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Emerging Multidrug Resistant Metallo-β-Lactamases (MBLs) Positive *Klebsiella* Species from Cloacal Swabs of Poultry Birds

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Abstract

The increasing prevalence of antibiotic resistant bacteria in the community is a public health issue because this phenomenon compromises our ability to effectively treat infectious diseases since these organisms such as those that express metallo-β-lactamase (MBL) are usually resistant to a wide variety of antibiotics. This study investigated the frequency of *Klebsiella* species from a local poultry farm that produced metallo-β-lactamase using phenotypic detection technique. Forty (40) samples from the cloacae of poultry birds were used for this study. Each sample was bacteriologically analyzed on MacConkey agar, and the isolated organism was identified by standard microbiology techniques. Susceptibility testing was done using disk diffusion technique, and the production of MBL was confirmed using disk diffusion technique in which EDTA was used as a chelating agent. Totally, 24 *Klebsiella* species isolates were isolated from the samples. High resistance of the *Klebsiella* species isolates was observed to oxacillin (100 %), ofloxacin (95.8 %), gentamicin (87.5 %), ertapenem (62.5 %), cefoxitin (58.3 %) and ciprofloxacin (87.5 %). The expression of MBL was only confirmed phenotypically in 5 (41.7%) *Klebsiella* species isolates. The indiscriminate use of antibiotics especially in the rearing of animals allows microbes to develop resistance through selective pressure. However, the timely and accurate detection of drug resistant microbes is critical to forestalling the emergence and spread these organisms in the community.

Keywords: MBLs; *Klebsiella* species; Carbapenems; Carbapenemases; Nigeria

Introduction

Metallo-β-lactamases (MBLs) are a type of carbapenemases that hydrolyze the carbapenems including imipenem, ertapenem and meropenem. MBLs are β-lactamases that belong to Ambler's class B type of enzymes, and they degrade a wide variety of β – lactams, penicillins and the carbapenems [1]. Pathogenic bacteria that produce MBLs are usually susceptible to aztreonam, a monobactam; and they are inhibited by chelating agents. 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 virtually all β–lactam drugs and some non β–lactam drugs like aminoglycosides, fluoroquinolones, and co-trimoxazoles used in clinical medicine today [1-4]. The presence of MBL genes in clinically important organisms threatens the efficacy of some available beta-lactam and non-beta-lactam agents [1,5]. Organisms producing MBLs in the community are of immense public health importance since they are usually resistant to a wide variety of antibiotics, and this makes these drugs to be less effective when used for therapy [6]. Such organisms harbouring genes for the production of MBLs and other multidrug resistant enzymes may cause serious infections. The emergence of multidrug resistant bacteria in both the hospital and non-hospital environments poses a serious public health challenge since these organisms defy the antimicrobial onslaught of some antibiotics. Thus, this study evaluated the occurrence of MBL-producing *Klebsiella* species using phenotypic detection technique.

Materials and Methods

Sample collection

Forty (40) samples from the cloacal region of poultry birds in a local poultry farm in Abakaliki metropolis were collected for this study. The samples were aseptically collected by inserting a sterile swab sticks in the cloacal region of the poultry birds at a depth of 3 cm and rotated at angle 360°. The swab sticks was returned to their respective containers, labeled and transported to the Microbiology Laboratory Unit of Ebonyi State University, Abakaliki for further bacteriological analysis.

Culture and identification of *Klebsiella* species

Each of the swab sticks were dipped in test tubes containing 5 ml of freshly prepared nutrient broth (Oxoid, UK) and the tubes were loosely covered with cotton wool, and incubated at 30°C for about 18-24 hours. Bacterial growth was indicated by the presence of turbidity in the tubes. Tubes showing turbidity (as indication of bacterial growth) were aseptically subcultured onto freshly prepared MacConkey agar (Oxoid, UK) plates and incubated at 30°C for 18-24 hours. Suspect colonies of *Klebsiella* species was subcultured onto freshly prepared

MacConkey agar plates for the isolation of *Klebsiella* species which form mucoid colonies on MacConkey agar. The identification of *Klebsiella* species was done using standard microbiology techniques [7].

