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CASE REPORT

Early-onset fulminant neonatal sepsis caused by Multi-Drug Resistant and ESBL producing *E. coli* (CTX-M gene) in a late-preterm neonate: case report and literature review

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SUMMARY

Neonatal sepsis is a systemic condition characterized by haemodynamic changes and other clinical manifestations due to a presence of pathogenic microorganisms (bacteria, viruses, or fungi) in normally sterile fluid that occurs in an infant younger than 90 days old. Neonatal sepsis may be divided into two types: early-onset neonatal sepsis (EOS) and late-onset neonatal sepsis (LOS). Gram-positive microorganisms are the etiological agents in 62% of EOS, and in 43% of the total the identified microorganism is *GBS*. Gram-negative microorganisms comprise 37% of the etiological agents of EOS, of which 29% are caused by *Escherichia coli*. ESBL-producing *Enterobacteriaceae* represent a major worldwide threat among drug-resistant bacteria in both hospital and community settings. ESBLs are often located on large plasmids that also harbour genes resistant to other antimicrobial classes, resulting in multidrug-resistant isolates. Plasmid-encoded ESBLs of the CTX-M-type are increasingly reported worldwide in Gram-negative rods and now account for most of the ESBLs found in *Enterobacteriaceae*.

We present one case of EOS by Multi Drug Resistant (MDR) and ESBL producing *E. coli* (CTX-M gene) in a neonate born to a mother recently immigrated from Africa. Maternal blood culture grew the same bacteria.

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INTRODUCTION

Neonatal sepsis is a systemic condition characterized by haemodynamic changes and other clinical manifestations due to a presence of pathogenic microorganisms (bacteria, viruses, or fungi) in normally sterile fluid that occurs in an infant younger than 90 days old. Worldwide, neonatal sepsis occurs in about 1 to 50 out of 1000 live births and accounts for 3 to 30% of infant and child deaths annually (Sherman MP, 2010). Neonatal sepsis may be divided into two types: early-onset neonatal sepsis (EOS) and late-onset neonatal sepsis (LOS). EOS is considered when the clinical condition appears within the first 72 h of life. The exception to this definition is neonatal sepsis caused by *Streptococcus agalactiae (GBS),* which, although having a perinatal aetiology, can occur within the first 7 days of life. LOS is defined when presenting after 72 h of life. The aetiological agents of early and late neonatal sepsis are quite distinct. EOS is acquired in the peripartum period, before or during childbirth; therefore, the microorganisms are usually acquired from the maternal genitourinary tract. The gold standard for the diagnosis of neonatal sepsis is a positive culture of blood, urine, cerebrospinal fluid, peritoneal fluid, or any other sterile tissues (Tam *et al*., 2017 and Cortese *et al*., 2016). Gram-positive microorganisms are the etiological agents in 62% of EOS, and in 43% of the total, the identified microorganism is *GBS*. Gram-negative microorganisms comprise 37% of the etiological agents of EOS, of which, 29% are caused by *Escherichia coli* (*E. coli*) (Stoll *et al*., 2011). LOS occurs most often in infants who remain hospitalized for long periods, such as preterm or full-term infants who require

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prolonged hospitalization and invasive procedures, with the most common microorganisms being those acquired in the hospital setting. In 79% of cases the identified microorganisms are Gram-positive bacteria, with coagulase-negative *Staphylococcus* occurring in 57% and *Staphylococcus aureus* in 12% of the total. Gram-negative microorganisms represent the remaining 19%, with *E. coli* being the most frequently identified among them, accounting for 7% of the total. Finally, fungi are found in 6% of cases of LOS (Greenberg *et al*., 2017).

ESBL-producing *Enterobacteriaceae* represent a major worldwide threat among drug-resistant bacteria in both hospital and community settings, especially in developing countries, with a pooled prevalence of 11% (Flokas *et al*., 2017). ESBLs are often located on large plasmids that also harbour genes resistant to other antimicrobial classes, resulting in multidrug-resistant isolates. Plasmid-encoded ESBLs of the CTX-M-type are increasingly reported worldwide in Gram-negative rods and now account for most of the ESBLs found in *Enterobacteriaceae (*Paterson *et al*., 2005).

We present a case of fulminant EOS by Multi Drug Resistant (MDR) and ESBL producing *E. coli* (CTX-M gene) in a neonate born to a mother recently immigrated from Western Africa.

