Original Research

Antibiotic susceptibility profiles of bacteria isolates obtained from patients presenting with diarrhoea in Machakos Distric Hospital, Kenya

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Abstract

Introduction: Bacterial diarrhoeal diseases are the cause of morbidity and mortality in Africa, but data on antimicrobial susceptibility patterns of enteric bacterial aetiologies are limited. This study was carried out in Machakos District Hospital from September 2010 to March 2013 on bacteria isolated from patients with diarrhoea where 300 patients were studied.
Methodology: Bacterial isolates were identified biochemically and *E. coli* pathotypes detected through Multiplex Polymerase Chain Reaction. Susceptibility testing was done following both disc diffusion and minimum inhibitory concentration methods.
Results: Bacterial isolates detected included: Enteroaggregative *E. coli* (EaggEC 13.7%), Enterotoxigenic *E. coli* (ETEC 11%), Enteroinvasive *E. coli* (EIEC 8.3%), Enteropathogenic *E. coli* (EPEC 4.3%), *Shigellae* (22.3%), *Salmonella* spp (0.3%), *Proteus* spp (0.6%) and *Klebsiella* spp (0.3%). The isolates were resistant to more than four antibiotics chloramphenical (28%), cotrimoxazole (78%), co-amoxilav (70%) erythromycin (98%), ciprofloxacin (4%), cefotoxime (18%) and tetracycline (56%).
Conclusions: The detection of high resistance found to commonly used antibiotics should serve as a warning call for close surveillance, identification and understanding of the epidemiology of the resistance with a view to setting up preventive strategies that can minimize or stop the emerging and spread of resistance to the antibiotic arsenal currently in use. For the first time in more than ten years, gentamicin has shown to be susceptible and can be utilized for management of diarrhoeal illness resulting from bacterial infection.

Key words: Antimicrobial resistance, *E. coli* pathotypes, Shigellae

Introduction

Bacterial antibiotic resistance is an emerging and serious public health concern due to the compromised efficacy of antimicrobial agents used in the treatment of infectious diseases [1]. The emergence and dissemination of antimicrobial resistance in bacteria has been well documented as a serious problem worldwide [2]. Selective pressure favoring antimicrobial-resistant phenotypes is applied whenever antimicrobials are used, including treating disease in clinical medicine and preventing disease and promoting growth in animal husbandry [1].

Reports have suggested the use of tetracycline, sulfa drugs, cephalosporins, and penicillins to be a major factor in the emergence and dissemination of antimicrobial-resistant *E. coli* [3-5]. However, a relative paucity of information exists regarding antimicrobial resistance in *E. coli* from nosocomial and non-hospital sources, especially those from animal sources. In this study, antimicrobial susceptibility profiles were determined for *Shigellae*, *Salmonella* spp, *Klesiella spp* and *E. coli* isolates of ETEC, EHEC, EPEC, EAEC and EIEC. Strains of *E. coli* pathotypes may cause urinary tract and enteric infections in humans and have been implicated in infections [1]. The isolates used in the present study, were collected from participants attending Machakos District Hospital in Kenya. None of the isolates were from food, water or animals.

Materials and Methods

Study patients: Three hundred study participants comprising of children as young as four months old and adults from Machakos County seeking medical attention during the period 2010 to 2013. The purpose and nature of the study was explained to the participants. Informed consent was obtained from the mature participants while parents/guardians gave signed consent for their children to participate in the study. Research ethical approval was granted by the Ethical Review Committee of Kenya Medical Research Institute (KEMRI SSC No. 989).
Sample collection: Stool samples were collected in a sterile stool container from

all patients who met the inclusion criteria and consented to participate in the study. For those patients who could not void stool at the time of their visit to the hospital, a rectal swab was obtained by qualified personnel and placed in Carry Blair transport medium for shipment to the laboratory for culture and identification.

