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Sources and Risk factors of a Novel MRSA Spa Type Circulating in Neonatal Intensive Care Unit

Rula Darwish [1*], Luay Abu-Qatouseh [2], Eman Badran [3]

[1] Department of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy, The University of Jordan, Amman, Jordan
[2] Department of Pharmacology and Biomedical Sciences, Faculty of Pharmacy, University of Petra, Amman, Jordan
[3] Department of Paediatrics, Division of Neonatology-Perinatology, School of Medicine, The University of Jordan, Amman, Jordan

ABSTRACT

Background: Methicillin-Resistant *Staphylococcus aureus* is a major source of colonization and infection affecting premature neonates in Neonatal Intensive Care Unit. **Objectives:** to determine the prevalence, risk factors, and resistant profiles in neonates, their mothers, and healthcare personnel. In addition, to confirm the clonal similarity of neonatal Methicillin-Resistant *S. aureus* to their mothers or healthcare personnel. **Materials and Methods:** Samples were screened and identified for Methicillin-Resistant *S. aureus* colonization by gram stain, biochemical tests, oxacillin disk diffusion test, and resistance pattern. PCR was used to detect the presence of **nuc** and **mecA** gene. DNA fingerprinting was conducted using a standard spa typing technique. **Results:** Healthcare personnel colonization was high (27.9%) compared to neonatal and maternal colonization (15F%, 9.7%, respectively) and was found to be the only significant risk factor for neonatal MRSA colonization. Fifteen different spa types were identified and the novel t12492 was predominant among neonates and was reported for the first time. There was no demonstrated distribution correlation with sources of colonization. Colonization appeared to originate from multiple sources. **Conclusion:** Findings suggest conducting periodical molecular investigations of colonization of MRSA in healthcare personnel. Surveillance, molecular analysis of strains, reinforcement of an inclusive infection control program and antibiotic control could be useful in preventing MRSA transmission.

Keywords: Neonatal Intensive Care; MRSA; Spa type; Risk-factors; Colonization.

INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is becoming a major source of colonization and infection affecting critically ill and premature neonates in Neonatal Intensive Care Units (NICU). This is linked with high morbidity and high financial burden (1). Data on MRSA colonization in middle and low- income countries is limited and usually reliant on studies from developed countries. Understanding how infants in high-risk settings become colonized with MRSA is important, as colonization is a risk factor for infection. Several risk factors for MRSA colonization and infection have been identified in some studies, including low gestational age, contact with healthcare personnel, mode of delivery, lower birth weight, multiple gestation, mechanical ventilation, endotracheal tube intubation, central venous catheterization, parenteral feedings and gavage and length of stay in the hospital (2, 3). MRSA colonization in neonates can happen in many ways most importantly, via contact with healthcare personnel (HCP) or the hospital

[*] ***Corresponding author: Rula Darwish***
rulad@ju.edu.jo
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environment or from mothers to their infants.

Many studies were done to determine risk factors that might predispose certain infants to colonization or infection (4-6). A wide variety of risk factors were identified including: low birth weight, prematurity, and multiple gestations. Procedures and devices required during neonates' hospital stay including; endotracheal tube intubation, mechanical ventilation, percutaneous central venous catheterization, and surgery (7) are also associated with increased risk of MRSA. Overcrowding and understaffing in NICU, have been associated with increased risk of healthcare-associated transmission and colonization, which may lead to epidemics of MRSA infection (8). Huang et al (9) found that infants who were colonized with MRSA had a significantly higher rate of MRSA infection (26%) as compared to those who were not colonized (2%). The best strategy for managing neonatal MRSA infections would be prevention rather than treatment. MRSA colonization usually precedes MRSA infections and according to Pierce R et al, each additional day of exposure to an untreated colonized infant may increase acquisition risks of MRSA by 6% (10). Therefore, decolonization may prevent MRSA infections by eliminating bacterial reservoirs. However, the progressively increasing resistance of MRSA to vancomycin poses a great challenge for anti-infective treatment of MRSA in neonates, especially that vancomycin is the empiric antibiotic of choice in neonates with sepsis and extensive skin infections (11). Acquiring information on MRSA transmission and their antibiotic susceptibility in high-risk settings in different countries is very important and useful to address specific targeted intervention strategies. Molecular typing processes, such as spa typing, and multilocus sequence typing are essential to control MRSA spread. In addition prevention of MRSA spread in health care settings would be possible when molecular typing is well-timed (12). Also, spa typing together with *mec A* gene subtyping can improve communication between laboratories on national and international levels for MRSA surveillance. This research aims to identify the prevalence of MRSA colonization, risk factors, resistant profile, sources, and spa types. Moreover, to verify if neonatal MRSA strains are related to their mothers or HCP in NICU to find potential areas for clinical intervention. This will aid in the control of MRSA clone spread among neonates by establishing preventive strategies such as a screening process.

