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Research Article

β-lactamase Genes Produced by *E. coli* Isolated from Water and Stool Samples in Mthata Region Eastern Cape Province of South Africa

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Abstract

Objective

1. To determine the antibiotic susceptibility patterns of *E. coli* isolated from water and stool samples.
2. To check for the presence of β-lactamase genes responsible for resistant strains.

Materials and Methods

A study was undertaken to determine the antibiotic susceptibility patterns and presence of β-lactamase genes from *E. coli* isolated from water and stool samples submitted to Mtahata Government hospital from January 2012-December 2012.

Results

A total of 500 water samples and 150 stool samples were screened for *E. coli*. *E. coli* was found in 80% of the water samples and 32% of his stools samples. Of the water samples, 40% of the isolated strains were β-Lactamase positive and 21% from the stool strains respectively.

The non β-lactamase producing isolates were susceptible to most of the antibiotics including meropenem, imipenem, ciprofloxacin and gentamicin. The ESBL strains were only susceptible to meropenem, imipenem, ciprofloxacin and gentamicin. Table 1 shows the comparisons of the susceptibility profiles of *E. coli* from water and stool samples. The results show a similar pattern in susceptibility profile of strains from water and stool samples.

The molecular characterization revealed that 29% isolates possessed the CTX-M genes alone while the CTX-M and SHV genes together were found in 51% isolates. 5% isolates which were genotyped, contained all the 3 types (CTX-M, SHV, TEM), while SHV had 22% and TEM had 16%. 48% were from environmental isolates and 19% were from clinical isolates shown in Table 4.

Conclusion

The results implicate a cross contamination of water sources by feacal material or that the population is drinking contaminated water. The presence of ESBLs in the water sources is of concern as this could indicate a serious source of contamination for the population using these water sources for their households.

Keywords: *β-lactamase; E. coli; Isolated strains; Enteropathogens; Infections*

Introduction

The emergency and spread of multi-drug resistant enteropathogens has become a major concern among public health officials and the public at large due to the changes in the genomic characteristics of bacterial pathogens. According to Obi., *et al*. [1], antibiotics are known to shorten the duration of diarrhea, decrease stool output and reduce some complications caused by diarrhea.

Enteric bacteria are one of the organisms associated with diarrheal infections due to contaminated water sources and Escherichia coli is the most troublesome in the group [2]. It is also used as an indicator organism in assessing faecal contamination of water sources. The major striking epidemiological feature is the low number of bacteria that may trigger disease, as one needs some 100 cells of *E. coli* to cause a clinical illness compared to Salmonella (10^7-10^8 cells) [3].

Enteric bacteria are one of the organisms associated with diarrheal infections due to contaminated water sources and Escherichia coli is the most troublesome in the group [2]. It is also used as an indicator organism in assessing faecal contamination of water sources. The major striking epidemiological feature is the low number of bacteria that may trigger disease, as one needs some 100 cells of *E. coli* to cause a clinical illness compared to Salmonella (10^7-10^8 cells) [3].

E. coli has been reported as an emerging infection due to HIV infections [2,4]. Previously, *E. coli* was mainly associated with infantile diarrhea and it would be excluded if isolated from and adult who has diarrhea, laboratories would not do any further test or carry out the antibiogram, but recently, reports have shown that an ESBL strain has emerged and it has proven to be very difficult to eradicate using the β-lactams and there is therefore need to analyze and identify the strains form the stools before ruling it out as insignificant [5].

Studies over the past few years have documented that *E. coli* and in particular ETEC is usually a frequent cause of diarrhea in infants younger than 2 years. In some African countries, it was found to be the most common cause of diarrhea with 70% of infants accounting for most of the infections, while males were more infected than females [5]. Of the six seropathogens, VTEC is associated with ruminant animal in particular cattle thus contaminated beef becomes the source of human infections. However, a wide variety of the other sources have been implicated, ranging from unpasteurized milk, cheese, beef, mushrooms, sprouts and salami [6]. Water born transmission occurs through swimming in contaminated lakes, pools, or drinking untreated water.

Direct contact with animal fecal material through recreational activities and also person - person contact. EIEC, EPEC and DAEC are mainly linked to contaminated water and food, with the most common foods associated with hamburger meat and unpasteurized milk, while EAEC is mainly associated with infant foodstuffs and formulae, milk and water are also implicated [7].

Material and Methods

Water samples were collected from January-October 2012 from different water sources around Mthata and surrounding towns and villages. Stool samples were those submitted to NHLS Mthata from Jan-October 2012.

Culture and isolation

Colilert method involving the screening of feacal contamination and selective media and 20E API biochemical test were used as they are the methods used in the NHLS Mthata. Positive water samples were then cultured onto MacConkey agar at 37°C over night.

Stool samples were inoculated onto DCA at 37°C for 24hrs and then lactose fermenting colonies were picked and subcultured onto MacConkey Agar. Colonies from both water and stool samples were inoculated onto the 20E API strip for biochemical test and incubated over night and the results were read using the API reader. β-lactamase strips were used to screen for ESBL strains on all the colonies and serological tests were done to identify the six seropathogens of *E. coli.*

DNA isolation, PCR and pulsed gel electrophoresis was done on the isolates [8].

Results

In the study period, specimens obtained from 150 water sources and 50 stool samples yielded positive cultures for *E. coli* (Table 1) Of these samples 87.5% of the water sources were positive for *E. coli* and 60% from stool samples. While 67% of these isolates displayed decreased susceptibility to third-generation cephalosporins and cefoxitin. They were further tested for β-Lactamase production using β-Lactamase E test strips. All β-Lactamase producing strains were then selected for Ampc phenotype expression. 67 samples of *E. coli* β-actamase producing and 33 from non β-lactamases shown in Table 2 and 4.

