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Prevalence of Ampc β-Lactamase-producing *Pseudomonas Aeruginosa* isolates from feacal matter of cow

Abstract

The possible contamination of food and/or food-producing animals with multidrug resistant bacteria including AmpC-producing *Pseudomonas aeruginosa* is considered a potential source for the wide dissemination of AmpC β-lactamase in the community. This portends public health risks for the populace – owing to the multidrug resistant nature of these organisms. There is paucity of data on the prevalence of AmpC-producing bacteria in Abakaliki, Nigeria – which was why this study was carried out. A total of 40 feacal swab samples from cow dung in a local abattoir were bacteriologically examined for the isolation and antimicrobial susceptibility testing of *P. aeruginosa* isolates using standard microbiological procedures on cetrimide selective agar and Kirby-Bauer disk diffusion method respectively. AmpC β-lactamase was phenotypically confirmed using the ceftazidime-imipenem antagonism test (CIAT). A total of 12 *P. aeruginosa* isolates were recovered from the samples; and they showed varied levels of resistance to the tested antibiotics especially to cefoxitin, ertapenem, oxacillin, amikacin and cefotaxime. Among the 12 *P. aeruginosa* isolates, AmpC β-lactamase was phenotypically detected in 3 (25%) isolates by the CIAT method. This study has presumptively shown that AmpC β-lactamase-producing *P. aeruginosa* isolates occur in abattoir. Thus, molecular characterization of the genes that encode AmpC β-lactamase production in this organism is crucial for a reliable epidemiological investigation into the possible emergence and dissemination of AmpC positive bacteria in the community.

Keywords: AmpC β-lactamase, gram negative bacteria, community acquired infections, abattoir, Nigeria

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Introduction

Environmental microorganisms represent the most relevant reservoir of resistance to antibiotics in the community, and this is due in part to the undue exposure of these organisms to antimicrobial agents. AmpC β-lactamases are clinically important cephalosporinases encoded on the chromosomes of bacteria in the *Enterobacteriaceae* family and some non-enteric bacteria including *Pseudomonas aeruginosa*, where they stimulate these organisms to be resistant to the cephamycins (cefoxitin for example).[1,2] Gram negative bacteria that produce AmpC β-lactamases have been implicated in many cases of hospital and community acquired infection.[1–3] For enteric organisms with the potential for high-level AmpC β-lactamase production by mutation, the development of resistance upon therapy especially to the cephamycins is a concern for public health since these agents are critical in the treatment and management of bacterial-related infections in humans.[4]

AmpC β-lactamase production in most *Enterobacteriaceae* including *Klebsiella pneumoniae* and *Escherichia coli* is low but the hyper-production of AmpC enzymes in these organisms is usually induced by the exposure of the bacteria to antibiotics.[3] AmpC β-lactamases can be expressed at high levels by mutation. The over expression of AmpC β-lactamases confers resistance to broad-spectrum cephalosporins including cefotaxime, ceftazidime, and ceftriaxone. It has been reported that transmissible plasmids have acquired genes for AmpC β-lactamases, which consequently can now appear in bacteria lacking or poorly expressing a chromosomal AmpC β-lactamase gene as is seen in *E. coli*, *K. pneumoniae*, and *Proteus mirabilis*.[5] The genes that confer or mediate the production of AmpC β-lactamases in Gram-negative bacteria are often chromosomally-borne rather than being plasmid encoded but these genes may still be plasmid-encoded in some bacteria.[6] The AmpC β-lactamases are clinically important beta-lactamases because they confer antimicrobial resistance to the narrow-spectrum, expanded-spectrum and the broad-spectrum cephalosporins and the penicillins; and their resistance is also expressed towards the β-lactamase inhibitors such as amoxycillin-clavulanic acid.[7,8] Techniques to identify AmpC β-lactamase-producing isolates are available but are still evolving and are not yet optimized for the clinical laboratory, which probably now underestimates this resistance mechanism in either the community or hospital environment. Nevertheless, the timely and accurate detection of organisms harbouring genes responsible for the production of AmpC β-lactamases is critical for total patient care. The occurrence of AmpC-producing *P. aeruginosa* from abattoir calls for effective monitoring and possibly a ban on the use of antibiotics in the production and/or rearing of food producing animals in order to assuage the emergence and spread of drug resistant bacteria in the community.[7,19,21] Thus, this study presumptively detected the occurrence of AmpC-producing *P. aeruginosa* isolates from feacal matter of cow in a local abattoir.

