Original Article

Broad spectrum β-lactam resistance in faecal *Escherichia coli* isolated from severely malnourished and nourished children attending Mbagathi district hospital, Nairobi: A case-control study

Abstract

Context: Severely malnourished children have increased risk of being put on antibiotics due to co-morbidities. **Aim:** The study's objective was to characterize the *Escherichia coli* β-lactamase mediated resistance to the broad spectrum β-lactam antimicrobials among this population and compare them with nourished children as controls. **Settings and Design:** In this case-control, hospital-based setup, 109 *E. coli* isolates were obtained from each group, one isolate per subject. **Materials and Methods:** Stool or anal swabs were collected, enriched in buffered peptone water and cultured on MacConkey and eosin methylene blue agars. Biochemical test were used to identify *E. coli*. antibiograms to determine phenotypic resistance were determined using a panel of 14 drugs. Only the isolates showing synergy between ampicillin-calvulanic acid and one or more third generation cephalosporins were picked as extended spectrum β-lactamase (ESBL) producers. **Statistical Analysis:** Differences in ESBL rates and susceptibility percentages between cases and controls were evaluated for significance using 2-tailed Fisher's exact test. **Results:** Prevalence of ESBL phenotype was higher in severely malnourished children (39%) as compared to the controls (7%). The plasmid–encoded AmpC's (pAmpC)-like phenotype was observed in 11% isolates. **Conclusions:** Isolation of ESBL-*E. coli* among severely malnourished children is high. Surveillance of ESBL producers, both in the community and hospital settings needs to be stepped up in Kenya.

Key words:
Antimicrobial resistance, diarrhea, Escherichia coli, extended spectrum β-lactamase-Escherichia coli, severely malnourished children, Kenya

Introduction

Severely malnourished children are at a higher risk of enteric infection, making them more prone to diarrhea than healthy children.[1,2] They often have other complications such as diarrhea, pneumonia and bacteremia.[3,4] This may warrant the empiric use of antimicrobials to boost their survival, but in the case of severe acute malnutrition complicated by diarrhea only, this predisposes a child to inappropriate antimicrobial use.[5,6]

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Antimicrobial resistance among *Escherichia coli* is of increasing global concern.[7] This has been associated with the emergence and spread of extended spectrum β-lactamase (ESBL)-producing *E. coli*, which are also frequently associated with resistance to quinolone and aminoglycosides.[8] Serious infections with ESBL producing *E. coli* are associated with high mortality rates as therapeutic options are limited to carbapenems.[9,10] ESBL producers are resistant to penicillins, oxyimino-cephalosporins,

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monobactams and are inhibited by β-lactamase inhibitor combinations.[11] ESBL are generally derived from temoneira (TEM) and sulfhydryl variable (SHV)-type enzymes.[12] Lately, cefotaximases (CTX-M) enzymes are substituting parent SHV and TEM enzymes as the more common type of ESBLs, mainly in community-acquired infections caused by *E. coli*.[13] ESBL's have been isolated before in in hospital settings Kenya.[10,14] Furthermore, plasmid–encoded AmpC (pAmpC) β-lactamases such as cephamycinases (CMYs) mediate resistance to most classes of β-lactams except to cefepime.[15]

Currently, no data exits showing the extent and consequence of drug resistant *E. coli* among severely malnourished children under 5 years of age in Kenya. This cross-sectional study therefore aimed to characterize *E. coli* antimicrobial resistance patterns to the broad spectrum β-lactam antimicrobials among severely malnourished children with diarrhea attending Mbagathi District Hospital and not to link *E. coli* with diarrhea.

