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Aneta Nitsch-Osuch[1, A–E], Irena Choroszy-Król[2, A–E], Ernest Kuchar[3, A–E], Krzysztof Korzeniewski[4, A–E], Katarzyna Życińska[1, A–E], Kazimierz Wardyn[1, A–E]

Microbiological Spectrum and Susceptibility Pattern of Clinical Isolates from the Neonatal Unit in a Single Medical Center

[1] Department of Family Medicine, Warsaw Medical University, Poland
[2] Department of Basic Sciences, Wroclaw Medical University, Poland
[3] Department of Pediatric Infectious Diseases, Wroclaw Medical University, Poland
[4] Department of Epidemiology and Tropical Medicine, Military Institute of Medicine, Warszawa, Poland

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Infections are a frequent and significant cause of morbidity and mortality in neonatal units. The bacterial pathogens and their susceptibility patterns should be monitored in hospital settings. The aim of the study was to describe the distribution of the bacterial agents and their antibiotic resistant and susceptibility patterns in the Special Neonatal Care Unit (SNCU).

Material and Methods. A retrospective analysis of results of microbiologically tested samples (blood, cerebrospinal fluid, urine, stool, eye excretions, external ear swabs, nasopharyngeal swabs and skin swabs) taken from newborns hospitalized in one SNCU in Warsaw (Poland) was conducted. The period analyzed was from July 1st, 2010 to December 31st, 2010.

Results. A total of 838 cultured samples were collected in the period analyzed. Three hundred seventy three of them (44.5%) were positive. The majority of the cultured microorganisms were classified as colonization: 338/373 (91%) strains. Gram negative bacteria were predominant colonizing flora: 227/338 (67%) strains. Gram positive bacteria were predominant causative agents in newborns with infections: 26/35 (74%) strains. 57.9% of *Escherichia coli* isolates were resistant to amoxicillin and ampicillin. 100% of *Klebsiella pneumonia*e were resistant to amikacin and netilmicin. *Staphylococcus aureus* methicillin resistant strains were cultured in 2.7% of cases.

Conclusions. Gram negative species continue to be predominant agents of neonatal colonizing flora while gram positive bacteria remain important causative agents for symptomatic infections. Continuous monitoring of bacterial flora and its antibiotic susceptibility pattern is necessary to provide a successful antibiotic policy. Current results may be used for future national and international comparison (**Adv Clin Exp Med 2015, 24, 1, 15–22**).

Key words: neonatal ward, infections, bacteria, antibiotic susceptibility.

Infections are a frequent and significant cause of morbidity and mortality in neonatal units. As many as 2% of fetuses are infected *in utero* and up to 10% are infected in the first month after birth [1]. With the increasing complexity of neonatal intensive care, prolonged ventilation, use of total parental nutrition and other modern neonatology methods, gestationally younger and lower birth weight newborns are surviving and remaining in an environment with a high risk of infection for longer [2]. Newborns are less capable of responding to infections due to immunological deficiencies involving the reticuloendothelial system, cytokines and antibody and cell mediated immunity. In addition, co-existing diseases such as hyaline membrane disease and acidosis also contribute to infections [1]. Pathogens causing neonatal infection and their antibiotic susceptibility patterns may change over time [3] and differ between units and countries [4]. It is extremely important to

diagnose infection cases, particularly including respiratory tract infections (pneumonia), sepsis and urinary tract infections, in time so that appropriate antibiotic treatment can be given. In addition, the bacterial pathogens responsible for infections and their susceptibility patterns should be monitored regularly in hospital environments [5].

The aim of the study is to describe the distribution of the bacterial agents and their antibiotic resistant and susceptibility patterns in a Special Neonatal Care Unit (SNCU).

Material and Methods

A retrospective analysis of the results of microbiologically tested samples taken from newborns hospitalized in one SNCU in Warsaw (Poland) was conducted. The period analyzed was from July 1st, 2010 to December 31st, 2010. Approval from the local Ethics Committee was obtained prior to the study.

