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Bacteriological Investigation of Antibiogram, Multiple Antibiotic Resistance Index and Detection of Metallo-B-Lactamase (MBL) in Klebsiella Species and Pseudomonas Aeruginosa of Abattoir Origin

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Abstract

To contain the emergence and spread of drug resistant bacteria including those that produce metallo-β-lactamases, it is important to curtail the use of antibiotics in non-hospital environment such as abattoir and poultry farms. Metallo-β-lactamase (MBL) are enzymes expressed by bacteria, and which mediate bacterial resistance to the carbapenems. The carbapenems are clinically used to treat serious bacterial infections including those caused by extended spectrum β-lactamase (ESBL)-producing bacteria. In this study, the antibiogram, multiple antibiotic resistance index (MARI) and frequency of Klebsiellas species and *Pseudomonas aeruginosa* isolates that were positive for MBL production was phenotypically investigated. Fifty (50) anal swab samples from ready-to-be-slaughtered animals were analyzed for the isolation and identification of Klebsiella species and *P. aeruginosa* isolates using standard microbiology techniques including Gram staining, citrate test, urease test and oxidase test. The susceptibility of the test isolates to selected antibiotics was carried out using the modified Kirby-Bauer disk diffusion technique. MBL production by the test bacterial isolates was phenotypically carried out using the modified Hodges (Cloverleaf) technique. *Klebsiella* species and *P. aeruginosa* isolates that were positive for MBL production was evaluated for multiple antibiotic resistance index (MARI). The Klebsiella species isolates were apparently resistant to ertapenem (75%), cefoxitin (100%), cloxacillin (100%) and ceftazidime (83.3%). *P. aeruginosa* isolates were resistant to ertapenem (65%), cefoxitin (100%), ofloxacin (35%) and cloxacillin (100%). All the 12 isolates of Klebsiella species (100%) recovered in this study were phenotypically positive for MBL production. Totally, 14 isolates of *P. aeruginosa* (70%) were phenotypically confirmed to be MBL positive by the modified Hodges test method. All isolates of Klebsiella species and P. aeruginosa that were phenotypically positive for MBL production were multiply resistant to 5 antibiotics out of the 7 antibiotics used for this study. This present study recorded high percentage of antibiotic resistance amongst the Klebsiella species and *P. aeruginosa* isolates which were also confirmed phenotypically to produce MBL. MBL-mediated resistance puts the use of the carbapenems at risk. The prompt detection and reporting of bacteria producing MBLs from either the hospital or non-hospital environment is crucial, and required to forestall any outbreak due to MBL-producing bacteria in this region.

Key words: *Gram negative bacteria; metallo-beta-lactamase (MBL); multiple antibiotic resistance index (MARI); antibiotic resistance*

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Introduction

The increase in antibiotic resistance among Gram negative bacteria including *Klebsiella* species and *Pseudomonas aeruginosa* especially through the production of metallo-β-lactamases puts the use of the carbapenems for therapeutic purposes at risk. Beta-lactamases are important components of the antimicrobial resistance mechanism found in Gram negative bacteria including *P. aeruginosa* and *Klebsiella* species [1,2]. However, mutation in these earlier beta-lactamases has led to the emergence of organisms that express multidrug resistance enzymes such as metallo-β-lactamases (MBLs), which give bacteria the exceptional ability to resist antimicrobial onslaught. Gram negative bacteria that produce MBLs are typically resistant to carbapenems and other beta-lactams, and this further compromises therapeutic option in the face of an infection or disease [2-5]. Metallo-β-lactamases (MBLs) are a type of carbapenemases that hydrolyze the carbapenems including imipenem, and render them inefficacious for treatment [6,7]. They are β-lactamases that belong to Ambler's class B type of enzymes, and they degrade a wide variety of β-lactams including penicillins, cephalosporins and carbapenems by hydrolyzing the amide bond of the β-lactam ring [6]. Pathogenic bacteria that produce MBLs are usually susceptible to aztreonam, a monobactam. However, bacterial organisms that express MBLs and other multidrug resistance enzymes are indeed a great threat and of clinical importance since these organisms are usually resistant to a wide variety of antibiotics especially the β–lactams which are important agents used clinically for the treatment of bacterial related infections [6,8]. The presence of MBL genes in clinically important organisms and even in environmental microbiota threatens the efficacy of some available beta-lactam and non-beta-lactam agents [6,9]. However, organisms producing MBLs in non-clinical isolates especially those from poultry and abattoir sources are of immense public health importance since they could serve as repertoires for the dissemination of MBL-producing organisms in the non-hospital environment. The genes responsible for MBL production may be chromosomal- or plasmid-mediated, and they pose a threat of horizontal gene transfer among other Gram-negative bacteria in their environment [3,6,10]. Antibiotic resistance is a public health phenomenon that bedevils our health care system around the world; and this puts antimicrobial therapy at risk – since drug resistant bacteria are notoriously resistant to some commonly available antibiotics. Studies have shown that zoonoses from abattoir wastes are yet to be fully controlled in more than 80% public abattoirs in developing countries inclusive of Nigeria [11,12]. We evaluated the antibiogram, multiple antibiotic resistance index and frequency of *Klebsiella* species and *P. aeruginosa* positive for metallo-β-lactamase (MBL) production was phenotypically investigated.

