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Predictors of the extended-spectrum-beta lactamases producing Enterobacteriaceae neonatal sepsis at a tertiary hospital, Tanzania

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ABSTRACT

The study was conducted to establish predictors of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) neonatal sepsis and mortality in a tertiary hospital, Tanzania. Between July and December 2016, blood culture was performed in neonates with clinical features of sepsis and neonates/mothers/guardians were screened for ESBL colonization. Selected isolates underwent whole genome sequencing to investigate relatedness. Logistic regression analysis was performed to determine predictors for ESBL-PE associated neonatal sepsis and mortality. Neonatal ESBL-PE sepsis was detected in 32(10.5%) of the 304 neonates investigated. Neonatal ESBL-PE sepsis was independently predicted by admission at the Intensive care Unit and positive mother and neonate ESBL-PE colonization. Deaths occurred in 55(18.1%) of neonates. Neonates infected with ESBL-PE, admitted at ICU, increased age and those transferred from other centres had significantly high mortality rates. Gram-negative bacteria formed the majority (76%) of the isolates, of which 77% were ESBL-PE. Virulent *Klebsiella pneumoniae* ST45 carrying $bla_{CTX\text{-}M\text{-}15}$ were commonly isolated from neonates. *Klebsiella pneumoniae* (ST45) were the predominant cause of ESBL-PE neonatal sepsis and mortality. Improved infection control and antibiotic stewardship are crucial in controlling the spread of resistant strains. Rapid diagnostic tests to detect ESBL-PE in low-income countries are needed to guide treatment and reduce ESBL-PE-associated mortality.

1. Introduction

Neonatal sepsis is one of the top three causes of morbidity and mortality during the neonatal period (Bhutta et al., 2010). Diagnosis and management of sepsis pose a great challenge for neonatologists in NICUs especially in low-income countries (Sharma et al., 2016). The increase of multidrug-resistant organisms in neonatal units limits the treatment options and delays effective treatment, resulting in increased morbidity and mortality (Patel and Saiman, 2010). In many low-income countries including Tanzania, extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) are the commonest cause of neonatal sepsis (Christopher et al., 2013; D'Andrea et al., 2013; Ghotaslou et al., 2007; Mshana et al., 2009; Thaver et al., 2009). Infections due to ESBL-PE are associated with increased morbidity and mortality (Blomberg et al., 2005; Kayange et al., 2010; Mhada et al., 2012). The antibiotics of choice for the treatment of neonatal sepsis due to ESBL-PE pathogens are not available in most settings and even when stocked, they are too expensive to be afforded (Vandijck et al., 2008). Varieties of ESBL-PE genotypes have been found in humans, animals and the environment in the city of Mwanza, Tanzania (Mshana et al., 2016). Despite ESBL-PE being prevalent in the city of Mwanza, the transmission pathways and risk factors of these pathogens in relation to neonatal sepsis is not well studied. This study was undertaken to investigate factors associated with ESBL-PE neonatal sepsis and mortality among neonates admitted at the Bugando Medical Centre (BMC), Mwanza, Tanzania. In addition, the study aimed to characterize selected isolates to show their virulence potential and transmission dynamics.

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2. Material and methods

2.1. Study duration, area and inclusion criteria

The study was conducted between July and December 2016 at the premature unit and neonatal Intensive care unit (NICU) of BMC. All neonates (0–28 days) with signs and symptoms of sepsis were enrolled. The sample size was estimated using the Kish Leslie formula for the cross sectional studies (Kish, 1965).

2.2. Data collection

Using signs and symptoms published by "The WHO Young infants Study group" (Margolis et al., 1999), a data collection tool was designed and used to obtain socio-demographic and clinical data together with other relevant factors related to risks for neonatal sepsis. At the time of enrollment, clinical data and blood samples were collected. Antenatal history such as use of antibiotics, presence of chronic diseases, febrile illness during pregnancy, labor history, duration of labor, history of premature rupture of membrane, mode of delivery were recorded. Neonates were subjected to a full clinical examination to assess temperature, respiration rate, presence or absence of cyanosis, jaundice, umbilical redness, convulsion, reduced movement and ability to feed.

2.3. Microbiological procedures

Umbilical-rectal swabs were collected using sterile cotton swabs in Amies transport medium (Biolab, HUNGARY) from all neonates to determine colonization status. Analysis of stool samples obtained from mothers/guardians provided data on colonization status. Swab and stool samples were inoculated on MacConkey agar (Oxoid, UK) supplemented with 2 μg/ml cefotaxime (Medochemie Ltd, Cyprus EU) and processed as previously described (Nelson et al., 2014). Using aseptic techniques approximately 1.5–2 ml of blood was obtained and inoculated directly into Brain Heart Infusion broth (BHI) (Oxoid Ltd, UK) in a ratio of blood to BHI of 1:10 and transported to the Catholic University of Health and allied Sciences (CUHAS) Microbiology laboratory for incubation and subsequent processing as previously described (Kayange et al., 2010). All blood culture isolates were tested for their antibiotic susceptibility pattern using disc diffusion methods and interpreted following the Clinical Laboratory Standard Institute guidelines (CLSI, 2015). Confirmation of the ESBL phenotype was performed using the disk approximation method (Mshana et al., 2009). All neonates enrolled in the study were assessed and managed adhering to the BMC neonatal unit protocols. The first line antibiotic treatment option included ampicillin and gentamicin, the second line included cefotaxime followed by the third line that incorporated the use of meropenem. All neonates were evaluated on a daily basis prior to discharge, after which they were monitored for a further 28 days.