Materials and Methods

In this hospital-based, case-control study, 109 non-duplicate *E. coli* isolates from severely malnourished children and 109 from matched healthy children were obtained in accordance with the National ethical standards on human experimentation (ERC Protocol No. 2382) using homogenous sampling. All the children were 2-60 months of age across both genders and patient categories (cases and controls). Severely malnourished children had a mid-upper arm circumference of less 110 mm (−3 *Z* scores from the mean) and were also recruited in the Cotrimoxazole Prophylaxis in Severely Malnourished Children (CTX)-clinical trial. All the children recruited in the study were from Kibera, an informal settlement in Kenya's capital city, Nairobi. Majority of the cases were hospitalized at Mbagathi District Hospital pediatric ward. Controls were children visiting out-patient department but requiring microbiology investigations. Specimens from cases were predominantly rectal swabs while stool samples were preferred for controls.

Rectal swab or stool sample was collected from each study participant, enriched overnight in 5% buffered peptone water and plated on MacConkey and Eosin Methylene Blue agar (Oxoid, Basingstoke, United Kingdom). Incubation was carried out for 18 h at 37°C. A single *E. coli* colony was picked from each specimen to yield one isolate per patient average. Antimicrobial susceptibility tests were performed using Kirby–Bauer disc diffusion technique.[16] This was carried out with antibiotic discs (Cypress diagnostics, Langdorp, Belgium) on Mueller Hinton agar (Oxoid). *E. coli* ATCC 25922 was included as a control strain on each test batch which in this case, was always susceptible to all the drugs. Antibiogram panel included; ampicillin (10 μg), cefpodoxime (30 μg), CTX (30 μg), ceftazidime (30 μg), cefepime (30 μg), cefoxitin (30 μg) represented, amoxicillin/clavulanic acid (20/10 μg), tazobactam/piperacillin (100/10 μg), gentamicin (5 μg), streptomycin (25 μg), ciprofloxacin (30 μg), nalidixic acid (30 μg), chloramphenicol (30 μg) and sulfamethoxazole/trimethoprim (23.75/1.25 μg). An isolate was defined as resistant to third generation cephalosporins, when the inhibition zone diameter of cefpodoxime (30 μg) and CTX (30 μg) was <17 mm and 22 mm, respectively.[17] Phenotypic ESBL detection was first performed by disk diffusion and double disk synergy test using CLSI 2012 guidelines. Only isolates showing synergy zones between amoxicillin/clavulanic and one- or more- third generation cephalosporins were picked as ESBL producers [Figure 1]. Antimicrobial susceptibility test results of all the isolates were analyzed using the WHONET 5.6 software. Fisher's exact test, 2-tailed was used to evaluate for significance on ESBL susceptibility profiles differences between cases and controls.

Results and Discussions

Antimicrobial resistance patterns

Although the purpose of the study was not to ascertain virulence or the pathotypes of the *E. coli* isolated, surveillance of drug resistance among these isolates is of equal importance. Out of the 109 *E. coli* non-duplicate isolates from each group, 43 (39%) and 8 (7%) were of ESBL phenotypes from cases and controls respectively [Figure 2]. In a similar study, fecal isolation rate of ESBL's among severely malnourished children in a pediatric re-nutrition center was slightly lower at 31% in 2011 compared with 39% in this study.[18] The increase could be a result of different antibiotic exposures between the two countries and/or an increase in the propensity of ESBL occurrence with time.

