Research article

Phenotypic Detection and Antimicrobial Profile of Metallo-Beta-Lactamase Producing *Pseudomonas aeruginosa* from Gunshot Wounds of In-Patients in Tertiary Hospitals in Maiduguri, Nigeria

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Background: *Pseudomonas aeruginosa* (*P. aeruginosa*) is the most common and significantly drug resistant bacteria isolated from all categories of in-patient wounds. The resistance is attributed to the production of metallo-ß-lactamases (MßLs) responsible for high morbidity and mortality.

Methods: *P. aeruginosa* was isolated and identified from wound samples using Bergey's manual of systemic bacteriology. Each wound swab was used to inoculate MacConkey agar and Cetrimide agar. The ethylenediaminetetraacetic acid (EDTA) disk testing method and modified Kirby-Bauer disc diffusion method was conducted to determine the phenotypic characterization and antimicrobial profile of MßL-producing *P. aeruginosa*.

Results: Out of the 100 wound swabs examined, 24 (24.0%) of the isolates were identified as *P. aeruginosa*. Among the 24 isolates identified as *P. aeruginosa*, 8/24 (33.3%) of those isolates were MßL-producing. Male in-patients had a higher prevalence of 19 (79.2%) for *P. aeruginosa* than females with 5 (20.8%) (P< 0.05). MßL-producing *P. aeruginosa* was of a higher prevalence in males with 7 (87.5%) than in females with 1 (12.5%) (P<0.05). Based on wound location, the lower limbs had a higher prevalence of 18 (75%) than those on the upper limbs 3 (12.5%), head 2 (8.3%) and abdomen 1 (4.2%) (P<0.05) for *P. aeruginosa*. MßL-producing *P. aeruginosa* also had higher prevalence for wounds on the lower limbs 7 (87.5%) compared with those on the upper limb 1 (12.5%), head and abdomen each with 0(0%) (P<0.05). Antimicrobial profiles of *P. aeruginosa* isolates indicated a 100% resistance against cefepime 30 µg and ceftazidime 30 µg and highest level of susceptibility (20.8%) for meropenem 10 µg, imipenem 10 µg and gentamicin 10 µg. While all the MßL-producing *P. aeruginosa* isolates recorded a 100% resistance against all the antibiotics used.

Conclusion and Recommendation: These results demonstrate that spread as well as the rate of drug resistance among the MßL-producing *P. aeruginosa* is concerning. Imipenem and meropenem are potential drugs of choice for treatment of infections caused by MßL-producing *P. aeruginosa*.

Key words: Metallo-Beta-Lactamases, *Pseudomonas aeruginosa,* Gunshot-wounds, In-patients, Antibiotic resistance, Maiduguri-Nigeria

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Introduction

Gunshot wounds are one of the most common cause of trauma worldwide and contribute significantly to economic burden, death and disability.[1,2] The wound is usually contaminated with foreign materials such as bullets and thus, promotes pathogenic colonization and breakdown of defense mechanisms.[3,4] The presence of colonizing microorganisms continue to provoke the immune system and interfere with normal healing process.[5] Moreover, antibiotic resistance in wound pathogens reduces the efficacy of the antibiotic treatment protocol.[4]

Pseudomonas aeruginosa (*P. aeruginosa*) is regarded as the most common agent of wound infection especially in developing countries.[6,7,8,9] The organism is intrinsically resistant to multiple antibiotics including ß-lactams (carbapenems), aminoglycosides, fluoroquinolones, and polymyxin B.[10,11] The prevalence of carbapenem resistance has been increasingly reported in several countries.[10,12]

In 2017, the World Health Organization (WHO) ranked carbapenem-resistant-*P. aeruginosa* as the second most critical-priority bacterium among 20 antimicrobial-resistant bacterial species.[13] This resistance is attributed to the production of metallo-ß-lactamases (MßLs) which cleave the amide bond of the ß-lactone ring, impermeability or loss of porin OprD, or increased expression of an efflux pump.[14,15] MBL–mediated resistance has been responsible for high morbidity and mortality among in-patients worldwide.[10,12,18,16,17,18] It is imperative to conduct the rapid detection of MßL-producing *P. aeruginosa* amongst gunshot in-patients in Maiduguri as an aid to management and empirical therapy.

