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Extended spectrum beta-lactamase producing Enterobacteriaceae causing bloodstream infections in rural Ghana, 2007–2012

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ABSTRACT

Background: High prevalence of Extended Spectrum Beta-Lactamase (ESBL) producing Enterobacteriaceae threatens treatment options for invasive bloodstream infections in sub-Saharan Africa.
Objectives: To explore the frequency and genotype distribution of ESBL producing Enterobacteriaceae causing bloodstream infections in a primary health care setting in rural Ghana.
Methods: Blood cultures from all patients with fever ≥38 °C within 24 h after admission (community-acquired) and from all neonates with suspected neonatal sepsis (hospital-acquired) were obtained. ESBL-producing isolates were characterized by combined disc test and by amplifying the *bla*CTX-M, *bla*TEM and *bla*SHV genes. Multilocus sequence typing (MLST) was performed for all ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates, and all *K. pneumoniae* isolates were differentiated by pulsed-field gel electrophoresis (PFGE).
Results: Among 426 Enterobacteriaceae isolated from blood cultures, non-typhoid *Salmonella* (n = 215, 50.8%), *S.* Typhi (n = 110, 26.0%), *E. coli* (n = 50, 11.8%) and *K. pneumoniae* (n = 41, 9.7%) were the most frequent. ESBL-producing isolates were restricted to the CTX-M-15 genotype and the species *K. pneumoniae* (n = 34, 82.9%), *Enterobacter cloacae* complex (n = 2, 66.7%) and *E. coli* (n = 5, 10.0%). The rates of ESBL-producers in *K. pneumoniae* were 55.6% and 90.6% in community-acquired and neonatal bloodstream infections, respectively. MLST and PFGE analysis identified four outbreak clusters among neonates.
Conclusions: Considering the rural primary health care study setting, the high proportion of ESBL-producing *Klebsiella pneumoniae* is worrisome and might be devastating in the absence of second line antibiotics. Therefore, enhanced diagnostic laboratories for surveillance purposes and sustainable hospital hygiene measures must be considered to prevent further spread of multidrug resistant bacteria within rural communities.

1. Introduction

Enterobacteriaceae that produce plasmid encoded Extended Spectrum β-lactamases (ESBL), are per definition resistant to all β-lactam antibiotics except carbapenems and cephamycins. ESBL-carrying strains frequently show parallel resistance to other antibiotic classes, such as the fluoroquinolones (Bush and Fisher, 2011). The global spread of the CTX-M genotype, in particular CTX-M-15, triggered a shift from clonal hospital outbreaks to the multi-clonal occurrence within and outside the hospital boundaries, making the distinction between nosocomial and community

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isolates increasingly difficult (Calbo and Garau, 2015; Pitout and Laupland, 2008). Intestinal colonization often precedes bacterial invasion (Pitout and Laupland, 2008), which, in case of bloodstream infections, has been associated in several clinical studies with increased mortality compared to infections with non-ESBL producers (Schwaber and Carmeli, 2007).

Notably, in sub-Saharan Africa, where the availability of effective antimicrobial therapies is limited, ESBL-producing bacteria narrow the range of treatment options and increase the likelihood of inadequate empiric treatment (Woerther et al., 2011). This emerging threat has been pointed out in numerous studies within communities in sub-Saharan Africa, which report considerably high intestinal ESBL carriage rates between 10% and 45% (Abdul Rahman and El-Sherif, 2011; Isendahl et al., 2012; Magoué et al., 2013; Schaumburg et al., 2013; Woerther et al., 2011). Data on bloodstream infections caused by ESBL bacteria in Africa are scarce and restricted to major tertiary care referral hospitals where the rate of ESBL isolates ranged between 0.7% (n = 1191) in a Malawian hospital to 75.8% (n = 185) in an intensive care unit in Egypt (Gray et al., 2006; Saied et al., 2011).

It was reported from the largest tertiary care hospital in Ghana (Korle-Bu Hospital, Accra) that 50% of the *Klebsiella pneumoniae* and 29% of the *Escherichia coli* bloodstream isolates were ESBL producers. However, the study did not distinguish between hospital or community acquired strains and genotyping was not performed (Obeng-Nkrumah et al., 2013). As data from small communities and hospitals within rural areas are not available, existing resistance data might not be representative.

