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

Daniel Eibach[a,*,§] (eibach@bnitm.de), Cristina Belmar Campos[b,*] (c.belmar-campos@uke.de), Ralf Krumkamp[a,c] (krumkamp@bnitm.de), Hassan M. Al-Emran[a] (al-emran@bnitm.de), Denise Dekker[a] (dekker@bnitm.de), Kennedy Gyau Boahen[d] (gyaukennedy@yahoo.com), Benno Kreuels[b] (b.kreuels@uke.de), Yaw Adu-Sarkodie[e] (sax@ghanatel.com.gh), Martin Aepfelbacher[b] (maepfelbacher@uke.de), Se Eun Park[f] (SeEun.Park@ivi.int), Ursula Panzner[f] (upanzner@ivi.int), Florian Marks[f] (fmarks@ivi.int), Jürgen May[a,c] (may@bnitm.de)

[a] Bernhard Nocht Institute for Tropical Medicine (BNITM), Bernhard-Nocht-Str. 74, 20359 Hamburg, Germany

[b] *University Medical Centre Hamburg-Eppendorf (UKE), Martinist. 52,* 20246 Hamburg, *Germany*

[c] *German Centre for Infection Research (DZIF), Hamburg-Borstel-Lübeck, Germany*

[d] *Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana*

[e] *Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana*

[f] *International Vaccine Institute, SNU Research Park, 1 Gwanak-ro, Gwanak-gu, Seoul, 151-742 Republic of Korea*

[*] contributed equally to this work

[§] corresponding author

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 24h 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.

Keywords: Ghana; Molecular epidemiology; Extended Spectrum β-lactamase; *Klebsiella pneumoniae;* Bloodstream infection

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 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=1,191) 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.

Methods

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 ≥38°C or a history of fever in the last 24 hours, were enrolled into the study. Additionally, on the neonatal ward all neonates (aged ≤ 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.

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.

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 disk 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)).

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/).

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

99 performed according to the PulseNet protocol for *E. coli*, using a single restriction enzyme
100 digestion with *Xba*I (http://www.cdc.gov/pulsenet/pathogens/ecoli.html). PFGE banding
101 patterns were analyzed with InfoQuest FP version 4.5 (Bio-Rad, USA).

102 *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 to 4 and >4 years. All data analyses were performed with Stata 14 (StataCorp LP, College Station, USA).

103 *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.

104 **Results**

105 *Identification of bacterial bloodstream infections*

In total, 7,172 blood cultures were performed within the study. Median age of the study participants was 3 years (IQR: 1–18 years) and 50.3 % (n=3,610) were female. Among all study participants, 564 (7.9%) were neonates. Out of 1,215 positive blood cultures, 568 (46.7%) contained a relevant bacterial pathogen. Environmental bacteria and bacteria belonging to the skin flora (e.g., coagulase negative staphylococci, Corynebacterium spp. and Bacillus spp.) were

considered as non-pathogenic contaminants. From those 568 blood culture isolates, 423 (74.5 %) bacteria of the Enterobacteriaceae family were cultured. Study participants with Enterobacteriaceae positive blood cultures had a median age of two years (IQR 1–8 years) and 54.8% (n=232) were female. The most frequently identified Enterobacteriaceae were non-typhoid Salmonella (50.8%, n=215) and S. Typhi (26.0%, n=110), followed by E. coli (11.8%, n=50) and K. pneumoniae (9.7%, n=41) (Table 1).

Antimicrobial susceptibility

ESBL production was detected in 9.7% (n=41) of the isolated Enterobacteriaceae whereby 82.9% (n=34), 66.7% (n=2) and 10.0% (n=5) of K. pneumoniae, Enterobacter cloacae complex and E. coli were ESBL positive, respectively (Table 1). None of the Salmonella isolates revealed any ESBL activity. Restricted to community-acquired infections, ESBL genotypes were found among 55.6% (n=5) K. pneumoniae and 8.7% (n=4) of E. coli.

