

STUDY OF MULTI-DRUG RESISTANT MICROORGANISMS ISOLATED FROM BLOOD CULTURES OF HOSPITALIZED NEWBORNS IN RIO DE JANEIRO CITY, BRAZIL

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ABSTRACT

The aims of this study were to determine the frequency and antimicrobial resistance patterns of microorganisms associated with sepsis cases in a public maternity hospital, and to analyze the main demographic data relating to these infected neonates over a two year period. We analyzed 255 positive blood cultures and the medical records of newborns admitted to the neonatal intensive care unit of the Hospital Maternidade Alexander Fleming II, Rio de Janeiro city, Brazil, from July 1997 to July 1999. Identification and antibiograms of the isolated strains were performed according to routine laboratory procedures. Demographics and microbiological data were analyzed using the Epi-Info program. The mean age of the newborns was 13.1 days, with an average of 1.2 strains isolated per patient. Antibiotics were administered to 207 (83.1%) patients before positive blood culture presentation. A total of 90.8% patients were premature; 83.9% had a gestational age of less than 36 weeks; 52.6% presented very low birth weight; 39.8% had asphyxia and 33.3% presented hyaline membrane disease. A total of 301 microorganisms were isolated with a predominance of *Klebsiella pneumoniae* (22.9%), coagulase-negative Staphylococci (17.3%), *Serratia marcescens* (15.9%) and *Pseudomonas aeruginosa* (10.6%). Gram-negative strains showed high resistance levels to cephalosporins and aminoglycosides. A total of 93.3% *Staphylococcus aureus* strains were methicillin-resistant. The newborns examined in this study presented several risk factors for acquiring hospital infection and the isolated microorganisms showed high levels of resistance to the majority of the antibiotics routinely used in the hospital.

Key words: epidemiology, newborns, hospital infection, sepsis, antimicrobial resistance

INTRODUCTION

Hospital-acquired infections (HI) in newborns are a serious problem in hospital units (25), and their prevention has been considered a priority by health professionals (15). Neonatal intensive care units (NICU) are classified as target areas in epidemiological vigilance models, due to the high risk conditions associated with newborns held in these facilities (9). In this context, NICU usually admit newborns that receive level III treatment, and often present one of the following risk conditions: umbilical catheter, ventilatory assistance, treatment of potentially serious infection, surgery and very low birth weight (VLBW; birth weight < 1500g) (13).

Technological advances in the treatment of newborns hospitalized in NICU have permitted the survival of premature babies (5,8), although treatment commonly involves invasive procedures such as: ventilatory assistance, catheters, blood transfusions, tracheal intubations, parenteral nutrition, and other factors which significantly increase the risk of infection for such patients (3,25).

Despite advances in neonatal care, sepsis is still considered the main cause of morbidity and mortality in hospitalized newborns (4,8,15). Sepsis increases the length of time spent in the hospital, and incurs costs relating to the involvement of clinical staff, antimicrobial drugs, laboratory tests and other factors depending on the treatment employed (2,24,25).

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Sepsis is a potential risk factor which increases mortality rates in hospital environment. Although high, the mortality rate associated with sepsis is extremely variable ranging from 12% to 81%, depending on the population and etiologic agent studied (18,25). The incidence of sepsis varies in accordance with the characteristics of the hospital and of the patients treated, as well as the type and duration of the invasive procedures used during treatment (7,17).

This study reports the frequency and antimicrobial resistance patterns of microorganisms associated with sepsis cases among newborns at a public maternity hospital in Rio de Janeiro city, Brazil during a two year period. In addition, it provides demographic data relating to the patients examined in this study.

MATERIALS AND METHODS

Hospital and patients

A total of 255 positive blood cultures were produced from individual hospitalized newborns, held in the NICU of the Hospital Maternidade Alexander Fleming II (HMAF), from July 1997 to July 1999; this number corresponds to all microbiologically confirmed sepsis cases among 1275 hospitalized newborns examined during this period in this NICU. The HMAF provides assistance and perinatal care including a neonatology intermediate care unit (NIU) with 40 beds and a NICU with 15 beds.

