ORIGINAL PAPER

Nig. J. Pharm. Res. 2015, 1, pp. 46-51
ISSN 0189-8434

A Preliminary Investigation of Prevalence of Extended Spectrum Beta Lactamases among Enterobacteriaceae Isolated from Poultry Farms in Ibadan, Nigeria

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background:-Antibiotic resistance and extended spectrum beta–lactamase (ESBL) producing enterobacteriaceae are global health concerns and major problems for the treatment of different infections caused by enterobacteriaceae.
Objective:-This study was carried out to determine the prevalence of phenotypically identified ESBL producers in enterobacteriaceae strains isolated from poultry farms in Ibadan.
Material and Methods :-Enterobacteriaceae were isolated from faecal samples of 45 chickens from 3 farms in Ibadan, Nigeria. The *E. coli* strains in the isolates were identified by biochemical methods. The susceptibility of all enterobacteriaceae strains to selected β lactam antibiotics were tested by disc diffusion method. ESBL production was tested by double disk synergy test and MIC determination (8–512 µg/ml)
Results:- A total of 40 Enterobacteriaceae strains were isolated and 20 of the strains were identified as *E. coli* while 20 were tagged other Enterobacteriaceae strains. The *E. coli* strains were generally susceptible to tested antibiotics while other Enterobacteriaceae were relatively resistant. All the tested Enterobacteriacea were susceptible to cefepine. 15% of *E.coli* isolates were resistant to amoxicillin/clavulaniz and 38.9% of other Enterobacteriaceace isolates were resistant to cefoxitin. 5% of *E. coli* strains and 25% of other Enterobacteriaceae produced ESBL with concentration range of <8 µg/ml and >512µg/ml for antibiotics used singly and in combination with clavulanic acid respectively.
Conclusions:- This study showed low occurrence of ESBL in *E. coli* strains but relatively high occurrence in other Enterobacteriaceae in poultries in Ibadan, Nigeria.. Therefore, there is need to control the use of antibiotics in poultry feeds and livestock production.

Keywords: Antibiotics, Enterobacteriaceae, Extended spectrum beta lactamases.

INTRODUCTION

Antibiotic resistance is a worldwide concern especially in veterinary medicine. The administration of antibiotics through feed to food animals is a complex food safety and public health issue. The antibiotics are used for therapy, prophylaxis and to increase animals` growth. From animals, the resistant bacteria can be transmitted to humans through direct contact, via food (Martin *et al*., 2013), and eventually, the environment and water supply can become contaminated (Chee-Sanford *et al*.,,2001). Antibiotic use in animals contributes to the selection and spread of resistant bacteria among animals, between herds and also between countries (McEwen, 2002) consequently affecting human health due to the development of drug resistant microbes which can be transferred from poultry to humans, also, residues of drugs in poultry birds may be ingested by human who predisposes to development of antibiotics resistance in the society (Duru *et al*., 2013).

A rapid development of resistance to extended spectrum cephalosporins has been observed in Enterobacteriaceae worldwide (Chanawong *et al*., 2002). *E. coli* and *Klebsiella species* are commonly found in the environment and the gastrointestinal tracts of a wide range animals (Haryani *et al*., 2007), especially raised for human consumption. Many studies have revealed that they can contaminate food of animal origin and contribute to disease and spoilage (Gundogan and Yakar *et al*., 2007; Haryani *et al*., 2007). Extended spectrum beta-lactamases (ESBLs) are often acquired plasmid mediated beta-lactamases that hydrolyze broader spectrum beta-lactams containing an oxyimino-group (e.g. ceftazidime, ceftriaxone, cefotaxime, aztreonam) as well as penicillins (Paterson *et al*., 2005). ESBL strains can be transmitted via the food chain. Fecal contamination might occur during animal slaughtering, milking, processing, and the growth of the contaminating bacteria may occur during the