Ciprofloxacin resistance was most frequently detected in K. pneumoniae (39.0%; n=16) and E. coli (26.0%; n=13), with 39% (n=16) of K. pneumoniae and 8% (n=4) of E. coli being non-susceptible to ciprofloxacin and concomitantly produced ESBL. In total, 95.1%, 97.6% and 51.2% of ESBL-producing Enterobacteriaceae exhibited parallel resistance to cotrimoxazole (39 out of 41 isolates), gentamicin (40 of 41 isolates) and ciprofloxacin (21 of 41 isolates), respectively. None of the isolates showed reduced susceptibility to carbapenems.

ESBL genotyping and subtyping

All ESBL-producing isolates harboured the CTX-M-15 ESBL gene. Among the 34 ESBL-producing *K. pneumoniae*, sequence types (ST) 13 (38.2%; n=13), ST25 (11.8%; n=4), ST36 (11.8%; n=4) and ST405 (8.8%; n=3) were predominant, while three (60.0%) ESBL-producing *E. coli* belonged to ST131 (Figure 1). PFGE was performed for all *K. pneumoniae* isolates and demonstrated clusters, which corresponded to the sequence type, except the two

ST502 isolates that showed distinct banding patterns (Figure 1). The ST13 cluster comprised community (n=2) as well as nosocomial (n=11) acquired isolates.

Age distribution and neonatal infections

While most K. pneumoniae (90.2%; n=37) bloodstream infections were observed among children below 4 years of age, the majority of E. coli (74%; n=37) infections were detected in adults and children above 14 years (Table 2). Thirty-two out of 39 Enterobacteriaceae (82.1%) isolated from neonates produced ESBL, the majority of which were K. pneumoniae (74.4 %).

These neonatal ESBL K. pneumoniae could be allocated to different clusters according to MLST and PFGE analysis, depending on different isolation dates during the study (Figure 1 and 2). Invasive bloodstream infections with K. pneumoniae ST25 clustered in March/April 2009 (4 cases), ST13 clustered in March-June 2011 (10 cases), ST36 clustered in September/October 2011 (4 cases) and ST334 clustered in November/December 2011 (2 cases).

Case fatality

Data on patient's outcome at discharge from the hospital was available for 329 out of 423 patients (77.8%) infected with Enterobacteriaceae. The case fatality of invasive bloodstream infections caused by ESBL-positive Enterobacteriaceae was, with an aOR of 3.0 (95%-CI: 1.2–7.3), significantly higher compared to infections with non-ESBL-producing Enterobacteriaceae. At the neonatology ward, outcome data was available for 40 out of 42 neonates diagnosed with Enterobacteriaceae, showing no mortality difference between bloodstream infections caused by ESBL and non-ESBL-producing Enterobacteriaceae (OR=0.6; 95%-CI: 0.1–3.7).

Discussion

The study results reveal an unequal distribution of ESBL producers among different species of Enterobacteriaceae causing invasive bloodstream infections. While the most commonly detected pathogens non-typhoid *Salmonella* and *S.* Typhi (77% of all isolated Enterobacteriaceae) showed no ESBL production, 82.9%, 66.7% and 10.0% of *K. pneumoniae*, *E. cloacae* complex and *E. coli* isolates presented with an ESBL genotype, respectively. A large meta-analysis on community-acquired bloodstream infections in Africa described *Salmonella enterica* as the predominant pathogen as well (Reddy et al., 2010). However, only a low but emerging number of ESBL-producing non-typhoid *Salmonella* in septicaemic patients was reported from other sub-Saharan countries, such as Burkina Faso (4.8%), the Democratic Republic of Congo (1.3%) and Tanzania (2.7%) (Blomberg et al., 2005; Lunguya et al., 2013; Maltha et al., 2014). Remarkably, 63.4% of non-typhoid *Salmonella* colonizing the gut of Malian children have been found to harbour ESBL genes (Boisramé-Gastrin et al., 2011).

The high proportion of community acquired ESBL-producing *K. pneumoniae* (55.6%) in Ghana is surprising for a rural primary health care hospital and of serious concern. In a study conducted at the largest hospital in Ghana in 2013, a slightly lower percentage (50.0%) of ESBL-producing *K. pneumoniae* was detected in blood cultures, not distinguishing between nosocomial and community acquired cases (Obeng-Nkrumah et al., 2013). The referral hospital in Kumasi reports 37–46% of community acquired *E. coli* and *K. pneumoniae* isolates being ESBL-producers (Ayisi and Adu-Sarkodie, 2015; Feglo et al., 2013).