All neonatal sepsis cases were defined by a single positive blood culture associated with appropriate clinical manifestations (one of the following clinical signs or symptoms: fever $>38^{\circ}\text{C}$, hypothermia $<36.5^{\circ}$, apnoea, bradycardia, tachycardia), with the exceptions of coagulase-negative Staphylococci (CoNS) and *Candida* sp., for which two successive positive blood culture were required.

Hospital infections were defined by standard Centers for Diseases Control and Prevention (CDC) definitions (14) adapted to neonatal pathology. In general, infections that occurred at least 48h post hospitalization were assumed to have been acquired in the NICU, but each case of infection was considered individually because of the possible late onset of some perinatally acquired infections.

Blood cultures, strain identification and susceptibility testing

Venous blood was obtained from NICU newborns by nursing staff, by means of aseptic techniques. Briefly, 0.2 ml of blood were drawn into bottles containing 10 ml of supplemented Trypticase Soy Broth (Roche) and incubated at 37°C . After 24h, blood cultures were used as inocula for Thioglycolate Broth (DIFCO) and plates of Blood Agar and Eosin-Methylene Blue Agar (EMB - DIFCO) with incubation at 37°C during a period ranged from 18 to 24 h. When the blood cultures were negative after incubation of 24 h, the inoculation into broth and plates above cited were repeated during a week. Gram-positive strain identification was performed according to Kloos and Bannerman

(20), using the catalase and coagulase tests. Gram-negative strains were characterized according to Ewing (11), and completed with the Crystal System of identification for fermenters and non-fermenters (BBL/Becton-Dickinson) if necessary. Yeast identification was performed using the Gram method.

Antimicrobial susceptibility testing was carried out using a disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations (22). Quality control employed standard strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27953) and *Staphylococcus aureus* (ATCC 25923). The following concentrations of antimicrobial drugs (CECON) were used: amikacin (30 μg), ampicillin (10 μg), carbenicillin (100 μg), cefepime (30 μg), cefoxitin (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefuroxime (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), imipenem (10 μg), oxacillin (1 μg), penicillin (10 U), tetracycline (30 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), vancomycin (30 μg). The detection of extended spectrum β -Lactamase (ESBL) producers among *Klebsiella pneumoniae* strains were performed using the E-test (AB BIODISK, Solna, SW).

Epidemiological analysis of patients

When a blood culture was required from a hospitalized newborn in the NICU, the responsible physician filled out a form to provide demographic data relating to the patients. These data included: mother's name, sex, weight, birth, age, gestational age, delivery type, antimicrobial use, risk factors and others. These epidemiological records together with the blood culture bottles were sent to the microbiology laboratory, where the results of culture and antimicrobial susceptibility tests were attached and filed. Simultaneously, these data were registered in the EXCEL 7.0 program (Microsoft) and subsequently analyzed using Epi-Info (version 6.04b; CDC, Atlanta, USA).

RESULTS

Table 1 shows the general demographic data relating to the hospitalized newborns presenting sepsis. The mean age was 13.1 days with a standard deviation of ± 4.3 days; males (60.2%) were more prevalent than females (39.8%); a higher frequency of HI was observed in vaginal delivery (74.7%) when compared with cesarean delivery (25.3%); a mean of 1.2 strains were isolated per patient; and a mean of 2.3 strains were isolated per case of polymicrobial infection (36 cases, 14.1%). A total of 207 (83.1%) patients received antimicrobial drugs before positive blood culture presentation with a mean of 2.9 antimicrobials used per patient and a mean of 8.7 days of therapy. A total of 15 different antimicrobials (data not shown) were used, with ampicillin (28.8%), amikacin (26.9%), oxacillin (13.8%) and cefotaxime (12.6%) predominating. The most significant risk

Table 1. Demographic characteristics of the hospitalized newborns in two years period (July 1997 to July 1999).