CASE PRESENTATION

A 34-week expected gestational age female was born to a 30-year-old Senegalese mother via spontaneous vaginal delivery, with rupture of membranes 11 weeks before delivery. She received many doses of ampicillin prior to delivery. Limited prenatal information was available, as prenatal care was provided predominantly by a midwife, and no laboratory tests were done in the final trimester of pregnancy. GBS status was unknown. Pregnancy was complicated by severe pre-eclampsia. The mother received two doses of betamethasone and magnesium sulphate. Apgar scores were 6 and 7 at 1 and 5 minutes, respectively. At 2 minutes of life she developed respiratory distress requiring continuous positive airway pressure and was transferred to the Neonatal Intensive Care Unit. At 3 hours of age the infant was intubated with worsening respiratory status and persistent apnoea. She started conventional ventilation and then high frequency ventilation within the next 10 hours. Chest x-ray showed generalized granular infiltration. An echocardiogram was performed to rule out cardiac malformation and showed a structurally normal heart, persistent ductus arteriosus with a bidirectional shunt, and signs of hypovolemia. At 4 hours of age, skin mottling, lethargy, abdominal distension, delayed capillary refill hypotension, and oliguria developed. A sepsis evaluation was started and ampicillin and netilmicin were begun. Laboratory results revealed elevated C reactive protein (10.1 mg/L), leukopenia (1,000/μL), neutropenia (100/μL) and severe metabolic acidosis with lactic acid of 12 mmol/L. Intravascular coagulation was present with pulmonary haemorrhage, thrombocytopenia, and coagulopathy. Multiple doses of fluid resuscitation did not improve the acidosis. The infant developed shock refractory to fluid boluses and vasopressors (noradrenaline, dobutamine and hydrocortisone). Due to her ethnicity, meropenem was added due to high suspicion for ESBL gram-negative sepsis. Lumbar puncture was deferred due to unstable clinical status and significant coagulopathy (international normalized ratio [INR] of 4.1). The infant continued to receive multiple blood products without improvement. She rapidly deteriorated despite vigorous resuscitative efforts and died 7 hours after onset of symptoms. Blood culture yielded CTX-M producing *E. coli* in-vitro, which was resistant to extended-spectrum cephalosporins, ciprofloxacin and amikacin and susceptible to fosfomycin, meropenem and colistin. Maternal blood culture grew the same bacteria.

DISCUSSION

Management of neonatal sepsis is always a challenge. Neonatal sepsis is a frequent cause of neonatal morbidity and mortality, especially in developing countries. Its diagnosis is difficult, since clinical signs are nonspecific and complementary exams have low accuracy. Continuous observation of the patient, knowing how to take into account clinical signs, and observing risk factors are essential for diagnostic suspicion. When neonatal sepsis is suspected, always collect samples for bacteriological analysis before starting empirical treatment. The decision to start empirical antibiotic therapy and the choice of the most appropriate treatment regimen are crucial (Renato Soibelmann Procianoy *et al*., 2020).

EOS is mainly caused by vertical transmission of organisms from mother to infant during labour and delivery. While GBS remains the most common aetiologic agent for EOS in industrialized countries, *Staphylococcus* species (chiefly *S. aures* and coagulase negative Staphylococci) and gram-negative bacteria such as *Klebsiella* and *E. coli* are the most frequent causative organisms responsible for EOS in most developing countries (Ganatra *et al*., 2010) (DeNIS, 2016). Furthermore, the prevalence of *E. Coli* is also increasing in industrialized countries, in particular in preterm infants (Barbara J. Stoll *et al*., 2020). EOS due to *E. coli* usually has a higher mortality rate than that caused by gram-positive bacteria (Simonsen *et al*., 2014). The incidence of invasive early-onset GBS disease in developed countries has significantly decreased due to the implementation of Intrapartum Antibiotic Prophylaxis (IAP) guidelines (Nanduri *et al*., 2019). However, the widespread use

of IAP for GBS disease has led to concerns about a potential adverse impact on *E. coli* incidence. Indeed, in an epidemiologic study from the United States, although the overall incidences of EOS due to *E. coli* over the last decade remained relatively stable, *E. coli* cases were found to be more common than GBS cases in some regions (Schrag *et al*., 2016).