The SNCU is a 9-bed 2nd level unit and cares for newborns born at the hospital and referred from 1st level units (for diagnostic and therapeutic procedures) and 3rd level units (for continuation of treatment and rehabilitation). The average number of live births at our hospital is 4,000, and approximately 5% of newborns are admitted to the SNCU (mainly preterm newborns older than 30 weeks of gestation, newborns with congenital infections, mainly pneumonia or sepsis, and congenital defects).

In the period analyzed, a total of 206 newborns were hospitalized in the SNCU. Most of these newborns were delivered at the hospital.

Results

A total of 838 cultured samples were collected in the period analyzed; 373 of them (44.5%) were positive. Samples included in the study were: blood, cerebrospinal fluid (CSF), urine, stool, eye excretions, external ear swabs, nasopharyngeal swabs and skin swabs (Table 1). Samples were mainly taken from the external ear (291 samples), the second most common cultured biological material was blood (222 samples). Samples of blood, cerebrospinal fluid, urine, skin swabs and eye excretions were taken from newborns suspected of having an infection, while samples taken from the nasopharynx, external ear canal, stool or rectum were used for the screening for colonization and collected from newborns born from infected mothers, newborns with a prolonged (> 7 days) stay in the SNCU and newborns admitted from other hospitals. Positive results were most often obtained from nasopharyngeal swabs (79%) and stool/rectal swabs (77%) (Table 1).

The majority of the cultured microorganisms were classified as colonizing flora, 338 of 373 (91%) strains, while 35 of 373 (9%) strains were found to be responsible for symptomatic infection. Gram negative bacteria were predominant among colonizing flora: 227 of 338 (67%) strains, while gram positive bacteria were found in 26 of the 35 (74%) cases of symptomatic infections (Table 2). The most common cultured bacteria in newborns from our ward were *Escherichia coli* and *Klebsiella pneumoniae* (Table 3). *Escherichia coli* was mainly cultured from urine, eye excretions, skin swabs, external ear swabs and nasopharyngeal swabs; *Klebsiella pneumoniae* was the most common pathogen cultured from stool/rectal swabs (Table 3). Gram positive germs consisted mainly of *Staphylococcus aureus* and coagulase negative staphylococci (Table 3). Gram positive bacteria *Streptococcus* sp. and *Enterococcus* sp. were mainly isolated from blood, cerebrospinal fluid, external ear and nasopharyngeal swabs (Table 3).

57.9% of *Escherichia coli* isolates were resistant to amoxicillin and ampicillin, but 98.2–100% were resistant to cefuroxime, cephazidime, amikacin and netilmicin. 100% of *Klebsiella pneumoniae* were resistant to amikacin and netilmicin, and 14.6% were resistant to piperacillin. *Staphylococcus*

Table 1. Distribution of positive cultures according to the source of the culture in hospitalized neonates

Source of culture	Number of samples (n = 838)	Number and proportion of positive results (n = 374)
Blood	222	9 (4%)#
Cerebrospinal fluid	8	2 (25%)#
Urine	56	9 (16%)#
External ear swabs	291	159 (55%)*
Nasopharyngeal swabs	66	52 (79%)*
Eye excretions	19	11 (58%)#
Skin swabs	11	4 (36%)#
Stool/rectal swabs	165	127 (77%)*
Total	838	373 (100%)

* colonization, # symptomatic infection.

Table 2. Distribution of gram positive and gram negative bacteria as causative and colonizing agents

	Number of isolates	% of isolates
Colonization (n = 338)		
Gram negative bacteria	227	67
Gram positive bacteria	111	33
Causative agents (n = 35)		
Gram negative bacteria	9	26
Gram positive bacteria	26	74

aureus methicillin resistant strains were cultured in 2.7% of cases, *Staphylococci* coagulase negative strains were resistant to methicillin in 25% of cases (Table 5). All *Staphylococcus* sp. was sensitive to vancomycin. No VRE (Vancomycin-Resistant *Enterococcus*), VISA (Vancomycin Intermediate *Staphylococcus aureus*) or KPC (+) (*Klebsiella pneumoniae* carbapenemase producing strains were isolated from our patients. MRSA, *Escherichia coli* ESBL (+) and *Klebsiella pneumoniae* ESBL (+) strains were isolated from stool/rectal swabs and were not responsible for symptomatic infections but classified as colonizing flora.