This study aims to explore the frequency and genotype distribution of ESBL-producing Enterobacteriaceae causing bloodstream infections in a primary health care setting in a rural community of Ghana.

2. Methods

2.1. Study site and study population

The study was conducted at the Agogo Presbyterian Hospital, situated in the Asante Akim North district of the Ashanti Region in Ghana. During two recruitment periods, from September 2007 to July 2009 and January 2010 to December 2012, patients of all age groups, who were hospitalized with a tympanic temperature $\geq$38 °C or a history of fever in the last 24 h, were enrolled into the study. Additionally, on the neonatal ward all neonates (aged $\leq$28 days) with suspected neonatal sepsis were included into the study. All bloodstream infections were regarded as community-acquired, apart from those identified among neonates, born in the same hospital, which were defined as nosocomial transmitted infections.

2.2. Detection and identification of pathogens

A blood culture was performed at hospital admission or in case of already hospitalized neonates, when a bloodstream infection was suspected. Eight to 10 millilitres of blood being inoculated into a blood culture bottle (BACTEC Plus Aerobic/F, Becton Dickinson, USA) and further processed using a BACTEC 9050 blood culture system (Becton Dickinson, USA) according to the manufacturer's instructions. If less than 3 millilitres of blood were available (e.g., from children) paediatric blood culture bottles were used (BACTEC Peds Plus/F, Becton Dickinson, USA). In case of bacterial growth, subcultures were performed on Columbia blood, chocolate and MacConkey agar (all Oxoid, Basingstoke, UK). All gram-negative rod shaped bacteria growing on MacConkey agar were identified biochemically by API 20E tests (bioMérieux, Marcy L'Etoile, France) and sent to Germany at −80 °C for species confirmation by MALDI-ToF MS (Bruker Daltonics, Bremen, Germany), antibiotic susceptibility testing and subtyping analysis.

2.3. Antimicrobial susceptibility testing

For all Enterobacteriaceae, antimicrobial susceptibility testing was performed with the VITEK 2 system using AST-N111 cards (bioMérieux, Marcy L'Etoile, France) according to the 2015 European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org). A positive ESBL phenotype was confirmed by the combined disc test with cefotaxime and ceftazidime alone and in combination with clavulanic acid (Becton, Dickinson and Company, Sparks, MD, USA) as described before by the EUCAST (The EUCAST guideline on detection of resistance mechanisms v 1.0 (2013-12-11)).

2.4. ESBL genotyping

All isolates with ESBL phenotypes were screened for the presence of *bla*CTX-M, *bla*TEM and *bla*SHV genes by Polymerase Chain Reaction (PCR) and subsequent sequencing, as described before (Belmar Campos et al., 2014). In order to further distinguish *bla*CTX-M positive isolates, group specific primers were used for amplification and sequencing, as previously published (Belmar Campos et al., 2014). The resulting sequences were identified by comparison with known sequences using the NCBI BLAST (http://blast.ncbi.nlm.nih.gov) and the Lahey Clinic Database (http://www.lahey.org/studies/).

2.5. Subtyping (MLST, PFGE)

Multilocus sequence typing (MLST) was conducted for all ESBL-producing *E. coli* and *K. pneumoniae* isolates according to previously published 7-loci protocols (Diancourt et al., 2005; Wirth et al., 2006). For all *K. pneumoniae* pulsed-field gel electrophoresis (PFGE) was performed according to the PulseNet protocol for *E. coli*, using a single restriction enzyme digestion with *Xba*I (http://www.cdc.gov/pulsenet/pathogens/ecoli.html). PFGE banding patterns were analyzed with InfoQuest FP version 4.5 (Bio-Rad, USA).

2.6. Epidemiological analysis

Categorical variables were described as frequencies and percentages. Continuous variables were described using medians and their corresponding interquartile ranges (IQRs). Case fatality was compared among different populations using the odds ratio (OR) along with the 95%-confidence interval (CI). If indicated, age adjusted ORs (aORs) were calculated using Mantel-Haenszel statistics and age categories of <1, 1–4 and >4 years. All data analyses were performed with Stata 14 (StataCorp LP, College Station, USA).

2.7. Ethical considerations

The Committee on Human Research, Publications and Ethics from the School of Medical Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana provided ethical approval for this study. All participants were informed about the study's purpose and procedures. Written informed consent was obtained prior to study enrolment from all participants or in case of children from their parents or guardian.