Study area

The study was conducted at the University of Maiduguri Teaching Hospital and Nigeria Army 7 Division Medical Services and Hospital, Maiduguri Nigeria from February 2022 to June 2022. Maiduguri, the capital of Bono State in Nigeria, is located in North-Eastern Nigeria and lies within latitude 11.15°N and longitude 30.05°E in the sudano-sahelian savanna zone.[19] The state has an area of 71.210sq km with the population of 4,151,193 according to National census conducted in 2006.[20]

Methodology

Samples population, collection, and processing

A total of one hundred (male: 76 and female: 24) wound swabs were aseptically collected using convenience purposive sampling from gunshot in– patients at the University of Maiduguri Teaching Hospital and the Nigerian army 7 Division medical services and Hospital Maiduguri. The samples were presserved on ice for investigation at the department of microbiology laboratory, University of Maiduguri. Informed consent for each sample collected was obtained and included the age of the patient, gender, and wound location.

Isolation and identification of Pseudomonas aeruginosa

Isolation and identification of the bacteria was conducted as described in Bergey's manual of systemic bacteriology.[21] Each wound swab was inoculated by spread plate on MacConkey agar and Cetrimide agar and incubated aerobically at 37°C for 24hrs. Isolates were identified using morphological and biochemical characteristics.[22]

Phenotypic test for detection of MßL-producing Pseudomonas aeruginosa

The phenotypic detection of MßL-producing *P. aeruginosa* isolates was conducted by EDTA disk testing. An overnight culture of the isolates was prepared in 2 mL of Mueller-Hinton broth (MHB), with turbidity 0.5 McFarland Standard (which is approximately 10^8 CFU/ml). The bacterial suspension was streaked evenly onto a 150-mm-diameter plate containing Muller Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, England). Two disks of imipenem 10mg, one of which was impregnated with 5 µL of 0.5 EDTA (Sigma, USA) solution were placed 10 mm apart from edge to edge on the surface of the same Muller Hinton Agar plates for 24 hours of incubation at 37°C. A difference of

≥7mm in diameter between the zones of inhibition for the EDTA impregnated imipenem disk and imipenem disk alone indicated the presence of MßL-producing *P. aeruginosa*.[23,24]

Antimicrobial susceptibility profile

The antibiotic susceptibility profile of the *P. aeruginosa* and MßL-producing *P. aeruginosa* isolates was evaluated using the Kirby-Bauer disk diffusion method as recommended by the Clinical Laboratory Standard Institute (CSLI) guidelines.[25,26] Bacterial suspensions were prepared in 2 mL of Mueller-Hinton broth (MHB), with turbidity 0.5 McFarland Standard (which is approximately 10^8 CFU/ml). The bacterial suspension was streaked evenly onto a 150-mm-diameter plate containing Muller Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, England). The anti-biogram was determined by comparing the zone of inhibition with the CSLI interpretative chart using meropenem 10 µg, imipenem 10 µg, ceftazidime 30 µg, cefepime 30 µg, gentamicin 10 µg and ciprofloxacin 5 µg.

Statistical analysis

Data obtained were presented as percentage prevalence and statistical significance was determined using the student "T" test to compare the prevalence of *P. aeruginosa* isolates and MßL-producing *P. aeruginosa* isolates from gunshot wounds of in-patients with respect to location of the wounds and gender of the patients. P –values equal to or less than 0.05 are regarded as significant.