Materials and methods

Sample collection and processing

A total of forty (40) swab samples were collected from feacal matter of cow in a local abattoir using sterile swab sticks. After collection, each of the swab sticks was returned into their respective containers, labeled and transported to the Microbiology Laboratory Unit of Ebonyi State University, Abakaliki for bacteriological analysis.

Isolation and identification of *Pseudomonas aeruginosa*

Cetrimide selective agar (Oxoid, UK) containing 10 ml of glycerol was used for the selective isolation of *P. aeruginosa* from the test samples. Each of the feacal swab samples was inoculated in 5 ml double strength of freshly prepared nutrient broth (Oxoid, UK) and incubated for 18-24 hours at 30oC. A loopful of the broth culture was aseptically streaked on cetrimide selective agar plates; and the plates were incubated at 30oC 18-24 hours. Suspected colonies were inoculated onto freshly prepared cetrimide selective agar for the isolation of pure cultures of *P. aeruginosa*. *P. aeruginosa* produces greenish/bluish pigmentation on cetrimide selective agar. The suspected *P. aeruginosa* isolates were identified using standard microbiological identification techniques.[9,10]

Susceptibility studies

Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disk diffusion method as per the guidelines of the Clinical Laboratory Standard Institute (CLSI) with single antibiotic disks comprising (disk concentration in µg): ceftazidime (CAZ; 30 µg), gentamicin (CN; 10 µg), cefoxitin (FOX; 30 µg), ciprofloxacin (CIP; 10 µg), ertapenem (ETP; 10 µg), meropenem (MEM; 10 µg), ceftriaxone (CRO; 30 µg), cefotaxime (CTX; 30 µg), ofloxacin (OFX; µg), amikacin (AK; 10 µg) and imipenem (IMP; 10 µg).Oxoid, UK. Each disk was aseptically placed on Mueller-Hinton agar plates containing the test isolates (adjusted to 0.5 McFarland turbidity standards); and the plates were incubated at 30oC for 18-24 hours. Susceptibility testing was carried out in triplicate for each isolate and the mean inhibition zone diameter (IZD) was recorded as the final IZD and reported as per the CLSI guidelines.[11,12] *P. aeruginosa* ATCC 25668 was used as quality control strain for the antimicrobial susceptibility studies.

Screening test for AmpC β-lactamase

The presence of AmpC β-lactamase was screened for in the test *P. aeruginosa* isolates according to the method of Singhal et al.[13]. A suspension of the test organism (adjusted to 0.5 McFarland turbidity standards) was swabbed on Mueller-Hinton agar plates and antibiotic disk of cefotaxime (30 µg) and ceftazidime (30 µg) were placed adjacent to cefoxitin (30 µg) disk at a distance of 20 mm from each other. Bacterial isolates showing blunting of ceftazidime or cefotaxime zone of inhibition adjacent to cefoxitin disk or showing reduced susceptibility to either of ceftazidime or cefotaxime and cefoxitin were considered possible AmpC β-lactamase producers.

