

**Etiology of early-onset neonatal sepsis and antibiotic resistance in Bukavu, Democratic Republic of the Congo.**

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**Summary:** The early-onset neonatal sepsis case fatality rate was 25.3% among hospitalized neonates in Bukavu. *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Serratia marcescens* were the pathogens most commonly retrieved. A total of 100%, 88.9% and 86.1% of these isolates were resistant to ampicillin, cefotaxime and gentamicin, respectively.

## Abstract

**Background.** The Democratic Republic of the Congo (DRC) ranks among the countries with the highest neonatal death rates (between 14 and 28‰). In the DRC, neonatal sepsis causes 15.6% of this mortality, but data on the bacterial etiology and associated drug susceptibility are lacking.

**Methods.** Hemocultures of 150 neonates with possible early onset neonatal sepsis (pEOS) were obtained at the Hôpital Provincial Général de Référence de Bukavu (HPGRB, Bukavu, DRC). The newborns with pEOS received an empirical first-line antimicrobial treatment (ampicillin, cefotaxime and gentamicin), based on the synopsis of international guidelines for the management of EOS which are in line with WHO recommendations. Isolates were identified by matrix-assisted laser desorption ionization - time of flight mass spectrophotometry (MALDI-TOF MS). Antibiotic resistance was assessed using the disk diffusion method.

**Results.** A total of 50 strains was obtained from 48 patients and identified. The three most prevalent species were *Enterobacter cloacae* complex (42%), *Klebsiella pneumoniae* (18%) and *Serratia marcescens* (12%). *Enterobacter cloacae* isolates were resistant to all first-line antibiotics. All *K. pneumoniae* and *S. marcescens* isolates were resistant to ampicillin, and the majority of the *K. pneumoniae* and half of the *S. marcescens* isolates were resistant to both cefotaxime and gentamicin. All *E. cloacae* complex strains, 89% of the *K. pneumoniae* and half of *S. marcescens* had an extended-spectrum  $\beta$ -lactamase (ESBL) phenotype.

**Conclusions.** The most prevalent pathogens causing EOS in Bukavu were *E. cloacae* complex, *K. pneumoniae* and *S. marcescens*. Most of these isolates were resistant to the WHO recommended antibiotics.

**Keywords:** Neonatal sepsis, Gram-negative, sub-Saharan Africa, extended-spectrum  $\beta$ -lactamase (ESBL), WHO guidelines

## Introduction

Neonatal sepsis is a leading cause of neonatal mortality [1, 2] and over 99% of cases occur in low- and-middle income countries [3]. Fleischmann-Struzek and co-workers (2018) estimated that 11-19% of the 3.0 million neonates who develop neonatal sepsis annually die [4]. In the Democratic Republic of the Congo (DRC), the early neonatal mortality rate is estimated between 14 [5] and 28 per 1000 livebirths [2], and 15.6% of cases are caused by neonatal sepsis [6].

Neonatal sepsis is a systemic infection, mostly caused by bacteria [7] and can be classified as either early-onset neonatal sepsis (EOS), occurring within less than 72 hours of life, or late-onset neonatal sepsis (LOS), presenting after the first 72 hours of life up to the age of three months [8]. Pathogens causing EOS are considered to be vertically transmitted from the maternal vagina to the fetus/newborn after breaking of the fetal membranes by an ascending infection or during passage through the birth channel. In contrast, pathogens causing LOS are considered to be hospital or community-acquired [8, 9].

The most common organisms causing EOS in high-income countries are group B streptococci (GBS, *Streptococcus agalactiae*) and *Escherichia coli*, accounting for over 70% of cases [7, 10, 11]. Most international guidelines in line with WHO recommendations for the empirical antibiotic treatment are based on the common antibiotic susceptibility of these predominant pathogens causing EOS in high-income countries [12]. These guidelines recommend hospitalization and antibiotic therapy with a combination of gentamicin and benzylpenicillin or ampicillin for at least 7–10 days for the management of serious bacterial infection in infants aged less than 2 months [13]. However, studies documenting the pathogens causing EOS in sub-Saharan Africa (SSA) are very limited, and so are data on antibiotic susceptibility. For example, a recent systematic review and meta-analysis only found reports on a total of 90 identified pathogens isolated from cases of neonatal sepsis in Central Africa in the period 2008-2018[14]. As such, data on the etiology and antibiotic resistance are urgently required to evaluate the adequacy of the empirical treatment and to tailor antibiotic regimens to local resistance patterns.

