

Surveillance of Pediatric Infections in a Teaching Hospital in Mato Grosso do Sul, Brazil

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Nosocomial infections (NI) result in considerably high mortality and morbidity rates, especially among pediatric patients. Considering current worldwide changes, information about the occurrence of pathogens and susceptibility tests are now seen as decisive for optimizing treatment. The purpose of this research was to determine the frequency of microorganisms, antimicrobial and genetic profiles, and risk factors associated with nosocomial infections in a teaching hospital in Campo Grande, Mato Grosso do Sul. From January 1998 to December 1999, 108 patients were characterized as having nosocomial infection, from which 137 pathogens were isolated. Identification and antimicrobial susceptibility was determined by conventional and automated techniques. *Staphylococcus aureus* and *Klebsiella pneumoniae* strains were characterized by Pulsed Field Gel Electrophoresis (PFGE). Pathogens were most often isolated from infants one-month old or younger, and bloodstream infections were the most frequent. The main isolated agents isolated were: coagulase-negative staphylococci (38), *Pseudomonas aeruginosa* (19), *S. aureus* (26), *K. pneumoniae* (18), and *Candida* spp. (13). The risk conditions that were most closely related to NI acquisition were: prolonged hospital stays (69.4%), prematurity (60.9%) and exposure to high-risk device procedures (95.4%). Ciprofloxacin and imipenem were the most effective drugs, inhibiting all or almost all of the *Enterobacteriaceae*, *P. aeruginosa* and *Acinetobacter calcoaceticus* isolates. Only 23% of the *S. aureus* samples were resistant to oxacillin. Genomic typing revealed 10 distinct patterns for *S. aureus* and 13 for *K. pneumoniae*, suggesting that most them did not belong to the same clone. PFGE was effective in differentiating the strains.

Key Words: Infant, nosocomial infection, antimicrobial susceptibility.

Nosocomial infections result in considerable mortality and morbidity, especially among pediatric patients [1-3]. In national and international research, special attention has been given to infant bacteremia, due to the risk factors to which they are exposed during their treatment and maintenance [1,4-7].

Considering ongoing worldwide changes, vigilance of predominant pathogens and susceptibility tests are

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now seen as decisive for optimizing treatment [7-9]. Surveillance data allow hospitals to take appropriate intervention measures to control the infection [5].

Phenotypic and genotypic methods have been used in epidemiologic studies. Recent reports consider them to be effective and useful tools for elucidating the means of dissemination and infection reservoirs [3,7-11].

We determined the frequency of microorganisms and the risk factors associated with nosocomial infections in a teaching hospital in Campo Grande, Mato Grosso do Sul and examined whether the bacteria involved in nosocomial infections at the pediatric units had the same antimicrobial and genetic profile as other bacteria isolated at the same unit.

Materials and Methods

Description of Hospital and Population. Bacterial strains were isolated from patients attended at pediatric units of the UFMS teaching hospital (Campo Grande-MS, Brazil). This hospital provides assistance to patients of all ages, from various parts of the city of Campo Grande in the State of Mato Grosso do Sul. From January 1998 to December 1999, 108 patients were characterized as having NI according to the Center for Disease Control (CDC) criteria [12]; in which 137 pathogens were isolated from these patients.

The data collected for each infection included the date, the site of infection and demographic characteristics of the patients.

Microorganisms, Identification and Susceptibility Testing. The culture and identification were made using the Vitek automated system [13]. Antimicrobial susceptibility was determined by the disk diffusion method, according to National Committee for Clinical Laboratory Standards recommendations – NCCLS, M100-S9 [14] for the drugs: CRO- ceftriaxone (30µg), TAZ- ceftazidime (30µg), TAX- cefotaxime (30µg) CLO – chloramphenicol (30µg) and TET- tetracycline (30µg). The MIC values (µg/mL) were determined with the Vitek System for AMP- ampicillin, CFL- cephalothin, TAZ- ceftazidime, TAX- cefotaxime, CPE- cefepime, FOX- cefoxitin, AZT- aztreonam; IMP- imipenem, TOB- tobramycin, GEN- gentamicin, AMI- amikacin CIP- ciprofloxacin, TSX – trimethoprim-sulfamethoxazole, OXA – oxacillin and VAN - vancomycin.

Quality control was carried out using standard strains of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27953) and *S. aureus* (ATCC 25923). For presumptive extended-spectrum β lactamase production we used the screening tests for *ESBL K. pneumoniae* recommended by NCCLS M100-S9: ceftazidime zone ≤0.22 mm, cefotaxime zone ≤0.27 mm and ceftriaxone zone ≤0.25 mm. ESBL production was determined by using E-test strips (AB Biodisk, Sona, SW). If MIC TZ/MIC TZL < 8, ESBL was considered negative and if the MIC of TZ/MIC TZL ≥ 8, ESBL was considered positive.

