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ORIGINAL ARTICLE

Faecal carriage of Extended Spectrum Beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in children from the community of Kwadedangendlale, KwaZulu-Natal, South Africa

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Abstract

Infections caused by ESBL producing organisms have been described as an emerging public health problem in many parts of the world. Traditionally, ESBL producing organisms have been associated with hospital acquired infections. Recent studies have shown an increase in these infections within the community. Faecal carriage is a significant reservoir of ESBL producing bacteria in the community and colonization of the intestinal tract has been shown to precede infection. Faecal carriage of ESBL producing organisms in children from the community in Africa has been poorly studied.

Stool samples were collected from 300 children from Kwadedangendlale, KwaZulu-Natal, South Africa, during the period July 2011 to May 2012. Samples were inoculated onto MacConkey agar. All Gram negative bacteria cultured underwent identification and antimicrobial susceptibility testing on the Vitek 2. Isolates reported as ESBL positive, underwent confirmatory testing by the CLSI recommended combined disk diffusion method. All duplicates were removed.

We report faecal carriage of ESBL producing enterobacteriaceae in 4.7% (14/300) of children from the community in KwaZulu-Natal, South Africa. ESBL producing *Klebsiella pneumoniae* (3.7%) was the predominant isolate, with ESBL producing *Escherichia coli* being detected in 1% of children.

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This is the largest study to report on faecal carriage of ESBL producing enterobacteriaceae in children from the community in Africa. There is an urgent need to perform regular surveillance of ESBL producing bacteria in the community. This information is needed in order to direct the appropriate use of antimicrobials for empirical treatment of community acquired infections.

Keywords: Beta-lactamases; *Escherichia coli*; *Klebsiella pneumoniae*; Faeces.

Background

The widespread and inappropriate use of antibiotics has resulted in a significant increase in antibiotic resistant bacteria worldwide.[1] A major contributor to this increasing resistance is the production of inactivating enzymes, in particular extended spectrum beta lactamases (ESBLs). There is no agreement on the exact definition of ESBLs. However, ESBLs are commonly defined as beta-lactamases that confer resistance to the penicillins; first, second, and third-generation cephalosporins and monobactams by hydrolysis of these antimicrobials. In addition, these enzymes are inhibited by beta-lactamase inhibitors such as clavulanic acid.[2]

ESBLs are encoded on transferrable conjugative plasmids, which facilitate widespread dissemination, not only between the same species of bacteria, but also across different species.[3,4] In addition, these plasmids code for resistance to other classes of potent antimicrobial agents, in particular, aminoglycosides and fluoroquinolones.[4] Carbapenems are currently the treatment of choice for serious infections, with fluoroquinolones being and an alternative choice, if the organism is shown to be susceptible. Infections with ESBL producing bacteria are not only extremely challenging to treat, but are also associated with increased mortality.[5]

Infections caused by ESBL producing organisms have been described as an emerging public health problem worldwide.[6] Traditionally, ESBL producing organisms have been associated with hospital acquired infections. However, recent studies have shown an increase in ESBL producing bacterial infections acquired within the community.[7,8] Recent publications have highlighted that faecal carriage is a significant reservoir of ESBL producing bacteria in the community. Colonization of the intestinal tract has been shown to precede infection and thus asymptomatic faecal carriage with ESBL producing organisms is of clinical significance.[9-12] Infections caused by *Escherichia coli* and *Klebsiella pneumoniae* are of particular concern. Recently the Infectious Diseases Society of America took the bold step of declaring that *E. coli* and *K. pneumoniae* be included in the list of six pathogens for which novel antimicrobials are urgently required.[13] Therefore there is a need to perform regular surveillance of ESBL producing bacteria in the community. This information is needed in order to direct the appropriate use of antimicrobials for empirical treatment of community acquired infections.

Faecal carriage of ESBL producing organisms has shown large variations worldwide. In Africa, faecal carriage of ESBLs has been poorly studied with only 6 published studies to date.[10-12,14-16] Only four were paediatric studies.[10,12,15,16] To the best of our knowledge, there is no published data from South Africa on the prevalence of community acquired faecal carriage of ESBL producing organisms in adults or children.

Aim

The aim of this study was to determine faecal carriage of ESBL producing *E. coli* and *K. pneumoniae* in children, aged 4-6 years, in the community of Kwadedagendlale, in KwaZulu-Natal, South Africa.

