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Prevalence of ESBLs and MBLs among *Escherichia coli* and *Klebsiella pneumoniae* Isolates from a Nigerian Abattoir

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Abstract

Background: Food products of animal origin play significant role in the transfer of antibiotic resistance. This work evaluated the antibiotic resistance profile and prevalence of beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in an abattoir in Awka, Nigeria.

Methods: One hundred swab samples were aseptically collected from the abattoir between January to April 2016 with sterile swab sticks and cultured in freshly prepared nutrient broth and MacConkey agar plates. *E. coli* and *K. pneumoniae* isolates were identified using standard microbiological identification techniques. The isolates were evaluated for antibiotic susceptibility and for the expression of ESBL, MBL and AmpC β-lactamases.

Findings: Ninety-four isolates comprising 60 *E. coli* and 34 *K. pneumoniae* were bacteriologically obtained from the abattoir samples. Their antibiotic resistances pattern was in the order of: erythromycin >cloxacillin > cefuroxime > augmentin>ceftriaxone>ceftazidime>ofloxacin>gentamicin (*E. coli* isolates) and erythromycin>cloxacillin>cefuroxime> augmentin> ofloxacin>ceftriaxone>gentamicin>ceftazidime (for *K. pneumoniae* isolates). Seven (12%) of the *E. coli* isolates and 15% *K. pneumoniae* isolates were confirmed phenotypically to be ESBL producers. None of the isolates was AmpC producing but 10% *E. coli* and 12% isolates were confirmed to be MBL-producers. Abattoir isolates of *K. pneumoniae* harbor resistance traits for the expression of ESBL and MBL-which are responsible for the MDR nature of Gram-negative bacteria and could serve as route via which these organisms can be transmitted through the food chain.

Keywords Beta-Lactamases; *Escherichia coli*; *Klebsiella pneumoniae*; Abattoir; Antibiotic resistance

Introduction

Food borne infections remain major causes of morbidity and mortality, especially in poor and developing countries where environmental hygiene is still in a pitiable state [1,2]. Food products of animal origin play prominent role in the transfer of antibiotic resistance [1,3]. This is because antibiotics are used in the rearing of livestock and poultry birds, and the antibiotic residues in these animals may cause the emergence of resistant bacteria via selective pressure. The irrational and off-label use of antibiotics in animal husbandry and in other agricultural practices allows bacteria to develop and acquire drug resistant genes over time through selective pressure, and this phenomenon impacts negatively on the efficacy of some available antibiotics [4].

Several studies have shown that the use of antimicrobial agents in animal husbandry has led to the emergence and spread of resistant bacteria through the food chain [3-5]. Antimicrobial drug resistance in food chain is an emerging public health problem that needs to be curtailed. The occurrence of drug resistant bacteria in food-producing animals presents a serious concern for infection control management both in the food chain and in healthcare system [4,5].

Food-producing animals have been reported as the primary reservoir of zoonotic food borne pathogens, including antimicrobial resistant bacteria [5,6]. The antibiotic resistance genes can be transferred among bacteria of varying taxonomic groups; and the transmission of resistant microbes from animals to humans is well established [7]. The most important mechanisms for resistance among the food borne pathogens include production of colistin resistance mechanism (mcr-1) gene, metallo β-lactamases (MBLs), AmpC enzymes and extended spectrum β-lactamases (ESBLs) [7-11]. Since the transmission to humans cannot be ignored, the increasing occurrence of multidrug resistant (MDR) microbes among food-producing animals has fueled interest in the genetics and mechanisms of resistance evolved by bacteria to counteract the effects of antibiotics ESBLs are a group of enzymes that break down β-lactam antibiotics including the penicillins and oxyimino-cephalosporins, and render them ineffective [11].

ESBLs are generally transmissible β-lactamases which are encoded and expressed by genes that can be exchanged between bacteria but can be inhibited by clavulanic acid, tazobactam or sulbactam [11-13]. While AmpC enzymes confer on bacteria the ability to resist the antimicrobial onslaught of the cephamycins (e.g. cefoxitin and cefotetan), MBLs help bacteria that produce them to be resistant to the carbapenems (e.g. imipenem, ertapenem and meropenem). Thus, beta-lactamase genes can be expressed in larger amounts and has high transmissibility to other bacterial species in any environment [5,10].