Methods

Study type and Settings

This study is a prospective cohort study. A neonatal sample size of 173 was chosen with a confidence interval of 90%, a proportion of 80%, and a margin of error of 5%. The study was conducted in high-risk settings at the NICU. in a teaching referral Jordan University Hospital in Amman, over 24 months' period. The unit is a level II/III NICU with 30 beds, including 21 intensive care and 9 intermediate care beds (30 beds in 6 rooms). It admits approximately 1000 inborn neonates to high-risk pregnant mothers every year. The nurse-to-patient ratio was 1: 4-5. The NICU is monitored for the implementation of infection prevention policies and protocols by the infection control committee. A total of 102 mothers and their 120 neonates were identified using a daily log of the birth records. Mothers were recruited at the time of their first visit to their infants in the NICU. HCP: Physicians (n=8) were recruited at the start of their work in NICU as well as nurses (n=35) in direct contact with the recruited neonates during the implementation of the study (figure 1). Those who were included in the study were; healthy mothers with the absence of mastitis who gave birth to single or twin infants within the time of data collection with their inborn neonates. The control group was neonates with negative laboratory tests for MRSA colonization. Also, HCP working in NICU during the study (n=43). Participants were considered as colonized with MRSA if they had MRSA with no signs of infection. The excluded participants included; mothers who refused nasal swab sampling (n=36), neonates with lethal congenital anomalies or refusal of their legal guardians to take nasal swab samples, incomplete neonatal health records, and out born neonates. HCPs who were unable or unwilling to participate in the survey (n=25) were excluded.

Methods

The routing hygiene care

Sponge bathing procedure using mild soap and water every three days by means of cotton cloth prepared for each infant. HCP washed his hands and used an antiseptic solution before and after handling the baby.

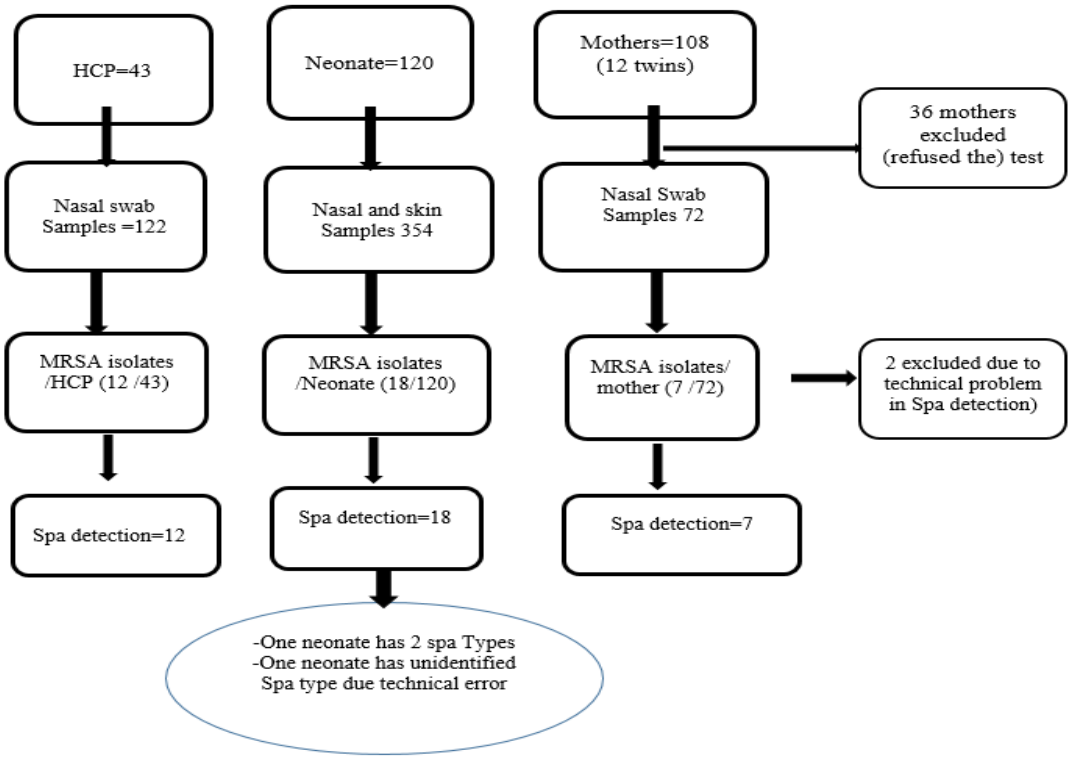
Data management and collection of sample