In the current study, we aimed to identify the pathogens causing EOS in Bukavu (DRC), to assess their antibiotic susceptibility patterns and to evaluate the applicability of the WHO guidelines for the management of EOS in the DRC.

## **Methodology**

### *Ethical approval*

This research was approved by the Review Board Committee of the Catholic University of Bukavu (reference number UCB/CIE/NC/016/2016) and endorsed by the Provincial Health Ministry (reference number 062/CD/DPS/SK/2017) in the DRC and by the Ethical Committee of the Ghent University Hospital (reference number PA2014/003). For each neonate for whom a parent or a close relative, accompanying the neonate, provided agreement for participation in the study, written informed consent was signed.

### *Study population and design*

This was a descriptive cross-sectional study carried out from June 2017 till July 2018 at the Provincial General Reference Hospital of Bukavu (HPGRB, *Hôpital Provincial Général de Référence de Bukavu*). The HPGRB's neonatal intensive care unit (NICU) serves for neonates born inside the hospital as well as for neonates referred from other health centers of the Bukavu area. Clinical data - history taken from the parents and physical examination report - were abstracted from the registers of the NICU and from the laboratory records. Neonates were considered as having possible early-onset neonatal sepsis (pEOS) when they had one of the following signs, according to the WHO recommendations: incapacity to feed, fever, hypothermia, tachypnea, severe chest indrawing, nasal flaring, grunting, lethargy, reduction of movements, poor capillary refill time, bulging fontanelle, convulsions, jaundice, skin pustules and/or unconsciousness [9, 15]. These newborns were admitted to the NICU where general resuscitation measures were taken, blood was sampled to assess C-reactive



protein and for hemoculture. A standard antibiotic regimen, consisting of a combination of ampicillin, cefotaxime (as an additive) and gentamicin was started according to the synopsis of international guidelines for the management of EOS [16] which are in line with WHO recommendations [13]. In case there was no clinical improvement, an empirical regimen of amikacin in combination with clindamycin or benzylpenicillin (based on availability) was initiated.

A minimum of 0.5 ml of blood was collected from each neonate admitted for suspicion of pEOS in the first 72 hours after birth, for hemoculture and for the assessment of C-reactive protein (CRP), except from neonates who began antibiotics before the admission or who had a congenital malformation or were undergoing a surgical procedure.

## Laboratory procedures

### *Hemoculture procedures and identification*

Neonatal blood samples were added to hemoculture bottles (BACT/ALERT PF plus, BioMérieux), which were incubated aerobically at 37 °C for up to seven days. In case bacterial growth was noticed, a small volume of hemoculture fluid was inoculated onto Tryptic Soy Agar plates with 5% sheep blood (Tryptic Soy Agar, Becton Dickinson, Erembodegem, Belgium; sheep blood from animals kept on campus) (blood agar plates) which were incubated aerobically for 2-7 days at 37 °C. Isolates were preserved in soft agar tubes, before shipment to the Laboratory Bacteriology Research (Ghent University, Ghent, Belgium). The colonies from the soft agar were regrown on blood agar plates and identified at the Department of Laboratory Medicine (Ghent University Hospital, Ghent, Belgium) by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) (Bruker, Bremen, Germany) using the direct spot method according to the manufacturer's protocol.

### *Antibiotic susceptibility testing*

The methodologies used for susceptibility using the disk diffusion method are consistent with those recommended by European Committee on Antimicrobial Susceptibility Testing (EUCAST)[17].

## **Results**

The flow chart of the hemocultures and isolate collection is presented in Figure 1. A total of 61 strains could be isolated from 59 neonates, of from the 61 strains that were preserved and shipped, 50 (82.0%), from 48 neonates, could be regrown (Figure 1). All strains that could not be recultured were found to be Gram-negative in the HPGRB laboratory.

>>Insert Figure 1<<

The clinical characteristics of the 150 neonates with pEOS are shown in Supplementary Information 1 (SII). Nearly all neonates (133/150, 88.7%) were admitted to the NICU between 24 and 72 hours after birth. The female to male ratio was about 1:1. Approximately two thirds of neonates were referred to the HPGRB. Less than half (46.0%) were born preterm and more than half (54.7%) weighed less than 2500 g at birth. Hypothermia (47.3%), hypotonia (34.0%) and incapacity to feed (8.7%) were the most frequently reported symptoms. Respiratory distress was the most frequently reported clinical sign at the admission in NICU (62.0 %). The EOS case fatality rate was 25.3% (38/150 patients) and the mortality was not associated with first-line treatment with antibiotic to which the pathogen was resistant (OR:4.7, p:0.14).