Confirmatory test for AmpC β-lactamase production

AmpC β-lactamase production in the test isolates was phenotypically confirmed using the ceftazidime-imipenem antagonism test (CIAT) which was performed using ceftazidime (30 µg) disk, cefoxitin (30 µg) disk and imipenem disk (10 µg) as described by Cantarelli et al.[14] Ceftazidime disc and imipenem disk were placed at a distance of 20 mm apart on Mueller-Hinton agar plate previously inoculated with a suspension of the test bacteria (adjusted to 0.5 McFarland turbidity standards) that showed reduced susceptibility to cefoxitin. A cefoxitin disk (30 µg) was also placed at a distance of 20 mm from the ceftazidime disk for comparison. All the susceptibility test plates were incubated at 30oC for 18-24 hrs. Antagonism indicated by a visible reduction in the inhibition zone around the ceftazidime disk adjacent to the imipenem or cefoxitin disk was inferred as a positive inducible AmpC β-lactamase production.[11,14]

Results

Table 1 shows the rate of recovery of *P. aeruginosa* isolates from the feacal swab samples of the feacal matter of cow. Out of the 40 feacal swab samples that was bacteriologically analyzed in this study, a total of 12 *P. aeruginosa* isolates was isolated on cetrimide selective agar incorporated with glycerol (Table 1). The result of antimicrobial susceptibility studies carried out on the isolated *P. aeruginosa* isolates is shown in Figure 1. The *P. aeruginosa* isolates were found to be mostly resistant to ceftriaxone (100 %), ertapenem (100 %), oxacillin (100 %), cefoxitin (100 %), amikacin (58.3 %) and cefotaxime (75 %). Meropenem, a carbapenem antibiotic also had little inhibitory activity against the *P. aeruginosa* isolates (Figure 1). However, the isolated *P. aeruginosa* isolates were found to be susceptible to the antimicrobial action of imipenem (100 %), ceftazidime (83.3 %), ofloxacin (83.3 %), gentamicin (91.7 %), and ciprofloxacin (91.7 %). Table 2 shows the frequency of AmpC β-lactamase-producing *P. aeruginosa* isolates phenotypically confirmed by the ceftazidime-imipenem antagonism test (CIAT). From a total of 12 *P. aeruginosa* isolates that was recovered from the feacal swab samples of cow dung's, AmpC β-lactamase production was phenotypically confirmed in 3 (25 %) *P. aeruginosa* isolates by the CIAT method. However, the other 9 isolates of *P. aeruginosa* isolates were not confirmed to produce AmpC β-lactamase as determined by the ceftazidime-imipenem antagonism test (CIAT).

Table 1 Recovery rate of *P. aeruginosa* on cetrimide selective agar

Sample source	No of samples	No (%) of isolates
Feacal matter of cow	40	12 (30)

Table 2 Frequency of AmpC β-lactamase producing *P. aeruginosa* isolates

Organism	Source	n(%) of isolates	Ampc positive n (%)	Ampc negative n (%)
P. aeruginosa	Feacal matter of cow	12	3(25)	9 (75)

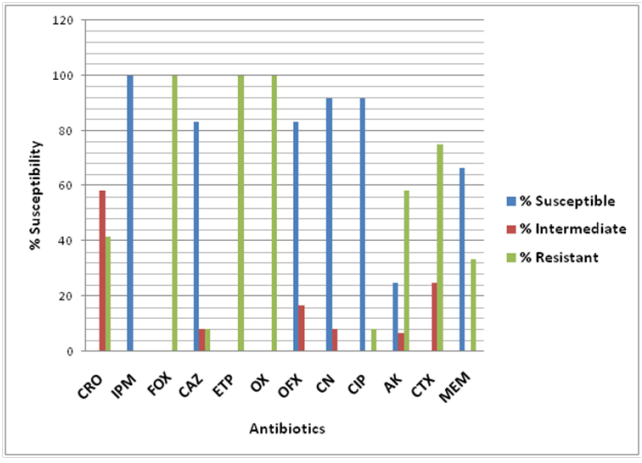

Figure 1 Antimicrobial susceptibility profile of the *P. aeruginosa* isolates.
Key: CRO: Ceftriaxone; IPM: Imipenem; FOX: Cefoxitin; CAZ: Ceftazidime; ETP: Ertapenem; OX: Oxacillin; OFX: Ofloxacin; CN: Gentamicin; CIP: Ciprofloxacin; AK: Amikacin; CTX: Cefotaxime; MEM: Meropenem.