Lower rates of ESBL-producing *K. pneumoniae* have been previously reported from East African tertiary hospitals in Kenya (13%), Tanzania (17%) and for all ESBL-producing Enterobacteriaceae in Malawi (0.7%).(Blomberg et al., 2005; Gray et al., 2006; Kohli et al., 2010) However, those data were collected between 2001 and 2008 and more recent data have

not been published from those countries. Indeed, a more recent study from a South African university hospital reports 76% of *K. pneumoniae* to be ESBL positive.(Dramowski et al., 2015)

Data from comparable rural primary health care settings are limited. In Gabon 49% of *K. pneumoniae* from different infection sites (bloodstream, ear-eye-nose-throat, surgical sites, soft tissue, UTI, wounds) were ESBL producers, while in rural Burkina Faso no ESBL positive *K. pneumoniae*, but 38% of ESBL-positive *E. coli* isolates causing bloodstream infections were reported (Alabi et al., 2013; Maltha et al., 2014).

Consistent with other epidemiological data, the study described here identified ESBL much more frequently in *Klebsiella* than in *E. coli* (Tansarli et al., 2014).

The present study underlines the preeminent role of CTX-M and in particular CTX-M-15. Throughout West and Central Africa CTX-M-15 has been shown to be the predominant genotype detected among all ESBL-producing Enterobacteriaceae species in stool samples, with 84%, 95% and 91% reported from Gabon, Guinea-Bissau and Niger, respectively (Isendahl et al., 2012; Schaumburg et al., 2013; Woerther et al., 2011). In Ghana, 98% of clinical ESBL isolates have been shown to carry the CTX-M-15 gene (Agyekum et al., 2016). Nosocomial bloodstream infections with multidrug resistant bacteria have been reported from Africa, particularly in vulnerable populations and high-risk areas, such as intensive care units and paediatric departments (Saied et al., 2011; Tansarli et al., 2014). Indeed, an exceptionally high rate (82%) of bloodstream infections among neonates was caused by ESBL Enterobacteriaceae in this study. MLST/PFGE analysis identified four ESBL *K. pneumoniae* clusters, which suggest a nosocomial transmission within the neonatology ward in Ghana. Even larger multi-clonal outbreaks between February and December 2011 presumably due to horizontal transfer of CTX-M-15 genes between different *Klebsiella* clones, as described before by Guyot et al., cannot be ruled out (Guyot et al., 2012). Clustering of nosocomial

ST13 outbreak isolates with community-acquired ST13 isolates in the PFGE analysis demonstrates the blending of hospital and community-acquired ESBL bacteria.

Multidrug resistant *Klebsiella* are known to cause hospital outbreaks among neonates in developing, but also developed countries (Mavroidi et al., 2014; Mshana et al., 2013). From a tertiary care hospital in Tanzania multiple outbreaks with CTX-M-15 *K. pneumoniae* among neonatal sepsis patients were reported (Mshana et al., 2013). The authors supposed patient-to-patient or mother-to-child transmission, contaminated equipment or health care workers as possible sources. A later study at the same hospital revealed that 60% of neonates acquired ESBL-producing *K. pneumoniae* and *E. coli* on day 1 after delivery (Nelson et al., 2014). Although mothers colonized with ESBL bacteria are a potential source of ESBL transmission, infection control measures must include health care personnel and environmental contamination. Inadequate hospital hygiene has been demonstrated in Niger and Gabon, where intestinal ESBL carriage rates increased from around 30% at admission to over 90% at hospital discharge (Schaumburg et al., 2013; Woerther et al., 2011). Apart from hospital hygiene, the lack of diagnostic tools, such as blood cultures and antibiotic susceptibility testing, results in the frequent administration of empirical antibiotic therapy and may lead to suboptimal therapy or overtreatment with antibiotics. A more restrictive and rational antibiotic use and control of low-quality antibiotics might help to alleviate the spread of ESBL-producing pathogens in sub-Saharan Africa. Permanent antimicrobial resistance surveillance systems, as established on a temporary basis in Ghana, would substantially improve local infectious disease management (Newman et al., 2011; Opintan et al., 2015).