Demographic data	Frequency
Mean age (\pm SD) ^a	13.1 days (\pm 4.3 days)
Male sex ^a	150/249 (60.2%)
Female sex ^a	99/249 (39.8%)
Vaginal delivery ^a	186/249 (74.7%)
Cesarean delivery ^a	63/249 (25.3%)
Twins ^a	20/249 (11.6%)
Mean of strains isolated per patient ^b	301/255 (1.2)
Polymicrobial infection cases ^b	36/255 (14.1%)
Mean of strains isolated in the polymicrobial infection cases ^c	82/36 (2.3)
Patients using antimicrobial drugs before positive blood cultures presentation ^a	207/249 (83.1%)
Mean of antimicrobial drugs use before positive blood culture presentation ^d	601/207 (2.9)
Mean of days of antimicrobial drugs use before positive blood culture presentation ^d	8.7 days
Underlying risk conditions	
Prematurity	226/249 (90.8%)
Low gestational age (LGA; < 36 weeks) ^a	209/249 (83.9%)
Very low birth weight (VLBW; < 1500g) ^a	131/249 (52.6%)
Asphyxia ^a	99/249 (39.8%)
Hyaline Membrane Disease (HMD) ^a	83/249 (33.3%)
Prolonged amniotic membrane rupture time (PAMR; > 24h) ^a	61/249 (24.5%)
Congenital infection ^a	7/249 (2.8%)
Others ^a	83/249 (33.3%)

SD – standard deviation; a – data available only from 249 patients; b – data obtained from 255 newborns; c – data obtained from 36 polymicrobial infection cases among 255 hospitalized newborns; d – data obtained from 207 patients who received antimicrobial drugs before positive blood culture presentation.

conditions encountered were prematurity (90.8%), low gestational age (LGA; 83.9%), VLBW (52.6%), asphyxia (39.8%) and hyaline membrane disease (HMD; 33.3%).

Three hundred and one strains were recovered from blood cultures (Table 2). Gram-negative strains accounted for 70% of isolated strains (71.6% fermenters and 28.4% non-fermenters), with *Klebsiella pneumoniae* (22.9%), *Serratia marcescens* (15.9%), *P. aeruginosa* (10.6%) and *Acinetobacter baumannii* (6%) being the most frequently encountered species. Among Gram-positive strains (25.7%), we found a predominance of CoNS (17.3%), *S. aureus* (5%), and one genus of yeast represented by *Candida* sp. (4.3%).

The Gram-negative strains were found to be highly resistant (70-100%) to several of the antimicrobial drugs examined

Table 2. Frequency of microorganisms isolated from 255 newborns blood cultures in two years period (July 1997 to July 1999).

Microorganisms	Frequency	(%)
<i>Klebsiella pneumoniae</i>	69	22.9
Coagulase negative Staphylococci	52	17.3
<i>Serratia marcescens</i>	48	15.9
<i>Pseudomonas aeruginosa</i>	32	10.6
<i>Acinetobacter baumannii</i>	18	6.0
<i>Staphylococcus aureus</i>	15	5.0
<i>Candida</i> sp	13	4.3
<i>Escherichia coli</i>	12	4.0
<i>Enterobacter aerogenes</i>	10	3.3
<i>Enterobacter cloacae</i>	10	3.3
<i>Streptococcus agalactiae</i>	5	1.7
<i>Streptococcus</i> sp. (α - hemolytic)	5	1.7
<i>Alcaligenes xylosoxidans</i>	4	1.3
<i>Pseudomonas putida</i>	3	1.0
<i>Burkholderia cepacia</i>	2	0.7
<i>Enterobacter asburiae</i>	1	0.3
<i>Morganella morganii</i>	1	0.3
<i>Stenotrophomonas maltophilia</i>	1	0.3
Total of samples	301	100