Pulsed Field Gel Electrophoresis – PFGE. Chromosomal DNA analysis of *S. aureus* (17) and *K. pneumoniae* (19) were performed by PFGE, according to procedures described by Sader et al., 1994 [8] and Weller et al., 1997 [9], respectively. DNA restriction was processed using Sma I for *S. aureus* and Xba I for *K. pneumoniae*. The fragments were separated using the CHEF DRIII pulsed field electrophoresis system (BioRad, Laboratories, Richmond, Calif.). The fragments were stained with ethidium bromide and photographed. All agarose gels were prepared in duplicate.

Band patterns were analyzed using Gel Compar II (Applied Maths, Belgium). The relation among fingerprints was determined by constructing a similarity matrix using Dice's coefficient with 1.5% position tolerance; a dendrogram was generated using the UPGMA clustering algorithm. The definition of the clone structure of isolates was made according to Tenover, 1997 [10].

Epidemiological Analysis of Patients. An epidemiological record with demographics and microbiological data was prepared for each NI case. Data were organized with Excel 7.0 software (Microsoft) and analyzed with Epi Info (version 6.04: CDC, Atlanta, USA)

Statistical Analysis. A univariate analysis was performed to determine significance in comparisons between groups. X² and Fisher tests were also used, where appropriate.

Results

Several parameters of the epidemiological data were analyzed. The general features of the 108 pediatric patients and the 59 controls group can be observed in Table 1. The average age of the patients was 4 months and 21 days for the NI group. Fifty-one percent of the infants were male. The pathogens were most often isolated from one-month- old or younger newborns. In this age range, bloodstream infections were the most frequent. The patients that had NI stayed in the hospital

for up to about 28 days. Of the 108 children, 20 (18.5%) died during hospitalization.

Microorganisms were most frequently isolated from blood samples (85), followed by catheter tips (23), wound secretions (11), eyes, ears or nose secretions (3) and others (3). Overall, 66 cases of bloodstream infection (BSI) were recorded (48.2%), 22 cases of pneumonia (PNE, 16.1%), 2 lower respiratory tract infections, excluding pneumonia (LRTI, 1.5%), 14 eye, ear and nose infections (EENI, 10.2%), 9 systemic infections (Sis, 6.6%), 8 gastrointestinal system infections (GSI, 5.8%), 6 cases of skin and soft tissue infection (SSTI, 6.44%), 7 surgical wound infections (SWI, 5.1%), 2 urinary tract infections (UTI, 1.5%) and 1 case of central nervous system infection (CNSI, 1.7%). The distribution of the site of the infection varied in the different pediatric units; BSI, pneumonia and GSI were most frequent among newborns kept in the neonatal intensive-care unit (NICU), while skin and soft tissue infection were most common in patients kept in infirmaries (Table 2).

The risk conditions that were mostly related to NI acquisition were prolonged hospitalization (75, 69.4%), prematurity (44, 60.9%) and exposure to high-risk devices and procedures (103, 95.4%) (Table 1). The most frequently isolated pathogens were coagulase-negative staphylococci (CoNS) (38), *P. aeruginosa* (19), *S. aureus* (26), *K. pneumoniae* (18), and *Candida* spp. (13) (Table 3). Death occurred in 7 (10.9%) of the 64 Gram-positive cocci (GPC), 14 (23%) of 60 Gram-negative bacilli (GNB), and 2 (15%) of the 13 fungal infection patients.

The resistance of GNB to the antimicrobials was determined (Table 4). Ciprofloxacin and imipenem were the most effective drugs, inhibiting all, or almost all, of the *Enterobacteriaceae*, *P. aeruginosa* and *A. calcoaceticus* isolates; resistance to ampicillin was more frequent (89.6%, MIC > 32 µg/mL). All of the *P. aeruginosa*, *K. pneumoniae* and *Serratia* isolates were resistant to this antibiotic.

The frequency of resistance of the *P. aeruginosa* isolates to tobramycin was 26.3% (MIC ≥ 16 µg/mL), followed by 15.8% to gentamicin (MIC ≥ 16 µg/mL) and 10.5% to amikacin (MIC > 32 µg/mL). The most

effective cephalosporin was ceftazidime, as only 15.8% of the isolates were resistant (MIC ≥ 32 µg/mL).

Almost ninety percent of the *K. pneumoniae* isolates were resistant to cephalothin (MIC ≥ 32 µg/mL) and resistance to other cephalosporins varied: 66.6% to ceftriaxone, 22.2% to ceftazidime (MIC ≥ 32 µg/mL) and 44.4% to cefepime (MIC ≥ 32 µg/mL). No strains were resistant to cefoxitin. The distribution of antimicrobial resistance, the frequency of *K. pneumoniae* producing ESBL and their genetic profile are shown in Table 5.

