Antibiogram and Detection of Metallo-Beta-Lactamase (MBL) positive *Escherichia coli* isolates from abattoir

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Abstract: Metallo-β-lactamases (MBLs) are group of β-lactamase enzymes that have zinc ion (Zn^+) in their active site and are active hydrolyzer's of carbapenems including meropenem, imipenem and ertapenem. However, MBLs are inactivated by chelating agents such as Ethylene diamine tetra-acetic acid (EDTA). This study evaluated phenotypically, the occurrence of Metallo-β-lactamase positive *Escherichia coli* from the anal swab of cows in an abattoir in Abakaliki metropolis, Ebonyi State, Nigeria. A total of 40 anal swab samples from cows were used for this study. The samples were bacteriologically analyzed in the microbiology laboratory of Ebonyi State University, Abakaliki using MacConkey agar, nutrient broth and eosin methylene blue (EMB) agar. The isolated *E. coli* were identified using standard microbiological techniques. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion technique. MBL production was phenotypically detected in the *E. coli* isolates using the inhibition based assay. A total of 32 *E. coli* isolates were recovered from the anal swabs of cow and they showed varying rates of susceptibility to the tested antibiotics. The isolates were particularly resistant to oxacillin (100 %), ceftriaxone (87.1 %), cefotaxime (87.1 %) and ceftazidime (83.9 %). However, most of the *E. coli* isolates were susceptible to the carbapenems, particularly imipenem (87.1 %) and meropenem (80 %). Only 14 (43.8 %) *E. coli* isolates out of the 32 isolates recovered were suspected to produce metallo-β-lactamase (MBL) in the screening test for MBL detection. However, only 4 (28.6 %) isolates of *E. coli* were phenotypically confirmed to be MBL producers. Proper detection of resistant bacteria from community samples and discouragement of the use of antibiotics as growth enhancers in animal production can help to prevent the spread of antibiotic resistant bacteria and thus, preserve the efficacy of available antibiotics.
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1. Introduction

Metallo-β-lactamases (MBLs) are group of β-lactamases (otherwise known as carbapenemases) that hydrolyze carbapenems, and have potent but variable enzymatic activity against other beta-lactam antibiotics except the monobactams such as aztreonam (Smith, 2004; Iyobe *et al.,* 2001). MBLs, which are a type of carbapenemases, are an emerging public health problem among clinically important Gram negative organisms and community isolates including *P. aeruginosa*, *A. baumannii* and the *Enterobacteriaceae* (Thompson, 2010; Franco *et al.*, 2010). The carbapenems are potent antimicrobial agents used for the treatment of serious Gram negative bacterial infections including those that are caused by bacteria harbouring extended spectrum beta-lactamases (ESBLs). The MBLs are known to confer variable range of high resistance to all beta-lactam antibiotics except the monobactams and their presence in both community and clinically important Gram negative bacteria have put the use of the carbapenems under threat (Tortola *et al*., 2005 and Thompson, 2010). Genetically, the MBLs are either plasmid-mediated or chromosomally-mediated, and those that are plasmid-mediated (or encoded by transferable genes or elements such as integrons and transposons) are found in more resistant bacteria such as *P. aeruginosa, A. baumannii,* and the *Enterobacteriaceae* while those that are chromosomally-mediated are found in bacterial strains such as *Bacillus cereus* and *Stenotrophomonas maltophilia* and in obscure non-clinical bacteria such as *Aeromonas* species (Walsh *et al*., 2005; Thompson, 2010 and Toleman *et al*., 2005). However, MBL genes are important resistance determinants considering the fact that most of these genes are carried as mobile gene cassettes (which can