Data on EOS from developing countries are relatively scarce, and the bacteria profile may be significantly different[4]. *E. coli* remains the leading bacterial pathogen for EOS despite GBS, emerging as equally important in recent years. Although the proportion of *E. coli* as pathogen for EOS has remained relatively stable over the last 2 decades, an increasing number of drug resistant *E. coli* due to ESBL production has been isolated as the responsible pathogen in Northern Africa, followed by Middle and Eastern Africa, and lowest in Southern and Western Africa. This poses a serious challenge regarding the selection of appropriate antibiotics for empirical therapy. In fact, ESBLs induce bacterial resistance by hydrolysing penicillins, first, second, and third generation cephalosporins and aztreonam, but not cephamycins or carbapenems. β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam usually inhibit them. Most ESBLs are derived from broad-spectrum β-lactamases TEM-1, CTX-M and SHV-1. Mutations of these genes result in alteration of the amino acid configuration around the active site of β-lactamases (Bradford, 2001). Genes for ESBL are frequently encoded by plasmids. CTX-Ms are the most predominant ESBL family worldwide. The CTX-M gene encodes an extended spectrum β-lactamase responsible for the hydrolysis of most β-lactams except cephamycins and carbapenems but often co-exists with other genes conferring resistance to different antimicrobial classes such as aminoglycosides, tetracyclines, sulphonamides and fluoroquinolones, as in our case (Kimberlin *et al*., 2018). Their success is based on their selective advantage in the presence of broad-spectrum cephalosporins and on the localization of the corresponding genes, mostly on highly diffusible plasmids. In addition, CTX-M genes are often associated with insertion sequences (ISs), such as ISEcp1, which further contribute to their dissemination. Thus, several factors have contributed to the spread of CTX-M genes across sectors, including humans, food and companion animals, wildlife and the food chain (Lupo *et al*., 2018).

ESBL-producing organisms were first described in Europe (Kliebe *et al*., 1985). In the U.S., the first cases of ESBL organisms were reported in 1988 (Jacoby *et al*., 1988) and the incidence has increased since then (Logan *et al*., 2014). A recent systematic review and meta-analysis have estimated a pooled prevalence of faecal colonization with ESBLs *Enterobacteriaceae* in healthy adults and children at 14% globally, with a higher prevalence of 22% in Southeast Asia and Africa (Karanika *et al*., 2016). Multiple countries report the emergence of community-associated infections with ESBL-producing *E. coli*. In the U.S., a recent prospective observational study showed an increase in the prevalence of ESBL-producing organisms, with the 36.8% of all ESBL infections community-acquired (Doi *et al*., 2013). A 2013 report of the Centers for Disease Control and Prevention classified ESBL-producing *Enterobacteriaceae* as a serious threat requiring prompt and sustained action. ESBL-producing organism infections in neonates have higher mortality rates than in other paediatric populations (Flokas *et al*., 2017) (Sehgal *et al*., 2007). The drug of choice for treatment of infections caused by ESBL-producing organisms is carbapenem. Among aminoglycosides, amikacin is the most active against ESBL-producing strains and can be used if the organism is susceptible (Kimberlin *et al*., 2018).

In 2018, Dolma *et al*. described three premature infants with EOS due to ESBL *E.coli* born by mothers immigrated from South and Southeast Asia. These cases were initially managed with β-lactams and aminoglycosides without improvement; only in their third case, the early use of meropenem led to clinical improvement and survival. The authors concluded by pointing out that infants with suspected sepsis, and whose mothers were from South and Southeast Asia, may have an increased risk of infection with ESBL-producing organisms. Furthermore, in 2019 Zhu *et al***.** focused on the changing pattern of antimicrobial resistance of *E. coli* responsible for EOS in perinatal centre in eastern China and found that ESBL-producing MDR *E. coli* emerged as a main pathogen responsible for EOS in their region. The authors pointed out that two thirds of *E.coli* isolated from preterm infants were resistant to third-generation cephalosporins, which is significantly higher than those isolated from term infants.

In conclusion, although the trend of increasing antibiotic resistance of *E. coli* is threatening the entire global population, it is more critical for neonates, since neonatal sepsis remains a major cause of neonatal mortality, especially in developing countries. Continuous surveillance for antibiotic susceptibility is needed to ensure proper empirical therapy. It is critical for clinicians to consider this trend and attempt to select proper effective antibiotics as the empirical treatment for early-onset neonatal sepsis.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report.

Conflict of interest

Nnone

Author Contribution

Aldo Naselli conceived of the paper, participated in

its design and coordination, and helped draft the manuscript. Elisabetta Venturini helped draft the manuscript. All authors read and improved the final manuscript.

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