

## ANTIMICROBIAL RESISTANCE BY BACTERIA THAT COLONIZE NEONATES WITH SUSPECTED SEPSIS

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**Objective** - The aim of this study was to determine the antibiotic resistance in bacteria that colonized neonates with suspected sepsis, born in the Department of Gynecology and Obstetrics, University Clinical Centre Tuzla, Tuzla, Bosnia and Herzegovina from December 1, 2008 to December 31, 2009.

**Methods** - The study included neonates who were discharged from the Maternity ward, who developed clinical signs of suspected sepsis after forty eight hours of life, and were therefore rehospitalized at the Department of Pediatrics in Tuzla.

**Results** - The study showed that 55 of 4299 newborns were diagnosed with suspected sepsis. The cumulative incidence of suspected sepsis was 1.3%. The bacteria that most often colonized neonates was *Klebsiella pneumoniae*, that was isolated in 42 (76.4%) newborns. In 14 cases (33.3%) *Klebsiella* was ESBL-producing. Moreover we found *Escherichia coli* in 5 neonates (9%), *Enterococcus faecalis* in 5 neonates (9%) and *Enterobacter Cloacae* in 3 neonates (5.4%). The bacteria that colonized newborns showed high resistance to ampicillin (91% of cases), slightly lower to cefotaxime (53.3%), gentamicin (43.6%), amikacin 12%, amoxiclav 14.3%, and imipenem and meropenem (2.1%).

**Conclusions** - The high degree of resistance among the enterobacteriaceae that were isolated from our neonates indicates the need to investigate the risk factors for colonization of newborns with antibiotic resistant bacterial strains. The promotion of breastfeeding could be a preventive intervention.

**Key words:** Newborn infants ■ Neonatal sepsis ■ Bacterial resistance

### Introduction

Nosocomial infections are a significant cause of neonatal morbidity and mortality, particularly in developing coun-

tries (1). The dissemination of antibiotic-resistant bacteria in hospital environments is becoming an increasing problem; it occurs as a result of several factors: the inappropriate use of antibiotics as a major cause, the lack of data on newborns treated for sepsis, and the delay in obtaining the results of microbiological tests.

Bacterial colonization of the mother by pathogenic bacteria, maternal infections during pregnancy, premature and/or prolonged amniorrhexis, prolonged maternal antibiotic therapy, and improper handling of neonates all are well known risk factors for infections (2).

Term babies have a shorter hospital stay in the nurseries, without invasive procedures, hence the most important risk factor for infection in these patients is exposure to health personnel. Gram-negative organisms are important nosocomial pathogens which colonize gastrointestinal, respiratory and other sites in hospitalized neonates, so they may represent a source of infection, especially in the absence of proper procedures for handwashing for example (3).

The increase in antibiotic resistance among bacterial species over time is the natural consequence of non rational, excessive use of antibiotics. According to statistics from the Center for Disease Control and Prevention (CDC), more than 70% of the bacteria that cause hospital-acquired infections are resistant to at least one of the antibiotics most commonly used to treat infections. The synthesis of antibiotic degrading enzymes is one of the mechanisms of antibiotic resistance, such as New Delhi metallo-beta lactamase (NDM1), which is an enzyme that confers resistance on one of the newer classes of antibiotics, the carbapenems (4).

The incidence of nosocomial sepsis in neonates, is 3-20 times higher in developing countries than in developed countries (1). On the other hand, bacterial resistance has reached an alarming level so that 70% of sepsis can no longer be treated with ampicillin and gentamicin (5, 6).

The aim of this study was to determine antibiotic resistance of bacteria isolated from different sites in newborns who were discharged from the Department of Gynecology and Obstetrics, University Clinical Centre Tuzla. After discharge, these neonates developed clinical symptoms suggestive of systemic infection, that required readmission to the Department of Pediatrics in Tuzla.

## **Subjects and Methods**

From December 1, 2008 to December 31, 2009, 4316 neonates were born at the Department of Gynecology and Obstetrics, University Clinical Centre Tuzla. Among these neonates 4299 were discharged and 17 died. After discharge 55 of the 4299 neonates developed clinical symptoms suggestive of infection. Therefore they were rehospitalized at the Department of Pediatrics in Tuzla and enrolled in this study. Among these neonates, 13/55 (24%) were preterm infants and 11/55 (20%) had a birth weight <2500 grams.

Clinical diagnosis of suspected sepsis was made according to the Center for Disease Control criteria (7). For each patient enrolled in the study we collected the following variables: maternal prolonged rupture of membranes (PROM) >18 hours, maternal chorioamnionitis, obstetric procedures in pre/perinatal age, gestational age, clinical signs of respiratory distress, presence of apnea lasting >20 seconds, tachycardia (heart rate >180 beats per minute) bradycardia (heart rate <100 beats per minute), poor perfusion, hypothermia (rectal temperature <36°C) hyperthermia (rectal temperature >38°C) irritability, lethargy, poor feeding and feeding intolerance, vomiting. The laboratory findings recorded were the following: blood C-reactive protein level >1.5 mg%, immature to total neutrophil ratio >0.2 absolute neutrophil count and erythrocyte sedimentation rate (8).

