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Original Article

Phenotypic Characterization of ESBL-Producing *Escherichia coli* from Animal Feacal Dung

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ABSTRACT

The beta-lactams is a large group of antibiotics that contain more than half of the drugs used in clinical medicine for the treatment of bacterial infections in humans, but the antimicrobial efficacy of this potent class of drugs is threatened by the production of beta-lactamases by pathogenic bacteria. Extended spectrum beta lactamases (ESBLs) are beta-lactamase enzymes that have the ability to hydrolyze and cause resistance to the cephalosporins and monobactams with the exception of carbapenems, clavulanic acid and cephamycins. A total of 300 environmental samples comprising of 150 feacal swab samples and 150 intestinal swab samples recovered from the carcass of animals slaughtered in different abattoirs in Abakaliki metropolis were phenotypically investigated for ESBL production by the double disk synergy test (DDST) method using cephalosporin/clavulanic acid combination disk as recommended by NCCLS. Seventy-one (71) isolates of *Escherichia coli* were recovered from the samples; and these showed varying rates of susceptibility and resistance to some available antibiotics. The *E. coli* isolates were highly resistant to cefotaxime, ceftazidime, sulphamethoxazole-trimethoprim, ciprofloxacin and ofloxacin. However, they were highly susceptible to imipenem and cefoxitin. Out of the 71 *E. coli* isolates, only 32 isolates of *E. coli* (45.1 %) were confirmed to produce ESBLs phenotypically by the DDST method used in this study. Our study presumptively shows that ESBL-producing *E. coli* is frequent in animal dung. Further sequencing studies and PCR analysis is required to characterize the ESBL phenotypes found in this environment. The detection of ESBL from both clinical and environmental samples is crucial in order to forestall any disease outbreak due to them.

1. INTRODUCTION

The increasing and irrational use of the 3rd-generation cephalosporins including ceftazidime, ceftriaxone and cefotaxime and other antibiotics in animal husbandry and other veterinary purposes may be linked to the current emergence and spread of extended spectrum beta-lactamase(ESBL) enzymes in the community. ESBLs are beta-lactamase enzymes that are produced

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by both Gram positive and Gram-negative bacteria, and which hydrolyze 3rd-generation cephalosporins and aztreonam with the exception of beta-lactamase inhibitors such as clavulanic acid.[1,2,3] The production of ESBLs is amongst the main resistance mechanisms that allow microbes to defy the antimicrobial onslaughts of some available drugs. The emergence and widespread distribution of pathogenic bacteria producing multidrug resistance enzymes inclusive of ESBLs in the community is well recognized, and a threat to the effective treatment and management of a variety of bacterial-related diseases.[4,5,6] Antimicrobial resistance is a global health phenomenon that is responsible for significant level of morbidity and mortality in both the community and health institutions and this development has made it difficult for physicians to effectively treat some microbial infections.[7,8,9] ESBLs are chiefly produced by pathogenic bacteria in the *Enterobacteriaceae* family including *Escherichia coli, Klebsiella pneumoniae*, and *Klebsiella oxytoca*; and they can also be produced by non-enteric bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[3,7,10,11] Feacal carriage is an important factor in the spread of ESBLs bacteria among human and animal populations.[12,13] Studies have shown that the occurrence and dissemination of ESBLs in food production units occurred through the feacal cross-contamination between individuals and animals, and food contamination may also occur during meat processing in abattoirs or poor handling of meat and food meant for human consumption in animal farms.[4,14,14,15] The shedding of pathogenic *E. coli* harbouring or producing ESBLs in animal feaces is an important factor for the dissemination of this all important enzymes in animal slaughter houses, and this could serve as routes via which susceptible human population could become infected. Thus, this study evaluated by phenotypic characterization method the production of ESBLs in *E. coli* isolated from animal dungs in Abakaliki metropolis, Ebonyi State, Nigeria.

2. MATERIALS AND METHODS

Study Area: This study was carried out in Abakaliki metropolis, Ebonyi State, southeastern Nigeria. Feacal swabs (n=150) and intestinal swabs (n=150) from the carcass of animals slaughtered in different abattoirs in Abakaliki metropolis were recovered and transported to the laboratory unit of Applied Microbiology Department, Ebonyi State University, Abakaliki where they were analyzed by standard microbiological techniques. Oral consent was sought from the authorities of the abattoirs for sample collection.

Isolation and characterization of *Escherichia coli*: The feacal swab samples (n=300) were cultured on MacConkey agar (MAC), Eosin methylene blue (EMB) agar and cystein lactose electrolyte deficient (CLED) medium for the selective isolation of *E. coli*. All media were purchased from Oxoid Limited (Oxoid, UK). Suspect colonies of *E. coli* were subcultured onto freshly prepared MAC, EMB and CLED medium, and then purified on nutrient agar plates. The *E. coli* isolates were identified using standard microbiological identification techniques.[16]

Detection of ESBLs by Double Disk Synergy Test (DDST) method: ESBL production was detected by the DDST method as was previously described.[5,7,11,17,18] Briefly, antibiotic disks of amoxycillin-clavulanic acid (20/10 µg) were placed at the center of a Mueller-Hinton agar plate previously inoculated with the test isolate. And single antibiotic disks containing cefotaxime (30 µg) and ceftazidime (30 µg) were each placed adjacently at a distance of 15 mm away from the center disk. The plates were incubated at 37^{o}C for 18-24 hr. And ESBL production was inferred phenotypically when the zones of inhibition of the cephalosporins (cefotaxime 30 µg and ceftazidime 30 µg) were expanded by the amoxycillin-clavulanic acid

disk. A ≥ 5 mm increase in the inhibition zone diameter for either of the cephalosporins (ceftazidime or cefotaxime) tested in combination with amoxycillin-clavulanic acid versus its zone when tested alone confirms ESBL production phenotypically.[2,5,10]