A research assistant was recruited and trained on sample collection, clinical data filling from medical records, microbiological processing, and DNA extraction. Neonatal swabs of anterior nares and umbilicus were taken once upon admission or on the third day of life nearest to working days. For non-colonized neonates in the first three days of life, further weekly sampling was performed until discharge. MRSA colonized infants were cohorted and kept in isolation until their discharge.

Maternal swabs were taken once from anterior nares in the first 48hrs of the newborn's life. Nasal swabs were obtained from 8 physicians at the start of their work in the NICU and were retaken if their one-month rotation was repeated. Nasal swabs were collected from nurses (n=35) on monthly basis during the study. A total of 548 nasal swabs were tested from the three groups. (figure 1)

[neonates =354/548 (64.5%); mothers =72/548 (13.3%) and HCP (8 doctors+35 Nurse) =122/548 (22.3%)].

Figure 1: Participants profile



Nasal swab samples were collected over 6 months. Swabs were immediately placed in a transport medium and subsequently sent to the microbiology lab in the School of Pharmacy / The University of Jordan for primary identification using gram staining and biochemical tests. For the determination of MRSA, an oxacillin disk diffusion test was used.

Strain characterization experiments conducted on the clinical isolates

Clinical samples were screened and identified for Methicillin-Resistant *Staphylococcus aureus* colonization by gram stain, biochemical tests, oxacillin disk diffusion test, and resistance pattern. PCR was used to detect the presence of nuc and mecA gene.

Antimicrobial resistance detection and molecular typing

MRSA strains were further tested for in vitro antimicrobial resistance patterns to eight conventional antibiotics (gentamicin, amikacin, ceftazidime, ceftriaxone, clarithromycin, and oxacillin) by Kirby-Bauer disk diffusion technique on Mueller-Hinton agar according to recommendations of the Clinical and Laboratory Standard Institute (13). Results were recorded after incubation for 18 hours at 37°C. Multidrug resistance (MDR) was defined as resistance to three or more unique antibiotic classes. The sensitivity of *S. aureus* strains to methicillin was determined by cefoxitin disk (30 µg, Himedia-India) and confirmed by *mecA* using polymerase chain reaction (PCR) as described below. *S. aureus* ATCC 25923 was used as a control strain for antibiotic susceptibility testing. Bacterial DNA was extracted using standard protocols and stored at a temperature of -80 ^{0}C. Spa typing was performed later on only 37 isolates. Genotypic identification of *S. aureus* was determined using standard methods of PCR for the presence of the **nuc** gene. The presence of **mecA** was determined by PCR using **mecA**-F (5'-AAA ATC GAT GGT AAA GGT TGG C-3') and **mecA**-R (5'-AGT TCT GCA GTA CCG GAT TTG C-3') primers (Macrogen, Seoul, South Korea). Then DNA fingerprinting of clinical isolates was conducted by standard spa typing technique (14). The polymorphic X region of protein A gene (15) was amplified from all **MRSA** isolates by using the primers spa-1113f (5′-TAA AGA CGA TCC TTC GGT GAG C-3′) and spa-1514r (5′-CAG CAG TAG TGC CGT TTG CTT-3′). All sequencing reactions were carried out with an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA). **Spa** types were determined and assigned using StaphType software (version 1.4; Ridom GmbH, Würzburg, Germany), as described by Harmsen et al (16).

Statistical analyses:

Statistical analysis was conducted using SPSS 22 (SPSS Inc., Chicago, USA). Analysis of contingency tables (chi-square test) was used to assess differences in frequencies. Fisher's exact test was applied at frequencies of less than 5. Differences were considered significant at $p < 0.05$.