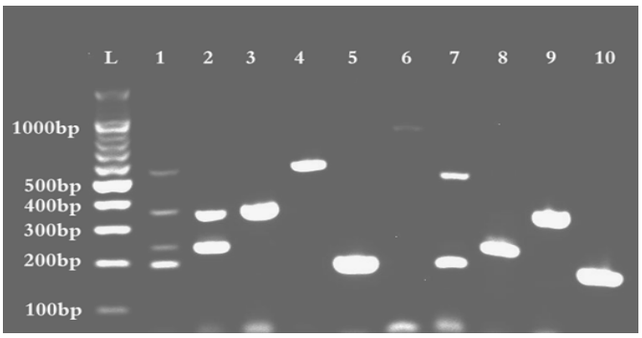
Detection of bacteria: Stool samples were plated on different freshly prepared and quality- controlled media (MacConkey, Xylose Lysine Desoxycholate (XLD) and sub cultured on nutrient agar following growth after 24 hours. Following 24 hours growth, different colonies from each media were selected as follows: Two to three colonies (black, medium and small size pink or clear) from XLD for suspected *Salmonella* spp and *Shigella* spp; 5-6 colonies (5-lactose and one non-lactose fermenters) from lactose MacConkey for different *E. coli* pathotypes including ETEC; both yellow and green colonies from TCBS for *Vibrio cholerae* and *Vibrio parahemolyticus* respectively, and one clear colony from Sorbital MacConkey to detect *E. coli* 0157:H7. Isolates confirmed as *E. coli* using TSI (A/A, -H_2S, ± gas), Simmon citrate (green), motile, and indole positive were confirmed for different pathotypes including ETEC by use of mPCR.

Multiplex Chain Reaction amplification of toxin genes for E. coli pathotypes: DNA extraction from *E. coli* strains was done by boiling the whole cell lysate. The complete multiplex PCR assay required 5 master mixes. For every toxin a master mix was made up of 15.2µl distilled water, 2.5µl of 10X PCR buffer, 1.5µl , 0.5µl dNTPs (25mM each nucleotide), primers, 0.3µl taq polymerase and 4 µl template DNA. Sterile distilled water and positive control strains of *E. coli* pathotype toxins were used as negative and positive controls, respectively. For amplification, the PCR program was set at 95^{0}C for 1min followed by 72^{0}C for 1 min for 5 cycles; 95^{0}C for 1 min followed by 62^{0}C for 1min and then 72^{0}C for 1 min for 20 cycles; and 72^{0}C for 5 min for final elongation of the amplified DNA product. The PCR products were then separated by agarose gel electrophoresis for 1hour. The DNA was visualized by exposing the gel to ultra violet light and photographed on alpha imager gel documentation machine.

Antibiotic susceptibility testing: The antibiotic susceptibility profiles were performed according to the Kirby-Bauer disk diffusion method. The following antibiotics were tested: ampicilin, ciprofloxacin, norfloxacin, nalidixic acid, amikacin, tetracycline, cefuroxime, cotrimoxazole, chloramphenicol and gentamycin. We used the standard strain of *E. coli* (ATCC 25922) for media quality and disk potency. In addition we performed Minimum Inhibitory Concentration for the same range of antibiotics.

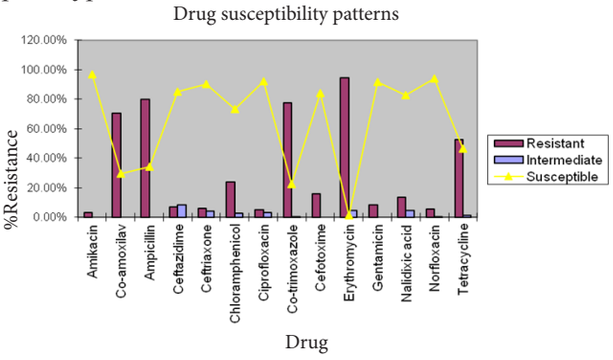
Results

Out of the 300 subjects recruited during the study period, it was observed that different pathotypes existed (Figure 1) and the isolates resisted more than four antibiotics (Figure 2) among them fluoroquinolones, cephalosporins and amino glycosides (Figure 2, Tables 1, 2 and 3).