All *E. coli* isolates were fully susceptible to imipenem, ciprofloxacin and chloramphenicol. The frequency of resistant to ceftazidime and cefoxitin was 12.5% and 11.1%, respectively. Resistance against the aminoglycosides ranged from 22.2% to 57.1%, while 65.5% of *E. coli* strains were resistant to trimethoprim-sulfamethoxazole and 100% were resistant to tetracycline.

Fifty percent of the *Enterobacter* strains were resistant to cefotaxime, cefoxitin, aminoglycosides, trimethoprim-sulfamethoxazole and to tetracycline. A high frequency of resistance (75%) was also found against other cephalosporins (cephalotin, ceftriaxone and ceftazidime), chloramphenicol and ampicillin.

The most active compounds against *Acinetobacter calcoaceticus* (100% susceptibility) were imipenem and ciprofloxacin. Among the cephalosporins, ceftazidime gave the lowest percentage of resistance (20%). All isolates were resistant to ampicillin and chloramphenicol, while the frequencies of resistance to tetracycline and tobramycin were 80% and 60%, respectively, *in vitro*.

All NFGNB isolates were resistant to cephalotin, ceftriaxone, cefotaxime, cefepime and chloramphenicol. Almost sixty-seven percent of these microorganisms were resistant to ceftazidime, ampicillin, aminoglycosides and tetracycline.

The two *Serratia* isolates were fully susceptible to the third and fourth generation antibiotics, including imipenem, ciprofloxacin, amikacin and gentamicin, and to trimethoprim-sulfamethoxazole.

According to the resistance profile of the *Staphylococci* species, 100% were sensitive to vancomycin (MIC ≤ 1 µg/mL). Twenty-three percent

Table 1. General features of 108 patients with nosocomial infection and 59 control patients

Demographic data	Study group	Control group	IC ₉₅	X ²	P value
Average age	4m21d ± 6m25d	3a10d ± 3a1m			
Male Sex	55(50.9%)	26(44.1%)	41.1% - 60.7% 31.2% - 57.6%	0.81	0.3667
Female sex	53(49.1%)	33(55.9%)	39.3% - 58.9% 42.4% - 68.8%	0.81	0.3667
Natural delivery	35/55(63.6%)	7/12(58.3%)	49.6% - 76.2% 27.7% - 84.8%	0.00	0.9882
Cesarian delivery	20/55(36.4%)	5/12(41.7%)	23.8% - 50.4% 15.2% - 72.3%	0.00	0.9882
Average hospitalization period	28d ± 19d	13d ± 11d			0.0000
Membrane rupture > 24h	7/30(23.3%)	1/6(16.6%)	9.9% - 42.3% 4.3% - 77.7%	0.03	0.8577
Antimicrobial average use	3.3	2.5			
Risk condition					
Invasive procedures	103/108(95.4)	43/59(72.9%)	74.9% - 89.8% 59.7% - 83.6%	17.55	0.0000
Hospitalization > 15 days	75/108(69.4%)	24/59(41.7%)	63.7% - 79.6% 28.6% - 55.1%	11.64	0.0006
Prematurity	44/69(60.9%)	8/14(57.1%)	51.2% - 78.8% 18.7% - 81.3%	0.07	0.7950
Low-birth-weight (< 1500g)	20/69(29.0%)	2/14(14.3%)	15.8% - 37.1% 1.7% - 40.5%	0.65	0.4213
Nasogastric sound use	70/108(65.4%)	15/59(25.4%)	49.0% - 67.3% 19.5% - 48.0%	24.83	0.0000
Mechanic ventilation use	46/108(42.6%)	7/59(11.8%)	28.8% - 46.8% 6.3% - 28.9%	16.63	0.0000
Oxygen catheter use	65/108(60.2%)	18/59(30.5%)	52.7% - 70.7% 25.7% - 55.7%	13.44	0.0002
Umbilical catheter use	30/108(27.8%)	3/59(5.1%)	13.8% - 29.0% 1.4% - 17.9%	12.39	0.0004
Drain use	11/108(10.2%)	11/59(18.7%)	4.7% - 15.8% 12.6% - 38.8%	2.39	0.1224
Parenteral nutrition use	44/108(40.7%)	6/59(10.2%)	35.9% - 54.3% 4.9% - 26.3%	17.00	0.0000
Chemo/Radiotherapy use	10/108(9.3%)	1/59(1.7%)	6.4% - 18.5% 0.1% - 11.5%	2.43	0.1194
Surgical procedure	27/108(25.0%)	7/59(11.8%)	18.4% - 34.9% 6.3% - 28.9%	4.06	0.0439

IC₉₅= 95% confidence intervals; x²= chi-square.