Microbiological analysis was performed on blood, urine, swabs from throat, nose, skin lesion and anal region specimens, by direct examination and culture. All samples were processed using standard microbiological techniques. Among the blood cultures, 12.7% were positive for growth of coagulase-negative Staphylococcus which we considered to be contamination in accordance with CDC criterions.

We used the antibiotic sensitivity of colonizing bacterial species to select the therapy. Isolation of the same agent from multiple cultures contributed to a greater likelihood of identifying the pathogen bacteria causing the infection. In some cases significant bacteriuria persisted after the discharge of the neonate from the hospital and 13% of them subsequently developed recidives of infections and were rehospitalized.

The sensitivity of isolated bacteria to antibiotics was tested at the Division of Microbiology, the University Clinical Center, Tuzla using the disc diffusion method. ESBL detection in each isolated strain of enterobacteriaceae was performed through discs of ceftazidime-amoxicillin/clavulanic acid-cefotaxime

involved in routine antibiogram. Phenotypic confirmation was performed using the automated system Vitek 2, "break point". Concentrations of the antibiotic higher than the "break point" confirmed the ESBL presence.

## Results

Fifty-five neonates with suspected sepsis were admitted to the Department of Pediatrics in Tuzla from December 1, 2008 to December 31, 2009. During the same period 4299 newborn were discharged from the Department of Gynecology and Obstetrics, University Clinical Centre Tuzla and the cumulative incidence of suspected sepsis was 1.3%.

The antimicrobial-resistant pathogens colonizing neonates with suspected sepsis are shown in Table 1.

*Klebsiella pneumoniae* was isolated in 42 (76.4%) neonates (14 or 33.3% were ESBL-strains), *Escherichia coli* in 5 (9.0%) neonates, *Enterococcus faecalis* in 5 (9%) neonates and *Enterobacter cloacae* in 3 (5.5%) neonates. Resistance to ampicillin was 91%, to cefotaxim 53.3%, to cefepim 36.1% to gentami-

**Table 1** Frequency of antimicrobial resistance in pathogens colonizing neonates with suspected sepsis

Antibiotic	Frequency of antimicrobial resistance				
	<i>Klebsiella pneumoniae</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>	<i>Enterobacter cloacae</i>	Total
	n	n	n	n	%
Ampicillin	42/42	3/5	2/5	3/3	91.0
Gentamicin	18/42	4/5	2/5	0/3	43.6
Amikacin	6/42	0/5	-	0/3	12.0
Cefotaxim	24/42	-	-	0/3	53.3
Cefepim	16/42	-	1/5	-	36.1
Bactrim	24/42	-	5/5	3/3	64.0
Nitrofurantoin	17/42	-	-	-	40.4
Eritromicin	-	4/5	-	-	80.0
Amoxicillin	6/42	-	-	-	14.3
Amoxicillin	-	-	2/5	-	40.0
Imipenem	1/42	-	0/5	-	2.1
Meropenem	1/42	-	0/5	-	2.1

cin 43.6%, to amikacin 12%, and to amoxiclav 14.3%. Resistance to carbapenems was 2.1%. *Klebsiella pneumoniae* - ESBL- strains were 100% resistant to ampicillin, cefotaxim, gentamicin, bactrim. Slightly lower resistance was observed to cefepime (57.2%), to amikacin (43%) and to nitrofurantoin (42.8%) to amoxiclav and carbapenems resistance was 7.2%.

## Discussion

In this study the incidence of suspected sepsis was 1.3%, higher than the reported incidence of infection among term, healthy infants (0.6%) and preterm neonates without the need for invasive procedures (9). The most common bacteria which colonized our infected neonates was *Klebsiella pneumoniae* (76.4%). This frequency was higher than the frequency shown by Zaidi et al. (1) and suggests that *Klebsiella pneumoniae* should be recognized as one of the most important pathogens, causing sepsis in neonates in developing countries. Its incidence is 6.3/1000 live births (1). In our study, the colonization incidence by *Klebsiella pneumoniae* in the newborn infants with suspected sepsis was 9.7/1000 live births. Newborns are commonly colonized by *Klebsiella pneumoniae* through the gastrointestinal and respiratory tract. Since the clinical picture of sepsis manifested after 48 hours from the birth, it could be assumed that the cause originated from the environment. To confirm this assumption one should investigate the incidence of colonization by *Klebsiella pneumoniae* in neonates born in the Department of Gynecology and Obstetrics in Tuzla.