Lanes L: 100 BP ladder, Lane 1; Pooled positive control, Lane 2: ST1 and ST2, Lane 3: LT,
Lane 4: CNF1, Lane5: eaeA, Lane6: negative control, Lane 7: ST2 and ipaH, Lane8: EAEC,
Lane 9: ST2, Lane 10: ST1.

Figure 1: MPCR amplification of different *E. coli* pathotypes toxins



Note: The figure shows increased susceptibility to gentamicin which has been resistant for more than ten years.

Figure 2: Drug susceptibility profiles on ETEC, other *E. coli* pathotypes and other enteric bacterial isolates from Machakos District Hospital

All *E. coli* pathotypes had raised MICs to different antibiotics ranging from cotrimoxazole to fluoroquinolones. However, there was reduced MICs to gentamicin.

Table 1: Minimum Inhibitory Concentration for 250 *E. coli* isolates

Drug	MIC 50	MIC 90	Ranges
STX	36	38	0.002 - 32
AM	16	16	0.16 - 256
GEN	4	6	0.16 - 256
CHL	24	64	0.16 - 256
CIP	0.08	0.16	0.002 - 4
CXM	16	16	0.16 - 256
CTX	32	32	0.16 - 32
CAZ	32	32	0.16 - 256
CRO	16	16	0.16 - 256
TET	8	24	1.5 - 256

Key: AMC= Augumentin, W= Trimethoprim SXT= Sulfamethoxazole, CN= Canamycin, CXM= Cefuroxime, CIP= Ciprofloxacin, AMP= Ampicilin, C= Chloramphenicol, NA= Nalidixic acid, TE= Tetracyclin, CAZ= Cefetazidine.

Shigellae isolates showed the highest raised minimum inhibitory concentrations compaired to ETEC and other *E. coli* pathotypes especially on cephalosporins ($P<0.05$, an indication of limited options in the treatment to reduce the shading of resistant strains from people infected with different *Shigellae* strains.

Table 2: Minimum Inhibitory Concentration ranges for shigellae isolates

Drug	MIC 50	MIC 90	Ranges
Contrimoxazole	>2/38	>4/38	0.002 - 32
Amikacin	>16	>16	0.16 - 256
Gentamicin	4	8	0.16 - 256
Chloramphenicol	>16	64	0.16 - 256
Ciprofloxacin	0.16	0.4	0.002 - 4
Cefuroxime	>16	>16	0.16 - 256
Cefotoxime	> 8	32	0.16 - 32
Ceftazidime	8	32	0.16 - 256
Tetracycline	32	64	1.5 - 256

The table shows raised minimum inhibition concentration to different antibiotics by 67 isolates of *Shigella* spp.

Table 3: Minimum Inhibitory Concentration ranges for 52 *Salmonellae* isolates

Drug	MIC 50	MIC 90	Ranges
Contrimoxazole	>2/38	>4/38	0.002 - 32
Amikacin	>16	>16	0.16 - 256
Gentamicin	4	8	0.16 - 256
Chloramphenicol	>16	64	0.16 - 256
Ciprofloxacin	0.16	0.4	0.002 - 4
Cefuroxime	>16	>16	0.16 - 256
Cefotoxime	> 8	32	0.16 - 32
Ceftazidime	8	32	0.16 - 256
Tetracycline	32	64	1.5 - 256

The table shows raised minimum inhibition concentration to different antibiotics by *Salmonella* spp.

All the isolates showed increased minimum inhibition concentrations at both MIC50 and MIC90 indicating that 50% or 90% of the microorganisms could be inhibited respectively. This indicates a possibility of limited choice in antimicrobial agents for management of bacterial diarrhoeal diseases especially in the curtailing of shedding of enteric pathogens.