Antibiogram: Antimicrobial susceptibility studies was carried out on Mueller-Hinton agar plates using the Kirby-Bauer disk diffusion technique in line with the criteria of National committee for Clinical Laboratory Standard (NCCLS), now Clinical Laboratory Standard Institute, CLSI.[19] The antibiotics used include cefoxitin (FOX 30 µg), ceftazidime (CAZ 30 µg), cefotaxime (CTX 30 µg), imipenem (IPM 30 µg), amikacin (AK 30 µg), sulphamethoxazole-trimethoprim (SXT 30 µg), tetracycline (TE 30 µg), ciprofloxacin (CIP 10 µg), and ofloxacin (OFX 30 µg) (Oxoid, UK). All test plates were incubated at 37^{o}C for 18-24 hr, and the zones of inhibition (IZDs) were measured to the nearest millimeter using a meter rule and recorded as per the guideline of NCCLS.

3. RESULTS

Out of the three hundred (300) samples comprising of 150 feacal swabs and 150 intestinal swabs recruited for this study, a total of 71 *Escherichia coli* isolates were isolated from the samples. Overall, 40 *E. coli* isolates were recovered from intestinal swabs (13.3 %) while feacal swabs produced only 31 *E. coli* isolates (10.3 %) as shown in Table 1. The results of the antimicrobial susceptibility studies are shown in Table 2. The *Escherichia coli* isolates produced varying rates of susceptibility and resistance to the test antibiotics. However, all the *E. coli* isolates were found to be resistant to sulphamethoxazole-trimethoprim (SXT) and tetracycline while imipenem was the most active antibiotic against the test *E. coli* isolates. And this was followed by amikacin and cefoxitin (Table 2). Ceftazidime and cefotaxime (both 3rd-generation cephalosporins) were poorly active against the *E. coli* isolates. A percentage susceptibility of 2.8 % was recorded for both ciprofloxacin and ofloxacin against the test isolates. The *E. coli* isolates were highly resistant to sulphamethoxazole-trimethoprim, ciprofloxacin, ofloxacin, tetracycline, cefotaxime and ceftazidime (Table 2).

Table 1: Source distribution of *Escherichia coli* isolates from the feacal samples

Organism	Intestinal Swab (n=150)	Feacal swab (n=150)
Escherichia coli	40 (13.3 %)	31 (10.3 %)

Table 2: Results of antimicrobial susceptibility studies

Antibiotics (µg)	No. Susceptible (%)	No. Resistant (%)
Cefoxitin	34 (47.9)	37 (52.1)
Ceftazidime	1 (1.4)	70 (98.6)
Cefotaxime	1 (1.4)	70 (98.6)
Imipenem	68 (95.8)	3 (4.2)
Amikacin	18 (25.4)	53 (74.6)
SXT	0 (0)	71 (100)
Tetracycline	0 (0)	71 (100)
Ciprofloxacin	2 (2.8)	69 (97.2)
Ofloxacin	2 (2.8)	69 (97.2)

SXT = sulphamethoxazole-trimethoprim

Out of the 71 *Escherichia coli* isolates, only 70 isolates were suspected to produce extended spectrum beta-lactamase (ESBL) enzymes (due to their low susceptibility to the cephalosporins ceftazidime and cefotaxime) by the screening test as recommended by NCCLS guidelines (Table 2). The results of our ESBL confirmatory test showed that only 32 *Escherichia coli* isolates out of the 71 *E. coli* isolates recovered from the test samples produced ESBL enzymes phenotypically (Table 3).

Table 3: Frequency of ESBL production among *E. coli* isolates

S/No	Frequency	Percentage (%)
ESBL Positive *E. coli*	32	45.1
ESBL Negative *E. coli*	39	54.9

Total	71	100

4. DISCUSSION

Bacterial organisms producing extended spectrum beta-lactamase (ESBL) enzymes has continued on the increase in both the hospital and community settings, and failure to detect these enzymes has also contributed to some of the failures recorded with the use of the cephalosporins for treatment purposes. The production of ESB by *Escherichia coli* and other enteric and non-enteric bacteria has continued to jeopardize the efficacy of some available drugs. In this study, 300 samples comprising of 150 feacal swabs and 150 intestinal swabs were investigated phenotypically for *Escherichia coli* isolates that produce ESBLs. The *E. coli* isolates were highly resistant to ceftazidime and cefotaxime. Similar resistance profiles of *E. coli* to the cephalosporins have also been reported elsewhere.[12,20,21,22] This present day study has revealed that 45.1 % of the 71 *Escherichia coli* isolates recovered and identified from non-clinical specimens (intestinal and feacal swabs) in Abakaliki metropolis, Ebonyi state of Nigeria were ESBL producers. The rate of ESBL production in the test organism though high, is in conformity with the work of Duru *et al*., [12] who in their recent study showed that 22.2 % of *E. coli* from poultry origin produces ESBLs. The prevalence of ESBL-producing *E. coli* has also been reported in the community elsewhere.[15] The prevalence of ESBL-producing bacteria including *E. coli* is an indication of the uncontrolled usage of the cephalosporins.[22] The use of antibiotics meant for human medicine in livestock production and animal husbandry also do not help matters as this gives room for some organism to develop resistance to them due to selective pressure. Conclusively**,** the results of this study has shown a high prevalence of the ESBL production amongst *Escherichia coli* isolates recovered from intestinal and feacal swabs of animals in this environment (Abakaliki metropolis, Nigeria). Since ESBL producing bacteria are important emerging nosocomial and community pathogens, it is therefore vital to screen both community and hospital pathogens for the production of these enzymes so as to contain any disease outbreak due to them.

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