Detection of ESBL-Producing *E. coli* Isolates from Selected Water Sources in Abakaliki, Nigeria

Ekeghalu Elsie Chidinma, PhD
Research and Statistics Unit, Administration Department
Alex Ekwueme Federal University Teaching Hospital Abakaliki
P.M.B 102 Abakaliki, Nigeria
Tel: +2437063429198
Tel: +2437018233935
Email:elsiehansom2@gmail.com

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Abstract

Recently, there has been concern that some strains of *Escherichia coli* can produce small proteins (enzymes) called extended-spectrum beta-lactamases (ESBLs). Hence, this study was designed to evaluate the ESBL production by multidrug resistant (MDR) *E. coli* isolates obtained from wells and borehole water samples using double disc diffusion synergy test (DDST). The resistance and susceptibility patterns of the isolates were determined by the Kirby and Bauer susceptibility test method. The results indicate that the *E. coli* isolates were highly resistant to Ceftazidine, Cefuroxime, Cefotaxime, Ceftriaxone and Amoxicillin/clavulanic acid which belong to the class Cephalosporins and Penicillins, thereby prompting the need for this test so as to ascertain if ESBL was responsible for their high resistance to the conventional antibiotics used. Out of the 36 isolates used for this study, only three isolates (8.3%) from the well water samples were positive for ESBL production. This study concluded that while the prevalence of ESBL producing E. coli isolates in these water samples is currently not very high, it may increase rapidly and may lead to a serious health problem, if not treated appropriately.

Keywords: Escherichia coli, extended spectrum beta lactamases, antibiotics, multidrug resistance, Double Disc Synergy Test

Introduction

This article has emerged from research that was conducted as a means of understanding the serious problem of water-borne disease outbreaks in Ebonyi State due to local conditions. The scientific explorations that follow in this article are from the author's doctoral research conducted in the period from 2015-2018. The author's research central to this article originally aimed at looking for ESBL-producing organisms in the water samples collected because they may cause antibiotic resistance in humans, thereby making treatment difficult. Therefore, the original research and this article are critically important for understanding factors around infectious disease prevalence. Such understanding is critical for microbiological scientific discoveries for the advancement of needed healthcare innovations and needed approaches to successful healing practices.

Beta-lactamases (β-lactamases) are enzymes produced by bacteria that provide multi-resistance to β-lactam antibiotics such as penicillins, cephamycins and carbapenems (ertapenem), although carbapenems are relatively resistant to beta-lactamase. Beta-lactamase provides antibiotic resistance by breaking the antibiotics' molecular structure. These antibiotics all have a common element in their molecular structure: a four-atom ring known as a β-lactam. Through hydrolysis, the lactamase enzyme breaks apart the β-lactam ring, deactivating the molecule's antibacterial properties. Beta-lactam antibiotics are typically used to treat a broad spectrum of Gram-positive and Gram-negative bacteria. Beta-lactamases produced by Gram-negative organisms are usually secreted when antibiotics are present in the environment (Neau, 1969).

ESBL (Extended Spectrum Beta-lactamases) are mostly produced by Gram-negative bacteria with *Escherichia coli* (some strains) and *Klebsiella* species being the most common ESBL producing bacteria (Abera et al., 2016). These enzymes breakdown commonly used antibiotics such as penicillins and cephalosporins and render them ineffective for treatment. The abbreviation--ESBL has now become common and describes resistance to β-lactams conferred through production of beta-lactamase enzymes which break down β -lactam antibiotic molecules (Sibhghatulla et al., 2015). If an ESBL-producing bacterium causes an infection, a different antibiotic may need to be used to treat the infection. People who carry ESBL-producing bacteria, without any signs or symptoms of infection, are said to be colonized. The most common ESBL-producing bacteria are some strains of *Escherichia coli* and *Klebsiella peumoniae*. ESBLs are spread via direct and indirect contact with colonized/infected patients and contaminated environmental surfaces. ESBLs are most commonly spread through unwashed hands of health care providers (PIC-NL., 2011)

The ESBLs are frequently plasmid encoded (Deepti *et al*., 2010). Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes (for example, aminoglycosides). Therefore, antibiotic options in the treatment of ESBL-producing organisms are extremely limited. Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms, yet carbapenem-resistant (primarily ertapenem resistant) isolates have recently been reported (P.H.A.C., 2010). ESBL-producing organisms may appear susceptible to some extended-spectrum cephalosporins. A number of studies have documented the presence of ESBL in food and water. These pathogens gain entry into humans by the faecal-oral route, with the common source being water contaminated with animal excreta and food contaminated with faecal pathogens (Walsh et al., 2011; Kluytmans et al., 2013).