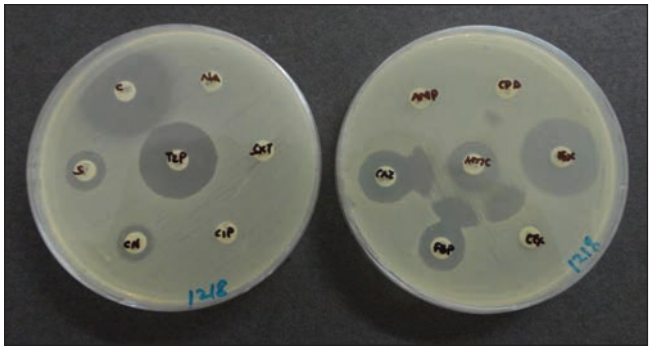


Figure 1: Phenotypic identification of extended spectrum β-lactam producing *Escherichia coli* (AMC – Amoxicillin-clavulanic acid, CPD – Cefpodoxime, FOX – Cefoxitin, CTX – Cefotaxime, FEP – Cefepime, CAZ – Ceftazidime, AMP – Ampicillin, SXT – Trimethoprim-sulfamethoxazole, CIP – Ciprofloxacin, TZP – Tazobactam-piperacillin, NA – Nalidixic acid, C – Chloramphenicol, S – Streptomycin, CN – Gentamicin. Occurrence of "ghost inhibition zones" between β-lactam/β-lactamase and β-lactam only antibiotic discs is suggestive of an ESBL phenotype)

ESBLs also confer co-resistance to quinolones and aminoglycosides.[19] This was also observed in this study with high rates of resistance to nalidixic acid, ciprofloxacin, gentamicin and chloramphenicol. There were significant differences in resistance antibiograms in ESBLs and non-ESBLs among cases and controls [Figures 3 and 4]. Differences in ESBL-*E. coli* antibiograms among cases and controls was only observed in cefotaxime, streptomycin and gentamicin [Table 1]. The incidence of co-resistance in ESBL producing *enterobacteriaceae* is an emerging public health concern due to their occurrence in both hospital and community setting, co-resistance with other classes of antimicrobials, higher cost and toxic treatment options in developing countries.[10,12,18,20-23]

A recent study carried out in Kibera showed a high

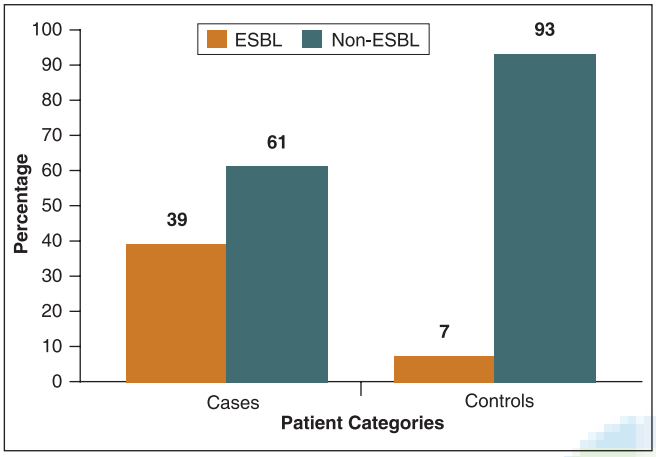
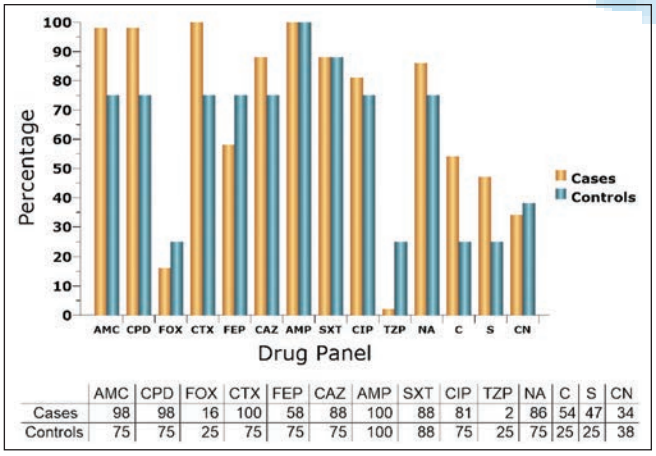


Figure 2: Distribution of ESBLæs among patient categories extended spectrum β-lactamase producing (ESBL) *Escherichia coli*, non-ESBL- *E. coli* isolates not exhibiting ESBL phenotype. Actual number of *E. coli* isolates from both cases and controls was 109 each



	AMC	CPD	FOX	CTX	FEP	CAZ	AMP	SXT	CIP	TZP	NA	C	S	CN
Cases	98	98	16	100	58	88	100	88	81	2	86	54	47	34
Controls	75	75	25	75	75	75	100	88	75	25	75	25	25	38