The research of Custovic et al. (10) related to nosocomial infections in all patients treated at the Department of Gynecology and Obstetrics, University Clinical Centre Tuzla, showed that gram-negative bacteria are the most common cause of nosocomial infections, and *Klebsiella pneumoniae* was represented in

24.6% of cases. This research also suggests that the gram-negative bacteria that colonize the infants resulted from the environment. Gram-negative bacteria as the causative agent of sepsis is common when delivery is performed without respect for hygienic measures, as suggested by Zaidi et al. (1) and rarely originates from mothers (11).

The occurrence of infection depends on many factors. The neonate is born without the endogenous microbial flora but is rapidly colonized by microorganisms from the maternal genital tract or from the environment. Since the immune system in the neonatal age cannot provide an effective defense against pathogens, the risk of developing an invasive infection is increased.

In our study, ESBL-*Klebsiella pneumoniae* strains were isolated in 33% of cases. In 100% of cases ESBL-*Klebsiella pneumoniae* strains were resistant to ampicillin, cefotaxime, gentamicin and bactrim. Similar results have been described in the research on neonatal pathogens in developing countries by Khassawneh et al. (12), Thaver et al. (5), all confirming that *Klebsiella pneumoniae* is often highly resistant to the antibiotics in current use. One of the possible consequences of this observation is that at least one child in three should start antibiotic therapy against sepsis with carbapenems. Resistance of enterobacteriaceae to antibiotics support the notion that the suggestion of the World Health Organization on the empiric treatment of sepsis with ampicillin and gentamicin can no longer be applied (6).

In our study, high rates of resistance of enterobacteriaceae to the most commonly used antibiotics (ampicillin, aminoglycosides and cephalosporins) indicate the need for close surveillance of nosocomial infections by monitoring pathogen resistance to the antibiotics used. Bacterial antibiotic resistance, resulting from often inappropriate and prolonged use of antibiotics, in turn leads to the use of newer and more potent molecu-

les, that worsen the situation and create new resistances, promoting a vicious circle from which it is difficult to escape. Vergnana's research supports this assertion (13). The spread of these bacterial strains in hospital wards, mostly through the hands of health personnel, and the effectiveness of proper hand hygiene procedures, has been known since the 19th century (14).

In our study the neonates showed no serious symptoms that would indicate systemic infection at discharge from the maternity ward. They had probably been colonized with enterobacteriaceae and the disease developed subsequently in those neonates with predisposing factors.

One of the simplest measures that may be implemented to reduce the colonization of neonates by pathogenic bacteria is the promotion of breast-feeding. The incidence of nosocomial infections, predominantly gastrointestinal and respiratory in children in developing countries is very high, ranging from 5% to 44%. There is no effective strategy to date for preventing these infections. In addition to nutritional and psychological benefits, it is well known that breast-feeding has a protective effect against infection. Human milk contains significant concentrations of secretory IgA and oligosaccharides, which provide protection against bacterial infection. In addition, the bifidus factor present in human milk stimulates the development of protective intestinal microflora in breast-fed infants. The protective effects of human milk against infection caused by rotavirus, enterobacteriaceae and *Streptococcus pneumoniae* are well known. Therefore the promotion and acceptance of breast-feeding as the ideal form of infant nutrition should be encouraged. Time spent in the promotion of breast-feeding is always time well invested, also in order to prevent colonization of neonates by pathogenic enterobacteriaceae (15).

Our study was performed on neonates with suspected sepsis. This fact constitutes a limitation because the diagnosis of sepsis was not confirmed by positive blood cultures. The reason is that 55% of mothers were under antibiotic therapy before, during and after childbirth, and 32.7% of newborns were also under antibiotic therapy, because of some clinical symptoms suggestive for infections that they showed in the nursery before discharge.

## Conclusions

This study showed that the cumulative incidence rate of suspected sepsis in neonates born in the Department of Gynecology and Obstetrics in Tuzla was 1.3%. The most common cause was *Klebsiella pneumoniae* (76.4% of cases) of which the ESBL-strain was present in 33.3%. Bacterial resistance to ampicillin was 91%, gentamicin 43.6%, cefotaxime 53.3% and for the carbapenems, 2.1%. The *Klebsiella pneumoniae* ESBL strain was 100% resistant to ampicillin, gentamicin, cefotaxime and carbapenems in 7.2% of cases. The high degree of resistance of isolated enterobacteria indicates that there is a need to investigate the risk factors for newborn colonization with antibiotic resistant bacterial strains.

## Authors' contributions:

Conception and design: IS and NA; Acquisition, analysis and interpretation of data: IS and SP; Drafting the article IS; Revising it critically for important intellectual content: IS and NA.

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**Conflict of Interest:** The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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