ESBL-producing microbes have a complex epidemiology, and they occur predominantly in members of the Enterobacteriaceae family such as *E. coli* and *K. pneumoniae* whose reservoirs are the environment (soil and water) and animals (farm, food, and pets) [11,14,15]. Therefore, the screening of abattoir samples for ESBL, AmpC and MBL positive bacteria is a useful epidemiological tool for the containment of possible disease outbreak due to these organisms. This was why this study presumptively evaluated the antibiotic resistance profile, prevalence and occurrence of *E. coli* and *K. pneumoniae* isolates that express MDR beta-lactamases from abattoir samples in Awka Metropolis, Nigeria.

Materials and Methods

Sample collection

A total of 100 swab samples were aseptically collected from a known abattoir in Awka metropolis during a four month period (January, 2016-April, 2016). Sterile swab sticks were used to collect the meat part of the freshly slaughtered animals and Meat seller's tables. The swab sticks were returned to their respective containers, labeled and transported to the Microbiology Laboratory Unit in the Department of Pharmaceutical Microbiology and Biotechnology, of Nnamdi Azikiwe University, Awka, Nigeria for bacteriological analysis. Each of the swab sticks was dipped and swirled into labeled test tubes containing 5 ml of freshly prepared nutrient broth (Oxoid, UK). The tubes were loosely covered with cotton wool and incubated at 37°C for 18-24 hours. Bacterial growth was indicated by the presence of turbidity in the tubes.

Isolation and identification of bacterial species

A loopful of the overnight broth culture from the test tubes was aseptically cultured on freshly prepared MacConkey agar (Oxoid, UK) plates and these were incubated at 37°C for 18-24 hr. Thereafter, they were sub-cultured by streaking onto freshly prepared MacConkey agar plates for the isolation of pure cultures of the organisms. The *E. coli* and *K. pneumoniae* isolates recovered from the culture plates were identified using standard microbiological identification techniques [16].

Antimicrobial susceptibility test

Susceptibility profiles of the bacterial isolates were evaluated using disk diffusion assay as described previously [17]. Briefly, a lawn culture of the test bacterial isolates (adjusted to 0.5 McFarland turbidity standards) was made with a standardized pre-incubated 18-24 hour culture. Each multiple disc (Abtek, UK) was carefully placed on the lawn and incubated at 37°C for 18-24 hrs, and the clear zone of inhibition was measured in millimeter, recorded and interpreted using the CLSI guidelines [18].

Double disk synergy test (DDST)

The isolates with diameter zone of inhibition of ≤ 22 mm for ceftazidine and ≤ 25 for ceftriaxone were further screened for ESBL production by DDST on Muller-Hinton (MH) agar (Oxoid, UK) plates as described by Ejikeugwu et al. [19].

The amoxicillin-clavulanic acid disk (30 µg) was placed aseptically at the center of a MH agar plate previously inoculated with the test organism (adjusted to 0.5 McFarland turbidity standards). Ceftazidime (30 µg) and cefotaxime (30 µg) single antibiotic disks were each placed adjacent to the central disk at a distance of 15 mm. The plates were incubated at 37°C for 18-24 hrs and the inhibition zone diameter (IZD) of the discs were recorded and interpreted. A ≥5 mm increase or difference in the IZD for either of the cephalosporins (ceftazidime or cefotaxime) tested in combination with amoxycillin-clavulanic acid compared to when tested alone phenotypically confirms ESBL production phenotypically [19].

Evaluation of Amp-C β-lactamase production

The isolates were screened for presumptive AmpC production by testing their susceptibility to cefoxitin (30 µg) using Kirby Bauer disk diffusion method [20]. The inhibition zone sizes were interpreted as per the CLSI guidelines [18]. The isolates with an IZD of ≤ 18 mm were further confirmed for AmpC enzyme production by the method of Barua et al. [21].

Evaluation of metallo-β-lactamase production

MBL was detected phenotypically by subjecting the imipenem resistant isolates to combined disc test. An organism was considered to be MBL positive if there was an increase of ≥7 mm in the zone of inhibition around the imipenem+EDTA disc as compared to imipenem disc alone after incubation at 37°C for 18-24 hours as was previously described [20,21].

Results

In this study 100 swab samples comprising 60 samples from freshly butchered meat and 40 samples from the meat-seller's tables were aseptically collected from the abattoir using sterile swab sticks and these were bacteriologically analyzed for the isolation of *E. coli* and *Klebsiella pneumoniae*. A total of 94 bacterial isolates comprising 60 isolates of *E. coli* and 34 isolates of *K. pneumoniae* were recovered from the abattoir samples analyzed in this study.

Result of antibiotic susceptibility studies

Figure 1 shows the percentage susceptibility profile of the *E. coli* isolates to the tested antibiotics. The isolates were resistant to erythromycin, cloxacillin cefuroxime and augmentin. Gentamicin and ceftazidime had the best antibacterial activity against the isolates.