Figure 3: Distribution of resistance in extended spectrum β-lactamase producing (ESBL) *Escherichia coli* (ESBL- *E. coli*) among cases and controls. (AMC – Amoxicillin-clavulanic acid, CPD – Cefpodoxime, FOX – Cefoxitin, CTX – Cefotaxime, FEP – Cefepime, CAZ – Ceftazidime, AMP – Ampicillin, SXT – Trimethoprim-sulfamethoxazole, CIP – Ciprofloxacin, TZP – Tazobactam-piperacillin, NA – Nalidixic acid, C- Chloramphenicol, S – Streptomycin, CN – Gentamicin. ESBL – Extended spectrum β-lactamase. Resistant isolates were grouped together with intermediate

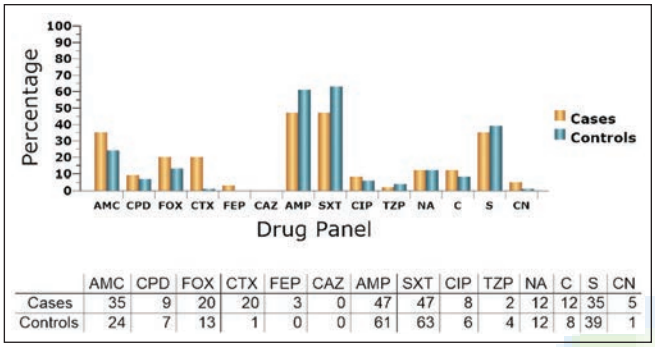
Table 1: Comparison of ESBL-*E. coli* antibiograms from cases and controls

Drug name and patient category	Resistant	Sensitive	*P* value	Comment
AMC				
Cases	39	4	0.2339	NS
Controls	6	2		
CPD				
Cases	42	1	0.0605	NS
Controls	6	2		
FOX				
Cases	7	36	0.6187	NS
Controls	2	6		
CTX				
Cases	43	0	0.0220	S
Controls	6	2		
FEP				
Cases	25	18	0.4564	NS
Controls	6	2		
CAZ				
Cases	38	5	0.3004	NS
Controls	6	2		
AMP				
Cases	43	0	–	–
Controls	8	0		
SXT				
Cases	38	4	1.000	NS
Controls	7	1		
CIP				
Cases	35	7	0.6231	NS
Controls	6	2		
TZP				
Cases	1	42	0.0605	NS
Controls	2	6		
NA				
Cases	37	6	0.5952	NS
Controls	6	2		
C				
Cases	23	20	0.2485	NS
Controls	2	6		
S				
Cases	30	13	0.0403	S
Controls	2	6		
CN				
Cases	34	9	0.0278	S
Controls	3	5		

AMC – Amoxicillin-clavulanic acid; CPD – Cefpodoxime; FOX – Cefoxitin; CTX – Cefotaxime; FEP – Cefepime, CAZ – Ceftazidime; AMP – Ampicillin; SXT – Trimethoprim-sulfamethoxazole, CIP – Ciprofloxacin; TZP – Tazobactam-piperacillin, NA – Nalidixic acid; C – Chloramphenicol; S – Streptomycin; CN – Gentamicin; ESBL-*E. coli* – Extended spectrum β-lactamase *Escherichia coli*; NS – Not significant; S – Significant