Discussion

Bacterial infection is still prevalent in newborns and it is a major medical problem [1–5]. Our experience shows that gram negative bacteria were the most common commensal microorganisms in neonates in our hospital while gram positive bacteria were mainly cultured from the blood of neonates with sepsis. This is not a surprising result because it has been previously well documented that, after birth, the neonate rapidly acquires commensal bacteria that colonize the skin and the mucous membranes, and the first micro flora is acquired from the mothers' birth canal during vaginal delivery [6].

There was a distribution of gram positive and gram negative bacteria as the agents cultured from neonates similar to that reported by Lee et al. [7]. However, other researchers observed another pattern of isolates from the neonatal unit (3rd level) – gram positive bacteria were cultured in 64% of cases, gram negative bacteria were cultured in 30.6% of cases and yeasts were present in 4.9% of samples [8].

Gram negative bacteria are the main etiologic agents for neonatal sepsis, meningitis and urinary tract infections. Monsef et al. studied the pattern of common bacterial pathogens in the neonatal ward (intensive care unit) and found that *Escherichia coli* and *Klebsiella pneumoniae* were the most frequent bacteria isolated from urine, eye excretions and blood cultures [9]. These findings were consistent with the results of Shaw et al. [10] and Aurangzeb et al. [11].

In our study, *Escherichia coli* were the most prevalent bacteria isolated from nasopharyngeal external ear swabs and urine, showing a high resistance to amoxicillin and ampicillin (57.9%), and a relatively low degree of resistance to cephalosporins (1.8–5.3%) and aminoglycosides (0–2.6%). Our results are consistent with previous studies: Bhat et al. also found that *Escherichia coli* and other gram negative *bacilli* isolated from newborns were susceptible to amikacin and netilmicin but

Table 3. Distribution of microorganisms isolated in culture positive newborns

Microorganism	Number of isolates (n = 373)	% of isolates
Escherichia coli	114*	30.5
Klebsiella pneumoniae	54**	14.4
Enterococcus faecalis	47	12.6
Staphylococcus aureus	37***	9.9
Klebsiella oxytoca	35	9.4
Staphylococcus sp. coagulase negative	28	7.5
Enterobacter cloacae	17	4.5
Streptococcus agalactiae	12	3.2
Streptococcus oralis/mitis	6	1.6
Acinetobacter baumannii	6	1.6
Citrobacter freundii	4	1
Enterobacter aerogenes	2	0.5
Morganella morganii	2	0.5
Proteus mirabilis	4	1
Pseudomonas aeruginosa	3	0.8
Streptococcus sp. beta haemolyticus gr. F	1	0.3
Streptococcus viridans	1	0.3

* 2 strains ESBL (+), ** 2 strains ESBL (+), *** 1 strain MRSA.

Table 4. Microorganisms isolated according to the source of the culture in hospitalized neonates