product transport and storage phases (Gundogan and Avci *et al*., 2013). Food animals are increasingly recognized as a reservoir for ESBL-producing strains thereby acting as a vehicle of transfer of β-lactam-resistant bacteria to the gastrointestinal tract of consumers (Overdevest *et al*., 2011). Some recent studies have documented frequent occurrence of ESBL-producing *E. coli* isolates in poultry (Kolar *et al*., 2010; Overdevest *et al*., 2011) and ESBL-producing Klebsiella isolates in dairy and meat products (Gundogan and Yakar, 2007; Gundogan *et al*., 2011). Because ESBL producers show resistance to a wide variety of β-lactams and some non-β-lactam drugs including fluoroquinolones, aminoglycosides and sulphamethoxazoles, failure to the therapeutic effectiveness of cephalosporins in clinical medicine has been attributed to ESBL-producing microorganisms and ESBL producers have been reported in both community and clinical environments (Ejikeugwu *et al*., 2013). The emergence and spread of ESBL organisms in the community constitutes a serious threat to the effective management and treatment of bacterial related infectious diseases and this compromises the efficacy of some available conventional antibiotics (Ejikeugwu *et al*., 2013). Meat and egg products are frequently contaminated with antimicrobial-resistant bacteria due to the intensive use of antimicrobial agents for food animal production. Thus, monitoring of ESBL-producing enterobacteriaceae should be done at various level (animals, human, and environment.. The continuing failure to do so may result in consequences such as treatment failure in patients who received inappropriate antibiotics or the uncontrolled spread of extended spectrum beta-lactamase-producing bacteria in the community setting. Therefore, the aim of the present study was to evaluate the occurrence of extended spectrum beta-lactamases producing *E. coli* and other Enterobacteriaceae in poultries in Ibadan, Nigeria.

MATERIALS AND METHODS

Isolation of Enterobacteriace

A total of 45 samples of poultry faeces were collected from three different poultry farms in Ibadan, Oyo State, Nigeria between September and October, 2014. All samples were cultured in MacConkey broth at 37 ^{O}C for 24 hours and later subcultured onto MacConkey agar. Non duplicated isolate was selected per sample for further characterization. Colonies showing characteristics morphology for Enterobacteriaceae on MacConkey agar were subcultured onto Eosin methylene blue (EMB) agar for further identification. Isolates were subjected to Methyl-red and Voges-proskauer (MR-VP) tests to differentiate between *E. coli* and other Enterobacteriaceae.

Antibiotic Susceptibility Testing of Enterobacteriaceae for Detection of ESBLs

The susceptibility of the Enterobacteriaceae to antibiotics was tested using disc diffusion method. The confirmed *E. coli* and other Enterobacteriaceae isolates were cultured overnight in Nutrient broth. Concentrations of the cultures which is equivalent to 0.5 McFarland standard (10^8 cfu/ml) were streaked on Muller Hinton agar. The following antibiotic discs (Oxoid, UK): Ceftazidime (CAZ) (30µg), Cefotaxime (CTX) (30µg), Amoxicillin/Clavulanic acid (30µg), Cefepime (FEP) (30µg) and Cefoxitin (FOX) (30µg) were placed firmly on the inoculated agar plates at 20mm apart from the centre. Amoxicillin/ clavulanic acid (30µg) (Oxoid, UK) was placed at the centre of the plate. The plates were incubated at 37 $^{\circ}$C for 24 hours. The diameter of zones of inhibition for the various antibiotics were measured in millimeter. Antimicrobial susceptibility test results were interpreted using Clinical Laboratory Standard Institute (CLSI) 2011. A characteristic 8 like shape between the central disk (Amoxicillin/clavulanic acid) and the other antibiotic disks was considered positive for ESBL production.

Determination of Minimum inhibitory concentration

The MIC of several antibiotics was studied in 9 isolates selected according to their observed resistance in disc diffusion method. MIC analysis by agar dilution method were used to determine the MIC to 2 cephalosporins, Cefotaxime (Nitaxim) and Ceftazidime (Betazidim) singly and in combination with Clavulanic acid with 8–512 µg/mL concentration range tested in all antibiotics. A characteristic 4 fold reduction in MIC of antibiotics used alone in comparism to antibiotics combined with Clavulanic acid denote positive confirmation for ESBL. (CLSI, 2011).