Table 2. Distribution of site of infection according to pediatric units

Site	Pediatric Units					Total
	Nursery	Infirmery	Emergency medical attendance	Pediatric intensive care unit	Neonatal intensive care unit	
BSI	10 (15.2%)	12 (18.2%)	5 (7.6%)	13 (19.37%)	26 (39.4%)	66 (48.2%)
PNE	5 (22.7%)	4 (18.2%)	2 (9.1%)	5 (22.7%)	6 (27.3%)	22 (16.1%)
LRTI	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (100.0%)	0 (0.0%)	2 (1.5%)
EENI	5 (35.7%)	2 (14.3%)	1 (7.1%)	1 (7.1%)	5 (35.7%)	14 (10.2%)
SI	1 (11.1%)	3 (33.3%)	3 (33.3%)	1 (11.1%)	1 (11.1%)	9 (6.6%)
GSI	1 (12.5%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	6 (75.0%)	8 (5.8%)
SWI	2 (28.6%)	2 (28.6%)	1 (14.3%)	2 (28.6%)	0 (0.0%)	7 (5.1%)
SSTI	2 (33.3%)	4 (66.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (4.4%)
UTI	1 (50.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	2 (1.5%)
CNSI	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (0.7%)
Total	27	28	13	25	44	137

BSI= bloodstream infection. PNE= pneumonia. EENI= eye, ear and nose infection; LRTI= lower respiratory tract infection; SI= systemic infection; GSI= gastrointestinal system infection; SSTI= skin and soft tissue infection; SWI= surgical wound infection; UTI= urinary tract infection; CNSI= central nervous system infection (Garner. 1988).

Table 3. Pathogens causing nosocomial infections in pediatric units in a teaching hospital during a period of two years, 1998 to 1999

Bacteria	Number	Percentage
Gram-positive (64)		
<i>Staphylococcus aureus</i>	26	19.0
<i>Staphylococcus epidermidis</i>	13	9.5
<i>Staphylococcus haemolyticus</i>	7	5.1
<i>Staphylococcus simulans</i>	3	2.2
<i>Staphylococcus hominis</i>	2	1.5
<i>Staphylococcus auricularis</i>	2	1.5
<i>Staphylococcus capitis</i>	1	0.7
Other CoNS	10	7.3
Gram-negative (60)		
<i>Pseudomonas aeruginosa</i>	19	13.9
<i>Klebsiella pneumoniae</i>	18	13.6
<i>Escherichia coli</i>	9	6.6
<i>Enterobacter</i> spp.	4	2.9
<i>Acinetobacter calcoaceticus</i>	5	3.6
Other NFGNB	3	2.2
<i>Serratia marcescens</i>	2	1.5
Yeasts (13)		
<i>Candida albicans</i>	4	4.4
<i>Candida tropicalis</i>	6	2.9
<i>Candida parapsilosis</i>	3	2.2
Total	137	100.0

CoNS= coagulase negative staphylococci; NFGNB= Non Fermenter Gram- negative bacilli.

of *S. aureus* isolates were resistant to oxacillin. The MIC value $\geq 4\mu\text{mL}$ for oxacillin was considered resistant for all *Staphylococci* species. All of the *S. simulans* (3) and *S. hominis* (2) isolates were resistant to this drug. A high frequency of resistance to oxacilina also observed among *S. epidermidis* (13, 84.6%), *S. haemolyticus* (7, 71.4%), *S. auricularis* (2, 50%) and other CoNS (10, 60%) strains. Overall, the mean percentage of CoNS and *Staphylococcus* species isolates resistant to oxacillin was 66.6% and 61.1%, respectively.

Genomic typing revealed 10 distinct patterns for *S. aureus* (Figure 1 A). PFGE pattern A was present in 4 strains, isolated from two wound secretions, one ocular secretion and one bloodstream sample. The infections occurred between June/98 and April/99 and the patients

were hospitalized in three different units: infirmary, nursery and the neonatal intensive care unit. PFGE pattern D was found in three isolates, responsible for BSI, SWI and skin infection. All the patients were interned in the same infirmary between November 98 and March 99. Clone C of *S. aureus* was found in two isolates from newborns treated in the nursery between March and November 99, with systemic and bloodstream infection, respectively. The dendrogram generated by Gel Compar II (Figure 1 B) revealed that 3 ORSA strains belong to two unrelated genetic clones.

We detected 13 different profiles (A – M) in the PFGE analysis of 18 *K. pneumoniae* (Figure 2A). PFGE pattern A was present in 3 isolates, responsible for three BSIs, which occurred between June and July

Table 4. Antimicrobial resistance percentage of Gram-negative strains isolated from pediatric patients with nosocomial infections during a two year period (January 1998 to December 1999), University Hospital, Campo Grande, MS, Brazil