It is essential that antibiotics are only used when necessary and, when they are needed, the full dose and full course of the antibiotic must be taken. This will help to reduce the number of bacteria that are becoming resistant to antibiotics.

Methods

Antimicrobial Susceptibility Studies

The resistance and susceptibility patterns of the isolates were determined by the Kirby and Bauer susceptibility test method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS), now the Clinical Laboratory Standard Institute (CLSI). An overnight culture of the test bacteria grown in nutrient broth (Oxoid, UK) was adjusted to 0.5 McFarland turbidity standards. The inoculum was aseptically inoculated on the surface of Mueller-Hinton (MH) agar plate(s) using sterile swab sticks. Fifteen (15) Single antibiotic disks from different classes were aseptically impregnated on the surface of the inoculated Mueller-Hinton agar. The antibiotics discs include tobramycin (10 µg), amikacin (30 µg), cefuroxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg) and cefotaxime (30 µg), imipenem (5 µg), meropenem (5 µg), ertapenem (5 µg), amoxicillin\clavulanic acid (30 µg), sulfamethoxazole\ trimethoprim (25 µg), nalidixic acid, (3 µg) ofloxacin (5 µg) and ciprofloxacin (5 µg) (Oxoid UK). The plates were incubated at 37°C for 18-24hrs, and the inhibition zone diameters (IZDs) produced by the antibiotic disks were measured with a meter rule and recorded and the inhibition zone diameter was compared to the standard breakpoints of the CLSI (CLSI, 2015).

Phenotypic Determination of ESBL Production by DDST

The *E. coli* isolates were highly resistant to Ceftazidine, Cefuroxime, Cefotaxime, Ceftriaxone and Amoxicillin/clavulanic acid which belong to the class Cephalosporins and Penicillins, thereby prompting the need for this test as to ascertain if ESBL was responsible for their high resistance to the conventional antibiotics used. The evaluation of ESBL production by the test isolates in this study was done using the double disc synergy test (DDST) as was previously described by Iroha et al. (2008).

Isolates suspected of producing ESBL after being screened with cephalosporins--ceftazidime (30µg), (30µg), and cefotaxime (30µg)--were swabbed on a Mueller-Hinton (MH) agar plates. A disk containing amoxicillin/clavulanic acid (30µg) was placed at the center of the MH agar plates and any of the above cephalosporins (ceftazidime and cefotaxime) was placed adjacent to the central disk at a distance of 15 mm. After an overnight incubation at 37°C, a ≥ 5 mm increase in the inhibition zone diameter for either of the cephalosporins tested in combination with the central disk versus its zone when tested alone confirms ESBL production phenotypically by the DDST method (Iroha et al., 2008).

