

Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Strains of Poultry Origin in Owerri, Nigeria

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Abstract: Extended spectrum β -lactamase (ESBL) producing *Escherichia coli* infections are a major clinical problem that has attained a global health concern. This study aimed to determine the presence of extended-spectrum β -lactamase (ESBL) - producing *E. coli* strains from poultry farms. A total of 45 *Escherichia coli* isolates recovered from 159 fecal samples of turkey, broiler and local fowls from 6 poultry farms in Owerri, southeast Nigeria were tested for antibiotic susceptibility and ESBL production by the double disk synergy test according to Clinical Laboratory Standard Institute guidelines. Location of resistant determinants of the ESBL isolates was established by plasmid curing and conjugation studies. ESBL was detected in 22.2% of *E. coli* isolates. The ESBL *E. coli* isolates were completely susceptible to imipenem, but resistant to cefepime, ceftriaxone, nalidixic acid, cefotaxime, ceftazidime, ampicillin, sulphamethoxazole-trimethoprim and ticarcillin. The ESBLs *E. coli* strains were all chromosomally-mediated indicating deficient transfer of antibiotic resistance traits to non-ESBL organisms. This study demonstrated the occurrence of ESBL *E. coli* strains in poultry and indicated an established reservoir in farm animals. Our finding warrants further molecular studies to prevent further transmission of ESBL organisms in this region.

Key words: Poultry • ESBLs • Antibiotic Susceptibility • *Escherichia coli* • Nigeria

INTRODUCTION

The increasingly usage of antimicrobial agents for both human and non-human purposes (e.g. in animal feedstock) in the hospital and community settings has over the times imposed selective pressure on bacterial organisms. This may have fostered the development of resistance to many readily available conventional antibiotics used for the treatment of infectious diseases. Extended-spectrum β -lactamases (ESBLs) are rapidly evolving group of plasmid-mediated β -lactamase enzymes with the ability to hydrolyze and cause resistance to oxyimino 3rd-generation cephalosporins (e.g. cefotaxime and ceftazidime) and monobactams [1-3]. ESBLs which are inhibited by β -lactamase inhibitors such as clavulanic acid were first described in the early 1980s and they are frequently produced by Gram negative bacteria especially the *Enterobacteriaceae* including *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* [1].

The production of ESBLs in pathogenic bacteria can be chromosomal in origin or plasmid-mediated and those that are plasmid-mediated can be transferred from one organism to another through means of genetic transfer such as conjugation [4]. The emergence and spread of ESBL producing bacteria in both the community and hospital settings is of importance since these pathogens are usually multidrug resistant, demonstrating resistance to β -lactams and non- β -lactams (e.g. fluoroquinolones and aminoglycosides) [5]. ESBLs have been increasingly reported worldwide and patients harboring ESBL producing bacteria are at risk of ineffective antibiotic therapy and increased morbidity and mortality [1, 2, 5-7]. Owing to the increased use of antibiotics in poultry farming and animal husbandry, there is high likelihood of zoonotic infections in humans as a result of resistant pathogens transferred to humans either directly or indirectly [8]. The use of antibiotics in poultry and livestock purposes affects human health due to the

development of drug resistant microbes which can be transferred from poultry to humans. Secondly, residues of drugs in poultry birds may be ingested by human who predisposes to development of antibiotics resistance in the society [9]. Presence of ESBL organisms in poultry farms portend great danger in livestock and humans in particular since the importation of ESBL-producing bacterial strains through this route has the potential to cause widespread dissemination of antibiotic resistant pathogens which cause community-acquired infections [10, 11]. The emergence and spread of ESBLs and other forms of antibiotic resistance is a global threat to optimal patients care; and the unregulated use of antibiotics is a contributing factor to this menace [12]. The primary objective of this study is to evaluate the prevalence of ESBL producing *E. coli* strains in poultry farms in Owerri, Nigeria.

MATERIALS AND METHODS

This study was approved as part of Master's program by the Postgraduate Committee of the School of Pharmaceutical Sciences and Microbiology and Ethics Committee of Anambra State University Teaching Hospital, Awka, Nigeria. Proprietors of six poultry farms in Owerri, Nigeria voluntarily participated in the study.