Ethical approval

This study was conducted according to "Good Clinical Practice", Declaration of Helsinki (2004; Tokyo), and "Good Laboratory Practice" for analysis of samples. Although the studied bacterial isolates were obtained during the daily care of preterm infants in NICU, ethical approval from the hospital was obtained as an institutional policy for the research (approval number:53/2011). All participants were provided with informed written consent after receiving full information about the purpose of the study, the voluntary participation, and the right to withdraw at any time.

Results

Rate of colonization of *Staphylococcus sp., S. aureus* MRSA among participants' swab samples

The distribution of *Staphylococcus aureus and MRSA* among the samples and the studied subgroups is summarized in Table 1. Total isolated colonized *Staphylococcus sp*. from all nasal swab samples of participants of the three groups occurred in 54.4% (298/548). Regarding *S. aureus* nasal swab distribution, there was a significant difference among the three groups (Table 1). It was significantly high among mothers' nasal swabs (24/72; 33.3%) with almost equal percentages among neonates (40/354; 11.3. %) and HCP (18/122; 14.8%) swabs. *S.aureus* infections were not identified in the blood of colonized neonates *Regarding* MRSA colonization swab distribution , the colonization rates among neonates (18/354; 5.1%) were lower than mothers (7/72; 9.7%) and HCP (12/122; 9.8%) which did not reach significant level (p- value > 0.05).

Table 1: Correlation between nasal swab samples of participants subgroups with Isolated Staphylococcus sp., *Staphylococcus aureus, and MRSA colonization and* Correlation of *MRSA colonization* with participants' subgroups

			Nasal swab sample source							
	Total nasal swab samples = 548		**Mothers' =72**		**Neonates** =354**		**HCW=122**		**Chi2**	**P value***
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**		
Isolated *Staphylococcus* sp. Colonization a									17.4	< 0.05
Yes	298/548	54.4	32/72	44.4	180/354	50.8	86/122	70.5		
No	250/548	45.6	40/72	55.6	174/354	49.2	36/122	29.5		
Isolated *Staphylococcus aureus colonization*									22.8	< 0.05
Yes	82/548	15.0	24/72	33.3	40/354	11.3	18/122	14.8		
No	466/548	85.0	48/72	66.7	314/354	88.7	104/122	85.2		
Isolated MRSA *colonization*									4.4	.12
Yes	48/548	8.80	7/72	9.7	18/354	5.1	12/122	9.8		
No	500/548	91.2	65/72	90.3	336/354	94.9	110/122	90.2		
Isolated MRSA *colonizatin anong Participants*	Total Number of participants N= 235		Mothers' n=72		Neonates** n =120		HCP n=43		13.45	< 0.001183
Yes	37/235	15.7	7/72	9.7	18/120	15	12/43	27.9		
No	198/235	84.	65/72	90.3	102/120	85	31/43	72.1		

* P-value <0.05 indicates that the proportion in at least one subgroup is statistically significantly different from the values in the other subgroups
** MRSA was identified in the first three days of admission for all colonized neonates

Rate *MRSA* colonization among participant's subgroups

Results indicates MRSA rates in HCP (12/43; 27.9%) and neonates (18/120; 15%) were significantly higher than those of mothers (7/72; 9.7%). Most MRSA colonized neonates (n=18) were detected in the first three days of life.

Risk factors for MRSA colonization in neonates

Table 2 summarized the correlation between risk factors that might be associated with increased rates of MRSA. Unexpectedly the only risk factor which had a significant association with MRSA colonization was HCP colonization p-value 0.0314 ($p < 0.05$). Other risk factors did not show a significant association with MRSA colonization among neonates.