Microorganisms (n) MICs ^a →	AMP	CFL	CRO	TAZ	TAX	CPE	FOX	IMP	TOB	GEN	AMI	CIP	CLO	STX	TET
	≥32	≥32	*	≥32	≥32	≥32	≥32	≥16	≥16	≥16	≥32	≥2	*	≥160	*
<i>Pseudomonas aeruginosa</i> (19)	100	100	78.9	15.8	77.8	31.6	100	5.3	26.3	15.8	10.5	10.5	83.3	89.5	94.7
<i>Klebsiella pneumoniae</i> (18)	100	89	66.6	22.2	55.6	44.4	0	0	93.8	88.9	16.7	0	56	50	73.3
<i>Escherichia coli</i> (09)	85.7	66.7	66.7	12.5	62.5	44.4	11.1	0	57.1	55.6	22.2	0	0	62.5	100
<i>Enterobacter</i> spp. (04) 75	75	75	75	50	25	50	0	50	75	25	0	75	50	50	50
<i>Acinobacter calcoaceticus</i> (05)	100	60	80	20	60	40	80	0	60	40	40	0	100	40	80
NFGNB (03)	66.7	100	100	66.7	100	100	100	33.3	66.7	66.7	66.7	0	100	0	66.7
<i>Serratia marcescens</i> (02)	100	50	0	0	0	0	50	0	50	0	0	0	100	0	100
Means resistance	89.6	77.2	66.7	30.3	58	40.8	55.9	5.51	57.7	48.9	25.9	1.5	73.5	41.7	80.7

NFGNB= Non Fermenter Gram-negative bacilli. AMP- ampicillin. CFL- cephalotin. CRO- ceftriaxone. TAZ- ceftazidime. TAX- ceftaxime. CPE- cefepime. FOX- cefoxitin. IMP- imipenem. TOB- tobramycin GEN- gentamicin. AMI- amikacin. CIP- ciprofloxaci. CLO- chloramphenicol. TSX- trimethoprim-sulfametoxazole. TT- tetracycline. a- MIC values (µg/mL) according to the VITEK System. * MIC not determined.

Table 5. Distribution of antimicrobial resistance. ESBL producing and genomic profile of *K. pneumoniae* strains isolated from pediatric patients

Resistance number profile	Antimicrobial resistance profile	Antimicrobial number	Number of strains (%)	ESBL	PFGE profile
1	AMP	1	1 (06.25)	Neg.	M
2	AMP/SXT	2	1 (06.25)	Neg.	D
3	AMP/CFL/CFZ/TAX/CRO/GN/TOB	7	1 (06.25)	Neg.	E
4	AMP/AM/AZT/CFZ/TAZ/CLO/GN/TSX	8	1 (06.25)	Neg.	B
5	AMP/CFL/CFZ/CLO/GN/TET/TOB/TSX	8	1 (06.25)	Neg.	C
6	AMP/CFL/CFZ/TAX/CRO/GN/TET/TOB	8	1 (06.25)	Neg.	A
7	AMP/CFL/CFZ/CRO/CLO/GN/TSX/TET/TOB	9	1 (06.25)	Neg.	C
8	AMP/AZT/CFL/CFZ/TAZ/CRO/CLO/GN/TOB	9	1 (06.25)	Neg.	J
9	AMP/AM/AZT/CFL/CFZ/CLO/GN/TET/TOB/TSX	10	1 (06.25)	Neg.	I
10	AMP/AM/AZT/CFL/CFZ/CPM/TAX/CRO/GN/TOB	10	1 (06.25)	Neg.	H
11	AMP/AZT/CFL/CFZ/CPM/TAX/CRO/CLO/GN/TOB	10	1 (06.25)	Neg.	L
12	AMP/AZT/CFL/CFZ/CPM/TAX/CRO/GN/TET/TOB	10	3 (18.75)	Pos./	A
				Pos./	A
				Neg.	G
13	AMP/AZT/CFL/CFZ/TAZ/CRO/CLO/GN/TSX/TET/TOB	11	1 (06.25)	Neg.	K
14	AMP/AZT/CFL/CFZ/TAX/TAZ/CRO/CLO/GN/TET/TOB/TSX	12	1 (06.25)	Neg.	F
15	AMP/AZT/CFL/CFZ/CPM/TAX/CRO/CLO/GN/TSX/TET/TOB	12	2 (12.50)	Pos./	B
				Neg.	B
	Total		18R		

AMP: ampicillin; AZT: aztreonam; CFL: cephalothin; CFZ: cefazolin; CPM: cefepime; TAX: ceftaxime; TAZ: ceftazidime; FOX: cefoxitin; CRO: ceftriaxone; CLO: chloramphenicol; GEN: gentamicin; TSX: trimethoprim-sulfamethoxazole; TT: tetracycline; TOB: tobramycin. A - L: Pulsed Field Gel Electrophoreses patterns; Pos.: positive; Neg.: negative.

98 and all these patients were in NICU. PFGE pattern B was found in three isolates, responsible for two BSIs and one ocular infection. One patient with BSI and another with an ocular infection were in the NICU between July and October 98, and the other BSI case occurred in the nursery ward in November 1999. A dendrogram constructed from all profiles showed that the ESBL producing strains with patterns A and H had 80% similarity (Figure 2B).