The identifications of the isolates are shown **Table 1**. The majority of isolates (82%) were Gram-negative species. The most prevalent species were *Enterobacter cloacae* complex (42.0%), *Klebsiella pneumoniae* (18.0%) and *Serratia marcescens* (12.0%).

>>Insert Table 1<<

The antibiotic susceptibility of *E. cloacae* complex, *K. pneumoniae* and *S. marcescens* isolates towards different antibiotics is shown in **Table 2**. The majority of these isolates had an ESBL phenotype, i.e. all *E. cloacae* complex, 8/9 (89%) of *K. pneumoniae* and 3/6 (50%) of *S. marcescens* isolates. Of the other Gram-negatives, only the *Proteus mirabilis* isolate had an ESBL phenotype.

>>Insert Table2<<

## Discussion

*Enterobacter cloacae* complex, *Klebsiella pneumoniae* and *Serratia marcescens* are the leading pathogens causing EOS in Bukavu

Our report is one of the few microbiological studies of EOS in the DRC. A recent systematic review and meta-analysis on the etiology and antimicrobial resistance in neonates in SSA reported on 90 cases from Central Africa, highlighting the absolute lack of data in this region [14]. Our data are somehow in line with these meta-data. We found that the majority of pathogens (82%) were Gram-negatives, in line with the reported 76% in the study of Okomo and colleagues [14]. *Enterobacter cloacae* complex (42%), *Klebsiella pneumoniae* (18%) and *Serratia marcescens* (12%) were identified as the most prevalent pathogens causing EOS in our study, whereas the meta-data reported *Klebsiella* (42%), *E. coli* (19%) and *S. aureus* (14%) to be the most prevalent pathogens, although it should be remarked that data of EOS and LOS are merged in the publication of Okomo et al (2019) . In the meta-analysis, *Enterobacter* was found in only 4% of the cases and *Serratia* was absent in Central Africa [14]. *Serratia* however is found in a minority of cases in Eastern, Southern and West Africa [14]. In contrast, Bunduki and coworkers (2019) documented the etiology of 18 cases of EOS in Butembo (DRC) and found *S. agalactiae* (n=6) to be the most prevalent pathogen, followed by *S. aureus* (n=5), *E. coli* (n=3) and *K. pneumoniae* (n=2).

GBS – causing most EOS cases in industrialized countries [18] - was only found in one case in our study, in line with the meta-analysis from Central Africa (2/90 cases) [14]. As we have only one GBS case among 660 neonates admitted at the NICU, the incidence of GBS EOS in Bukavu appears lower

than the estimated 1.3 cases per 1000 live births in SSA [19]. GBS vaccination has been suggested by the WHO as a potential control strategy in low-and-middle-income countries to reduce GBS EOS [20]. According to our results, GBS vaccination likely would not substantially reduce the burden of neonatal sepsis mortality in Bukavu.

In our study, we could document only two cases of EOS caused by *Staphylococcus aureus* and two by *Escherichia coli*, the latter pathogen causing most EOS mortalities in high-income countries [21].

*Evaluation of the current empirical antimicrobial regimen for the treatment of EOS in Bukavu (DRC) based on antibiotic resistance patterns*

In our study, the case fatality rate of neonates with culture-confirmed EOS was substantial (25.3%) and nearly three times higher compared to the 9.8% in low-income countries, reported by Seale and coworkers [22]. For the treatment of EOS in the HPGRB, ampicillin, gentamicin and cefotaxime were used in the first line based on the synopsis of international guidelines for the management of EOS [16] which are in line with WHO recommendations [13] and of the American Academy of Pediatrics for the management of EOS [23]. In case no improvement was noticed in the first 72 hours, ampicillin was replaced by clindamycin.

In our study, all *Enterobacter*, *Klebsiella* and *Serratia* isolates were resistant to ampicillin as would be suspected and to clindamycin because these species are intrinsically resistant to clindamycin [24-26]. Moreover, all *Enterobacter* and most *Klebsiella* and *Serratia* isolates were resistant to gentamicin and cefotaxime. These high resistance rates of the most prevalent pathogens in our study population towards the first-line antibiotics likely contributed substantially to the high EOS mortality rate in our study population.