The *E. coli* isolates were found to be resistant to many of the antibiotics used with a majority completely resistant to cloxacillin and erythromycin (at 100%). The *E. coli* isolates were resistant to augmentin (88%), cefuroxime (91%), and they also showed moderate sensitivity to ceftazidime (56%), ofloxacin (46%), ceftriaxone (63%), and gentamicin (46%).

The antibiotic susceptibility profile of the *K. pneumoniae* isolates is shown in Figure 2. The isolates were resistant to erythromycin, cloxacillin, cefuroxime and augmentin. Ceftazidime, ceftriaxone, gentamicin and ofloxacin were active against the isolates. The *K. pneumoniae* isolates were also resistant to cloxacillin (100%), erythromycin (100%), ceftazidime (32%), cefuroxime (94%), ofloxacin (44%), ceftriaxone (44%), gentamicin (44%).

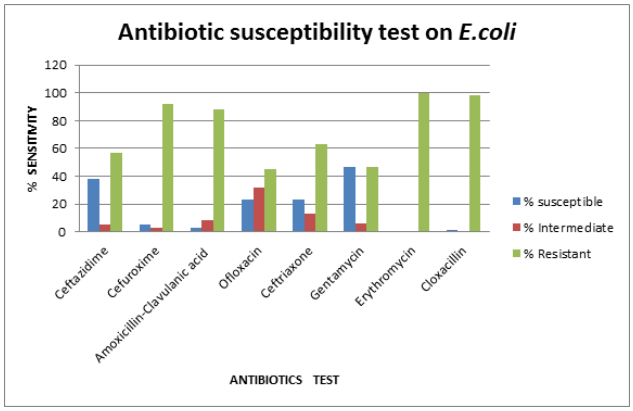

Figure 1: Antibiotic susceptibility pattern of *E. coli.*

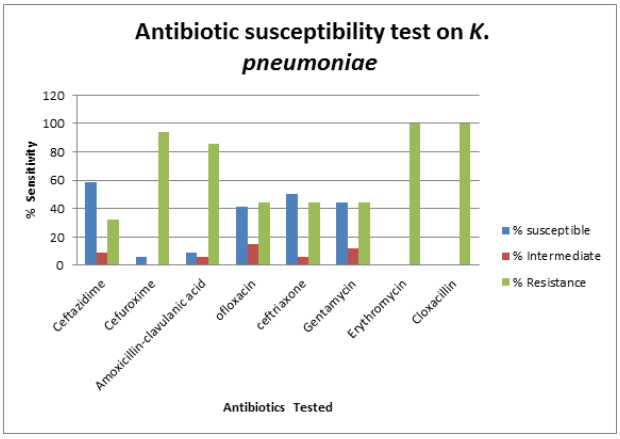

Figure 2: Antibiotic susceptibility pattern of *K. pneumoniae.*

Results of ESBL screening studies

The result of the screening tests for possible ESBL production showed that 27 (45%) isolates of the *E. coli* and 13 (38.2%) isolates of the *K. pneumoniae* showed reduced susceptibility to the cephalosporins (ceftazidime and cefotaxime). However, only 7 (12%) isolates of *E. coli* and 5 (15%) isolates of *K. pneumoniae* were confirmed phenotypically to be ESBL producers (Table 1).

Bacterial Isolates	% ESBL Screen positive	% ESBL producing
E. coli	45% (27)	12% (7)
K. pneumoniae	38.2% (13)	15% (5)

Table 1: Result of ESBL isolates.

Amp-C β-lactamase detection

A total of 11 isolates of *E. coli* and 4 isolates of *K. pneumoniae* showed reduced susceptibility to the cephamycin, cefoxitin but none of these isolates was confirmed to be AmpC producers (Table 2).

Isolates	Amp-C Screen positive	% Amp-C producing
E. coli	20% (12)	0%
K. pneumoniae	11.8% (4)	0%

Table 2: Results of Amp-C β-Lactamase detection.

Metallo-β-lactamase (MBL) detection

A total of 8 *E. coli* isolates and 5 *K. pneumoniae* isolates were found to be resistant to imipenem or meropenem with IZDs of ≤ 23mm or ≤ 27mm. But only 6 (10%) isolates of *E. coli* and 4 (12%) isolates of *K. pneumoniae* were confirmed to be MBL-producers (Table 3).

Bacterial Isolates	MBL screen positives	MBL non-producing	MBL producing
E. coli	13.3% (8)	3.3% (2)	10% (6)
K. pneumoniae	14.8% (5)	3% (1)	11.8% (4)

Table 3: Results of Metallo-β-lactamase (MBL) detection (%).