Methods

This was a laboratory based study conducted at the NHLS microbiology laboratory at Inkosi Albert Luthuli Central Hospital [IALCH]. It was an ancillary study of the much larger epidemiological study called the ASENZE study. This study was conducted by the Department of Paediatrics and Child Health. The study involved investigating the health profile of children aged 4-6 years, from the area of Kwadedangendlale. As part of this study, stools and urine were obtained from all children

between the ages of 4-6 years in the community, irrespective of whether they attended school or not. These were thereafter sent to our laboratory to determine the prevalence of soil transmitted helminths and urinary bilharzia. We utilized these same stool samples to determine the prevalence of ESBL producing *E. coli* and *K. pneumoniae*.

Study population

The study sample included 300 children aged 4-6 years, from five areas of Kwadedangendlale, namely, Molweni, Kwangcolisi, KwaNyuswa, Qadi and Embo. Stool samples were collected from every child during the period July 2011 and May 2012.

Sampling strategy

Research assistants under the supervision of the principal investigator were based at the ASENZE study site. They made visits to schools participating in the study on a scheduled basis and collected stool samples. Specimen containers were kept in a cooler box until they were transported to the NHLS microbiology laboratory at IALCH, usually within 24 hours.

ESBL detection

Stool samples were inoculated onto MacConkey agar plates, which were incubated in ambient air at 35°C for 24 hours. Plates demonstrating no growth on primary examination were incubated for a further 24 hours. Different colonial morphotypes resembling Gram negative bacteria, were initially confirmed by Gram staining, and thereafter underwent identification and antimicrobial susceptibility testing on the Vitek 2 (BioMérieux, France).

Thereafter, only isolates that were reported as ESBL positive by VITEK 2, underwent further testing by the CLSI recommended combined disk diffusion method using 30µg ceftazidime (CAZ) and 30µg cefotaxime (CTX) discs with and without the inhibitor clavulanic acid (10 µg) [Mast laboratories] on Mueller Hinton agar to confirm the presence of ESBL. Plates were incubated at 37°C in ambient air for 16-18 hours. ESBL production was indicated by an increase in a zone diameter of ≥ to 5 mm for either antimicrobial agent tested in combination with clavulanic acid, compared to zone diameters obtained with these antibiotics alone. All duplicate isolates from the same patient were removed from the final analysis.

Ethical approval was granted separately for the ASENZE study, and our laboratory based study by the Biomedical Research Ethics Committee, UKZN. Confidentiality of all data was maintained throughout the study.

Results

Although not the main focus of this study, the majority of stools grew enterococci. Gram negative organisms that were isolated are listed in Table I. *E. coli* and *K. pneumoniae* were cultured from the stools of 97 and 12 patients respectively. ESBL production was only found in the *E. coli* and *K. pneumoniae* isolates. Eleven (3.7%) yielded ESBL producing *K. pneumoniae* and 3 (1%) yielded ESBL producing *E. coli*, giving a total of 14 (4.7%) of ESBL producing enterobacteriaceae. ESBL producing enterobacteriaceae were isolated in 9 females and 5 males. This difference is not statistically significant (p=0.5146).

Eleven of the 12 (92%) *K. pneumoniae* isolated from the 300 stool samples, were ESBL producing; in contrast only 3 of the 97 (3.1%) *E. coli* isolated were ESBL producing. This finding is statistically significant (p<0.0001, Table II). Of note, there were no stool samples that had both ESBL producing *E. coli* and *K. pneumoniae* present together.

Table I. Gram negative bacteria isolated from stool samples from 300 children in the community

Bacteria	No (%)
*Escherichia coli**	97 (32.3%)
Sphingomonas spp.	24 (8%)
*Klebsiella pneumoniae**	12 (4%)
Pantoea spp.*	9 (3%)
Aeromonas spp.	3 (1%)
Brucella melitensis	2 (0.67%)
*Routella planticola**	1 (0.33%)

* Members of the Enterobacteriaceae family

Table II. Faecal carriage of ESBL producing and ESBL non –producing *Escherichia coli* and *Klebsiella pneumoniae* in stool samples from 300 children in the community

Organism No (%)		Males	Females
ESBL +ve *K. pneumoniae*	11(3.7%)	5	6
ESBL -ve K. *pneumoniae*	1(0.3 %)	1	0
ESBL +ve *E. coli*	3 (1%)	0	3
ESBL -ve *E. coli*	94 (1, 3 %)	45	49
TOTAL ESBL + enterobacteriaceae	14 (4.7%)	5	9

With regard to the antibiograms obtained on Vitek 2, ESBL producing *E. coli* and ESBL producing *K. pneumoniae* isolates were uniformly susceptible to the carbapenems, fluoroquinolones, piperacillin-tazobactam, aminoglycosides, nitrofurantoin, tigecycline, cefoxitin and colistin.