Collection of fecal Samples: Fecal samples (159) were obtained from six (6) poultry farms using sterile swab sticks during the period (between January and September of 2012). Samples were cultured on Eosin Methylene Blue agar, Nutrient agar, MacConkey agar and Cystein lactose electrolyte deficient medium (Oxoid, UK) in triplicates. Suspected isolates were identified using standard microbiology techniques as described previously [13].

Antibiogram: Antibiogram was evaluated as per the Clinical Laboratory Standard Institute (CLSI) guidelines [14] using ofloxacin (OFX-5 µg), cefepime (FEP-30 µg), sulphamethoxazole-trimethoprim (SXT-25 µg), amoxicillin-clavulanic acid (AMC-30 µg), ceftazidime (CAZ-30 µg), ceftriaxone (CRO-30 µg), ampicillin (AMP-10 µg), imipenem (IMP-10 µg), nalidixic (NA-30 µg) and ticarcillin (TIC-30 µg) (Oxoid, UK)14. *Escherichia coli* ATCC 25922 was used as the Quality Control Strain for antibiotic susceptibility testing.

Phenotypic Detection of Extended-spectrum Beta-Lactamase (ESBL) Isolates: Isolates that showed reduced susceptibility to any of the indicated cephalosporins

using ESBL screening breakpoints (ceftazidime ≤ 22 and cefotaxime ≤ 27) were considered possible ESBL producers and were further tested for phenotypic ESBL confirmation. ESBL production was confirmed phenotypically by the double disk synergy test (DDST) method as described by Zhang *et al.* [15]. Overnight suspension of the test bacteria adjusted to 0.5 McFarland turbidity standards was swabbed aseptically on a Mueller Hinton (MH) agar (Oxoid, UK) plate using sterile swab sticks. A combination disk of amoxicillin-clavulanic acid, AMC (20/10 µg) was placed at the center of the MH agar plate and cefotaxime (30 µg) and ceftazidime (30 µg) were placed on either sides of the central disk (AMC-20/10 µg) at a distance of 15 mm. The plates were incubated for 18-24 h at 37°C. After incubation, a ≥ 5 mm increase in zone diameter for either of the cephalosporins (CAZ and CTX) tested in combination with AMC (20/10 µg) compared to its zone when tested alone confirms ESBL production in the test bacteria [15].

Plasmid Curing Experiment: Curing experiment was undertaken to determine the location (plasmid or chromosomal) of resistant determinants in our ESBL positive strains as described previously [16]. Briefly, overnight cultures of the ESBL positive strains were grown in double strength MH broth supplemented with 0.5 mg/ml of the mutagen acridine orange (Merck, Germany) at 37°C for 24 h. After incubation, each mutagen exposed isolate was seeded on drug-free MH agar plates and these were incubated again for 24 h at 37°C. Three colonies were aseptically picked using sterile swab sticks and tested for loss of antibiotic resistance by exposure to antibiogram studies.

Conjugation Studies: Conjugation test was carried out as previously described [16]. ESBL positive strains (donor) and ESBL negative *K. pneumoniae* strains (recipient) were grown separately in MH broth at 37°C for 24 h. The donor strains and recipient strains (which are susceptible to resistance markers that included: SXT, CN, CIP and AMP) were then mated in a ratio of 1:10 (donor to recipient respectively) and these were grown in MH broth at 37°C for 3 h. Samples of this mixture was plated aseptically on MacConkey agar plates (which served as the selection plate) and incubated at 37°C for 24 h. Transconjugates growing on the selection plate were subjected to susceptibility studies to confirm the transfer of resistance markers. Samples from donor and recipient strains were used as control.

Table 1: Fecal samples and *Escherichia coli* isolates

Source	No. of fecal sample	No of <i>E. coli</i> isolates	ESBL positive
Turkey	48	9	1
Broiler	96	36	9
Local fowl	15	0	0
Total	159	45	10

RESULTS

Of the 159 samples processed, 48 were from turkey, 96 from broiler and 15 fecal samples from local fowls (free grazing fowls) (Table 1). All fecal samples were obtained from six (6) different poultry farms in Owerri, Imo state, Southeast Nigeria. A total of 45 *Escherichia coli* strains were isolated from the fecal samples. (Table 1).

Detection of ESBL: Ten or 22.2% of the *E. coli* isolates were ESBL producing *E. coli*. ESBL was detected in nine broiler fowls and one turkey. None was isolated from free grazing domestic fowl.