Discussion

The production of AmpC β-lactamases inclusive of extended spectrum β-lactamases (ESBLs) and metallo β-lactamases (MBLs)

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is one of the mechanisms of drug resistance in Gram negative bacteria especially those in the *Enterobacteriaceae* family and some non-enteric bacteria such as *P. aeruginosa*. Since the detection of multidrug resistant bacteria including those that produce AmpC β-lactamases is fundamental for gathering sound epidemiological data (needed for infection control measures for example) and proper patients management, this study was designed to presumptively detect the possible occurrence of AmpC-producing *P. aeruginosa* in feacal matter of cow from a local abattoir. In this study, the frequency of *P. aeruginosa* isolates from the 40 feacal swab samples of cow dung's analyzed was 30 %; and this represents a total of 12 *P. aeruginosa* isolates that was isolated in this study. *P. aeruginosa* is a nosocomial and opportunistic Gram negative bacterium that is notorious in causing both community and hospital acquired infection/ diseases; and the bacterium is intrinsically resistant to antimicrobial agents.[15] It was also observed that the *P. aeruginosa* isolates were highly resistant to the cephamycin, cefoxitin. Ertapenem and oxacillin also had no inhibitory activity against the *P. aeruginosa* isolates. The *P. aeruginosa* isolates was also found to be highly resistant to amikacin, cefotaxime and meropenem. However, the carbapenem, imipenem showed satisfactory level of antimicrobial activity against the *P. aeruginosa* isolates from the feacal samples of animal dung. Ceftazidime, ofloxacin, ciprofloxacin and gentamicin showed appreciable level of antimicrobial activity against the *P. aeruginosa* isolates at the rate of 83.3 %, 83.3 %, 91.7 %, and 91.7 % respectively. Reports around the world show that *P. aeruginosa* is notorious for its resistance to antibiotics.[16-18] Our result on the antimicrobial susceptibility patterns of *P. aeruginosa* isolates to antibiotics is similar to the work of Franco et al.[19], in which high-level resistance of *P. aeruginosa* isolates to the carbapenems was reported in Brazil. Our result is also similar to the work of Aibinu et al.[20] & Olutayo and Abimbola.[21] - who reported similar resistance patterns of *P. aeruginosa* isolates in southwest Nigeria. Bashir et al.[22] and Akinduti et al.[23]also reported in their study carried out in Kashmir and Abeokuta respectively that *P. aeruginosa* is a multidrug resistant organism that is notoriously resistant to several antibiotic classes. And the multidrug resistant nature of *P. aeruginosa* isolates has been confirmed by Fernandez et al.[16] in the Americas as well. The prevalence of AmpC β-lactamase positive *P. aeruginosa* isolates was 25 %. This rate of prevalence of AmpC-producing *P. aeruginosa* isolates is similar to the work of Akinduti et al.[23] carried out in southwest Nigeria. The prevalence of AmpC-positive *P. aeruginosa* isolates in this study is lower than the report of Abd El-Baky et al.[24] who reported higher prevalence of AmpC-positive *P. aeruginosa* (72.4 %) in their study. The occurrence of antibiotic resistant bacteria from the community portend public health risk to mankind owing to the fact that drug resistant bacteria could easily be transmitted in human population through the food chain since antibiotics now serves as an important factor in the rearing and production of livestock and even poultry birds. Conclusively, proper surveillance measures aimed at detecting and taming this menace is critical to bring the emergence and spread of AmpC β-lactamase-producing *P. aeruginosa* in the community under control.

Conflicts of interest

There is no conflict of interest.

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