2.4. Whole genome sequencing and in silico analyses

Fifty-three (16 from blood and 37 from colonized neonates) ESBL-PE isolates were subjected for whole genome sequencing (WGS). These isolates were serially selected as they were isolated, no other criteria were used, and aim was to sequence the first 50 isolates. DNA was isolated using PureLink® Pro 96 Genomic DNA Purification Kit (Thermo-Fisher Scientific, Dreieich, Germany) according to the manufacturer's instruction. WGS was performed on an Illumina NextSeq 500 instrument (Illumina, San Diego, CA, USA) using an Illumina Nextera XT library with 2 × 150bp paired-end reads. The data was assembled using SPAdes (version 3.6.2) (Bankevich et al., 2012).

Sequences were analyzed for their multi locus sequence types, transferrable resistance genes, plasmid replicon types and pMLST using MLST 1.8, ResFinder, Plasmidfinder and pMLST software of the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/), respectively (Carattoli et al., 2014; Larsen et al., 2012; Zankari et al., 2012). The presence/absence of *Klebsiella pneumoniae* virulence genes was assessed using blastn following Holt et al (Holt et al., 2015).

The SNP analysis of isolates depicting identical STs was performed using SAMtools (Li, 2011). Regions of high recombination (> 50 SNPs per 1 kb) were excluded manually from the analysis.

The raw data of all sequenced ESBL-PE isolates are available at the European Nucleotide Archive (ENA) under the project number PRJEB20875.

2.5. Ethics approval

This study obtained clearance from the Joint CUHAS-BMC Ethics and Scientific Review committee with certificate no: CREC/162/2016. Written informed consent was obtained from parents/guardians of the study participants.

2.6. Statistical analysis

Data was entered using Microsoft Excel 2007 and processed using STATA version 13.0 (Stata Corp, College Station, TX, USA). Categorical variables such as history of antibiotic use, history of admission, colonization status etc., were presented proportionally and compared using chi squared test or Fisher's exact test. Continuous variables such age, gestation age, length of hospital stay, weight etc., were described as medians (inter quartile range/ IQR)). To determine predictors of ESBL-PE neonatal sepsis, ESBL-PE neonatal colonization and the outcome, univariate followed by multivariate logistic regression analysis was performed. All factors with p value of less than 0.2 were further subjected to multivariate logistic analysis and controlled for both age and sex. To estimate survival rates and hazard ratios, a Cox regression model was used. All factors with p value less than 0.05 at 95% confidence interval (CI) were considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics

A total of 304 neonates were enrolled in the study. Male neonates formed the majority 192 (63.2%) of study participants. The median age of the enrolled neonates was 6 days (IQR: 3–9). A total of 94 (30.1%) neonates had birth weights of below 2.5 kg. The median gestation age was 39 weeks (IQR: 37–40) and the median body temperature measured was 37.9 °C (IQR-37-38.2 °C). A total of 57 (14.1%) of all neonates were delivered by cesarean section (C/S) and 16 (5.3%) were delivered at home (Table 1).

3.2. ESBL-PE neonatal sepsis, colonization, isolates and genotypes

Of 304 neonates, 32 (10.5%, 95%CI: 7.1–13.9) were infected by ESBL-PE. Neonatal ESBL-PE colonization occurred in 166 cases (54.6%; 95%CI: 49–60.1). In total, 86 (28.3%, 95%CI: 23–33.3) of mothers/guardians were carriers of ESBL-PE. Of the 32 neonates infected by ESBL-PE, *Klebsiella pneumoniae* was the most common pathogen (65.6%) (Table 2). This was also the case for neonate colonization, where *Klebsiella pneumoniae* isolates was the most common ESBL-PE (n = 112, 67.5%). On the other hand, of 86 mothers/guardians colonized by ESBL-PE, only 26 (30.2%) were colonized by ESBL-producing *Klebsiella pneumoniae*. Neonates were significantly more often infected/colonized by *Klebsiella pneumoniae* than guardians/mothers ($P < 0.001$). Other bacterial species isolated from the blood of the neonates were *Enterococcus* spp., *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Acinetobacter baumannii*, *Salmonella* spp., *Citrobacter* spp., and *Enterobacter* spp. (Table 2). The proportion of the *Klebsiella pneumoniae* isolates (n = 26) resistant to ampicillin,