RESULTS

A total of 40 Enterobacteriaceae strains were isolated from the faecal samples of 45 chicken from 3 poultry farms in Ibadan. Twenty of the strains were identified as *E. coli* through positive result in MR test and green metallic sheen on EMB agar while the remaining 20 were tagged other Enterobacteriaceae strains.

The *E. coli* strains were generally susceptible to all tested β lactam antibiotics, only 25% exhibited resistance to 1 antibiotic and none show resistance to more than 1 antibiotic. However, 55% of other Enterobacteriaceae were resistant to at least 1 β lactam antbiotic, 30% were resistant to at least 2 antibiotics, 15% were resistant to at least 3 antbiotics, 5% were resistant to at least 4 antbiotics while none were resistant to all tested antibiotics (data not shown). 15% of *E.coli* isolates were resistant to amoxicillin/clavulaniz and. 16.7%, 22.2%, 38.9% of other Enterobacteriaceace isolates were resistant to ceftazidime, cefotaxime, and both amoxicillin/clavulanic acid and cefoxitin respectively (Fig I and II).

All the tested Enterobacteriacea were susceptible to cefepine.5% of *E. coli* strains and 25% of other Enterobacteriaceae produced ESBL (data not shown). Minimum inhibitory concentration was determined to confirm the production of ESBL using ceftazidime and cefotaxime singly and in combination with clavulanic acid, a 4 fold reduction in MIC of ceftazidime/clavulanic acid compared to MIC of ceftazidime alone confirms Enterobacteriaceae Q3, Enterobacteriaceae H2, Enterobacteriaceae O3, Enterobacteriaceae S3, *E. coli* B1 as ESBL producers (Table I). The results from double disc

method generally correlate with the results obtained with MIC for detection of ESBL using ceftazidime. MIC method with cefotaxime only confirms Enterobacteriaceae D3 as ESBL producer.

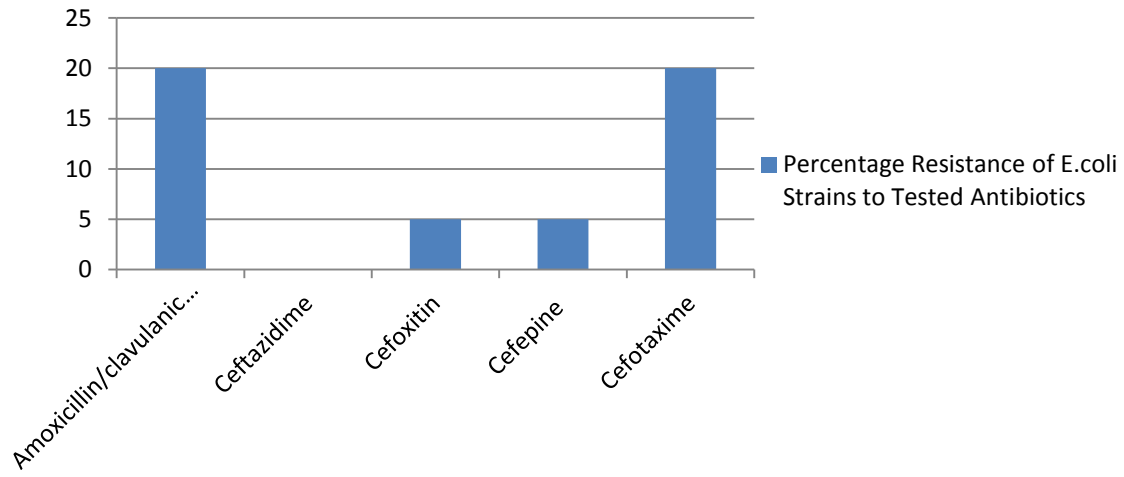

Figure 1: Percentage Antibiotic Resistance of E. coli Strains to Tested Antibiotics

