Infection and Colonization by *Staphylococcus aureus* in a High Risk Nursery of a Brazilian Teaching Hospital

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Neonates are susceptible to nosocomial infections due to immunological immaturity, prolonged hospital stay and the use of invasive procedures. We evaluated the incidence of infections and the prevalence of colonization by MRSA (Methicillin-resistant *Staphylococcus aureus*) and MSSA (Methilin-susceptible *Staphylococcus aureus*), as well as colonization risk factors. Staphylococcal infections were observed by analyzing medical records in the HICS (Hospital Infection Control Service) and the HRN (High Risk Nursery). Additionally, four inquiries concerning colonization prevalence were made for *S. aureus*, from January/2000 to December/2002. Clinical specimens from the nostrils, mouth and anus were cultivated in mannitol-salt agar plates and identification was made through standard methods. The frequency of neonates colonized by *S. aureus* was 49%. MSSA was more prevalent (57%) than MRSA (43%). Risk factors related to the acquisition of MRSA were: low weight and antibiotic use. , Hospital stay was the only variable significantly associated with colonization by *S. aureus*. The incidence of infections by *S. aureus* during the last three years was 2.18% (159 cases). Nine of them (5.5%) were associated with MRSA and 150 (94.5%) with MSSA. Staphylococcal infections were considered as invasive (sepsis) and non-invasive (conjunctivitis, cutaneous), corresponding to 31% and 69%, respectively. The MRSA phenotype in infection was rare compared with methicillin-susceptible samples, although *S. aureus*, MRSA and MSSA colonization rates were high.

Key Word: *Staphylococcus aureus*, neonates, infection, colonization and risk factors.

Nosocomial infections result in considerable morbidity and mortality among neonates, especially those in neonatal intensive care units [1-3].

The most frequently found microorganism involved in these infections is Gram-positive *Staphylococcus aureus*; it is considered the most important agent of endemic infections as well as outbreaks, followed by *S. epidermidis*, associated with central venous catheters [4,5].

Although -methicillin-susceptible *S. aureus* (MSSA) continues to be the most frequent pathogen in hospitalized neonates, the resistant phenotype (MRSA) is of great importance, as it is associated with many outbreaks [6,7].

The umbilical cord, the perineum and the groin are the first sites to be colonized by *S. aureus* in neonates, the colonization of these mucocutaneous sites being an important risk factor for *S. aureus* infections [8]. Though the neonate can be colonized through contact with the mother, many population-based studies provide good evidence that the nursery staff usually spreads this microorganism, and that the hands are the most important route of transmission [8,9]. Previous colonization is the most important risk factor for staphylococcal infections [10], being followed by premature birth, low weight, immunodeficiency, prolonged hospital stay, antimicrobial use, invasive methods and surgical interventions [7,11,12].

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We evaluated the rates of infection, incidence, and colonization prevalence by MRSA and MSSA, as well as colonization risk factors in colonized neonates, from January, 2000, to December, 2002.

Materials and Methods

Hospital. This study was made at the High Risk Nursery (HRN) of the Clinics Hospital, Federal University of Uberlândia (HC-UFU), with approximately 45 beds, which attends at a tertiary level. This study was approved by the Commission of Ethics of this University.

Study design. Staphylococcus aureus infection data was obtained by analyzing neonate medical records of the Hospital Infection Control Service (HICS) and the HRN.

Four prevalence inquiries (25/10/2000, 11/09/2001, 05/02/2002 and 23/08/2002) were made of *S. aureus* colonization. Clinical specimens were collected from all the neonates in the unit and an individual record was filled out with the demographic data, clinical, and intrinsic and extrinsic risk factors.

Microbiological techniques. Clinical specimens were collected by swabs, from the anus, mouth and nostril. The samples were taken to the laboratory in tubes containing "Trypticase Soy Broth" (TSB). They were cultivated in mannitol-salt agar plates and incubated at 37^0 C during 24 to 48 hours. The colonies were characterized as *Staphylococcus* through Gram staining and by the catalase test. *Staphylococcus aureus* identification was made by mannitol-salt agar fermentation. The cultures were kept in stock agar tubes containing 20% glycerol in a freezer (-20^0 C). A sample of S. *aureus* ATCC 25923 were used as a control. MRSA samples were characterized through cultivation in screening agar containing 4% NACl and 6mg oxacillin /mL [13].