Source of cultures	Isolated microorganisms	Number/proportion of isolated microorganisms
Blood (9 positive isolates from symptomatic patients)	*Staphylococcus aureus MSSA* *Escherichia coli* *Streptococcus viridans* *Streptococcus mitis* *Staphylococcus* sp. coagulase negative: *Staphylococcus epidermidis MRCNS* *Staphylococcus epidermidis MSCNS* *Staphylococcus haemolyticus MRCNS* *Staphylococcus warneri* *Staphylococcus hominis MSCNS*	1 (11. %) 1 (11.1%) 1 (11.1%) 1 (11.1%) 1 (11.1%) 1 (11.1%) 1 (11.1%) 1 (11.1%) 1 (11.1%)
Cerebrospinal fluid (2 positive isolates from symptomatic patients)	*Enterococcus faecalis* *Streptococcus mitis*	1 (50%) 1 (50%)
Urine (9 positive isolates from symptomatic patients)	*Escherichia coli* *Enterococcus faecalis* *Klebsiella oxytoca*	4 (44.4%) 2 (22.2%) 3 (33.3%)
Eye excretions (11 positive isolates from symptomatic patients)	*Escherichia coli* *Staphylococcus* spp. coagulase negative *Enterococcus faecalis* *Streptococcus mitis/oralis* *Enterobacter cloacae*	3 (27%) 3 (27%) 2 (18%) 2 (18%) 1 (9%)
Skin swabs (4 positive isolates from symptomatic patients)	*Escherichia coli* *Morganella morganii* *Pseudomonas aeruginosa* *Enterococcus faecalis*	1 (25%) 1 (25%) 1 (25%) 1 (25%)
External ear swabs (159 positive isolates – colonization)	*Escherichia coli* *Enterococcus faecalis* *Staphylococcus* sp. coagulase negative *Streptococcus agalactiae* *Klebsiella pneumoniae* *Staphylococcus aureus* *Enterobacter cloacae* *Streptococcus oralis* *Pseudomonas aeruginosa* *Citrobacter freundii* *Streptococcus* sp. beta *haemolyticus* gr. F	84 (52.89%) 39 (24.5%) 13 (8.2%) 7 (4.4%) 4 (2.5%) 3 (1.9%) 3 (1.9%) 2 (1.2%) 2 (1.2%) 1 (0.6%) 1 (0.6%)
Nasopharyngeal swabs (52 positive isolates – colonization)	*Escherichia coli* *Staphylococcus aureus* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Acinetobacter baumannii* *Enterobacter cloacae* *Enterococcus faecalis* *Staphylococcus* sp. coagulase negative *Streptococcus agalactiae*	13 (25%) 12 (23%) 8 (15.3%) 7 (13.4%) 4 (7.7%) 3 (5.8%) 2 (3.8%) 2 (3.8%) 1 (1.9%)
Stool/rectal swabs (127 positive isolates – colonization)	*Klebsiella pneumoniae* *Staphylococcus aureus* *Klebsiella oxytoca* *Enterobacter cloacae* *Proteus mirabilis* *Streptococcus agalactiae* *Citrobacter freundii* *Acinetobacter baumannii* *Enterobacter aerogenes* *Morganella morganii* *Escherichia coli* *Staphylococcus epidermidis*	42* (33%) 21** (16.5%) 25 (19.7%) 10 (7.8%) 4 (3.1%) 4 (3.1%) 3 (2.3%) 2 (1.6%) 2 (1.6%) 1 (0.8%) 8 (6.3%)*** 5 (4%)

* – 2 strains *Klebsiella pneumoniae* ESBL (+), ** – 1 strain MRSA, *** – 2 strains *Escherichia coli* ESBL (+).

remarkably less sensitive to ampicillin [12]. Similar results were reported by Hyde et al. [13] and Patzer et al. [14]. Monsef et al. [9] reported a higher resistance of *Escherichia coli* cultured from neonatal ward patients to cephalosporins and aminoglycosides.

In our study, gram positive bacteria were predominantly bacteria cultured form blood samples, present in 89% of patients with sepsis. Our findings are in agreement with results obtained by Gray et al. [15], Burnie et al. [16] and Gupta et al. [17]. In contrast, Mahmood et al. found that the

Table 5. Antibiotic sensitivity and antibiotic resistance of the most commonly isolated bacteria from positive cultures in neonates