A meta-analysis reports that infections with ESBL-producing Enterobacteriaceae are associated with nearly twice the mortality compared to non-ESBL producers (Schwaber and Carmeli, 2007), which corresponds to the present data. Nevertheless, case fatality seems to be highly dependent on patient age and the high case fatality rate among neonates in the study hospital applies to bloodstream infections regardless of the pathogen’s ESBL activity.

This study is limited by the fact that the definition of nosocomial and community-acquired cases is simplistic. As number and lengths of previous hospital stays are often unknown, Enterobacteriaceae detected in blood cultures at the day of admission might still result from a previous hospitalization, which consequently underestimates nosocomial-acquired cases. Neonatal infections caused by ESBL-producing pathogens are commonly classified to be acquired in the hospital (Mshana et al., 2013).

Conclusion

This study reveals a high proportion of invasive bloodstream infections with community acquired ESBL-producing Enterobacteriaceae in a rural community in Ghana, with numbers exceeding those found in university hospitals in sub-Saharan Africa. Beyond community transmission, ESBL-positive *K. pneumoniae* represent a serious threat in neonatal care. Considering the limited availability of second line drugs (e.g. carbapenems) in rural hospitals within sub-Saharan Africa the spread of ESBL-producing Enterobacteriaceae might be devastating. In many parts of sub-Saharan Africa laboratory surveillance of multidrug resistant strains is still impaired by the absence of routine culture and susceptibility testing. The establishment of diagnostic laboratories in rural areas in combination with the application of sustainable hospital hygiene measures must be advanced to control and prevent the dissemination of multidrug resistant bacteria.

Acknowledgements

This work was supported by the Bill & Melinda Gates Foundation. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation. The funders had no role in study design,

data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare no conflicts of interest.

References

Abdul Rahman, E.M., El-Sherif, R.H., 2011. High rates of intestinal colonization with extended-spectrum lactamase-producing Enterobacteriaceae among healthy individuals. J. Investig. Med. 59, 1284–6. doi:10.231/JIM.0b013e318238748e

Agyekum, A., Fajardo-Lubián, A., Ansong, D., Partridge, S.R., Agbenyega, T., Iredell, J.R., 2016. blaCTX-M-15 carried by IncF-type plasmids is the dominant ESBL gene in Escherichia coli and Klebsiella pneumoniae at a hospital in Ghana. Diagn. Microbiol. Infect. Dis. 84, 328–33. doi:10.1016/j.diagmicrobio.2015.12.010

Alabi, A.S., Frielinghaus, L., Kaba, H., Kösters, K., Huson, M.A.M., Kahl, B.C., Peters, G., Grobusch, M.P., Issifou, S., Kremsner, P.G., Schaumburg, F., 2013. Retrospective analysis of antimicrobial resistance and bacterial spectrum of infection in Gabon, Central Africa. BMC Infect. Dis. 13, 455. doi:10.1186/1471-2334-13-455

Ayisi, L.A., Adu-Sarkodie, Y., 2015. Extended-Spectrum-Beta-Lactamase (ESBL) production among Escherichia coli and Klebsiella species in Kumasi, Ghana. J. Nat. Sci. Res.

Belmar Campos, C., Fenner, I., Wiese, N., Lensing, C., Christner, M., Rohde, H., Aepfelbacher, M., Fenner, T., Hentschke, M., 2014. Prevalence and genotypes of extended spectrum beta-lactamases in Enterobacteriaceae isolated from human stool and chicken meat in Hamburg, Germany. Int. J. Med. Microbiol. 304, 678–84. doi:10.1016/j.ijmm.2014.04.012

Blomberg, B., Jureen, R., Manji, K.P., Tamim, B.S., Mwakagile, D.S.M., Urassa, W.K., Fataki, M., Msangi, V., Tellevik, M.G., Maselle, S.Y., Langeland, N., 2005. High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. J. Clin. Microbiol. 43, 745–9. doi:10.1128/JCM.43.2.745-749.2005