Discussion

Infections with ESBL producing bacteria are not only becoming more challenging to treat, but are also associated with increased mortality. Widespread resistance to beta - lactams and many other first line antimicrobials, has resulted in treatment choices being limited to the carbapenems and fluoroquinolones. In addition, increasing resistance to fluoroquinolones and emerging resistance to carbapenems has exacerbated the problem.

In hospital settings, patients colonised with community acquired ESBL producing organisms constitute an important source of transmission to other patients. Studies to date have focused on faecal carriage of ESBL producing enterobacteriacae in healthcare-associated outbreaks.[4] There is a paucity of studies conducted during non-outbreak situations. The intestinal tract is reported to be a major reservoir of ESBL producing bacteria and faecal carriage has been shown to precede infections. Studies investigating faecal carriage of ESBLs in the community are limited, with even fewer studies focusing on faecal carriage of ESBLs specifically in children in the community.

In Africa, faecal carriage of ESBL producing bacteria has been poorly studied with only six published studies to date.[10-12,14-16] Two studies were conducted in adults with reported prevalence of 10.1% and 6.7% respectively.[11,14] Four of these studies involved children, which is the focus of further discussion in this article. Two of these paediatric studies looked at hospital admissions,[10,15] and the other two focused on faecal carriage of *E. coli* in the community.[12,16] One of these latter two studies was carried out in Senegal among 20 children living in a remote village and reported a prevalence of 10% (2/20). The second, more recently published study, was carried out in Libya, and reported a prevalence of 13.4% (18/134) in children aged 3-12 years old presenting at clinics. These children, however, presented with diarrheal disease which may have resulted in the higher prevalence. Thus from Africa, our study is the largest study carried out in healthy children from the community. Furthermore, in contrast to most published studies, we identified and performed susceptibility testing on all Gram negative organisms isolated. A predominance of *E. coli* followed by *Sphingomonas paucimobilis*, *Klebsiella pneumoniae*, *Pantoea agglomerans*, *Acinetobacter baumannii*, *Aeromonas* spp., *Enterobacter cloacae*, *Brucella melitensis* and *Routella* spp. was found. ESBL production was detected in 4.7% of eneterobacteriacae (*E. coli* and *K. pneumoniae* isolates only).

There are many reports of faecal carriage of ESBL producing organisms from other parts of the world, but as indicated previously, only a few studies were carried out in children in the community. In Europe, the highest prevalence of ESBL producing *E. coli* was reported from Spain (24.0%, 30/125), in infants aged 8 to 16 months.[17] A study carried out in Portugal

found a prevalence of 2.7% of ESBL producing *E. coli* (3/112).[18] A French study looking at 411 children aged 6-24 months presenting either for a routine check-up or otitis media, reported a prevalence of 4.6% ESBL producing enterobacteriaceae. In this latter study, a contributing factor was the history of recent third-generation oral cephalosporin exposure which was associated with a higher risk of ESBL carriage.[9]

In Bolivia and Peru, two studies carried out in healthy children reported prevalence rates of ESBL producing *E. coli* of 0.1% (4/3208) and 1.7% (50/3193) respectively.[19,20] A prevalence of 12.4% (60/482) ESBL producing *E. coli* was reported in a third study carried out in healthy children from Bolivia and Peru. This higher prevalence may have been attributed to the use of antibiotics which was reported as an independent risk factor.[21]

A study carried out in China differed from all the previous studies in that it was conducted in hospitalised children and their household contacts. Overall, the prevalence of faecal carriage of CTX-M-producing *E. coli* was very high at 43.5% (37.7% hospitalised children, 20.7**%** household children, and 50.3% household adults).[22] This highlights the transmission of ESBLs from hospitalised children to healthy contacts within the community setting.

In a study carried out in France, faecal carriage of ESBL producing organisms has also been described in children who were adopted from developing countries. ESBL producing enterobacteriaceae were detected in 96% (24/25) of adopted children.[23] This highlights the additional challenge posed by asymptomatic faecal carriage of multi drug resistant organisms in adopted children from developing countries.

Most studies to date have reported ESBL production predominantly in *E. coli*.[24] In contrast, our study reports a much higher ESBL production in *K. pneumoniae* (3.7%) rather than *E. coli* (1%). Although our study yielded a small number of *K. pneumoniae* isolates, 92% were ESBL producing. In contrast, only 3.1% of a larger number of *E. coli* isolates were ESBL producing. This finding is of major concern as it would suggest that the probability of ESBL production in *K. pneumoniae* is significantly higher than in *E. coli* in our setting. To the best of our knowledge, the children in our study were otherwise healthy and had not received antibiotics or were hospitalised previously. Ethical approval for this laboratory based study did not allow us to obtain additional useful information such as risk factors associated with carriage of resistant bacteria. This information could have possibly helped to explain why ESBL production was higher in the *K. pneumoniae* isolates.