Discussion

We observed that the frequency of infection was inversely related to the age of the patient; this was quite similar to what was found in some previous studies [1,3]. The duration of hospitalization was longer for children with NI than for the control group (28 versus 13 days).

We found that the frequency of BSI (48.2%) was high, similar to the Raymond study [16], and respiratory diseases were more frequent (16.1%) than previously reported [5].

BSIs were most frequent in newborns (36, 52.2%), and 26 (70.6%) of these subjects had an umbilical catheter. Univariate analysis revealed that invasive procedures, such as catheters, ventilatory support and parenteral nutrition, as well as nasogastric sounds are associated with a risk of nosocomial infections. In addition, prolonged hospitalization may have favored the occurrence of nosocomial infections, as described by other researchers [1-4,17]. In recent years, several reports [4,6] have emphasized that the knowledge of risk factors is useful, so that precautions can be taken to protect patients identified as highly susceptible to infection, to reinforce rational means to administrate and to adequately dose antibiotics, and to avoid invasive procedures that can provoke bacteremia.

The most frequently isolated microorganisms: CoNS, *P. aeruginosa*, *S. aureus*, *K. pneumoniae* and *Candida* spp. were also described in previous reports as NI agents [2,4,16,18]. Coagulase-negative staphylococci and *Candida* spp. are emerging as important nosocomial pathogens. We found that *P. aeruginosa* and *K. pneumoniae* strains were

responsible for many BSI cases, and were associated with prematurity (3/7 and 3/5, respectively) and high mortality rates (2/7, and 4/9, respectively).

The results of this *in vitro* research demonstrate that carbapenem and fluoroquinolone have broad activity against many common aerobic Gram-negative bacteria. These data are in general agreement with other previously reported studies [17,19]. Even though fourth generation cephalosporin is generally effective against *Enterobacteriaceae* and *P. aeruginosa*, we found a higher percentage of resistant isolates (29.1%) than the 18.6% observed by Mendes, 2000 [20]. Frequencies of resistance vary greatly among countries, hospital units and even patients [16].

We found cefoxitin, ciprofloxacin and imipenem to be the most effective agents for treatment of *K. pneumoniae* at the UFMS University Hospital; however these antibiotics are used in our institution only as a last option, since imipenem very quickly induces ESBL production [4] and fluoroquinolone is not yet licensed for use in children, because of its toxicity to cartilage in animal models [21].

ESBL production was detected in 4 *K. pneumoniae* isolates (23.5%), which is lower than levels found in other Brazilian hospitals [7,20,21]. Despite the small number of strains, this finding is clinically significant because it may result in clinical failure when cephalosporins are used for treatment. The continuous use of ampicillin and gentamicin, which is a policy of our hospital, can lead to selective pressure able to promote resistance to these drugs (Table 4). The *K. pneumoniae* isolates gave 15 different antimicrobial resistance profiles (ARPs) in which 12 and 15 ARPs were related to a specific genetic profile (A and B, respectively).

Oxacillin-resistant *S. aureus* is an important agent of NI and it is frequently transmitted in hospital and perinatal units [22,23]. In our study, *S. aureus* oxacillin-resistance was less frequent (23.1%) than found in other hospitals in Brazil [7,17] and none of these isolates were vancomycin-resistant. The percentage resistance of CoNS against oxacillin was higher (66.6%), similar to results of other researchers [4,7,16].

Genomic typing revealed considerable genetic variety; there were 10 distinct patterns for *S. aureus*

Figure 1A. λ : lambda ladder pulsed field gel electrophoresis marker (50-1000Kb). Lanes 1-10. PFGE patterns of *S. aureus* produced by Sma I restriction. Lane 1 = ORSAs (Sa1 and 2); lane 2 = A (Sa7, 11, 37 and 39); lane 3 = ORSA (Sa8); lane 4 = B (Sa10); lane 5 = C (Sa18 and 33); lane 6 = D (Sa24, 29 and 34); lane 7 = E (Sa36); lane 8 = F (Sa44); lane 9 = H(Sa49); lane 10= G (Sa50).