Table 2 Correlation between Risk factors with MRSA colonization among neonates

		MRSA Colonization					**95%CI**		
	Total N=120	**Colonized 1**		**Not Colonized N =102**		**OR**		**Upper CI**	**P-value**
	Total N(%)	N	%	N	%				
Gender						.962	.353	2.619	0.939
Male	59/120(49.2)	9/18	50.0	50/102	49.0				
female	61/120(50.8)	9/18	50.0	52/102	51.0				
Mode of delivery						.436	.158	1.204	0.103
Cesarean	74/120(61.7)	8/18	44.4	66	64.7				
vaginal	46/120 (38.3)	10	55.6	36	35.3				

		MRSA Colonization					95%CI		
	Total N=120	**Colonized 1**		**Not Colonized N =102**		**OR**		**Upper CI**	**P-value**
Birth weight						1.645	.196	13.839	0.644
Less 2.5 Kg	110 /120(91.7)	17	94.4	93	91.2				
More or equal 2.5	10/120(8.3)	1	5.6	9	8.8				
Total	120	18							
Preterm						1.3	.475	3.56	0.609
Less 37 week	60/120(50)	10	55.6	50	49				
More equal than 37 week	60/120(50)	8	44.4	52	51				
Duration of hospitalization						0.585	0.204	1.680	0.315
Less /or =one week	53/120(44.2)	6	33.3	47	46.1				
More than week	67/120(55.8)	12	66.7	55	53.9				
PROM*						0.786	0.163	3.793	0.767
Yes	16/120 (13.3)	2	11.1	14	13.7				
No	104/120 (86.7)	16	88.9	88	86.3				
Total									
SGA						2.231	0.694	7.173	0.170
yes	20/120(16.7)	5	27.8	15	14.7				
No	100/120(83.3)	13	72.2	87	85.3				
Neonatal Death						0.941	0.107	8.317	0.957
yes	7/120(5.8)	1/18	5.6	6	5.9				
No	113/120 (94.2)	17	94.4	96	94.1				
Maternal colonization									.818756
yes	9/120(7.5)	2/18	11.1	7/102	6.9				
no	63/120(52.5)	9/18	50.0	54/102	52.9				
ND	48/120(40)	7/18	38.9	41/102	40.2				
Health care personnel colonization						4.57	1.15	18.24	0.0314
yes	10/120(8.3)	4/18	22.2	6/102	5.9				
no	110/120(91.6)	14/18	77.8	96/102	94.1				
Central line						0.8	.26	2.47	.697885
yes	91/120(75.8)	13/18	72.2	78/102	76.5				
no	29/120(24.2)	5/18	27.8	24/102	23.5				
Use of invasive Mechanical ventilator						0.3005	0.0166	5.4360	0.4157
yes	8 /120 (6.7)	0	0	8	6.7				
No	112/120(93.33)	18	100	94	92.2				

P-value was considered significant at $p < 0.05$ within subgroup analysis ***PROM Premature Rupture of Membranes**

Susceptibility of MRSA isolates

MRSA isolates were tested for their susceptibility to different types of antibiotics (Table 3). There was no statistical difference in resistance against clindamycin and vancomycin among the three groups. An alarming vancomycin resistance occurred in 10 % of the nasal swab samples. MDR was significantly higher in neonates 89.6% when compared with mothers and HCP (22.2%,41.6% respectively). Most of the MRSA isolates in neonates were resistant to macrolides and third-generation cephalosporins including cefotaxime and ceftriaxone.

Table 3: Correlation between antibiotic-resistant patterns for MRSA isolates and subgroups

Antibiotics	MRSA isolates Total N=37		Neonates N=18		Mothers N=7		HCP N=12		Chi2	P-value
	N	%	N	%	N	%	N	%		
Gentamycin	16/37	43.2	10/18	55.6	2/7	28.6	4/12	33.3	2.2059	0.331
Amikacin	4/37	10.8	2/18	11.1	1/7	14.3	1/12	8.3	0.1657	0 .920
Erythromycin	22/37	59.5	15/18	83.3	2/7	28.6	5/12	41.6	10.9677	0.004
Clarithromycin	20/37	54.1	13/18	72.2	2/7	28.6	5/12	41.6	4.967	0.094
Ceftazidime	25/37	67.6	17/18	94.4	2/7	28.6	6/12	50	12.4811	0.002
Ceftriaxone	22/37	59.5	14/18	77.8	2/7	28.6	6/12	50	5.721	0 .057
Clindamycin	8/37	21.6	4/18	22.2	2/7	28.6	2/12	16.7	.3772	0 .828
Vancomycin	4/37	10.8	1/18	5.6	1/7	14.3	2/12	16.7	1.03	0.598
MDR*	23/37	62.2	15/18	88.9	2/7	28.6	5/12	41.6	8.6027	0.014
P-value was considered significant at $p < 0.05$ within subgroup analysis										