Statistical analysis. The epidemiological data were analyzed by X^2, *t* Student and exact Fisher tests by using the Epi-Info 5.0 program [14], considering $P< 0.05$ as significant.

Results

During the inquiries, 143 neonates were analyzed. The frequency of colonization by *S. aureus* was 49% (70/143). MSSA was more prevalent than MRSA, with colonization rates of 57% and 43%, respectively. MRSA was more frequent only in the second inquiry (79%) (Table 1).

Risk factors associated with colonization by MRSA and MSSA, as well as among children colonized by both when compared with children without *S. aureus* (control group) colonization are in tables 2 and 3.

Hospital stay was the only variable significantly associated with colonization by *S. aureus* ($P<0.05$, Table 2). It was found that weight inferior to 1500 grams and antibiotic use were more frequent ($P < 0.05$) in the group with MRSA than in the neonates colonized by MSSA (Table 3).

The incidence of *S. aureus* infections was 2.2%, corresponding to 159 cases, with nine cases (5.5%) associated with the MRSA phenotype and 150 (94.5%) with MSSA (Table 4).

The clinical forms of staphylococcal infections were divided into invasive infections (31%), including cases of sepsis, and non-invasive (69%), including conjunctivitis and cutaneous infection, which were the most prevalent forms (Table 4).

Discussion

The epidemiology of nosocomial infections by MSSA and MRSA presents several different aspects. Infection by MRSA is more commonly associated with adult patients, particularly in those with the following risk factors: prolonged hospital stay, antimicrobial use, invasive procedures, surgeries and patients submitted to the hemodialysis or dialysis peritoneal [15]. MSSA infections are more prevalent in neonates, especially those with the following risk factors: premature birth, low weight, breathing syndromes, immunodeficiency, antimicrobial use, prolonged hospital stay, invasive methods and surgical interventions [12].

Risk factors associated with infections are the same as those related with colonization. The latter is

Tab1e 1. Colonization by methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) in neonates, based on four prevalence inquiries made from January 2000 to December 2002

Date	Neonates	Colonized neonates		Prevalence/Colonization			
				MRSA		MSSA	
	N	N	%	N	%	N	%
10.25.2000	35	13	37	3	23	10	77
09.11.2001	35	19	54	15	79	4	21
02.05.2002	36	20	56	4	20	16	80
08.23.2002	37	18	49	8	44	10	56
Total	**143**	**70**	**49**	**30**	**43**	**40**	**57**

Table 2. Risk factors for acquiring *Staphylococcus aureus* colonization in neonates hospitalized from January 2000 to December 2002

Risk factor	Colonized (n=70)		Not colonized (n=73)		P	OR (CI)
	N	%	N	%		
Sex						
Male	44	63	36	49	0.143	1.74 (1.85-3.59)
Weight						
≤1500g	25	36	21	29	0.477	1.38 (0.64-2.96)
Apgar						
0-4	5	7	4	5	0.948	1.33 (0.29-6.21)
5-7	51	73	44	60	0.156	1.77 (0.83-3.81)
8-10	14	20	25	34	0.084	0.48 (0.21-1.09)
Weeks of gestation						
≤ 36 weeks	39	56	37	51	0.663	1.22 (0.60-2.49)
Antibiotic use	22	33	25	34	0.856	0.88 (0.41-1.88)
Length of Hospitalization[1]						
≥ 7 days	57	81	47	64	0.035*	0.41 (0.18-0.95)
Incubator care	30	43	32	43	0.959	0.96 (0.47-1.96)
Mechanical ventilation	11	16	10	14	0.917	1.17 (0.42-3.26)
Invasive devices	35	50	38	52	0.937	0.92 (0.45-1.87)

OR: Odds rates, CI: Confidence intervals, [1]Time between hospitalization and taking the samples.
* P≤0.05 - significant.

Table 3. Risk factors for acquiring MRSA and MSSA colonization in neonates interned from January 2000 to December 2002

Risk factor	MRSA (n=30)		MSSA (n=40)		P	OR (CI)
	N	%	N	%		
Sex						
Male	16	53	28	70	0.238	0.49 (0.16-1.47)
Weight						
<1500g	16	53	10	25	0.029*	3.43 (1.11-10.78)
Apgar						
0-4	4	13	1	2.5	0.203	6.00 (0.57-149.37)
5-7	21	70	30	75	0.846	0.78 (0.24-2.55)
8-10	5	17	9	22	0.762	0.69 (0.17-2.66)
Weeks of gestation						
<36weeks	19	63	20	50	0.385	1.73 (0.59-5.10)
Antibiotic use	14	46	8	20	0.034*	3.50 (1.08-11.58)
Length of Hospitalization[1]						
≥ 7 days	27	90	30	75	0.198	0.33 (0.06-1.52)
Incubator care	15	50	15	37	0.422	1.67 (0.57-4.88)
Mechanical ventilation	4	13	7	17	0.886	0.73 (0.16-3.20)
Invasive devices	19	63	16	40	0.090	2.59 (0.88-7.76)

OR: Odds rates, CI: Confidence intervals, [1]Time between hospitalization and taking samples.
* P≤0.05 - significant.