Discussion

Food-producing animals serve as reservoirs and or routes for the spread of antibiotic resistant bacteria in the community through the food chain [1,4]. In Nigeria, there is a heavy usage of antibiotics to optimize animal production. The heavy and off-label use of antibiotics has been reported to be a risk factor for the development and spread of beta-lactamase producing organisms [14,22]. In south-eastern Nigeria, the local and regional epidemiological studies on beta-lactamases-producing Enterobacteriaceae and their potential risks in animal-derived food chain are lacking. In this study, we screened for beta-lactamase production in *E. coli* and *K. pneumoniae* isolates from raw/freshly slaughtered animals and Meat seller's tables in a local abattoir. The results demonstrate high prevalence of *E. coli* isolation than *K. pneumoniae*. The result of the prevalence of *E. coli* in abattoir complies with the report of this organism as a prominent cause of food borne infection [23]. *K. pneumoniae* though not a common known bacteria found in animal intestine, has been reported as an opportunistic pathogen of humans, animals, and a common contaminant of retail meats [24]. In 2005, multidrug-resistant *K. pneumoniae* was prevalently isolated from turkey, cattle, and chicken farms and retail meat products in Oklahoma [25]. However, *K. pneumoniae* naturally occurs in the soil and is mostly implicated in soil contamination [3]. Thus its high prevalence in our study points to poor hygienic practice among the meat handlers. Furthermore, the high colonization rate could be attributed to cross contamination of meats in abattoirs particularly during slaughtering. The processes of slaughtering are potential risk factors that may exacerbate the transmission rate of beta-lactamase producing *E. coli* resistant strains [26]. The result of antimicrobial susceptibility testing revealed an interesting pattern with resistance rates observed in the majority of antimicrobial agents tested especially amongst the beta-lactam and macrolides groups. Most of the isolates obtained were multi-drug resistant. Since majority of the resistance were against β-lactam antibiotics the resistance pattern might be by the inactivation of β-lactam ring by the β-lactamases as most of these enzymes are constitutive in Gram-negative organisms [12]. A total of 7 (12%) isolates of *E. coli* and 5 (15%) isolates of *K. pneumoniae* were confirmed phenotypically to be ESBL producers.

This observed ESBL prevalence is of public health concerns because it indicates a health risk for the meat consumers in the studied locality. The colonization of the food animals might lead to a risk of infection and colonization of the human with ESBL-producing *E. coli* especially when these meats are consumed without proper cooking/processing. Secondly, resistance caused by ESBLs is often associated with resistance to other classes of antibiotics, and this makes it difficult to choose effective therapy [27]. ESBL-producing *E. coli* associated mortality has been reported to be three-times higher than non-ESBL producing *E. coli* [15,28]. Our ESBL result differed from what Tekiner et al. reported in Brazil where 80% of *E. coli* and 3.6% *K. pneumoniae* recovered from foods of animal origin were ESBL producers [15]. A total of 10.99% (21/191) isolates of *E. coli* in foods of animal origin in India were reported presumptive ESBL producers by Bhoomika et al. while 20% ESBL-producing bacteria were found from minced meat in Austria [29,30]. The prevalence and distribution of MDR organisms varies widely in food-animal reservoirs and thus the extent of transmission from food animals to humans may vary by geographic region [31]. None of the isolates resistant to cefoxitin were positive for AmpC production. Contrary to a study in a local abattoir in Ebonyi State, Nigeria where 20% of *E. coli* isolates recovered from anal region of cows was AmpC producers [7]. In addition, majority of these isolates were sensitive to imipenem showing that they could be effectively treated with carbapenems. Some of the isolates were thoroughly resistant to meropenem. Among the tested isolates, 10% strains were MBL producing while 11.8% of the *K. pneumoniae* strains were MBL producing strains [4,6]. These findings reflected a high prevalence of MBL-producing *E. coli* and *K. pneumoniae* from Kwata abbatior with a great risk and possibility of other forms of antibiotic resistance. This result differed from 28.6% MBL producing *E. coli* recorded from a slaughter house in a neighboring state in Nigeria [6]. The shortcoming of the study is that Genotyping of beta-lactamases for confirmation was not done.

Conclusion

Conclusively, this study shows that the abattoir is a reservoir for food borne pathogens that are multidrug resistant in nature. And the high prevalence of these organisms in our study coupled with their high antibiotic resistance profile reflects poor handling of the meat products and undue use of antibiotics in the production of these animals. Efficient and periodic surveillance programmes should be encouraged to monitor ever shifting prevalence and antibiogram patterns.

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