Antibiotics Susceptibility Studies: All isolates were susceptible to imipenem. This was followed by ofloxacin (80%), cefepime (33.3%), amoxycillin-clavulanic acid (26.7%) and nalidixic acid (26.7%) (Figure 1). Imipenem, a carbapenem was the most effective agent against all our ESBL *E. coli* isolates. This was followed by ofloxacin (70%). High resistance was recorded against ticarcillin (100%), nalidixic acid (100%), ceftazidime (100%), cefotaxime (100%), amoxicillin/clavulanic acid (90%), ceftriaxone (90%) and cefepime (90%) (Figure 2).

Conjugation Studies: There was no loss of antibiotic resistance in the *E. coli* positive ESBL isolates when they were exposed to acridine orange curing (0.1 mg/ml). All retained relatively high resistance rates to the tested antibiotics. This therefore suggests a possible chromosomal location of all recorded *E. coli* ESBL phenotypes since chromosomally located genetic determinants cannot be transferred through non-replicating bacteria by conjugation.

DISCUSSION

Bacterial organisms producing extended spectrum β -lactamases (ESBLs) in human populations have been studied for over three decades and their presence in animal population have also been recently reported [5, 8, 11]. In this study, a total of 159 fecal samples from turkey, free grazing and broiler fowls were investigated for the presence of ESBL positive *E. coli*. The antimicrobial susceptibility studies of all the 45 *E. coli* isolates showed that imipenem was the most active antibiotic showing 100% susceptibility on all the isolated strains of *E. coli*. Surprisingly, sulphamethoxazole-trimethoprim, ticarcillin, ceftazidime and ampicillin were the least active agents as they showed no antibacterial effect against the *E. coli* isolates. Cefepime, amoxicillin/clavulanic acid, nalidixic acid and ofloxacin demonstrated susceptibility of 33.3, 26.7, 26.7 and 80 percent against the *E. coli* isolates respectively (Figure 1). Previous reports in Turkey and Italy indicated that *E. coli* isolates of fecal animal origin

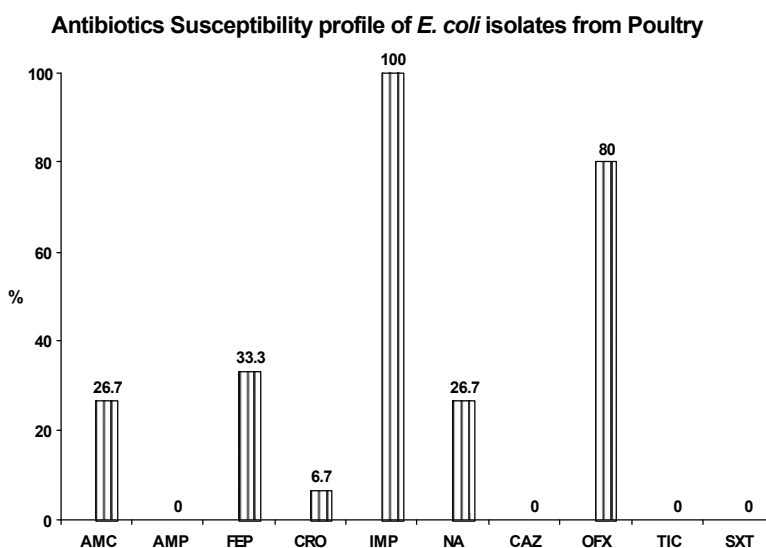


Fig. 1: Antibiotic susceptibility profile of *E. coli* isolates from poultry.

AMC-Amoxycillin-clavulanic acid, AMP-ampicillin, FEP-cefepime, CRO-ceftriaxone, IMP-imipenem, NA-nalidixic acid, CAZ-ceftazidime, OFX-ofloxacin, TIC-ticarcillin, SXT-sulphamethoxazole-trimethoprim