Discussion

From the current study, multiple antimicrobial resistance patterns of the *Shigella* spp, *E. coli* and *Salmonella spp* to the drugs most frequently used in Kenya that is, amoxicillin / ampicillin, trimethoprim/sulfamethoxazole and chloramphenicol was observed. The results are similar to those from other studies [2,6-8]. However, the current study differs from other studies in that the isolates were sensitive to gentamycin while in the other four studies the organisms were shown to be resistant. The current study also found 4% resistance to ciprofloxacin whereas Sang *et al*. [7] and Kariuki *et al.* [2] reported zero resistance. The data also indicate that tetracycline, ampicillin, augmentin, trimethoprim/sulfamethoxazole and chloramphenicol are least likely to be effective in the treatment of infections due to diarrhoea in Machakos County. Similar observations have been reported from different regions in other parts of this country [2,6,7]. Similarly, high proportions of multidrug-resistant *E. coli, Shigella, Salmonella Klebsiella* isolates in children and adults have also been reported from Lagos and Ogun states in Nigeria [1,9], Accra region of Ghana [10] and Lima in Peru [11]. In contrast to the studies in Ghana, the Kenyan isolates were not only resistant to the drugs mentioned but also to quinolones and third generation cephalosporins.

Susceptibility testing of *E. coli* isolates revealed high resistance to the locally used antibiotics comparable to those shown by the closely related Shigellae. This underlines the usefulness of *E. coli* as a surveillance tool for infections due to gram negative bacteria. A good percentage of *E. coli* isolates 507/1450 (34.9%) from patients belonged to diarrhoeagenic strains and possessed virulent genes. More than half of the *E. coli* isolates in this study presented as normal stool *E. coli* since bacteria isolated from both children and adults as nonpathogenic *E. coli* were also resistant to the same antibiotics.

Moreover, both diarrhoeagenic and commensal resistant *E. coli* may constitute a potential reservoir for resistance genes that can be transmitted horizontally to other bacteria. The high proportions of resistant bacteria particularly those resistant to tetracycline, which is generally not used in children less than 5 years indicate the acquisition of resistant bacteria by the children rather than resistance induced through antimicrobial treatment. The findings from the current study agree with those from a population-based study, which indicate that children acquire resistant *E. coli* isolates from household contacts [12, 14]. Resistance to β-lactam antibiotics or chloramphenicol was observed more frequently among isolates obtained from infants below 5 years when compared with older children that were between 6-10 and 11-16 years. The association was statistically significant ($X^2 = 3.38$; $P<0.05$) and this group may be representative for the overall harboring of resistant enteric bacteria by children in the County. These data suggest that infants may acquire the commensal enteric flora from their parents who have likely been more exposed to antibiotics than their older children. Notably, a high prevalence of resistant *E. coli* has been shown for adults from other

parts of Africa [12-16]. With increasing age, the children may lose some of the resistant strains acquired originally. This view was supported by findings from older children between the ages of 11-16 years for whom *E. coli, Shigella* spp and *Salmonella* spp showed reduced resistance.

The data also indicate that the commonly used antibiotics can no longer be considered as first- line treatment options for infections due to *E. coli, Shigella* and *Salmonella* in people with diarrhoeal illness in Machakos County. In addition, 18/300 (6%) isolates produced extended-spectrum β-lactamases. Similar findings have also been reported in Cameroon by Gangoue [16], though their figures were 2% higher than those reported in the current study.

Conclusion and recommendations

Due to the high prevalence of resistance to most of the antibiotics used in Machakos County, their continued use may not be appropriate for the treatment of infections caused by *E. coli*, *Shigellae* or *Salmonella.* Other effective drugs should be identified and adopted for use on a regular basis. The data underlines the importance of regular antimicrobial surveillance in district hospital settings such as Machakos. Consequently, the required laboratory infrastructure and protocols for surveillance of resistance must be established, monitored, evaluated and sustained. Since over-the-counter sale of commonly used antibiotics without prescription is one of the contributing factors for the spread of resistance, the practice must be curtailed if the antibiotic arsenal available to physicians is to be effective.

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