Distribution of the spa types

Among 18 MRSA colonized neonates,18 spa types were detected in 17 infants. Spa typing of MRSA isolates from the groups revealed fifteen different types (Table 4). The novel type t 12492 was the most common (60%; 9/15) followed by t934 (33.3%; 5/15) and t253 (26.7%; 4/15). t 044 and t11023 were equally distributed among spa types (20%; 3/15). t021and t3534 were similarly spread among MRSA isolates (13.3%;2/15). t012, t214, t233, t338, t3767, t 5075, t5634, and t668 were the least detected with a percentage of 6.7% (1/15). Tables 4 demonstrated the distribution of spa type among mothers and their neonates and HCP. Spa typing of MRSA isolates of mothers and infants suggested potential mother-to-infant transmission within one mother-infant pair (cases 18; table 2) sharing the MRSA clone t12492. No similarities in spa types were observed among isolates from mothers and infants of remaining cases. MRSA clone t044, t934, t253 were shared between HCP and infants of cases 5,11,14,21, and the unique t12492 of cases 2,8,9,19, 22. Two MRSA clones were isolated from the same infant (case 5), in two different sampling times, t934 (week 1) t11023(week 2); indicating different clonal persistence.

Table 4: Comparison of MRSA colonization by SPA type among neonates and their mothers

Infant Case Number	Neonate MRSA colonization	neonate SPA type	Maternal MRSA colonization	Maternal Spa type
1	Yes	Not assigned	Yes	t253
2	Yes	t12492***	ND**	-
3	Yes	t012	NO	-
4	Yes	t11023	ND**	-
5 (sample1)	Yes	t934	No	-

Infant Case Number	Neonate MRSA colonization	neonate SPA type	Maternal MRSA colonization	Maternal Spa type
5 (sample2)	Yes	t11023		
6	No	-	Yes	t044
7	Yes	t5075	No	-
8	Yes	t12492***	No	-
9	Yes	t12492***	No	-
10	No	-	Yes	t021
11	Yes	t253	No	-
12	Yes	t3534	ND**	-
13	No	-	Yes	t223
14	Yes	t044	No	-
15(Twin 1)	No	-		
16(Twin 2)	No	-	Yes	t253
17	No	-	Yes	t11023
18	Yes	t12492***	Yes	t12492***
19	Yes	t12492***	ND**	-
20	Yes	t3534	ND**	-
21	Yes	t934	ND**	-
22(Twin 1)	Yes	t12492***		-
23 (Twin 2)	Yes	t3767	No	

*: BD (bad quality for Spa detection)
**: ND Not done (maternal refusal)
*** unique spa type
- No MRSA

Discussion

The *S. aureus* including MRSA colonization is dynamic and can be changed from negative to positive or vice versa in individuals across a period of time. In 43 HCWs, a total of 122 nasal swab samples were taken which indicated ~2.8 samples were taken for one HCPs. In comparison the rate was 2.95 in 120 neonates and the rate was 1 among mothers. This study reported that rates of *S. aureus* swabs colonization among neonates (11.3%) and HCP (14.8%) was significantly lower than that of mothers (33.3%). Findings of high rates of *S. aureus* colonization in mothers can impact babies' health, healthcare system, and community. In one study, colonization rates of *S. aureus* were reported to be 40–50% during the first 8 weeks of life, followed by a gradual decrease to around 20% at 6 months of age (17).Regarding the rate of colonization of MRSA among participants' a significantly high MRSA colonization rate was noticed with a rate of 27.9% in HCP while neonates and mothers were 15% and 9.7% respectively. This concurs with findings of Jimenez-Truque N et al. who indicated that maternal MRSA colonization occurred in 10- 17% of mothers, with the highest prevalence at enrollment and 20.9% in infants (18). Similarly, a study by Balamohan et al. showed the colonization rate to be 14.5 % (19) .Other studies (20) showed even lower frequencies of MRSA colonization in a range from 1- 4% in infants and mothers. MRSA colonization shown in our study could be due to many factors like misuse of antibiotics, overcrowding, low nurse to patient ratio (1: 4-5), etc. Behari P et al. stated that mothers can be a potential reservoir for MRSA as a source of transmission to their neonates in the NICU (21).This study reported a higher rate of MDR in neonates and HCP compared to mothers. In agreement with results reported