(Table 3), particularly the cephalosporins. Intermediate resistance (35-70%) was observed to aminoglycosides, tetracycline, chloramphenicol and trimethoprim-sulfamethoxazole. In addition, a small number of bacterial strains were resistant to imipenem and ciprofloxacin. The *K. pneumoniae* strains were highly susceptible to ceftazidime, imipenem and ciprofloxacin; intermediate susceptibility was shown to tetracycline; and 60.9% (42/69) were ESBL producers. The *S. marcescens* strains were highly susceptible to imipenem and ciprofloxacin, and intermediate resistance was found to chloramphenicol and trimethoprim-sulfamethoxazole. Most *P. aeruginosa* strains were susceptible to gentamicin, amikacin, imipenem and ciprofloxacin, but resistant strains were detected for these antimicrobials; and showed high resistance levels to the other antimicrobial drugs. *Enterobacter* spp. were highly susceptible to imipenem, gentamicin and ciprofloxacin. And the *A. baumannii* strains presented high susceptibility only to imipenem (these strains showed high resistance levels to ciprofloxacin).

Gram-positive strains showed 63% of resistance to oxacillin (Table 4), examples of this included the following species: *Streptococcus* sp. (α -hemolytic) (100%), *S. aureus* (93.3%) and CoNS (58.8%); resistance to vancomycin was not detected; and the other antimicrobial drugs tested (cephalotin, ampicillin, amikacin, erythromycin and penicillin) showed resistance percentages ranging from low to high, depending on the bacterial species analyzed (Table 4).

Table 3. Antimicrobial resistance percentage of the Gram-negative strains isolated from newborns blood cultures in two years period (July 1997 to July 1999).

Microorganisms	CFL	CFO	CRO	CRX	CAZ	CPM	CAR	IMP	GEN	AMI	CIP	CLO	SX	TET
<i>Klebsiella pneumoniae</i>	92.8	8.7	94.2	94.2	-	-	-	0	82.6	94.2	0	92.8	89.8	43.5
<i>Serratia marcescens</i>	100	89.6	79.2	100	-	-	-	0	85.4	91.7	0	56.2	52.1	85.4
<i>Pseudomonas aeruginosa</i>	100	100	96.9	100	81.2	68.8	75.0	6.2	15.6	12.5	3.1	93.7	90.6	78.1
<i>Acinetobacter baumannii</i>	100	100	100	100	-	-	-	0	55.6	72.2	77.8	100	66.7	72.2
<i>Escherichia coli</i>	75.0	8.3	66.7	91.7	-	-	-	0	58.3	75.0	0	83.3	91.7	83.3
<i>Enterobacter aerogenes</i>	100	100	100	100	-	-	-	0	0	90.0	0	90.0	90.0	10.0
<i>Enterobacter cloacae</i>	100	100	90.0	100	-	-	-	0	0	80.0	0	90.0	70.0	30.0
<i>Alcaligenes xylosoxidans</i>	50.0	0	50.0	100	-	50.0	-	0	100	100	0	0	0	0
<i>Pseudomonas putida</i>	66.7	66.7	66.7	100	50.0	100	-	0	66.7	66.7	0	66.7	33.3	33.3
<i>Burkholderia cepacia</i>	100	100	100	100	100	-	-	0	50.0	50.0	0	0	0	50.0
<i>Enterobacter asburiae</i>	100	100	100	100	-	-	-	0	0	0	0	100	0	100
<i>Morganella morganii</i>	100	100	100	100	-	-	-	0	0	0	0	0	0	0
<i>Stenotrophomonas maltophilia</i>	100	100	100	100	-	-	-	100	0	0	0	100	0	100
Resistance mean	91.1	74.9	88.0	98.9	77.1	72.9	75.0	8.2	39.6	56.3	6.2	67.1	44.9	52.7

CFL – cephalothin, CFO – ceftoxitin, CRO – ceftriaxone, CRX – cefuroxime, CAZ – ceftazidime, CPM – cefepime, CAR – carbenicillin, IMP – imipenem, GEN – gentamicin, AMI – amikacin, CIP – ciprofloxacin, CLO – chloranphenicol, SXT - thrimetoprim – sulfametoxazole, TET – tetracycline; - Non tested.

Table 4. Antimicrobial resistance percentage of the Gram-positive strains isolated from newborns blood culture in two years period (July 1997 to July 1999).