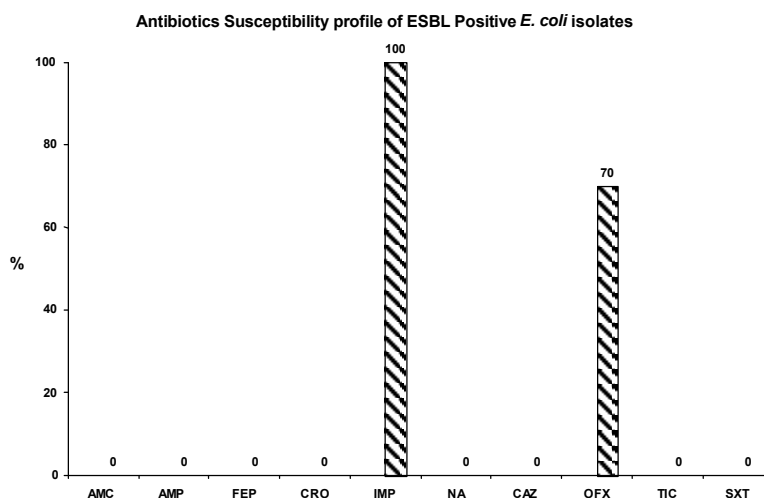


Fig. 2: Susceptibility profile of *E. coli* positive ESBL Isolates

AMC-Amoxycillin-clavulanic acid, AMP-ampicillin, FEP-cefepime, CRO-ceftriaxone, IMP-imipenem, NA-nalidixic acid, CAZ-ceftazidime, OFX-ofloxacin, TIC-ticarcillin, SXT-sulfamethoxazole-trimethoprim

are resistant to commonly used antibiotics such as ampicillin, sulphamethoxazole-trimethoprim, fluoroquinolones and some cephalosporins [8, 12]. In another development, Oyinloye and Ezekiel [17] and Girlich *et al.* [18] reported that *E. coli* isolates from poultry origins in southwestern Nigeria and France respectively are resistant to cephalosporins, fluoroquinolones and aminoglycosides [17,18]. These results are similar to the findings of this study.

The use of antibiotics in livestock and poultry production has also been reported in studies from Spain, USA and Nigeria [6, 8, 19]. This practice however, could be the reason for the rising incidence of antibiotic resistant bacteria in the community because resistant pathogens can be passed on to humans following the consumptions of these animals.

This study demonstrated the presence of ESBL in fecal strains of *Escherichia coli* from six (6) poultry farms in Owerri, south eastern Nigeria. Overall, 10 out of the 45 *E. coli* isolates were found to be ESBL positive by the double disk synergy test (DDST). ESBL was detected in 9 broiler fowls and 1 turkey. The occurrence of ESBL in this study (22.2%) is similar to another study conducted in southwestern Nigeria that reported 18% ESBL positive *E. coli* isolates of poultry origin [17]. Also in France, 12 *E. coli* isolates that were recovered over a four month period were found to produce ESBL [18]. Another study in southeast Nigeria (Enugu state precisely) reported 9.4% ESBL production amongst *E. coli* isolates from chicken [9]. Higher frequency of ESBL production has also been reported in poultry farms in Czech Republic [20].

The results of this study and other previous studies have demonstrated the widespread distribution of ESBL in Nigeria and other parts of the world. This is of immense concern because ESBL bacterial pathogens are multidrug resistant and are responsible for a number of community and nosocomial infections. Also, this study demonstrated that imipenem; a carbapenem was the most effective agent against all ESBL *E. coli* isolates. This further confirms that the carbapenems (including imipenem, meropenem and ertapenem) are the drug of choice for the treatment of ESBL and other multidrug resistant *E. coli* infections [1, 2, 4, 5]. ESBL positive *E. coli* isolates demonstrated absolute resistance against other antibiotics except ofloxacin (Figure 2). According to Chah *et al.* [9] ampicillin is widely used in poultry production in Nigeria and this may provide a selective pressure favoring the emergence of resistance genes including those that produce ESBLs. High resistance rates of ESBL positive *E. coli* strains have also been reported in other parts of the world [8, 12].

ESBL production is a major problem in clinical health settings owing to their multidrug resistance. Their presence in poultry farms is worrisome because it serves as a route through which they can be transferred to other bacteria and humans through consumption of these animals. The absence of strict regulations in the use of antibiotics in poultry farms and over-the-counter availability of drugs in Nigeria coupled with poor-infection control measures in most of our hospitals may foster the spread of ESBL producing Gram negative organisms. The study further demonstrated

the importance of instituting necessary regulations for effective use of antibiotics in poultry farms. Further molecular studies are warranted to determine the genetic profile of our ESBL *E. coli* strains and other multidrug resistance (MDR) genes in other Gram negative organisms from this region.

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