Table 4. Incidence of infections by MRSA and MSSA and forms of infection observed in neonates interned at the HRN/UFU-HC from the first semester of 2000 to the second semester of 2002

Semester/ year	Staphylococcal infections		Infection							
			MRSA		MSSA		Invasive		Non invasive	
	N	%	N	%	N	%	N	%	N	%
1/2000	14	1.22	2	0.17	12	1.05	6	43	8	57
2/2000	27	2.11	6	0.47	21	1.64	9	33	18	67
1/2001	35	2.43	-	-	35	2.43	4	11	31	89
2/2001	33	2.65	1	0.08	32	2.57	8	24	25	76
1/2002	32	2.72	-	-	32	2.72	15	47	17	53
2/2002	18	1.78	-	-	18	1.78	8	44	10	56
Total	**159**	**2.18**	**9**	**5.5**	**150**	**94.5**	**50**	**31**	**109**	**69**

considered the most important risk factor for illness [10].

Studies carried out in the USA concerning infection by *S. aureus* show that it causes clinically-significant infections in 10% to 60% of the hospital patients who acquire carriage [16]. On the other hand, studies performed in the United Kingdom demonstrated infection rates far less than 60%, and probably less than the lower level of 10% [17,18].

Our results are similar to those found in the UK, less than 5% infection in colonized patients. In the case of MRSA, only nine (6%) infected patients were detected, even though 43% of the neonates were found to be colonized. Adock [19] described similar results in the USA. However, Loureiro et al. [11] found 50% of the neonates to be colonized by MRSA, in a study made in a public hospital in Rio de Janeiro.

Brazilian HRNs and NICUs are always crowded, which contributes to cross infections [20]. Neonate hospital stay is usually prolonged, facilitating *S. aureus* colonization. This was an important observation in our study because hospital stay was the only variable significantly related to colonization by *S. aureus* [11,21].

Resistant bacteria are more common in ICUs [22] than in other hospital units, due to greater risk factors and more antibiotic use [23,24]. We also found antibiotic use to be an important risk factor for colonization by MRSA.

Staphylococcus aureus is the second most common microorganism isolated from infections in NICUs [25]; methicillin-susceptible samples being the most common [26]. Such infections predominantly involve sepsis, pneumonia, cutaneous infection and conjunctivitis [4].

A recent study made in Rio de Janeiro by Loureiro et al [27] found 5.6% infection, which was similar to our results. On the other hand, Tapia-Rombo et al. [2] observed 23.8% infection in Mexico.

Most of infections we observed (69%) were classified as non invasive (cutaneous, conjunctivitis); among the sepsis cases (31%) the MRSA phenotype contributed only 10%. Loureirol et al. [27] found that 93.3% of the cases of sepsis by *S. aureus* were caused by MRSA.

There is considerable use of antibiotics in HRNs, being stimulated by difficulties in the clinical diagnosis of sepsis and the high mortality in these units [22,28]. Approximately 75% of the neonates in NICU receive antibiotics until the third day of life, being more frequent in those of low weight [29].

Nagata et al. [21] in Londrina /PR, found that 46% of the neonates used antibiotics, which was more than in our study (34%). Of the neonates colonized by MRSA, 47% were using antibiotics, while just 20% of the MSSA infected neonates used them.

In the control of infection/colonization in neonatal units a restriction in the use of antibiotics should be considered, with the implantation of educational measures [29].

The most effective methods for the reduction of infections are not always the most expensive ones, such as washing hands, considered the most important procedure in the prevention of nosocomial infections [21].

Our results show that while the policy to restrict the use of antibiotics , educational measures, prevention practices and rigid hand-washing policies adopted in the NICU has resulted in a low frequency of neonates infected by MRSA, on the other hand the situation is complex since about half of the children were found to be colonized by *S. aureus.*

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