Antibiotic susceptibility testing

Susceptibility testing was done on Mueller-Hinton (MH) agar (Oxoid, UK) plates by disk diffusion method as per the Clinical Laboratory Standard Institute guideline using imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), amikacin (10 µg), ofloxacin (5 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ciprofloxacin (10 µg), oxacillin (1 µg), cefoxitin (30 µg) and gentamicin (10 µg). Briefly, the MH agar plates were swabbed with the test isolates (adjusted to 0.5 MacFarland turbidity standards); and each of the antibiotic disk was aseptically placed on the MH agar plates at a distance of 15 mm. The susceptibility test plates were incubated at 30°C for 18-24 hours and the zones of inhibition diameter was measured, recorded and interpreted according to the CLSI criteria [6,8].

MBL screening test

To screen bacterial isolates for the production of MBLs, it has been recommended to use any of the carbapenems including meropenem and imipenem since MBL positive bacteria are resistant to the carbapenems [6,8]. The Kirby-Bauer disk diffusion technique was used, and each of the carbapenems including meropenem, ertapenem and imipenem was placed at a distance of 20 mm apart on MH agar plates already inoculated with the test bacterial isolates and the plates were incubated at 30°C for 18-24 hours. MBL production was suspected when the test organism showed reduced susceptibility to any of the carbapenems. As per the CLSI criteria, isolates showing inhibition zone diameter (IZD) of ≤ 23 mm were suspected to produce MBL and these isolates were subjected to phenotypic confirmation test.

Inhibition based assay for confirmation of MBL production

Test organisms found to be resistant to imipinem or meropenem (as indicated in the screening test) was evaluated phenotypically for the production of MBLs as was previously described [4,6]. Standard antibiotic disks of imipenem (10 µg) and meropenem (10 µg), impregnated with EDTA were aseptically placed on MH agar plates already swabbed with the test bacterial isolates (adjusted to 0.5 MacFarland turbidity standards), and supplementary imipenem (10 µg) and meropenem (10 µg) disks without EDTA were also placed alongside antibiotic disks impregnated with EDTA. All the plates were incubated at 30°C for 18-24 hours and zones of inhibition were measured, recorded and interpreted as per the CLSI criteria. A difference of ≥ 7 mm between the zones of inhibition of any of the carbapenem disks with and without the chelating agents infers metallo-β-lactamase production phenotypically [6,9].

Results

This study phenotypically evaluated the occurrence of *Klebsiella* species producing metallo-β-lactamase from the cloacal swabs of poultry birds in a local poultry farm in Abakaliki metropolis of Ebonyi State, Nigeria. The frequency of isolation of *Klebsiella* species from the cloacal swab samples of the poultry birds is shown in Table 1. A total of 24 isolates of *Klebsiella* species were isolated from the cloacal swab samples of the poultry birds over a period of one month (July, 2016), and these isolates were positive for urease test and citrate test (Table 1).

Table 2 shows the result of antimicrobial susceptibility profile of the isolated *Klebsiella* species to some commonly available antibiotics. The antimicrobial susceptibility pattern revealed that the *Klebsiella* species isolates were more susceptible to amikacin (87.5%), imipenem (95.8%), meropenem (91.7%), ceftriaxone (66.7%), cefotaxime (62.5%) and ceftazidime (58.3%). However, the isolates showed high resistance to ertapenem, ofloxacin, ciprofloxacin, oxacillin, gentamicin and cefoxitin (Table 2).

Sample source	No of samples	n (%) of *Klebsiella* species	Urease test	Citrate test	Gram staining reaction
Cloacal swabs of poultry birds	40	24 (60)	+	+	Gram negative

Table 1: Frequency of *Klebsiella* species isolation.

Antibiotics (µg)	n (%)Resistance	n (%) Intermediate	n (%) Susceptibility
AK (10)	1 (4.2)	2 (8.3)	21 (87.5)
CRO (30)	8 (33.3)	0 (0.0)	16 (66.7)
CAZ (30)	9 (37.5)	1 (4.2)	14 (58.3)
FOX (30)	14 (58.3)	4 (16.6)	6 (25.0)
CTX (30)	9 (37.5)	0 (0.0)	15 (62.5)
IPM (10)	0 (0.0)	1 (4.1)	23 (95.8)
CN (10)	21 (87.5)	0 (0.0)	3 (12.5)
OX (1)	24 (100)	0 (0.0)	0 (0.0)
MEM (10)	0 (0.0)	2 (8.3)	22 (91.7)
OFX (5)	23 (95.8)	0 (0.0)	1 (4.2)
ETP (10)	15 (62.5)	0 (0.0)	9 (37.5)
CIP (10)	21 (87.5)	2 (8.3)	1 (4.2)