easily be integrated into the chromosomes of other susceptible organisms) on class one integrons with the potential to spread to other non-MBL-producing bacteria in either the community or hospital environment. And because the MBL genes are mainly plasmid-borne, there spread to the population of pathogenic organisms is of great concern and a menace to our ability to fight and treat a wide variety of Gram negative infections (Toleman *et al*., 2005 and Carfi *et al*., 1995; Walsh *et al*., 2005). Opportunistic organisms from the environment are also known to ubiquitously express MBLs chromosomally, and the reason for this is still arcane (Walsh *et al*., 2005 and Tortola *et al*., 2005). The MBLs belong to a group of beta-lactamases which requires divalent cations (e.g. zinc ions) as cofactors for their enzyme activity, and enzymes in this category are similar in that they require zinc ions for enzyme activity and they are inhibited by EDTA and other chelating agents (Toleman *et al*., 2005 and Varaiya *et al*., 2008). The advent of MBL-producing bacteria calls for concerted effort to detect and contain their spread in either the community or hospital environment in order to sustain the efficacy of some available potent antimicrobial agents. It is in this direction that this study evaluated the occurrence of MBL-producing *Escherichia coli* isolates from abattoir.

2. Material and Methods

Sample collection and processing: A total of 40 anal swab samples were collected from cows in a local abattoir in Abakaliki metropolis, Ebonyi State, Nigeria using sterile swab sticks. The sterile swab sticks was inserted to about 3 cm deep into the anus of the cow and rotated at an angle of 360°. The swab sticks was returned to their respective containers and labeled. And they were transported to the microbiology laboratory of Ebonyi State University, Abakaliki for bacteriological analysis. Each of the collected samples was inserted into 5 ml of freshly prepared nutrient broth and the tubes were loosely covered with cotton wool. The tubes were arranged on test tube rack and were incubated at 30^oC for 18-24 hours. Bacterial growth was identified by the presence of turbidity or cloudiness in the tubes after incubation. Tubes that showed turbidity were further subcultured onto solid culture media plates for the isolation of the primary bacterium.

Culture: A loopful of the turbid growth in the tubes was aseptically inoculated onto freshly prepared MacConkey agar and eosin methylene blue (EMB) agar plates. The plates were properly labeled and incubated at 30^oC for 18- 24 hours. Culture plates that showed bacterial growth were subcultured onto freshly prepared MacConkey agar and EMB agar plates for the isolation of pure cultures of *Escherichia*

coli prior to biochemical tests. *E. coli* produces pinkish colonies on MacConkey agar and colonies with metallic sheen on EMB agar. All the suspect *E. coli* isolates were further identified using standard microbiological techniques (Cheesbrough, 2000).

Antibiotic susceptibility testing: Susceptibility testing was done on Mueller Hinton agar plates (Oxoid, UK) using the Kirby-Bauer disk diffusion method as per the criteria of Clinical Laboratory Standard Institute (CLSI, 2015). The standard antibiotic disks used include: imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), amikacin (30 μg), ofloxacin (5 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), ciprofloxacin (30 μg), oxacillin (1 μg), and cefoxitin (30 μg). All the antibiotic disks used were procured from Oxoid limited (Oxoid, UK). A loopful of the test organism (adjusted to 0.5 McFarland turbidity standards) was streaked on freshly prepared Muller-Hinton agar plates; and the plates were allowed to stand for 15 minutes. The antibiotic disks were placed at a distance of 15 mm apart and the susceptibility plates were incubated at 30^oC for 24 hours (Ejikeugwu *et al*., 2014; CLSI, 2015). The zones of inhibition diameter were measured according to the CLSI criteria.

Screening for the presence of metallo-beta-lactamase (MBL): All test *E. coli* isolates were screened for the production of MBL by determining their susceptibility to any of the carbapenems including imipenem (IPM), meropenem (MEM) and ertapenem (ETP) as was previously described (Ejikeugwu *et al*., 2014). The Kirby-Bauer disk diffusion technique was used, and each of the antibiotics disk was placed at a distance of 20 mm apart and the plates were incubated at 30^oC for 18-24 hours. MBL enzyme-producing isolates was suspected when the test organism(s) showed reduced susceptibility to any of the carbapenems used in the screening test. As was previously described, isolates showing inhibition zone diameter (IZD) of ≤ 23 mm were suspected to produce MBL and these isolates were subjected to phenotypic confirmation test (Ejikeugwu *et al*., 2014; Varaiya *et al*., 2008).