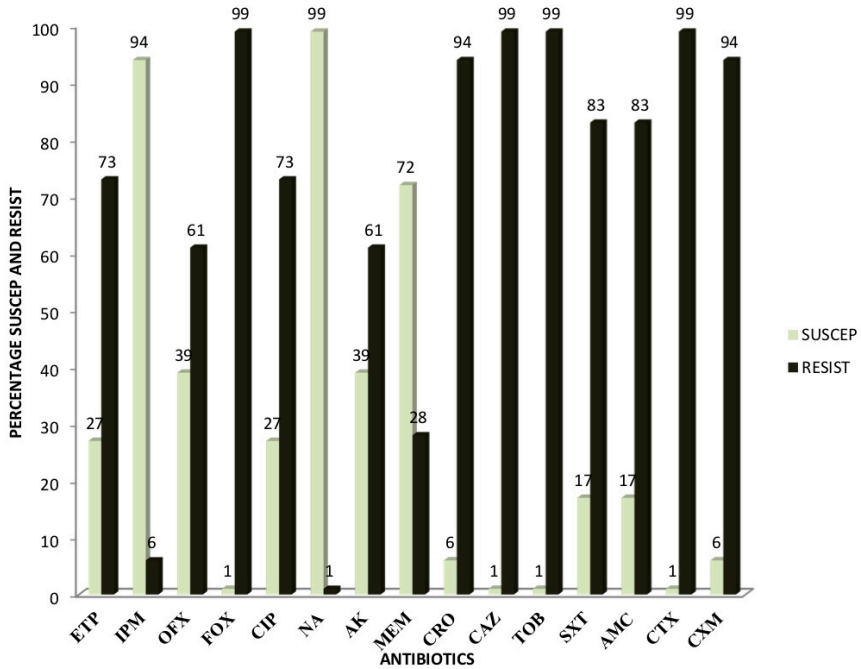
Isolation, Identification and Characterization of Escherichia coli

Pour plate method was used to determine the total viable count of bacteria. Serial dilutions of the water samples (Boreholes and Well water) were carried out aseptically up to 10^4 dilutions. Dilutions were plated out for enumeration and isolation of bacteria on nutrient agar and were

incubated for 18-24 hrs at 37°C. Colony growth were sub-cultured to obtain pure culture and were plated out on different selective media such as Eosin methylene blue (EMB), MacConkey agar and Cystine Lactose Electrolyte Deficient agar for further characterization and identification (Chessbrough, 2006).Standard microbiological techniques were used to identify and characterize the bacteria isolates which includes the following: gram staining, motility test, catalase test, Voges-Proskauer test, indole test, oxidase test and sugar fermentation test.

Results

E. coli is one of the most common ESBL producing bacteria. Because the *E. coli* in the collected samples was highly resistant to the antibiotics used in this study (especially cephalosporins), the isolates from both water samples were screened for ESBL production. Three isolates from the well water samples produced ESBL and none from the borehole water samples. The following figure and two tables from the study provide highlighted detail for these results.



KEY: ETP = Ertapenem, IPM = Imipenem, OFX = Ofloxacin, FOX = Cefoxitin, CIP = Ciprfloxacin, NA = Nalixidic acid, Ak = Amikacin, MEM = Meropenem, CRO = Ceftriazone, CAZ = Ceftazidime, TOB = Tobramycin, SXT = Trimethroprim/Sulfamethoxazole, AMC = Amoxicillin/clavulanic acid, CTX = Cefotaxime and CXM = Cefuroxime.

Figure 1. Antibiotics susceptibility pattern of E. coli isolated from well water samples collected from different locations in Abakaliki Metropolis.

Table 1. Frequency of isolation of ESBL producing E. coli from borehole water samples collected from different locations within Abakaliki Metropolis.

SAMPLE CODE	ESBL NEGATIVE	ESBL POSITIVE
AB_3	-	
AB_2	-	
AB_3	-	
AGB_1	-	
AGB_2	-	
KPB_1	-	
KPB_3	-	
UB_1	-	
UB_2	-	
UB_3	-	
UB_4	-	

KEY: AB = Azuiyiokwu, KPB = Kpirikpiri, UB = Udensi, AG=Aguogboriga

Table 2. Frequency of isolation of ESBL producing E. coli from well water samples collected from different locations within Abakaliki Metropolis.

SAMPLE CODE	ESBL NEGATIVE	ESBL POSITIVE	SAMPLE CODE	ESBL NEGATIVE	ESBL POSITIVE
AW_1		+	KP W_1		+
AW_2		+	KP W_2	-	
AW_3	-		KP W_3	-	
AW_4	-		KP W4	-	
AW_5	-		U W_1	-	
AW_6	-		U W_2	-	
AW_7	-		U W_3	-	
AW_8	-		U W_4	-	
AW_9	-				
AW_{10}	-				
AW_{11}	-				
AW_{12}	-				
AW_{13}	-				
MW_1	-				
MW_2	-				
MW_3	-				
MW_4	-				
MW_5	-				