Microorganism	Number/proportion of sensitive isolates	Number/proportion of reduced sensitivity isolates	Number/proportion of resistant isolates
Escherichia coli (n = 114)			
Ampicillin/amoxicillin	43 (37.7%)	5 (4.4%)	66 (57.9%)
Piperacillin	71 (62.3%)	5 (4.4%)	38 (33.3%)
Amoxicillin with clavulanic acid	89 (78.1%)	20 (17.5%)	5 (4.4%)
Cefalotin	87 (78.1%)	19 (16.7%)	8 (7%)
Cephazolin	102 (89.5%)	6 (5.3%)	6 (5.3%)
Cefuroxime	112 (98.2%)	0 (0%)	2 (1.8%)
Ceftazidime	112 (98.2%)	0 (0%)	2 (1.8%)
Amikacin	111 (97.4%)	3 (2.6%)	3 (2.6%)
Netilmicin	114 (100%)	0 (0%)	0 (0%)
Klebsiella pneumoniae/oxytoca (n = 89)			
Ampicillin/amoxicillin	0 (0%)	0 (0%)	89 (100%)
Piperacillin	64 (71.9%)	12 (13.5%)	13 (14.6%)
Amoxicillin with clavulanic acid	79 (88.8%)	5 (5.6%)	5 (5.6%)
Cefalotin	86 (96.6%)	2 (2.2%)	3 (3.4%)
Cephazolin	84 (94.4%)	5 (5.6%)	5 (6.7%)
Cefuroxime	86 (96.6%)	0 (0%)	3 (3.4%)
Ceftazidime	86 (96.6%)	0 (0%)	3 (3.4%)
Amikacin	89 (100%)	0 (0%)	0 (0%)
Netilmicin	89 (100%)	0 (0%)	0 (0%)
Morganella morganii/ Enterobacter spp./*Citrobacter freundii* (n = 29)			
Ampicillin/amoxicillin	–	–	–
Piperacillin	27 (93.1%)	2 (6.9%)	2 (6.9%)
Amoxicillin with clavulanic acid	–	–	–
Cefalotin	–	–	–
Cephazolin	–	–	–
Cefuroxime	–	–	–
Ceftazidime	29 (100%)	0 (0%)	0 (0%)
Amikacin	29 (100%)	0 (0%)	0 (0%)
Netilmicin	29 (100%)	0 (0%)	0 (0%)
Staphylococcus aureus (n = 37)			
Methicillin	36 (97.3%)	0 (0%)	1 (2.7%)
Erythromycin	28 (75.7%)	0 (0%)	9 (24.3%)
Clindamycin	29 (78.4%)	0 (0%)	8 (21.5%)
Ciprofloxacin	35 (94.6%)	0 (0%)	2 (5.4%)
Gentamicin	37 (100%)	0 (0%)	0 (0%)
Amikacin	37 (100%)	0 (0%)	0 (0%)
Netilmicin	37 (100%)	0 (0%)	0 (0%)
Chloramphenicol	37 (100%)	0 (0%)	0 (0%)
Trimethoprim	36 (97.3%)	0 (0%)	1 (2.7%)
Doxycycline	37 (100%)	0 (0%)	0 (0%)

Microorganism	Number/proportion of sensitive isolates	Number/proportion of reduced sensitivity isolates	Number/proportion of resistant isolates
Staphylococci sp. coagulase negative (n = 28)			
Methicillin	21 (75%)	0 (0%)	7 (25%)
Erythromycin	17 (61%)	0 (0%)	11 (39%)
Clindamycin	16 (57%)	0 (0%)	12 (43%)
Ciprofloxacin	25 (89%)	0 (0%)	3 (11%)
Gentamicin	22 (79%)	0 (0%)	6 (21%)
Amikacin	22 (79%)	0 (0%)	6 (21%)
Netilmicin	22 (79%)	0 (0%)	6 (21%)
Chloramphenicol	27 (96%)	0 (0%)	1 (4%)
Trimethoprim	21 (75%)	0 (0%)	7 (25%)
Doxycycline	24 (85.7%)	2 (7.1%)	2 (7.1%)
Enterococcus faecalis (n = 47)			
Ampicillin	47 (100%)	0 (0%)	0 (0%)
Amoxicillin with clavulanic acid	47 (100%)	0 (0%)	0 (0%)
Penicillin	47 (100%)	0 (0%)	0 (0%)
Gentamicin	45 (96%)	0 (0%)	2 (4%)
Chloramphenicol	36 (92%)	0 (0%)	3 (8%)
Erythromycin	6 (13%)	33 (70%)	8 (17%)

majority of isolates causing neonatal sepsis were gram negative rods [5].

The epidemiology of neonatal sepsis in developed and developing countries shows some important differences in the pattern of etiological bacteria and antibiotic susceptibility [12, 18]. In the industrialized world, group B *streptococci* (GBS) caused neonatal sepsis predominantly, *Escherichia coli* was the second most common etiologic agent [19, 20], but following GBS prophylaxis, a decreasing incidence of GBS and an increased rate of *Escherichia coli* infections has been reported [13, 17]. There is no doubt that, throughout the years, there has been a shift in the microorganisms responsible for neonatal septicemia; this was shown by Freedman et al. [21]: in the 1950's, staphylococci became a major cause of nursery outbreaks throughout the world. Since the 1980's, coagulase negative *staphylococci*, commonly known as *S. epidermidis,* have assumed considerable importance as troublesome nosocomial pathogens in neonatal units. This organism is more commonly seen in premature infants who require prolonged hospitalization, total parental nutrition, central vascular catheters and thoracostomy tubes. Treatment of these infections is also complicated by the high frequency of penicillin and gentamicin resistant strains, yet most strains remain sensitive to vancomycin [21].