In children, risk factors for faecal carriage of ESBL producing organisms in the community have not been well defined. Birgy *et al.* looked at the use of antibiotics between 7 days to 3 months before enrolment, daycare modalities, hospitalisation during the previous 6 months and immunization history as possible risk factors. Exposure to third-generation oral cephalosporins and age over 1 year were the only significant risk factors identified.[9]

Although data published does not adequately reflect the total number of adults and children with faecal carriage of ESBL producing organisms, it has shown large variations worldwide. The highest carriage rates have been seen in developing countries. This finding has been linked to higher population densities, poverty and poor access to drinking water. Water has been documented as a reservoir for ESBL dissemination. Interestingly, not only waste water has been implicated.[25-27] Studies have shown that river water, sea water, aquatic ecosystems and even water from the Antarctic were found to harbour ESBL producing organisms.[28]

In addition, it has recently been reported that the food chain may be another source of ESBL dissemination, and molecular typing strongly suggests dissemination from animals to humans.[29,30] Even in Switzerland, which follows strict antibiotic policies, colonisation rates as high as 15% in pigs and 63% in chickens has been reported.[31] Reports have also indicated that companion animals may be reservoirs for CTX-M-15-producing *K. pneumoniae*.[32]

The area of KwaDedangendlale is a semi-rural area located 40 kilometres northwest of Durban in the province of KwaZulu-Natal. Access to basic services such as water, sanitation, electricity, roads and

telephones is limited. We can only postulate that contaminated water, the food chain or companion animals could possibly represent sources of faecal carriage of ESBL producing *E. coli* and *K. pneumoniae* reported in the children from this community.

In our study, the predominant ESBL producing organism in children in the community was *K. pneumoniae*. Studies in South Africa have shown that *K. pneumoniae* is also the most common enterobacteriaceae causing healthcare-associated infections.[33-35] A recently published study of *K. pneumoniae* isolates from bacteraemic patients submitted by sentinel laboratories in five regions of South Africa from mid-2010 to mid-2012 reported that 68.3% of 2774 isolates were ESBL-positive, and 46.5% of isolates were resistant to ciprofloxacin and 33.1% to piperacillin-tazobactam and 5% were also resistant to carbapenems.[36] Several outbreaks of ESBL producing *K. pneumoniae* infections have been reported from South Africa.[37-39] We hypothesize that the community acquired ESBL producing *K. pneumoniae* could possibly serve as the precursor of the hospital acquired infections.

The community isolates of ESBL producing *E. coli* and *K. pneumoniae* from children in the present study were susceptible to fluoroquinolones, piperacillin-tazobactam, aminoglycosides and nitrofurantoin, in addition to carbapenems, tigecycline and colistin. Our results contrast with the community acquired ESBL producing isolates reported in other published studies, which were generally more resistant.[14]

Although we did not have access to molecular typing, our isolates showed higher minimal inhibitory concentrations to cefotaxime than ceftazadime., suggesting that the isolates were of the CTX-M type.[2] Previous characterisation of ESBLs from South Africa has revealed TEM and SHV types (especially SHV-2 and SHV-5)[34,35,40] with more recent studies reporting CTX-M types.[36]

There are currently no screening and treatment guidelines for ESBL carriers admitted to hospital. Screening for ESBL carriage upon admission was shown not to be cost effective. It is well recognised that *K. pneumoniae* tends to be more frequently cross-transmitted than *E. coli* within hospitals. This is particularly important in our setting, as we found a predominance of ESBL producing *K. pneumoniae*. The need for strict adherence to standard contact precautions, including simple hand washing, remains pivotal in order to prevent the spread of community acquired ESBL producing Enterobacteriaceae within the hospital setting.

Conclusion

The present study is the largest study from Africa that focused on children in the community. We report faecal carriage of ESBL producing enterobacteriaceae in 4.7% (14/ 300) of children from the community in KwaZulu-Natal, South Africa. ESBL producing *K. pneumoniae* (3.7%) was the predominant isolate, with ESBL producing *E. coli* being detected in 1% of children. There is an urgent need to perform regular surveillance of ESBL producing bacteria in the community. This information is needed in order to direct the appropriate use of antimicrobials for empirical treatment of community acquired infections. In addition, it is essential to reinforce strict infection control measures in order to prevent further spread within the community and from the community into hospital settings.

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