Phenotypic detection of MBL: Test organisms found to be resistant to imipenem or meropenem (as indicated in the screening test) was evaluated phenotypically for the presence of metallo β-lactamase (MBL) as was previously described (Varaiya *et al*., 2008; Ejikeugwu *et al*., 2014). The test bacteria isolates (adjusted to 0.5 McFarland turbidity standards) were aseptically swabbed on Mueller-Hinton (MH) agar plates; and standard antibiotic disks of imipenem (10 μg) and meropenem (10 μg) impregnated with EDTA (1 μg) was aseptically placed on MH agar plates. Supplementary imipenem (10 μg) and meropenem (10 μg) disks without EDTA were

also placed alongside the antibiotic disks impregnated with the chelating agent (EDTA) at a distance of 20 mm apart. The chelating agents were initially tested on the test bacteria prior to the phenotypic assay to ensure they had no inhibitory effect on the test organisms. All the plates were incubated at 30°C for 18-24 hours and zone of inhibition were recorded after incubation. A difference of ≥ 7 mm between the zones of inhibition of any of the carbapenem disks with or without the chelating agents infers metallo-beta-lactamase production phenotypically (Ejikeugwu *et al.*, 2014).

3. Results

Table 1 show the frequency of isolation of *Escherichia coli* from the environmental samples. A total of 40 samples from the anal swab of cow collected from a local abattoir in Abakaliki metropolis were used for this study. A total of 32 (80) *E. coli* isolates was recovered from the 40 anal swab samples

bacteriologically analyzed in this study. Table 2 shows the antimicrobial susceptibility pattern of the *E. coli* isolates to some commonly used antibiotics. It was observed in this study that the *E. coli* isolates showed varying rates of susceptibility and resistance to the tested antibiotics. The test *E. coli* isolates was found to be highly resistant to oxacillin (100 %), cefotaxime (84.4 %), ceftazidime (81.3 %), cefoxitin (78.1 %), ertapenem (71.9 %) and amikacin (31.3 %). However, they showed susceptibility to imipenem (84.3 %), ofloxacin (50 %), gentamicin (78.1 %), meropenem (68.8 %), ciprofloxacin (34.4) and amikacin (53.1 %). Table 3 show the occurrence of MBL-positive *E. coli* isolates in this study. A total of 32 *E. coli* isolates were screened for MBL production. And 14 (43.8%) isolates of *E. coli* were suspected to produce MBL (Table 3). Only 4 (28.6 %) isolates of *E. coli* were phenotypically confirmed to produce MBL enzymes in this study (Table 3).

Table 1: Frequency of *Escherichia coli* isolation

Sample	No of samples	No (%) of *E. coli*
Anal swabs of cow	40	32 (80)

Table 2: Susceptibility profile of the *Escherichia coli* isolates

Antibiotics (µg)	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
CRO (30)	2 (6.3)	3 (9.4)	27 (84.4)
FOX (30)	4 (12.5)	2 (6.3)	25 (78.1)
IPM (10)	27 (84.3)	3 (9.4)	2 (6.3)
CAZ (30)	1 (3.1)	4 (12.5)	26 (81.3)
ETP (10)	5 (15.6)	3 (9.4)	23 (71.9)
OX (1)	0 (0.0)	0 (0.0)	32 (100 .0)
OFX (5)	16 (50.0)	9 (28.1)	6 (18.8)
CN (5)	25 (78.1)	0 (0.0)	6 (18.8)
CIP (5)	11 (34.4)	11 (34.4)	9 (28.1)
AK (30)	17 (53.1)	4 (12.5)	10 (31.3)
CTX (30)	3 (9.4)	1 (3.1)	27 (84.4)
MEM (10)	22 (68.8)	1 (3.1)	8 (25.0)

Key: CRO = Ceftriaxone, FOX =Cefoxitin, IPM =Imipenem, CAZ = Ceftazidime, ETP = Ertapenem, OX = Oxacillin, OFX = Ofloxacin, CN = Gentamicin, CIP = Ciprofloxacin, AK = Amikacin, CTX = Cefotaxime, MEM = Meropenem