In our study, only one strain (2.7%) of *Staphylococcus aureus* was methicillin resistant, but 7 strains (25%) of *Staphylococcus* sp. coagulase negative were methicillin resistant. All strains of *Staphylococci* sp. were sensitive to vancomycin. Aftab et al. also reported an increasing number of staphylococci strains resistant to cephalosporins, which may result in difficulties relative to the empiric treatment of neonatal infections [22]. In our study, we found 12 cases of *Streptococcus agalactiae* (GBS) – all of them were considered colonizing flora, not causative agents of infections. This may be attributed to an increased awareness of GBS carrying mothers and the use of prophylactic intrapartum antibiotics and the rapid screening and treatment of babies [17].

"Alarm" pathogens, including MRSA (methicillin resistant *Staphylococcus aureus*) and *Enterobacteriaceae* ESBL (+) (extended spectrum beta-lactamases) were cultured in only 5 neonates: 1 case of MRSA, 2 cases of *E. coli* ESBL(+), 2 cases of *Klebsiella pneumoniae* ESBL (+). All of them were classified as gastrointestinal tract colonization and cultured from rectal swabs.

In our study, we did not observe high antibiotic resistance from the most common isolated bacteria, except in the case of *Escherichia coli* resistant to ampicillin and amoxicillin (described also by other Polish researchers [23]), and a small number of cultured alarm pathogens. We think it may be a result of the organization and special character of our neonatal ward. It is not a tertiary intensive neonatal intensive care ward, there are no very preterm newborns hospitalized here with very low and extremely low body mass requiring prolonged mechanical ventilation and other highly specialized procedures – these newborns are transferred to other hospitals. The same explanation may be

given for the low number of positive blood cultures in our material (4%), while the frequency of sepsis in intensive care neonatal units has been reported as being higher – 13% [24]. However, there have been single cultures of MRSA and *Enterobacteriaceae* ESBL (+) isolates. All came from rectal swabs and were classified as colonization of the gastrointestinal tract. However, it should be remembered that the local environment, possibly contaminated with these alarm pathogens, may also be an important source of bacteria for other neonates and may result in severe and dangerous outbreaks in the neonatal unit. It is worth noting that our hospital has a good and effective hospital infection control team, which consists of a physician (epidemiologist), a nurse (specialized in infection control procedures) and a microbiologist. The infection control team actively monitors infections (mainly nosocomial) and runs educational activities focused on decreasing the risk of spreading infections among patients (hand hygiene, good adherence to infection control measures, including avoiding crowding babies and mothers, avoiding a low number of medical staff (mainly nurses) and proper use of medical instruments, etc.). An antibiotic policy has also been introduced at the hospital (glycopeptides and carbapenems and third generation cephalosporins may only be administrated after approval from the ward manager). We conclude that the previously introduced infection control measures could have resulted in a low number of nosocomial infections and low number of multi-resistant bacteria.

The bacterial spectrum of neonatal infections and colonization could be different in different hospital wards [25, 26]. Continued surveillance of neonatal infections and colonization should be mandatory for each hospital ward due to temporal changes in the causative organisms and their antibiotic susceptibility. Periodic evaluations not only show the trend of resistance to commonly-used antibiotics but also help with the implementation of a rational empirical treatment strategy. This study indicated that gram negative species continue to be the predominant agents of colonizing flora among newborns and they present a low susceptibility to commonly used antibiotics like ampicillin – which is a cause for concern. Gram positive bacteria are the main causative agents in neonatal septicemia and strains of *Staphylococcus* sp. resistant to methicillin are also a problem.

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Address for correspondence:

Aneta Nitsch-Osuch
Department of Family Medicine
Warsaw Medical University
Banacha 1a
02-097 Warszawa
Poland
Tel.: +48 22 59 92 190
E-mail: anitsch@amwaw.edu.pl

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