Water sources **Sampled treated**	**Antimicrobial agents tested**										
	AMI	**AMP**	**ATM**	**CET**	**CTX**	**CIP**	**GEN**	**IMP**	**NAL**	**SUT**	**TC**
S	15	4	15	11	15	15	15	15	10	9	4
I	0	2	0	4	0	0	0	0	3	0	0
R	0	9	0	0	0	0	0	0	2	6	11
Community taps											
S	25	9	25	23	25	25	25	25	24	24	21
I	0	2	0	2	0	0	0	0	1	0	1
R	0	14	0	0	0	0	0	0	0	1	3
Open water sources											
S	22	9	22	17	22	22	22	22	7	4	5
I	0	0	0	1	0	0	0	0	0	2	0
R	0	28	0	4	0	0	0	0	15	16	17

Table 1: *Susceptibility profile of E. coli strains from water.*

MI: amikacin; GEN: gentamicin; IMP: imipenem; CET: cephalothin; CTX: cefotaxime; CIP: ciprofloxacin; ATM: aztreonam; AMP: ampicillin; NAL: nalidixic acid; SUT: sulfametoxazol-trimetoprim; TC: tetracycline

	+ve β-lactamase	**-ve β-lactamase**
Stool isolates	30%	70%
Water isolates	60%	40%
Water&stool isolates	67%	33%

Table 2: *ESβL/β Lactamase of bacterial isolates from human and environmental sources.*

***E. coli* isolates**	**Number of tested isolates**	**PCR products**		
		CTX-M	***SHV***	***TEM OXA negative***
Water isolates	65	18	18	12 0 17
Clinical Isolates	35	11	4	4 0 16
Total isolates	**100**	29	22	16 0 33

Table 4: *Distribution of TEM, SHV, OXA and CTX-M ESBL types among 100 isolates of E.coli.*

The molecular characterization revealed that 29% isolates possessed the CTX-M genes alone while the CTX-M and SHV genes together were found in 51% isolates. 5% isolates which were genotyped, contained all the 3 types (CTX-M, SHV, TEM), while SHV had 22% and TEM had 16%. 48% were from environmental isolates and 19% were from clinical isolatesas shown in Table 4.

The non β-lactamase producing isolates were susceptible to most of the antibiotics including meropenem, imipenem, ciprofloxacin and gentamicin. The ESBL strains were only susceptible to meropenem, imipenem, ciprofloxacin and gentamicin. Table 1 shows the comparisons of the susceptibility profiles of *E. coli* from water and stool samples and ESBL strains. The results show a that there is an increased resistance in water samples with 48% of the isolates showing presence of β-lactamase genes While 19% of the clinical isolates presented with the presence of β-lactamase genes also as shown in Table 4.

Discussion

The most obvious finding from this survey was that there was a high degree of heterogeneity among ESBL producing strains. The presence of these strains was not due to emergence of one specific clone, but seemed to be due to the spread of mobile genetic elements (e.g. plasmids and transposons) harboring $bla_{CTX\text{-}M15}$ as well as other β-lactamase resistance genes (bla_{SHV} and bla_{TEM}).

The emergence of plasmid mediated ESBLs among the members of Enterobacteriaceae have increased worlwide.the incidence of ESBLS has been noted to be on the increase and this study also showed a significant rise among the *E. coli* isolates from both water and clinical samples which confirmed with the β-lactamase colorimetric test used to check for the presence of β-lactamase production in the isolates [9-10].

The present study also showed that these β-lactamase producers were also resistant to other non beta-lactam antibiotics like fluroquinolones and aminoglycosides with a significant value of 0.001 as shown in Table 3. It was also interesting to note that specific ESBLs appeared to be present mainly in the water isolates. The data which is presented here demonstrates the presence of CTX-M, SHV, TEM genes in water and clinical samples which could mean cross contamination.

	Antimicrobial agents	ESBL producers	NonESBL producers	P. Value
1	Gentamicin	50.74%	20.00%	< 0.001
2	Amikacin	20.15%	10.31%	< 0.001
3	Ciprofloxacin	80.22%	60.50%	< 0.001
4	Ciphalothin	84.55%	50.80%	< 0.001
5	Chloramphenicol	56.23%	30.76%	< 0.05
6	Cefotaxime	50%	20.73%	< 0.001
7	Ampicillin	84.90%	56.51%	< 0.001

Table 3: *Antimicrobial resistance pattern among ESBL producers and non ESBL producers.*

Our findings emphasized the increasing roles of the multiple ESBLs genes in antibiotic resistance and this has led to the of an emperical treatment for the infections which are caused by coliforms, especially in immunocompromised individuals.

This study emphasized the alarming role of β-lactamases, especially ESBLs in antibiotic resistance in diarrheagenic *E. coli* strains. It gave us an insight into the current prevalence and genetic backgrounds of these strains.

Conclusion

It can be concluded from this study, that microorganisms especially *E. coli* have also been evolving towards an antimicrobial drug resistance and this has been considered as a major challenge in hospitals. The use of broadspectrum antibiotics such as quinolones, the second, third and fourth generation cephalosporins, beta-lactams combined with beta-lactamase inhibitors and hospitalization are some of the risk factors for the infections which are caused by ESBLs producing enterobacteriaceae.

The presence of the ESBL genes in water samples is of concern as most of the rural population use these water sources for household including as drinking water at most times.

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