by Carey AJ et al which showed healthcare-associated strains tend to be resistant to multiple antibiotics (22). The context of HCP and babies carried more MDR MRSA than mothers indicate that MRSA circulating in babies could have hospital origins, which in turn suggest a horizontal transmission from HCP to babies. In context of direct contact of HCP with neonates thus spreading MRSA ; literature revealed that MRSA colonization spread horizontally through contact with HCP or hospital environment (23). Many antibiotics have demonstrated reduced efficacy against MRSA. This study confirms this with most MRSA isolates in neonates being resistant to macrolides and third-generation cephalosporins including cefotaxime and ceftriaxone. This is in concordance with findings of Kaur and Chate (24) who reported that MRSA isolates were resistant to routinely tested antibiotics however none of their isolates were resistant to vancomycin. The study revealed quite high astonishing resistance to vancomycin mainly among HCP and mothers as compared to neonates but this difference did not reach a significant level. Moreover, clindamycin resistance was also high in the three groups with no significant difference. Further investigation is needed to verify the MIC of VRSA and to correlate neonatal spa types with the antimicrobial resistance to find potential areas of clinical interventions. Understanding how infants in NICU become colonized with MRSA is important, as colonization is a risk factor for infection. Surprisingly, the results of this study showed that low birth weight and low gestational age did not reveal a significant difference in MRSA colonization rate. This contradicts other studies that showed low birth weight and prematurity are risk factors for neonatal MRSA colonization and infection (7). The study did not show the contribution of other risk factors including central line insertion, prolonged rupture of membrane, age of starting feed, invasive ventilation, mode of delivery, gender, length of hospitalization stay with rates of neonatal colonization although they have been described as risk factors by other (25). Possible explanations for this are the strict monitoring of the infection control committee over the implementation of their policies and protocols and the small participants size of the study. In concordance with Giuffrè, Mario, et al.(26) this study showed that most MRSA colonization occurs in the first week. Many studies measuring maternal and neonatal colonization, showed strong evidence for increased possibilities of surface colonization in newborns of colonized mothers, supporting indications that there is a direct transfer from mother to newborn during delivery through contact. On the contrary maternal colonization in this study was an insignificant risk factor for neonatal colonization. Nevertheless, it should be taken into consideration that laboratory procedure, may underestimate several factors that can affect results including nasal swab samples' volumes, procedures used, and dilution methods (27). The current study showed that colonized HCP rather than maternal colonization was a source for neonatal MRSA colonization. This concurs with the WHO initiative in addressing HCP as a risk factor, which gained importance to a point that has led to the introduction of the term "healthcare-associated infection"(28). Literature showed that the incidence of MRSA associated with HCP has increased over the last years in NICU (29). In support of this, the present work reports correlation between MRSA isolates spa types recovered from HCP, mothers, and their neonates. It also highlights the emergence of a unique MRSA spa type and looks at infant colonization as it relates to maternal and HCP colonization in NICU. The genetic diversity of 15 different spa types detected in our study including a new one registered as t12492 in Ridom Spa Server is noteworthy (https://spaserver.ridom.de/spa-t12492.shtml). Furthermore, the sharing of the MRSA clone t044, t934, t253 between HCP and infants of cases 5,11,14,21 and the unique t12492 of cases 2,8,9, 19, 22 was also evidenced. Similarly, MRSA clone t12492 was the same clones within one mother–infant pair suggesting a potential mother-to-infant transmission.

MRSA isolates recovered from HCP displayed several

genetic fingerprinting similarities using spa typing as compared to colonized neonates, making nasal flora of HCP a likely reservoir for MRSA transmission. Differences in genetic fingerprinting for the other isolates might suggest that MRSA is also transmitted from infant to infant or from the environment. Also, the same practices that facilitate transmission between neonates could promote self-colonization. Clinical evidence, resistance profiles, and spa typing suggested a more relevant role of horizontal dissemination to this cohort of neonates. These results are per a study by Ulrich Nu¨be et al (30) who further suggested integration of epidemiological and genomic data to enable studying specific MRSA transmission routes within the NICU(31).

