All isolates were *mecA* positive.
b See table 2

Table 2. Patterns of the antibiogram

	Antibiogram pattern [a]	**Isolate code**
I	OX; PEN; AMP; AMC; CFZ; FEP; CTX; CXM; CF; IMP; MER; TIC	17, 70
II	OX; PEN; AMP; AMC; CFZ; FEP; CTX; CXM; CF; IMP; MER; TIC; SXT	43
III	OX; PEN; AMP; AMC; CFZ; FEP; CTX; CXM; CF; IMP; MER; TIC; GM, E	47, 29
IV	OX; PEN; AMP; AMC; CFZ; FEP; CTX; CXM; CF; IMP; MER; TIC; GM; E; TE	72, 84
V	OX; PEN; AMP; AMC; CFZ; FEP; CTX; CXM; CF; IMP; MER; TIC; GM; SXT; E; CC	31,41,85, 6,4, 63,16,34,45, 35, 50,49, 39,30,67, 76,61,90,62
VI	OX; PEN; AMP; AMC; CFZ; FEP; CTX; CXM; CF; IMP; MER; TIC; GM; E; CC, C	66, 88
VII	OX; PEN; AMP; AMC; CFZ; FEP; CTX; CXM; CF; IMP; MER; TIC; GM; CC, C; SXT	32

a The minimal inhibitory concentration (MIC) was determined using MicroScan POS Combo Panel Type PC 1A (Dade Behring; MicroScan, Sacramento, California, USA).
OX: oxacillin; PEN: penicillin; AMP: ampicillin; AMC: amoxicillin/ clavulanic acid; CFZ: cefazolin; FEP: cefepime; CTX: cefotaxime; CXM: cefuroxime; CF: cephalothin; IMP: imipenem; MER: meropenem; TIC: ticarcillin; SXT: trimethoprim/sulfamethoxazole; E: erythromycin; CC: clindamycin; GM: Gentamicin; C: Chloramphenicol, TE: tetracycline

relative size < 0.5- 1.0 Kb (data not shown) and resulting in only four different RAPD patterns (Table 1).

The RAPD results in Table 1 show that five *S. epidermidis* isolates (31, 47, 29, 41, and 85) shared identical banding patterns using both primers. In addition, the results of RAPD typing corresponded with antibiogram typing for three of these five isolates (Tables 1, 2). *S. epidermidis* strains with a particular RAPD genotype that share an identical antibiotype have been reported earlier [12].

In conclusion, RAPD and antibiogram types detected among the *S. epidermidis* isolates over the examined time period did not indicate any common clones, except for one RAPD genotype shared by five *S. epidermidis* isolates. The diverse clonal origin of the tested isolates indicates that there was no dissemination of a single *S. epidermidis* strain among neonates in the NICU setting.

Recommendations were set to limit infection in the NICU and included the use of sterile gowns when entering the NICU, changing gloves when handling different neonates, and hand hygiene using soap and drying with paper towel or rubbing with an alcohol-based product or chlorhexidine gluconate 0.4%, monitored by a surveillance camera placed in front of the dispensing unit.

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