During the study, a combination of amikacin with clindamycin or with benzylpenicillin was introduced. Amikacin was chosen because of its killing effectiveness to multidrug-resistant Gram-negative aerobes. In the current study, all *E. cloacae* complex isolates and *S.*

*marcescens* strains were susceptible to amikacin, as were more than half of the *K. pneumoniae* strains.

In this study, the *Bacillus*, *Corynebacterium* and *S. epidermidis* isolated could be contaminants rather than true pathogens[27], and cautious interpretation is warranted. We could not determine that the resistance to usual antibiotic was an independent risk factor of neonatal mortality. Although it is known that prevalent EOS pathogens may be vertically acquired from the vagina, data about bacterial vaginal colonization of sick neonates' mothers were not collected during this study. Also, neonates who could not reach the referral hospital were missed.

### *Future perspectives*

The high prevalence of antibiotic resistance and ESBL phenotypes in our study population is worrisome. We did consider the possibility of an outbreak, but matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)-based typing[28] (data not shown) and epidemiological data did not support this hypothesis. The widespread use of over-the-counter antibiotics within the community, fueled by a lack of knowledge by healthcare providers [29] likely is an important contributor. Our study clearly highlights the importance of a sustained monitoring system of (the antibiotic susceptibility patterns of) pathogens causing neonatal sepsis in the DRC. The current challenges are to address the lack of quality-assured laboratories equipped for bacteriological monitoring and surveillance of EOS, the lack of adequate training in infection prevention and appropriate control of access to essential antibiotics.

## NOTES

**Acknowledgments.** We would like to thank Eric Hendwa, Faustin Kinunu, Faustin Masirika, the staff of HPGRB NICU, the staff of the HPGRB Department of Obstetrics and Gynecology, and all neonates and their parents for their helpful support of the study.

**Funding.** This work was supported the Flemish Interuniversity Council (VLIR-UOS) [grant number ZIUOS2012AP024].

**Potential conflict of interest.** All authors do not report any conflicts of interest.

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**Table 1.** Isolates of 48 cases of possible early-onset neonatal sepsis.

Species	Number of isolates <sup>#</sup>	Fatality numbers per species
	n	n
<b>Gram-negatives</b>	<b>41</b>	<b>12</b>
<i>Enterobacter cloacae</i> complex	21	5
<i>Klebsiella pneumoniae</i>	9	5
<i>Serratia marcescens</i>	6	0
<i>Escherichia coli</i>	2	1
<i>Acinetobacter towneri</i>	1	0
<i>Proteus mirabilis</i>	1	0
<i>Pseudomonas stutzeri</i>	1	1
<b>Gram-positives</b>	<b>9</b>	<b>3</b>
<i>Staphylococcus aureus</i>	2	1
<i>Staphylococcus epidermidis</i> <sup>##</sup>	2	1
<i>Aerococcus viridans</i>	1	0
<i>Bacillus</i> sp. <sup>##</sup>	1	0
<i>Corynebacterium callunae</i> <sup>##</sup>	1	1
<i>Enterococcus faecalis</i>	1	0
<i>Streptococcus agalactiae</i>	1	0

<sup>#</sup> isolated from the blood of 48 neonates (i.e., two neonates had a polymicrobial infection)

<sup>##</sup> likely being contaminants based on Hossain et al. 2016 [27] .

Table 2. Percentage of antibiotic resistances in *Enterobacter cloacae* complex, *Klebsiella pneumoniae* and *Serratia marcescens*.

Antibiotic	<i>Enterobacter cloacae</i> complex (N = 21)	<i>Klebsiella pneumoniae</i> (N = 9)	<i>Serratia marcescens</i> (N = 6)
	% resistant	% resistant	% resistant
Amoxicillin/clavulanic acid	100	56	100
Ampicillin <sup>a</sup>	100	100	100
Cefotaxime <sup>a</sup>	100	89	50
Ceftazidime	29	78	50
Cefuroxime	100	44	100
Meropenem	0	0	0
Piperacillin/tazobactam	0	11	0
Amikacin	0	44	0
Colistin	0	0	100
Fosfomycin	10	89	17
Gentamicin <sup>a</sup>	100	78	50
Levofloxacin	0	12	0
Nitrofurantoin	5	11	0
Temocillin	0	44	0
Tigecycline	0	0	0
Tobramycin	100	78	67
Trimethoprim/sulfamethoxazole	43	100	50

<sup>a</sup> Antibiotics used as first-line empirical treatment.

**Figure 1. Flow chart of the hemocultures and isolate collection.**

# isolated from the blood of 48 neonates (i.e., two neonates had a polymicrobial infection).

Figure 1