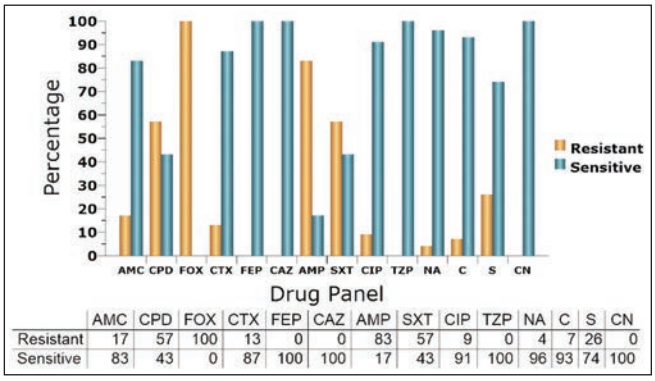
percentage of environmental *E. coli* being resistant to commonly used drugs; tetracycline, ampicillin and sulphamethoxazole/trimethoprim.[24] Malnutrition increases the risk of entero-toxigenic *E. coli* colonization which is associated with diarrheal illness.[1] In addition, malnutrition aggravates enteric infections affecting morbidity, mortality and therapy.[25,26] Diarrhea was found to a common morbidity among severely malnourished children in Dhaka, Bangladesh.[27]

ESBL *E. coli* isolates were from 20 male (47%) and 23 female (53%) subjects in cases. Controls constituted five females (63%) and three males (37%). AmpC CMYs are β-lactamase enzymes that hydrolyze cephamycins (cefoxitin) and third



	AMC	CPD	FOX	CTX	FEP	CAZ	AMP	SXT	CIP	TZP	NA	C	S	CN
Cases	35	9	20	20	3	0	47	47	8	2	12	12	35	5
Controls	24	7	13	1	0	0	61	63	6	4	12	8	39	1

Figure 4: Resistance profiles of the non-extended spectrum β-lactamase producing (none-ESBL's) *Escherichia coli* among cases and controls AMC – amoxicillin-clavulanic acid, CPD – Cefpodoxime, FOX – Cefoxitin, CTX – Cefotaxime, FEP – Cefepime, CAZ – Ceftazidime, AMP – Ampicillin, SXT – Trimethoprim-sulfamethoxazole, CIP – Ciprofloxacin, TZP – Tazobactam-piperacillin, NA – Nalidixic acid, C – Chloramphenicol, S – Streptomycin, CN – Gentamicin. Resistant isolates were grouped together with Intermediate



	AMC	CPD	FOX	CTX	FEP	CAZ	AMP	SXT	CIP	TZP	NA	C	S	CN
Resistant	17	57	100	13	0	0	83	57	9	0	4	7	26	0
Sensitive	83	43	0	87	100	100	17	43	91	100	96	93	74	100

Figure 5: Antibiograms of 23 *Escherichia coli* isolates resistant to FOX showing no extended spectrum β-lactamase (ESBL) production. AMC–Amoxicillin-clavulanic acid, CPD–Cefpodoxime, FOX – Cefoxitin, CTX – Cefotaxime, FEP – Cefepime, CAZ – Ceftazidime, AMP – Ampicillin, SXT – Trimethoprim-sulfamethoxazole, CIP – Ciprofloxacin, TZP – Tazobactam-piperacillin, NA – Nalidixic acid, C – Chloramphenicol, S – Streptomycin, CN – Gentamicin

generation cephalosporins.[28] They are either plasmid or chromosomally encoded, but our interest was on pAmpC's enzymes.[28] Majority of plasmid mediated cephamycin resistance is characterized by resistance to cefoxitin and susceptibility to cefepime: A fourth generation cephalosporin.[15] Cefoxitin resistance using disc diffusion has 97% sensitivity and 64% specificity.[29] Out of the 23 cefoxitin resistant *E. coli* isolates from the study, 23 (100%) showed resistance to cefoxitin but susceptibility to cefepime [Figure 5]. Cefoxitin resistance observed in the 23 isolates is more likely to be plasmid mediated than chromosomally induced. Amp-C β-lactamase are found throughout the world but are not prevalent as ESBL's.[15]

Conclusion

The prevalence of ESBL-*E. coli* among severely malnourished children is unusually high. ESBL's also manifest co-resistance to other classes of antimicrobials rendering treatment a toxic and costly affair. Therefore, ESBL screening among severely malnourished children is of great importance since it affects treatment outcome. ESBL surveillance should be stepped up by creation of sentinel sites throughout the Country.

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