Materials and Methods

Samples: Aanal swab samples (n = 50) were collected from the anal region of cows using sterile swab sticks. All samples were labeled and transported to the Microbiology Laboratory Unit of Ebonyi State University, Abakaliki within one hour of collection for analysis. They were each inserted into 5ml of freshly prepared nutrient broth (Oxoid, UK) and incubated at 30°C overnight. Bacterial growth was identified by the presence of turbidity in the broth culture.

Culture: Loopfuls of suspensions from the turbid solution was plated aseptically onto MacConkey (MAC) and cetrimide selective agar (CSA) (Oxoid, UK) plates, and incubated at 30°C overnight. Suspect colonies of *P. aeruginosa* and *Klebsiella* species were sub cultured onto freshly prepared MAC and CSA plates for the isolation of pure cultures of *Klebsiella* species and *P. aeruginosa* respectively. All isolates of Klebsiella species and P. aeruginosa were further characterized using standard microbiology identification techniques including Gram staining, urease test, citrate test and oxidase test [13].

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing (AST) was carried out on Mueller-Hinton agar plates using the Kirby-Bauer disk diffusion technique as per the guidelines of the Clinical and Laboratory Standard Institute (CLSI). The antibiotic disks used comprises: amikacin (AK, 10 µg), cefoxitin (FOX, 30 µg), cloxacillin (OB, 10 µg), ceftazidime (CAZ, 30 µg), ofloxacin (OFX, 5 µg),

ertapenem (ETP, 10 µg) and imipenem (IPM, 10 µg) (Oxoid, UK). Susceptibility plates were incubated at 30°C overnight; and inhibition zone diameters (IZDs) were recorded and interpreted using the standard antibiotic breakpoint of CLSI [8,14].

Confirmatory Test for MBL Production: Bacterial isolates that showed reduced susceptibility to imipenem (IPM, 10 µg), meropenem (MEM, 10 µg) and ertapenem (ETP, 10 µg) [Oxoid, UK] were confirmed for MBL production phenotypically [10,14,15]. The Cloverleaf (Hodges) test was used to phenotypically confirm MBL production in the bacterial isolates. All the plates were incubated at 30°C overnight; and macroscopically observed for indentation and the growth of the test bacteria towards the imipenem (10 µg) susceptibility disk. Presence of indentation and growth of test bacteria towards the imipenem disk is indicative of metallo-β-lactamase (MBL) production phenotypically [10,15].