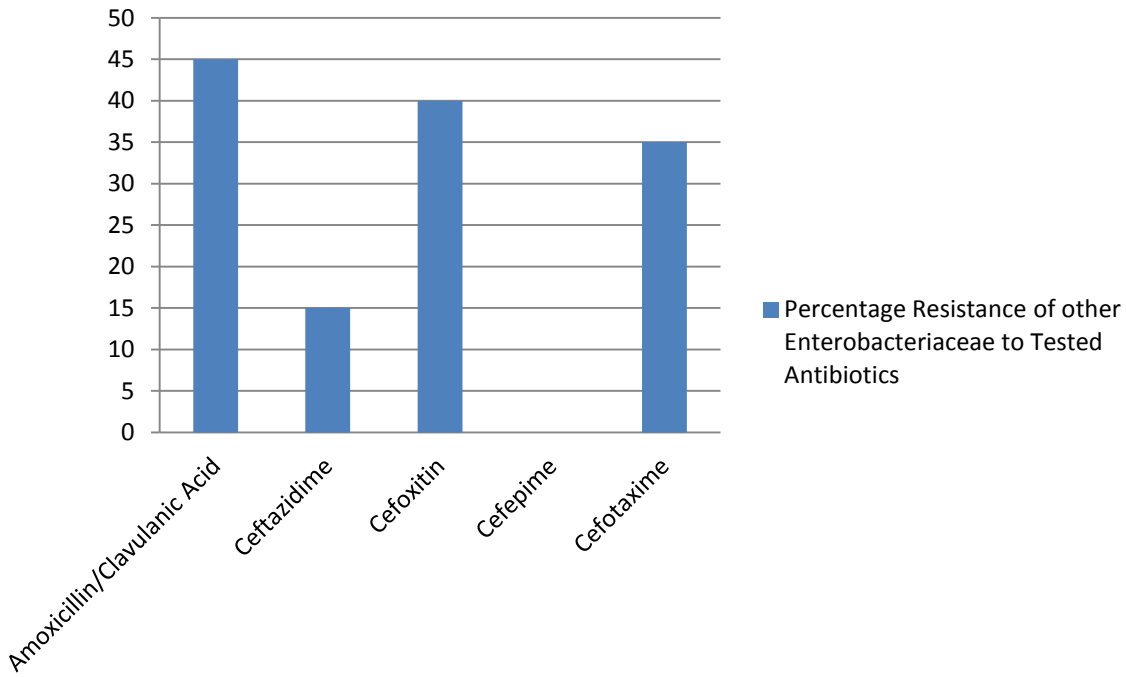

Figure 2: Percentage Antibiotic Resistance of other Enterobacteriaceae to Tested Antibiotics

Table I. Analysis of Minimum Inhibitory Concentration of Enterobacteriaceae Strains to Selected Antibiotics

Antibiotics	MIC	
Ceftazidime	CAZ+CA	CAZ
Enterobacteriaceae Q3	<8 µg/ml	>512 µg/ml
Enterobacteriaceae H2	<8 µg/ml	>512 µg/ml
Enterobacteriaceae O3	<8 µg/ml	>512 µg/ml
Enterobacteriaceae S3	64 µg/ml	>512 µg/ml
E. coli B1	<8 µg/ml	>512 µg/ml
Cefotaxime	CTX+CA	CTX
Enterobacteriaceae D3	<8 µg/ml	>512 µg/ml

Note:
CAZ = Ceftazidime
CTX= Cefotaxime
CA=Clavulanic Acid

DISCUSSION

Overuse of antibiotics has contributed to the emergence of antibiotic-resistant bacteria. Third generation cephalosporins have become common antibiotics in veterinary practice (Hassan, 2013). β- lactam antibiotics are among the most frequently prescribed antibiotics worldwide, and as such their use is subject of the problems associated with microbial resistance. Over the years, resistance to cephalosporins among members of Enterobactericeae has increased mainly due to the spreading of extended-spectrum beta-lactamases (ESBL) (Oyinloye and Ezekiel, 2011).