Conclusions

Our results confirm previous studies reporting multiple strains of MRSA circulating in NICUs during a non-outbreak setting. One of our major findings is the presence of significantly high MRSA colonization rate that was noticed with a rate of 27.9% in HCP as compared with neonates and their mothers (15% and 9.7% respectively) and the emergence of a novel predominant spa type (t12492) among MRSA strains from neonates in Jordan. Colonization by MRSA of infants appeared to originate from multiple sources and not from a single point. In an attempt to identify all colonized MRSA neonates as part of infection control, we suggest that conducting active periodical screening investigation for colonization of MRSA in neonates and HCP. Molecular analysis of strains, reinforcement of an inclusive infection control program and antibiotic control could be useful in preventing MRSA transmission.

This paper highlights the importance of molecular typing to trace transmission routes and to identify intervention strategies. An alarming vancomycin resistance occurred in 10 % of the total sample's swabs.

The findings would have an impact of infection control program and antibiotic control as useful too in preventing MRSA transmission. As antibiotics, disinfection, and antiseptic policies in NICUs. We recommend conducting a periodical molecular investigation of the colonization of MRSA in the HCP of the NICU. The increasing frequency and use of molecular typing methods could provide an adjunctive solution to reduce transmission of MRSA in the NICU. Colonized neonates in NICU may contribute to a growing population of infants at risk for MRSA colonization and subsequent infections.

Conflict of interest The authors declare that they have no conflict of interest

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Authors' contribution: RMD, LFQ, EFB have made a substantial contribution to the conception, design, and conduction of the study in addition to drafting the article and revising it critically.

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مصادر والعوامل المحددةلانتشار نوع MRSA Spa الجديد المنتشر في وحدة العناية المركزة لحديثي الولادة

رلى مدحت درويش [1]* ، لؤي أبو قطوسة [2]، إيمان بدران[3]

[1]قسم الصيدلانيات والتقنية الصيدلانية، كلية الصيدلة، الجامعة الأردنية، عمان، الأردن

[2] قسم الصيدلة والعلوم الطبية الحيوية، كلية الصيدلة، جامعة البترا، عمان، الأردن

[3] قسم طب الأطفال، قسم حديثي الولادة، طب الفترة المحيطة بالولادة، كلية الطب، الجامعة الأردنية، عمان، الأردن

ملخص

المكورات العنقودية الذهبية المقاومة للميثيسيلين هي مصدر رئيسي للتمركزالبكتيري والعدوى التي تصيب الخدج في وحدة العناية المركزة لحديثي الولادة. الهدف من الدراسة: تحديد مدى الانتشار وعوامل الخطر وملامح المقاومة لدى الولدان وأمهاتهم والعاملين في مجال الرعاية الصحية. أيضًا للتحقق من مدى ارتباط المكورات العنقودية الذهبية المقاومة للميثيسيلين بتلك لدى أمهاتهم أو موظفي الرعاية الصحية. المواد المستخدمة و الطرق المتبعة: تم فحص العينات وتحديدها لتمركز المكورات العنقودية الذهبية المقاومة للميثيسيلين عن طريق صبغة الجرام والاختبارات البيوكيميائية واختبار انتشار قرص الأوكساسيلين ونمط المقاومة. تم استخدام PCR للكشف عن وجود جين nuc و mecA كما تم إجراء بصمة الحمض النووي. النتائج: كان تمركز البكتيريا في العاملين في الرعاية الصحية مرتفعًا (27.9٪) مقارنة بحديثي الولادة والأمهات (15٪ ، 9.7٪ على التوالي) ووجد أنه عامل الوحيد المهم لاحتمالية الانتقال لحديثي الولادة. تم تحديد خمسة عشر نوعًا مختلفًا وكانت t12492 هي السائدة بين الولدان وتم الإبلاغ عنها هنا لأول مرة. لم يكن هناك ارتباط واضح توزيع مع مصادر التمركزالبكتيري. يبدو أن التمركزالبكتيري نشأ من مصادر متعددة. الاستنتاج: تشير النتائج إلى أهمية إجراء تحقيقات جزيئية دورية ل MRSA في موظفي الرعاية الصحية. كما وانه يمكن أن تكون المراقبة والتحليل الجزيئي للسلالات وتعزيز برنامج شامل لمكافحة العدوى ومكافحة المضادات الحيوية مفيدة في منع انتقال MRSA

الكلمات الدالة: العناية المركزة لحديثي الولادة، MRSA ،السلالة عوامل الخطر، التمركز البكتيري.

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* ***المؤلف المراسل: رلى مدحت درويش***

rulad@ju.edu.jo

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