Multiple Antibiotic Resistance Index (MARI): MARI was calculated for *Klebsiella* species and *P. aeruginosa* isolates that were positive for MBL production [7]. This was achieved by dividing the number of antibiotics to which the isolate is resistant to by the total number of antibiotics tested in this study.

$$\text{MARI} = \frac{(\text{number of antibiotics to which isolate is resistant to})}{(\text{total number of antibiotics used})}$$

Results

Isolation, Identification and Antibiogram

(Table 1) show the distribution of the isolated Klebsiella species and *P. aeruginosa* isolates that was bacteriologically recovered from the rectal/anal swab samples. There were more isolates of *P. aeruginosa* recovered from the anal swab samples than isolates of *Klebsiella* species. The result of the antimicrobial susceptibility of the isolates of *Klebsiella* species is shown in (Table 2). Imipenem, amikacin and ofloxacin were the best performing antibiotics in terms of their antimicrobial onslaught against the isolates of Klebsiella species and *P. aeruginosa* used in this study. The result of the antimicrobial susceptibility profile of the isolated *P. aeruginosa* isolates is shown in (Table 3). The *P. aeruginosa* isolates were resistant or intermediately resistant to ertapenem (65%), ofloxacin (35%) and amikacin (40%). However, none of the *P. aeruginosa* isolates were susceptible to cefoxitin and cloxacillin (Table 3).

Bacteria	Sample (n)	Number (%)	Morphological Appearance	Gram reaction	Biochemical reaction
Pseudomonas aeruginosa	Anal swabs (25)	20 (80)	Colonies with greenish pigmentation on cetrimide selective agar	Gram negative	Oxidase positive
Klebsiella species	Anal swabs (25)	12 (48)	Mucoid colonies on MAC; non-metallic green sheen colonies on EMB	Gram negative	Citrate positive Urease positive

N-Number,

%-Percentage;

EMB-Eosin Methylene Blue;

MAC-MacConkey

Table 1: *Isolation of Klebsiella species and Pseudomonas aeruginosa.*

Antibiotics (µg)	Resistance n (%)	Susceptible n (%)
Imipenem (10)	0 (0)	12 (100)
Cefoxitin (30)	12 (100)	0 (0)
Amikacin (10)	0 (0)	12 (100)
Ofloxacin (10)	2 (16.7)	10 (83.3)
Ertapenem (10)	9 (75)	3 (25)
Cloxacillin (10)	12 (100)	0 (0)
Ceftazidime (30)	10 (83.3)	2 (16.7)

Table 2: *Antibiogram of isolates of Klebsiella species.*

Antibiotics (µg)	Resistance n (%)	Susceptible n (%)
Ertapenem (10)	13 (65)	7 (35)
Cefoxitin (30)	20 (100)	0 (0)
Imipenem (10)	0 (0)	20 (100)
Ceftazidime (30)	1 (5)	19 (95)
Ofloxacin (10)	7 (35)	13 (65)
Cloxacillin (200)	20 (100)	0 (0)
Amikacin (10)	8 (40)	12 (60)

Table 3: *Antibiogram of isolates of P. aeruginosa.*

MARI and Incidence of MBL-Producing *Klebsiella* Species and *P. Aeruginosa*

Table 4 shows the prevalence of MBL-producing isolates of *P. aeruginosa* and *Klebsiella* species recovered in this study. All the 12 isolates of *Klebsiella* species recovered in this study were phenotypically positive for MBL production. A total of 14 isolates of P. *aeruginosa* out of the 20 *P. aeruginosa* isolates were phenotypically confirmed to be MBL positive by the Hodges test method. On average, the result of the multiple antibiotic resistance profile of the MBL-producing isolates of *Klebsiella* species and *P. aeruginosa* bacteriologically recovered in this study revealed that the bacterial isolates were found to be resistant to about 5 antibiotics out of the 7 antibiotics that were tested in this study.

Phenotype	*Klebsiella* species n (%)	*P. aeruginosa* n (%)
MBL positive	12 (100)	14 (70)
MBL negative	0 (0)	6 (30)