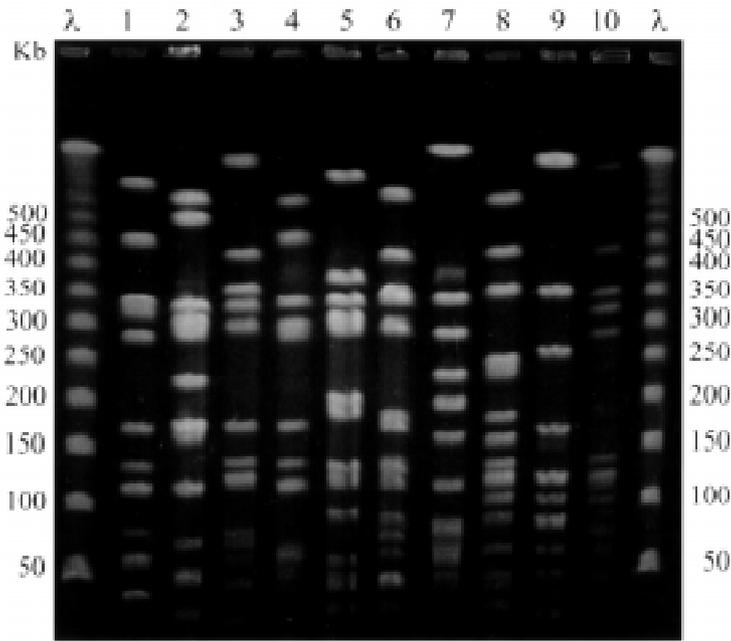


Figure 1B. Dendrogram showing 10 different profiles of *S. aureus* strains isolated from pediatric patients at HU-UFMS during a two year period (01/1998 to 12/1999). OSSA= *S. aureus* oxacillin-sensitive; ORSA= *S. aureus* oxacillin-resistant

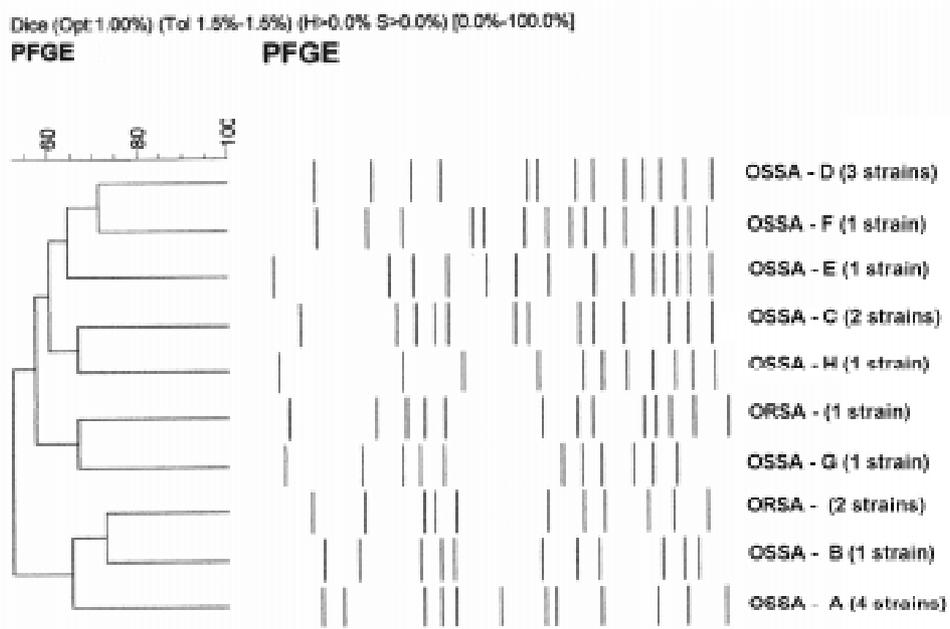


Figure 2A. PFGE of *K. pneumoniae* strains isolated from pediatric patients at HU-UFMS during a two-year period. λ . lambda marker (50-1000 Kb); Lanes 1-13. PFGE patterns detected in 18 *K. pneumoniae* isolates. Lane 1= L (Kpne1 strain); lane 2= C (Kpne2 and Kpne4 strains); lane 3= D (Kpne5 strain); lane 4= A (Kpne6, Kpne7 and Kpne9 strains); lane 5= B Kpne10, Kpne11 and Kpne23 strains); lane 6= E (Kpne12 strain); lane 7= H (Kpne13 strain); lane 8= K (Kpne14 strain); lane 9= G (Kpne15 strain); lane 10= F (Kpne16 strain); lane 11= J (Kpne19 strain); lane 12= I (Kpne21 strain) and lane 13= M (Kpne22 strain).