Microorganisms	CFL	AMP	AMI	ERY	OXA	PEN	VAN
Coagulase negative <i>Staphylococci</i>	13.0	90.2	35.3	35.3	58.8	94.1	0
<i>Staphylococcus aureus</i>	63.6	100	73.3	66.7	93.3	100	0
<i>Streptococcus agalactiae</i>	0	0	100	0	0	0	0
<i>Streptococcus sp.</i> (α -hemolytic)	100	25.0	100	100	100	75.0	0
Resistance mean	44.1	53.8	77.2	50.5	63.0	67.3	0

CFL- Cephalotin, AMP- ampicillin, AMI- amikacin, ERY- erythromycin, OXA- oxacillin, PEN- penicillin, VAN- vancomycin.

DISCUSSION

The newborns examined in this study presented risk factors for contracting HI. The most predominant factors were: 1- submission to invasive procedures as: tracheal intubations, mechanical ventilation, airway aspiration, intravascular catheters, parenteral nutrition (data not shown); and 2- presence of conditions such as: prematurity, asphyxia, HMD, VLBW, LGA, prolonged amniotic membrane rupture time (PAMR) and congenital infection. Greenberg *et al.* (15) in a study performed at a university hospital in Negev, Southern Israel, concluded that the risk factors of prematurity and VLBW significantly

influenced the incidence of sepsis in comparison to that seen in neonates from the general population (prematurity 59% v.s. 7%; VLBW 42% v.s. 1%). In relation to birth weight and gestational age, Fanaroff *et al.* (12) related a significant decrease in the risk of septicemia with increasing birth weight and gestational age in a study performed with infants weighing between 501 to 1500g at birth from eight participating centers in the USA. The observations reported in our study concerning invasive procedures and other conditions are in accordance with those of several authors, who described similar risk conditions for acquisition of HI and death in NICUs (3-5,8,13,17)

During certain periods in the present study, the NICU was overcrowded and we cannot discount the possible breakdown in the application of aseptic techniques, especially hand washing during manipulation of newborns, which have may facilitated cross-infections. These factors have also been mentioned in reports from other hospital units (4,16,21,26,30).

The microorganisms most frequently identified from blood culture in this work were: *K. pneumoniae* (22.9%), CoNS (17.3%), *S. marcescens* (15.9%), *P. aeruginosa* (10.6%), *A. baumannii* (6%) and *S. aureus* (5%). All species isolated in the present study are in accordance with those reported in previous studies (4,12,15,18,24,25,27,30).

The data presented in Table 3 highlights a high levels of resistance to cephalosporins and intermediate resistance levels to aminoglycosides among Gram-negative isolates, suggesting the presence of a variety of resistance mechanisms, such as: reduced permeability of antimicrobials through the outer membrane, enzyme production and efflux pump mechanisms, as described by other authors (1,2,6,10,19,21,27,30).

The high resistance rates found in this study may be associated with the high frequency at which these antimicrobial drugs were used for both prophylactic and therapeutic treatment of hospitalized newborns. This practice have may exerted selective pressures leading to the emergence of multi-drug resistant strains (8,16,26,30), which in turn may have stimulated the acquisition of genes encoding resistance mechanisms via horizontal transfer mechanisms between bacterial strains within the hospital environment (16,29). It is of particular interest, that in this study a significant number of patients (83.1%) received antimicrobial drugs before positive blood culture presentation (empiric use), due to clinical manifestations suggestive of sepsis.

Our data revealed that 60.9% of the *K. pneumoniae* strains were ESBL producers. Some authors (9,23) state that cephalosporin resistance may be mediated by ESBL production, with these enzymes resulting from mutations in SHV₁, TEM₁, or TEM₂ genes. Other authors (23,30) have reported that plasmids carrying sequences encoding for β -lactamases production are commonly found in *E. coli* and members of the KES group (*Klebsiella* spp., *Enterobacter* spp. e *Serratia* spp.), and frequently carry other resistance factors, including resistance to aminoglycosides.

The KES group is composed by pathogens of significant morbidity and mortality in immunocompromised and debilitated patients, as well as newborns, and these bacterial species frequently transfer their resistance genes to susceptible strains during outbreaks (23,26).