Boisramé-Gastrin, S., Tandé, D., Münck, M.-R., Gouriou, S., Nordmann, P., Naas, T., 2011. Salmonella carriage in adopted children from Mali: 2001-08. J. Antimicrob. Chemother. 66, 2271–6. doi:10.1093/jac/dkr307

Bush, K., Fisher, J.F., 2011. Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from gram-negative bacteria. Annu. Rev. Microbiol. 65, 455–78. doi:10.1146/annurev-micro-090110-102911

Calbo, E., Garau, J., 2015. The changing epidemiology of hospital outbreaks due to ESBL-producing Klebsiella pneumoniae: the CTX-M-15 type consolidation. Future Microbiol. 10, 1063–75. doi:10.2217/fmb.15.22

Diancourt, L., Passet, V., Verhoef, J., Grimont, P.A.D., Brisse, S., 2005. Multilocus Sequence Typing of Klebsiella pneumoniae Nosocomial Isolates. J. Clin. Microbiol. 43, 4178–4182. doi:10.1128/JCM.43.8.4178-4182.2005

Dramowski, A., Cotton, M.F., Rabie, H., Whitelaw, A., 2015. Trends in paediatric bloodstream infections at a South African referral hospital. BMC Pediatr. 15, 33. doi:10.1186/s12887-015-0354-3

Feglo, P., Adu-Sarkodie, Y., Ayisi, Lord, Jain, R., Spurbeck, R.R., Springman, A.C., Engleberg, N.C., Newton, D.W., Xi, C., Walk, S.T., 2013. Emergence of a novel extended-spectrum-β-lactamase (ESBL)-producing, fluoroquinolone-resistant clone of extraintestinal pathogenic Escherichia coli in Kumasi, Ghana. J. Clin. Microbiol. 51, 728–30. doi:10.1128/JCM.03006-12

Gray, K.J., Wilson, L.K., Phiri, A., Corkill, J.E., French, N., Hart, C.A., 2006. Identification and characterization of ceftriaxone resistance and extended-spectrum beta-lactamases in Malawian bacteraemic Enterobacteriaceae. J. Antimicrob. Chemother. 57, 661–5. doi:10.1093/jac/dkl037

Guyot, K., Biran, V., Doit, C., Moissenet, D., Guillard, T., Brasme, L., Courroux, C., Maquelin, K., van Leeuwen, W., Vuthien, H., Aujard, Y., De Champs, C., Bingen, E., 2012. Raman spectroscopic analysis of the clonal and horizontal spread of CTX-M-15-producing Klebsiella pneumoniae in a neonatal intensive care unit. Eur. J. Clin. Microbiol. Infect. Dis. 31, 2827–34. doi:10.1007/s10096-012-1636-4

Isendahl, J., Turlej-Rogacka, A., Manjuba, C., Rodrigues, A., Giske, C.G., Nauclér, P., 2012. Fecal carriage of ESBL-producing E. coli and K. pneumoniae in children in Guinea-Bissau: a hospital-based cross-sectional study. PLoS One 7, e51981. doi:10.1371/journal.pone.0051981

Kohli, R., Omuse, G., Revathi, G., 2010. Antibacterial susceptibility patterns of blood stream isolates in patients investigated at the Aga Khan University Hospital, Nairobi. East Afr. Med. J. 87, 74–80.

Lunguya, O., Lejon, V., Phoba, M.-F., Bertrand, S., Vanhoof, R., Glupczynski, Y., Verhaegen, J., Muyembe-Tamfum, J.-J., Jacobs, J., 2013. Antimicrobial resistance in invasive non-typhoid Salmonella from the Democratic Republic of the Congo: emergence of decreased fluoroquinolone susceptibility and extended-spectrum beta lactamases. PLoS Negl. Trop. Dis. 7, e2103. doi:10.1371/journal.pntd.0002103

Magoué, C.L., Melin, P., Gangoué-Piéboji, J., Okomo Assoumou, M.-C., Boreux, R., De Mol, P., 2013. Prevalence and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae in Ngaoundere, Cameroon. Clin. Microbiol. Infect. 19, E416–20. doi:10.1111/1469-0691.12239