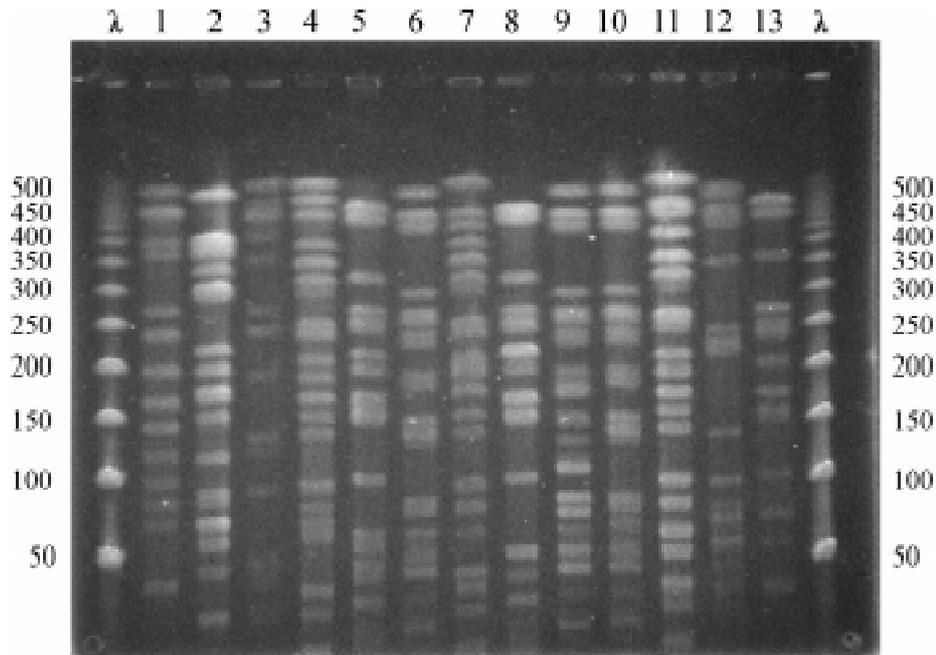
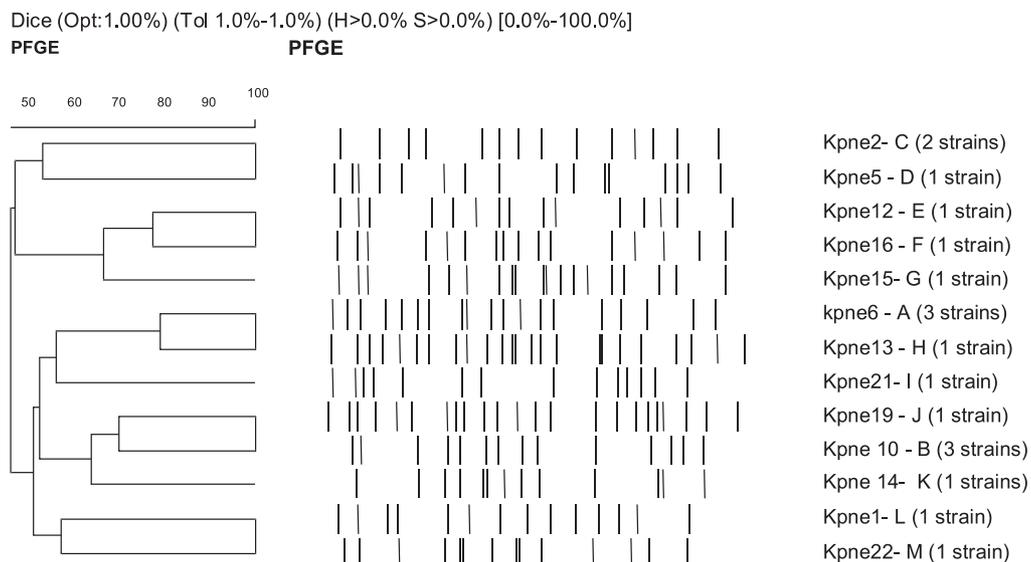


Figure 2B. A comparative dendrogram and schematic representation of 13 PFGE patterns (A - M) detected in 18 *K. pneumoniae* strains isolated from pediatric patients at HU-UFMS during a two year period (01/1998 to 12/1999)



and 13 for *K. pneumoniae*, suggesting that most did not belong to the same clone, however we observed small outbreaks of both genera in our study.

Different clones of *S. aureus* identified with the PFGE technique (A, B, E and F) had the same antimicrobial profile; this was also found by other authors [23]. Consequently, an identical profile does not imply that the same clone is involved, as was once thought. Analyzing the clinical data of the patients and the PFGE patterns of the *S. aureus* strains, little or no relation between infection, locality or period of hospitalization was found.

Genetic similarity was estimated by comparing PFGE patterns. There was a low degree of similarity between the ORSA strains (67-71%), indicating distinct infection sources. The similarity of OSSA isolates varied from 53% to 75%, indicating a high degree of genetic diversity. The two strains of ORSA, which had the same genetic profile, had a 75% similarity with the OSSA of group B.

Klebsiella pneumoniae infections did not represent outbreaks; rather they were a series of sporadic infections caused by different strains. However, there was possibly a small outbreak caused by strains with closely related A patterns in patients in the NICU.

Two ESBL producing *K. pneumoniae* (Kpne7 and 9) shared the same ARPs and genetic profile (A). Nevertheless, within these same PFGE A patterns, there was one *K. pneumoniae* isolate that did not produce the ESBL enzyme. These results are in agreement with those of other researchers who reported strains of the same clone that have different ARPs [7,9,11].

We conclude that it is important to screen pediatric patients for NI agents and that the PFGE method is useful for typing pathogens in epidemiological studies.

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