Regarding the 13 cases of sepsis from which *Candida* sp. were isolated (Table 2), the newborns were treated with antimicrobials before positive blood culture presentation, most frequently ampicillin and amikacin (61.5%; 8/13 patients) followed by cefotaxime and oxacillin (38.5%; 5/13). Some patients were submitted to parenteral nutrition (46.2%; 6/13), mechanical ventilation (46.2%; 6/13) and vascular catheters (23.1%; 3/13) (data not shown). Some authors (5, 24) have stated that *Candida* sp. have emerged as important nosocomial pathogens since the 70s, principally in VLBW newborns (under 1500g), perhaps as a result of prolonged vascular catheterization, parenteral hyperalimentation, ventilatory support, broad-spectrum antimicrobial therapy and increased steroid usage.

In the current study, *S. aureus* presented a high level of resistance to oxacillin (93.3%), being conventionally classified as methicillin-resistant *Staphylococcus aureus* (MRSA). These strains are considered a public health problem in neonatology because of their strong potential for dissemination in the wards, high levels of antibiotic resistance and an association with high rates of morbidity and mortality (7,28).

In the last decade, CoNS have been recognized as true pathogens and the most common microorganisms isolated in newborns sepsis cases, principally when the patients are using

vascular devices or receiving parenteral nutrition or mechanical ventilation. These strains generally show resistance towards many antimicrobials including oxacillin (2,3,15,16,24,25). CoNS was the most prevalent Gram-positive strain recovered in this study and presented high levels of resistance to ampicillin and penicillin, intermediate resistance to amikacin, erythromycin and oxacillin, and a low level of resistance to cephalotin. Interestingly, some patients received mechanical ventilation (44.2%; 23/52), parenteral nutrition (34.6%; 18/52) or used vascular catheters (9.6%; 5/52) as part of their therapeutic treatment.

The Committee for control of Hospital Infection at the HMAF has used the results of this study to control new cases, optimize the use of antimicrobial therapy in the hospital, implement antibiotic policy programs and guide antimicrobial choice in cases when prophylactic or therapeutic therapy must be started before blood culture and antibiogram results are available, due to the clinical conditions of the patients.

RESUMO

Estudo de microrganismos resistentes a múltiplas drogas isolados de hemoculturas de neonatos hospitalizados na cidade do Rio de Janeiro, Brasil

Os objetivos deste estudo foram determinar a frequência e perfis de resistência a antimicrobianos de microorganismos associados com casos de sepsis em um hospital maternidade pública, e analisar os principais dados demográficos relacionados a estes neonatos infectados durante um período de dois anos. Analisamos 255 hemoculturas positivas e prontuários médicos de neonatos admitidos na unidade de tratamento intensivo neonatal do Hospital Maternidade Alexander Fleming II, Rio de Janeiro, Brasil, de Julho de 1997 a Julho de 1999. As Identificações e antibiogramas das cepas isoladas foram realizadas de acordo com os procedimentos de rotina laboratorial. Os dados demográficos e microbiológicos foram analisados utilizando o programa Epi-Info. A idade média dos neonatos foi 13,1 dias, com média de 1,2 cepas isoladas por paciente. Antibióticos foram administrados a 207 (83,1%) pacientes antes de apresentarem hemocultura positiva. Um total de 90,8% pacientes eram prematuros, 83,9% tiveram idade gestacional menor que 36 semanas, 52,6% apresentaram peso de nascimento muito baixo, 39,8% tiveram asfixia e 33,3% apresentaram doença de membrana hialina. Um total de 301 microrganismos foram isolados com uma predominância de *Klebsiella pneumoniae* (22,9%), *Staphylococcus coagulase-negativo* (17,3%), *Serratia marcescens* (15,9%) e *Pseudomonas aeruginosa* (10,6%). As cepas Gram-negativas mostraram altos níveis de resistência para cefalosporinas e aminoglicosídeos. Um total de 93,3% *Staphylococcus aureus* foram metilino-resistentes. Os neonatos examinados neste estudo apresentaram