Table 2: Antimicrobial susceptibility profile of 24 *Klebsiella* species isolated from the cloacal swabs of poultry birds. Key: CN=gentamicin, OX=oxacillin, OFX=ofloxacin, ETP=ertapenem, CIP=ciprofloxacin, AK=amikacin, CRO=ceftriaxone, CAZ=ceftazidime, IPM=imipenem, FOX=cefoxitin, MEM=meropenem, CTX=cefotaxime.

Organism (n=24)	Sample source	Suspected MBL producers n (%)	MBL positive n (%)	MBL negative n (%)
Klebsiella species	Cloacal swabs of poultry birds	12 (50)	5 (41.7)	7 (58.3)

Table 3: Distribution of MBL positive *Klebsiella* species and MBL negative *Klebsiella* species from cloacal swabs of poultry birds.

Table 3 shows the occurrence of MBL positive *Klebsiella* species in this study. Out of the 24 isolates of *Klebsiella* species screened for the production of MBLs, 12 *Klebsiella* species isolates were suspected to express MBLs phenotypically. However, only 5 (41.7 %) isolates of

Klebsiella species were phenotypically confirmed to produce MBLs by the inhibition-based assay technique used in this study (Table 3).

Discussion

The expression of carbapenemases including metallo-β-lactamases (MBLs) is one of the major mechanisms of carbapenem resistance in Gram negative bacteria; and the production of this enzyme gives the organism the ability to ward-off the antimicrobial action of the carbapenems – which are considered potent agents for the treatment of infections caused by bacteria that produce extended spectrum β-lactamases (ESBLs). In this study, the antimicrobial susceptibility profile and production of MBLs by *Klebsiella* species from the cloacal swabs of poultry birds was investigated phenotypically. Out of the 40 cloacal swab samples that were bacteriologically analyzed in this study, a total of 24 *Klebsiella* species was isolated. All the isolated *Klebsiella* species showed high resistance to the tested antibiotics. The *Klebsiella* species isolates was resistant to ciprofloxacin (87 %), ofloxacin (95.8 %), oxacillin (100 %), gentamicin (87.5 %), cefoxitin (58.3 %), ceftazidime (37.5 %) and ceftriaxone (33.3 %). The Klebsiella species were also resistant to ertapenem but susceptible to imipenem and meropenem. The high resistance profile of the isolated *Klebsiella* species in this study is in agreement to a similar and recent work carried out in Japan by Okazaki *et al.* [10] in which *K. pneumoniae* isolated from a non-hospital environment was reported to be resistant to some non-beta-lactam and beta-lactam antibiotics including gentamicin, amikacin and cefotaxime. Totally, 12 isolates of *Klebsiella* species were suspected to express MBL when tested. However, only 5 (41.7 %) *Klebsiella* species isolates was phenotypically confirmed to express MBL by the inhibition-based assay. The frequency of MBL positive *Klebsiella* species as reported in this study is similar to a previously conducted work by Ejikeugwu *et al.* [6] in which MBL expression was phenotypically confirmed in *Klebsiella* species. In Nepal, Bora *et al.* [11] also reported that *Klebsiella pneumoniae* from clinical isolates expressed MBLs. In another related study, Enwuru *et al.* [12] in Lagos, Nigeria also reported that *Klebsiella* species from hospital environments expressed MBL phenotypically, and that these organisms are resistant to some commonly available antibiotics. The emergence and spread of MBL-producing *Klebsiella* species in the community as reported in this study may portend public health issues if these organisms are transmitted to humans via the food chain. Conclusively, this study reports the occurrence and expression of metallo-β-lactamase (MBL) in *Klebsiella* species from a non-hospital source, particularly from poultry birds; and the isolates were found to be resistant to some commonly used antibiotics. We therefore recommend the timely and accurate detection of organisms producing MBLs since such measures will help to prevent the dissemination of MBL positive bacteria in either the community or hospital environment.

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