Maltha, J., Guiraud, I., Kaboré, B., Lompo, P., Ley, B., Bottieau, E., Van Geet, C., Tinto, H., Jacobs, J., 2014. Frequency of severe malaria and invasive bacterial infections among children admitted to a rural hospital in Burkina Faso. PLoS One 9, e89103. doi:10.1371/journal.pone.0089103

Mavroidi, A., Liakopoulos, A., Gounaris, A., Goudesidou, M., Gaitana, K., Miriagou, V., Petinaki, E., 2014. Successful control of a neonatal outbreak caused mainly by ST20 multidrug-resistant SHV-5-producing Klebsiella pneumoniae, Greece. BMC Pediatr. 14, 105. doi:10.1186/1471-2431-14-105

Mshana, S.E., Hain, T., Domann, E., Lyamuya, E.F., Chakraborty, T., Imirzalioglu, C., 2013. Predominance of Klebsiella pneumoniae ST14 carrying CTX-M-15 causing neonatal sepsis in Tanzania. BMC Infect. Dis. 13, 466. doi:10.1186/1471-2334-13-466

Nelson, E., Kayega, J., Seni, J., Mushi, M.F., Kidenya, B.R., Hokororo, A., Zuechner, A., Kihunrwa, A., Mshana, S.E., 2014. Evaluation of existence and transmission of extended spectrum beta lactamase producing bacteria from post-delivery women to neonates at Bugando Medical Center, Mwanza-Tanzania. BMC Res. Notes 7, 279. doi:10.1186/1756-0500-7-279

Newman, M.J., Frimpong, E., Donkor, E.S., Opintan, J.A., Asamoah-Adu, A., 2011. Resistance to antimicrobial drugs in Ghana. Infect. Drug Resist. 4, 215–20. doi:10.2147/IDR.S21769

Obeng-Nkrumah, N., Twum-Danso, K., Krogfelt, K.A., Newman, M.J., 2013. High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance. Am. J. Trop. Med. Hyg. 89, 960–4. doi:10.4269/ajtmh.12-0642

Opintan, J.A., Newman, M.J., Arhin, R.E., Donkor, E.S., Gyansa-Lutterodt, M., Mills-Pappoe, W., 2015. Laboratory-based nationwide surveillance of antimicrobial resistance in Ghana. Infect. Drug Resist. 8, 379–89. doi:10.2147/IDR.S88725

Pitout, J.D.D., Laupland, K.B., 2008. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet. Infect. Dis. 8, 159–66. doi:10.1016/S1473-3099(08)70041-0

Reddy, E.A., Shaw, A. V, Crump, J.A., 2010. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet Infect. Dis. 10, 417–32. doi:10.1016/S1473-3099(10)70072-4

Saied, T., Elkholy, A., Hafez, S.F., Basim, H., Wasfy, M.O., El-Shoubary, W., Samir, A., Pimentel, G., Talaat, M., 2011. Antimicrobial resistance in pathogens causing nosocomial bloodstream infections in university hospitals in Egypt. Am. J. Infect. Control 39, e61–5. doi:10.1016/j.ajic.2011.04.009

Schaumburg, F., Alabi, A., Kokou, C., Grobusch, M.P., Köck, R., Kaba, H., Becker, K., Adegnika, A.A., Kremsner, P.G., Peters, G., Mellmann, A., 2013. High burden of extended-spectrum β-lactamase-producing Enterobacteriaceae in Gabon. J. Antimicrob. Chemother. 68, 2140–3. doi:10.1093/jac/dkt164

Schwaber, M.J., Carmeli, Y., 2007. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. J. Antimicrob. Chemother. 60, 913–20. doi:10.1093/jac/dkm318

Tansarli, G.S., Poulikakos, P., Kapaskelis, A., Falagas, M.E., 2014. Proportion of extended-spectrum β-lactamase (ESBL)-producing isolates among Enterobacteriaceae in Africa: evaluation of the evidence--systematic review. J. Antimicrob. Chemother. doi:10.1093/jac/dkt500

Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L.H., Karch, H., Reeves, P.R., Maiden, M.C.J., Ochman, H., Achtman, M., 2006. Sex and virulence in Escherichia coli: an evolutionary perspective. Mol. Microbiol. 60, 1136–51. doi:10.1111/j.1365-2958.2006.05172.x

Woerther, P.-L., Angebault, C., Jacquier, H., Hugede, H.-C., Janssens, A.-C., Sayadi, S., El Mniai, A., Armand-Lefèvre, L., Ruppé, E., Barbier, F., Raskine, L., Page, A.-L., de Rekeneire, N., Andremont, A., 2011. Massive increase, spread, and exchange of extended spectrum β-lactamase-encoding genes among intestinal Enterobacteriaceae in hospitalized children with severe acute malnutrition in Niger. Clin. Infect. Dis. 53, 677–85. doi:10.1093/cid/cir522

Tables

Table 1. Frequency and antibiotic resistance of Enterobacteriaceae causing invasive bloodstream infections (n=423).

Enterobacteriaceae	total (n)	ESBL production [n (%)]	Ciprofloxacin non-susceptibilty [n (%)]	Concomitant ESBL production and ciprofloxacin non-susceptibility [n (%)]
Non-typhoid *Salmonella*	215	0 (0.0)	14 (6.5)	0 (0.0)
S. Typhi	110	0 (0.0)	0 (0.0)	0 (0.0)
Escherichia coli	50	5 (10.0)	13 (26.0)	4 (8.0)
Klebsiella pneumoniae	41	34 (82.9)	16 (39.0)	16 (39.0)
Enterobacter cloacae	3	2 (66.7)	1 (33.3)	1 (33.3)
Proteus mirabilis	3	0 (0.0)	0 (0.0)	0 (0.0)
Leclercia adecarboxylata	1	0 (0.0)	0 (0.0)	0 (0.0)

Table 2. Age distribution of patients with invasive bloodstream infection caused by *Enterobacter cloacae* complex, *Escherichia coli* or *Klebsiella pneumoniae*.

	E. cloacae		*E. coli*		*K. pneumoniae*	
agegroups	frequency	ESBL production n (%)	frequency	ESBL production n (%)	frequency	ESBL production n (%)
0-7 days	3	2 (66.7)	2	0 (0.0)	30	27 (90.0)
8-28 days	0	0 (0.0)	2	1 (50.0)	2	2 (100.0)
29-365 days	0	0 (0.0)	2	0 (0.0)	3	2 (66.7)
1-4 years	0	0 (0.0)	6	0 (0.0)	2	2 (100.0)
5-14 years	0	0 (0.0)	1	0 (0.0)	0	0 (0.0)
15-59 years	0	0 (0.0)	21	2 (9.5)	3	0 (0.0)
≥ 60 years	0	0 (0.0)	16	2 (12.5)	1	1 (100.0)

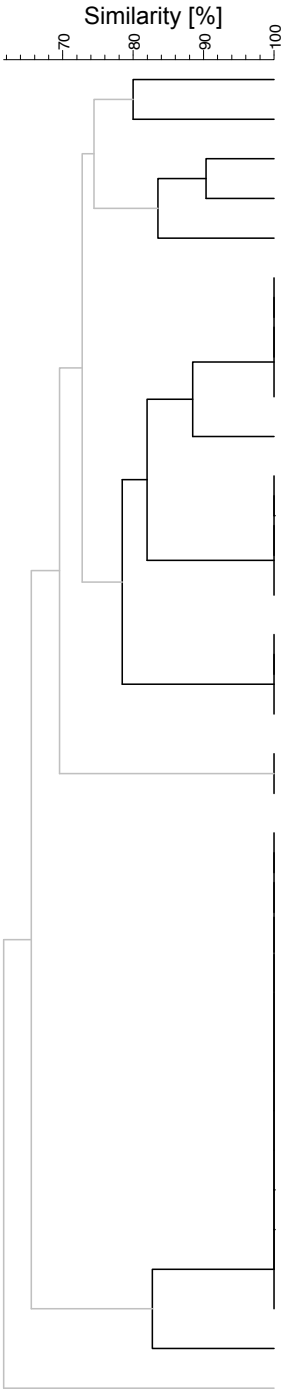
Figures

Figure 1. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) of all ESBL-producing *Klebsiella pneumoniae* isolates (n=34). PFGE clusters correspond to the MLST sequence type, except for two ST502 isolates that show distinct banding patterns. The ST13 cluster comprises community and nosocomial-acquired isolates.