Table 4: *Occurrence of MBL-producing isolates.*

Discussion

Metallo-beta-lactamases (MBLs) are expanded spectrum enzymes that gives Gram negative bacteria the exceptional ability to resist the antimicrobial onslaught of some potent and available antimicrobial agents especially the carbapenems such as imipenem, ertapenem and meropenem. Carbapenems are used for the treatment of serious bacterial related infections including those caused by pathogenic bacteria that produces extended spectrum beta-lactamase (ESBL). However, some Gram negative bacterial strain including but not limited to Klebsiella species and *P. aeruginosa* now possess the ability to resist the antimicrobial prowess and potentials of the carbapenems [3,4,6,7]. In this study, the antibiogram and occurrence of MBL-producing isolates of Klebsiella species and *P. aeruginosa*

was bacteriologically and phenotypically investigated in rectal/anal swab samples obtained from ready-to-be-slaughtered cows in an abattoir in Abakaliki, Nigeria. The result of the bacteriological analysis of the anal/rectal swab samples showed that *P. aeruginosa* (n = 20; 80%) was isolated more from the samples than Klebsiella species (n = 12; 48%). *P. aeruginosa* is usually an opportunistic bacterium implicated in a number of infectious diseases while Klebsiella species is a member of the human normal flora that can also be implicated as a causative agent of some bacterial infections in humans [16,17]. The result of the antimicrobial susceptibility testing showed that the isolated *Klebsiella* species was resistant or intermediately resistant to ertapenem (75%), ceftazidime (83.3%), cloxacillin (100 %) and cefoxitin (100%). Ertapenem and cefoxitin are broad spectrum antibiotics that are used for the treatment of serious bacterial infections. The antibiotic resistant nature of *Klebsiella* species isolated from the anal/rectal swab samples of ready-to-be-slaughtered cows in this region portends serious health challenge because of their reduced susceptibility to the tested antibiotics. The isolates of *P. aeruginosa* were also found to be resistant or intermediately resistant to ertapenem, cefoxitin, cloxacillin, ofloxacin and amikacin, which are used to treat bacterial infections caused by *P. aeruginosa*. Imipenem was found to be the most active antibiotic in this study given that all the isolates of Klebsiella species and *P. aeruginosa* were found to be completely susceptible to this carbapenem. This was followed by amikacin which was only active against the isolates of the Klebsiella species. The antibiotic resistance profile of the *Klebsiella* species and *P. aeruginosa* isolates recovered in this study is worrisome – owing to the fact that some of the antibiotics to which these organisms are resistant to are often used as both first line and second line or even last line antibiotics for antimicrobial therapy in hospitals. The observed resistance of bacteria to these antibiotics as obtainable in our study should therefore be checkmated through proper antimicrobial susceptibility testing on bacterial isolates from the non-hospital environment in order to keep antibiotic resistant bacteria at bay. In an earlier report of ours, we reported similar resistance profile amongst Gram negative bacteria from both the hospital and non-hospital environment [7,8,15]. The production of metallo β-lactamase (MBL) was phenotypically confirmed in all isolates of the Klebsiella species (n = 12; 100%) investigated in this study. MBL production was phenotypically confirmed in only 14 (70%) isolates of *P. aeruginosa* out of the 20 isolates recovered in this study. Samah and Noha [2] also reported high prevalence of MBL production in Gram negative bacteria recovered from clinical samples of patients with haematological conditions. Also in Brazil and India, MBL production has also been reported in *P. aeruginosa* isolates that were found to be imipenem resistant in nature [9, 10]. The *Klebsiella* species and *P. aeruginosa* isolates that were positive for MBL production were multiply resistant to 5 antibiotics out of the 7 antibiotics used in this study. This indicates that these isolates are multidrug resistant in nature since they were found to be serially and/or multiply resistant to some available antibiotics as used in this study.

Conclusion

This present study phenotypically confirmed the occurrence of MBL producing *Klebsiella* species and *P. aeruginosa* from anal/rectal swabs of ready-to-be-slaughtered animals in Abakaliki, Nigeria. Also, the isolated *Klebsiella* species and *P. aeruginosa* isolates were multiply resistant to some available antibiotics.

Recommendations

Antibiotic usage in animal husbandry and for poultry production should be replaced with proper sanitation, vaccination and other acceptable practices that are devoid of antibiotics application, since the singular use of antibiotics promotes resistance development in bacteria. The emergence and dissemination of antibiotic resistant bacteria in the non-hospital environment should be monitored and kept under check through surveillance and proper detection of these organisms. Finally, the government and health policy makers should step up and embark on periodic public awareness creation on antibiotic resistance and the proper usage or non-usage of antibiotics in agriculture, poultry production and animal husbandry.

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