Table 3: Occurrence of MBL-positive *Escherichia coli*

Organism	No of isolates screened	Suspected MBL producers	MBL-positive n (%)	MBL-negative n (%)
Escherichia coli	32	14	4 (28.6)	10 (71.4)

4. Discussions

Metallo beta-lactamase (MBL) production is one of the mechanisms of resistance among bacterial isolates especially the members of *Enterobacteriaceae* and other Gram negative bacteria such as

Pseudomonas aeruginosa and *Klebsiella* species. This mechanism of resistance has tremendous public health implications as they limit treatment options for bacterial infections caused by multidrug resistant bacteria (Bashir *et al* 2011). The result of this research

showed high prevalence of *Escherichia coli* (80 %) from anal swab samples of cow analyzed. *E. coli* is a common bacterial organism that is associated with a variety of human infections including those that are nosocomial in origin and those that are acquired from the community. Poor handling of meat in abattoirs could predispose consumers to the acquisition of pathogenic *E. coli* that may harbour drug resistance genes. All the *E. coli* isolates showed varying rates of resistance and susceptibility to the tested antibiotics. Among the antibiotics tested, the *E. coli* isolates were particularly resistant to oxacillin (100 %), ceftriaxone (87.1 %), cefotaxime (87.1 %) and ceftazidime (83.9 %). However, most of the *E. coli* isolates were susceptible to imipenem (87.1 %) and meropenem (80 %). This high level of susceptibility of the *E. coli* isolates to the carbapenems (imipenem and meropenem) could be the reason why the carbapenems have remained the drug of choice in the treatment of infections caused by resistant strains of Gram-negative bacilli including those mediated by ESBLs (Dahiya *et al.*, 2015; Walsh *et al.*, 2005; Toleman *et al.*, 2005; Thompson, 2010; Tortola *et al.*, 2005). The *E. coli* isolates were totally resistant to oxacillin; and the isolates also showed reduced susceptibility to cefotaxime (84.4 %), ceftazidime (81.3 %), cefoxitin (78.1 %), ertapenem (71.9 %) and amikacin (31.3 %). Ofloxacin, gentamicin, ciprofloxacin and amikacin were other non-beta-lactam agents that had activity on the test *E. coli* isolates. Similar rates of susceptibility and resistance of *E. coli* isolates from environmental samples (as obtainable in this study) have been previously reported (Moore *et al.*, 2014; Ejikeugwu *et al.*, 2014; Ejikeugwu *et al.*, 2016). MBL production was phenotypically detected in only 4 (28.57%) *E. coli* isolates out of the 32 isolates of *E. coli* screened for the enzyme in this study. The occurrence of MBL positive *E. coli* in the community amongst Gram-negative bacilli have previously been reported; and they also account for the spread of drug resistance genes in these settings (Johnson *et al.*, 2013; Ejikeugwu *et al.*, 2014; Walsh *et al.*, 2005). In Abakaliki Ebonyi State, south east Nigeria, where this research was conducted, there is paucity of report on MBL positive *E. coli* isolated from anal region of cow. The rapid spread of resistance among bacteria may be attributed to the widespread and inappropriate use of antibiotics in the environment. Further still, the non-medical uses of antibiotics in such areas as animal husbandry, fish farming as feed additives and in the treatment of certain plant diseases may also have contributed to the spread of antimicrobial resistance in the community (Claude, 2013). Appropriate preventive measures are advocated so as to prevent the emergence of newer resistance mechanisms among

bacteria species in the community; and top among these preventive measures include proper detection and reporting of resistant bacteria from community samples. Conclusively, the result of this study showed high frequency of *E. coli* from anal region of cow; and some of these isolates were confirmed to be MBL-producers. These *E. coli* isolates showed varying susceptibility and resistance to the tested antibiotics. This may be due to the undue use of antibiotics in animal husbandry.

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