Key: AW = Azuiyiokwu well water, MW = Mile 50 well water, KPW = Kpirikpiri well water,

UW = Udensi well water

Discussion

Of the 36 *E. coli* isolates from both borehole (11) and well (25) water samples, only three (3) isolates from well water samples were ESBL positive while others were ESBL negative. The decreased susceptibility of ESBL producing *Escherichia coli* to the tested antibiotics may be due to the multidrug resistance gene in plasmids that they are harbouring (Rooney et al., 2009). However, the non ESBL producing *Escherichia coli* were also significantly ($P<0.05$) resistant to the various antibiotics tested. All ESBL positive *E. coli* strains were resistant to cefotaxime, ceftazidime and ceftriaxone. This result obtained in this study is in agreement with the study done by Islam et al.(2014) and Sharma et al.(2013) that also identified *E.coli* isolates which were resistant to cefepime, cefotaxime, ceftazidime and ceftriaxone but remained susceptible to imipenem. This study is also in line with the study of Sompolinsky et al. (2015) whose findings also showed that identified *E. coli* isolates were resistant to cefepime, cefotaxime, ceftazidime and ceftriaxone and after performing phenotypic confirmation test on these isolates, they were confirmed ESBL producers.

ESBL positive isolates also showed high degree of resistance to other antibiotics like cefoxitin, cefuroxime, nalidixic acid, ciprofloxacin and ofloxacin. ESBL positive strains being resistant to cefoxitin is abnormal, as they may be harboring ESBL and Amp-C, where Amp-C is more expressed than ESBL, they become resistant to cefoxitin. Antimicrobial resistance surveillance done by Nepal Public Health Laboratory (NPHL) found that ESBL *E. coli* were susceptible to imipenem (98.5 %), and amikacin (96.1 %). High percentages of isolates were susceptible to the carbapenems. The study done by Kader and Angamuthu (2005) revealed more than 89 % of the ESBL producers were susceptible to imipenem and meropenem, however this study is slightly in contrast to the reports of Mekki et al. (2010) who found 100 % MDR *E.coli* isolates to be sensitive to the carbapenems.

The isolation of *E. coli* expressing ESBL enzyme should be considered as a signal of an urgent need for proper sanitary method and inspection of all water producing municipal stations to enforce that water production is according to WHO standards (2006). This will help in eradicating the existence of these resistance organisms that could lead to a very serious public health problem in the near future. ESBL producing organisms are known worldwide to harbor multidrug resistance genes in plasmids, which confer resistance to wide range of antibiotics. The majority of these wells are poorly constructed and sited near pit latrines as we observed making them vulnerable to contamination. The presence of *E.coli* detected in the various well water sources could possibly be due to heavy contamination of faecal bacteria origin emanating from heavy rainfall patterns resulting into floods and indiscriminate disposal of garbage which poses a potential public health hazard (Ramphal and Ambrose, 2006).

Conclusion

The results showed that *E. coli* isolates concealing ESBL enzymes are multi-drug resistant and may have substantial therapy challenges. Organisms may easily transfer ESBL-containing plasmids to other organisms because bacteria readily exchange drug resistance plasmids amongst themselves, the 8.3% ESBL samples from wells could swiftly increase if given the opportunity, such as in a flood. Results of antibiotic sensitivity tests in our study revealed that ESBL-producing isolates were more resistant to certain members of cephalosporins, floroquinolones and aminoglycoside antibiotics than carbapenems. Antibiotic resistance has been reported

in different parts of the world where *E. coli* was found to be resistant to all fluoroquinolones, aminoglycoside and some beta lactam antibiotics (Pruss et al., 2002).

In conclusion, the result of this study confirmed the presence of ESBL producing *E. coli* isolates that are multi-drug resistant and thus, are difficult to treat also while the prevalence of ESBL producing *E. coli* isolates in these water samples is currently not very high, it may increase rapidly and may lead to a serious health problem, if not treated appropriately. This is an important screening procedure for water safety in communities. It will inform proper placement of wells and latrines.

Photo by: Uncle Udeaku 2016

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