Figure 2. Distribution of *Klebsiella pneumoniae* sequence types (n=88). The figure shows ESBL (coloured boxes) and non-ESBL (dark grey boxes) *Klebsiella pneumoniae* sequence types causing invasive bloodstream infections among all recruited infants on the neonatal ward (age ≤ 28 days) throughout the study period (black line). Clusters can be observed for ST13, ST25, ST36 and ST334.

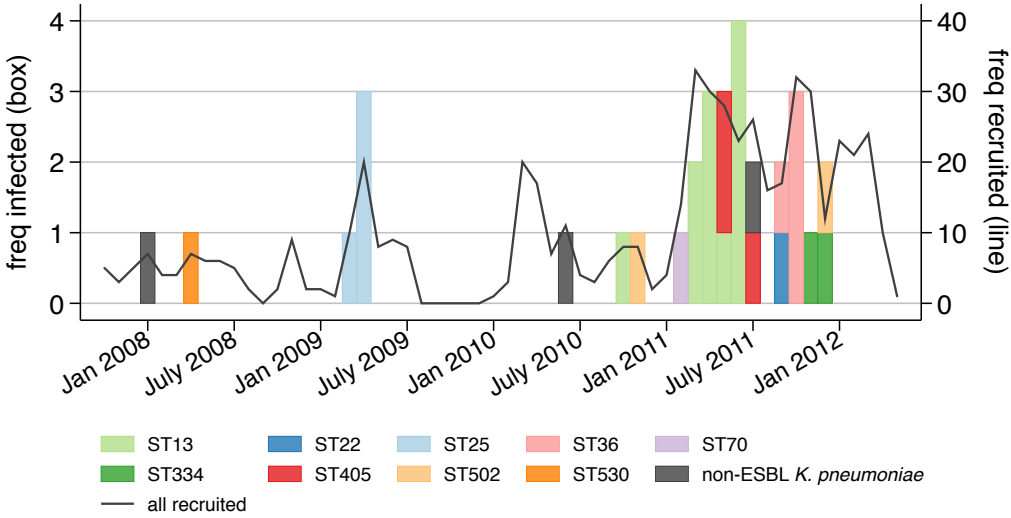
Figures

Figure 1



Key	MLST	Transmission
105156	ST110	Community
112113	ST414	Community
113512	ST502	Nosocomial
115579	ST502	Nosocomial
115037	ST22	Nosocomial
111947	ST25	Nosocomial
111999	ST25	Nosocomial
112004	ST25	Nosocomial
112029	ST25	Nosocomial
300112	ST15	Community
115142	ST36	Nosocomial
115148	ST36	Nosocomial
115149	ST36	Nosocomial
115183	ST36	Nosocomial
114226	ST405	Nosocomial
114282	ST405	Nosocomial
114636	ST405	Nosocomial
115422	ST334	Nosocomial
115604	ST334	Nosocomial
113446	ST13	Nosocomial
113875	ST13	Nosocomial
113896	ST13	Nosocomial
114059	ST13	Community
114066	ST13	Nosocomial
114097	ST13	Nosocomial
114104	ST13	Nosocomial
114136	ST13	Nosocomial
114359	ST13	Nosocomial
114466	ST13	Nosocomial
114478	ST13	Nosocomial
114480	ST13	Nosocomial
114571	ST13	Community
113792	ST70	Nosocomial
107361	ST530	Nosocomial

Figure 2


freq infected (box)
freq recruited (line)
0
1
2
3
4
0
10
20
30
40
Jan 2008
July 2008
Jan 2009
July 2009
Jan 2010
July 2010
Jan 2011
July 2011
Jan 2012
ST13
ST22
ST25
ST36
ST70
ST334
ST405
ST502
ST530
non-ESBL K. pneumoniae
all recruited