Extended spectrum Beta-lactamase-producing bacteria are frequently present in the gastrointestinal tract of animals and have been isolated from swine, cattle, turkey, cats, dogs, poultry, wild animals and horses (Carattoli, 2008). The gastrointestinal tract of animals is seen as an important reservoir for bacteria that produce beta-lactamases, and a potential source for human pathogens to take up these resistance genes (Akinlabi *et al*., 2008).

Chicken may serve as a reservoir of ESBL-producing *E. coli* strains which could be transferred to man and other animals (Gaze *et al*., 2008). In this study, a total of 45 faecal samples from chicken and turkey were investigated for the presence of ESBL. Twenty isolates were *E. coli* and 1 out of the 20 (5%) isolates produced ESBL. Oyinloye and Ezekiel, 2011 reported that 18.2% of the *E. coli* isolates produced ESBL Another study in southeast Nigeria reported 9.4% ESBL production among *E. coli* isolates from chicken (Duru *et al.,* 2013). Resistance and susceptibility studies of all the studied 20 *E. coli* isolates showed that cefoxitin and cefepime, which are 2nd and 4th generation cephalosporins respectively, were still effective; both showing 95% susceptibility on all the isolated strains of *E. coli*. Duru *et al*., 2013 reported that cefepime and amoxilin/clavulanic acid showed susceptibility of 33.3 and 26.7 percent against the *E. coli* isolates respectively. Laudy *et al*., 2015 clarified that the main mechanism of resistance to cefepime of the majority of *Enterobacteriaceae* is β-lactamase production and also suggest that MDR efflux pumps are responsible for the low and moderate resistance to cefepime of ESβL-positive *P. aeruginosa* strains. Therefore susceptibility of our strains to cefepime is an indication of non production of beta lactamases in the isolates.

The *E. coli* isolates were not resistant to ceftazidime. Ejikeugwu *et al*., 2013 reported that the resistance rates of the *E. coli* isolates to cefotaxime and ceftazidime were 97.2% and 91.7% respectively, which is in contrast with this study. Gundogan and Avcin, 2013 reported that resistance rate of *E. coli* isolates to cefotaxime is 33.3% and to ceftazidime 8.9%.

The other Enterobacteriaceae used in this study were generally resistant to the studied β lactam antibiotics with 55% of them resistant to at least 1 antbiotic and 25% being ESBL producers. Increasing resistance to third generation cephalosporins has become a cause for concern about Enterobacteriaceae (Okesola and Makanjuola, 2009). ESBL genes are located on plasmids which enable them to spread very rapidly (Naseer and Sundsfjord, 2011). Since the introduction of cephalosporins in health institutions, resistance by members of the Enterobacteriaceae, especially *Escherichia coli, Klebsiella and Salmonella species* have been on the increase globally. The prevalence of resistance to third generation cephalosporins in *E. coli* isolates from blood cultures was 4.8% in 2003, which increased to 11.7% in 2005 (Oyinloye *et al.,* 2011).

The presence of ESBL in poultry farms serves as a route through which these enzymes can be transferred to other bacteria and humans through contact and consumption of carrier animals. The intensive use of antibiotics for food animal production causes contamination of meat and milk products with antibiotic-resistant bacteria; resulting in the increasing prevalence of resistance in the isolates from animal origin which may have some therapeutic implications. This resistance increases morbidity and mortality in infected individuals by hampering the adequate provision of effective chemotherapy therefore making treatment more costly.

From this study, a low prevalence of phenotypically identified ESBL producers in *E. coli* strains but relatively high prevalence in other Enterobacteriaceae were observed. The fact that any ESBL producer was detected call for stringent measures to control the use of antibiotics in poultry feeds and livestock production. Increased awareness on ESBL-producing E. coli and other enterobacteriaceae isolates in animals is vital to prevent the emergence and spread of ESBL organisms within the community. Further studies are on going to determine the prevalence of ESBL producing Enterobacteriaceae in poultry in South West Nigeria and to molecularly characterised the ESBL producing Enterobacteriace.

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Conflict of Interest: None declared

Received: 4 May, 2015

Accepted: 29 July, 2015