Results and Discussion

ß-lactams, including carbepenems are considered the most potent agents for treatment of infections caused by *P. aeruginosa* .[27] Resistance to MßL among *P. aeruginosa* is increasing and has been reported in several countries.[28,29]

In this study, out of the total of one hundred (100) (male: 76 and female: 24) gunshot wound samples collected, a prevalence of 24 (24%) were recorded for *P. aeruginosa*. Of these, 8 (33.3%) were recorded as MßL-producing *P. aeruginosa* by imipenem-impregnated EDTA test. (Figures 1,2) However, the result revealed that male in-patients had a higher prevalence rate of 19 (79.2%) for *P. aeruginosa* than females with 5 (20.8%), while male had a higher prevalence rate of 7(87.5%) for MßL-producing *P. aeruginosa* than females with 1(12.5%). (Figures 3, 4) Based on wound location, the lower limbs had a higher prevalence rate of 75% (18/24) for *P. aeruginosa* than the upper limbs 12.5% (3/24), head 8.3% (2/24) and abdomen 4.2% (1/24). (Figure 5) The lower limbs had a higher prevalence rate of 87.5% (7/8) for MßL-producing *P. aeruginosa* than the upper limb 12.5% (1/8), head 0% (0/8) and abdomen 0% (0/8). (Figure 6) The variation in the patient's gender and wound location in relation to the prevalence of *P. aeruginosa* and MßL-producing *P. aeruginosa* infection was statistically significant (P<0,05).

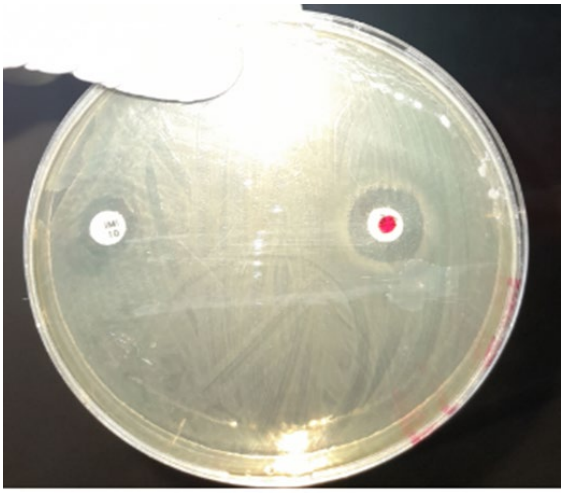

Figure 1. EDTA Disk Testing method showing enhanced inhibition zone of >7mm around IPM + EDTA disc indicating MßLs positivity.

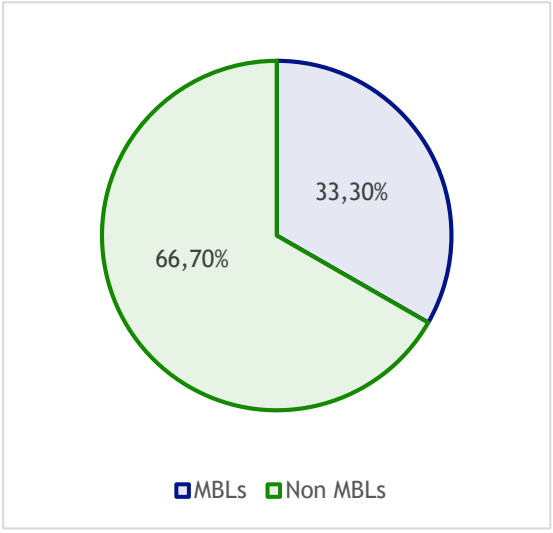

Figure 2: Percentage prevalence of metallo-ß lactamase (MBL) producing *P. aeruginosa*

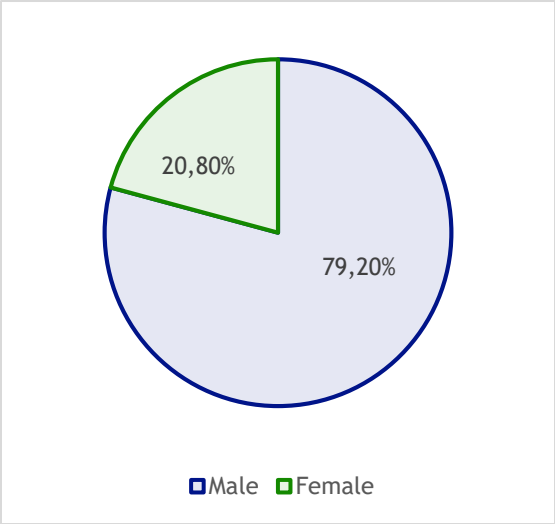

Figure 3: Percentage prevalence of *P. aeruginosa* based on gender of in-patients

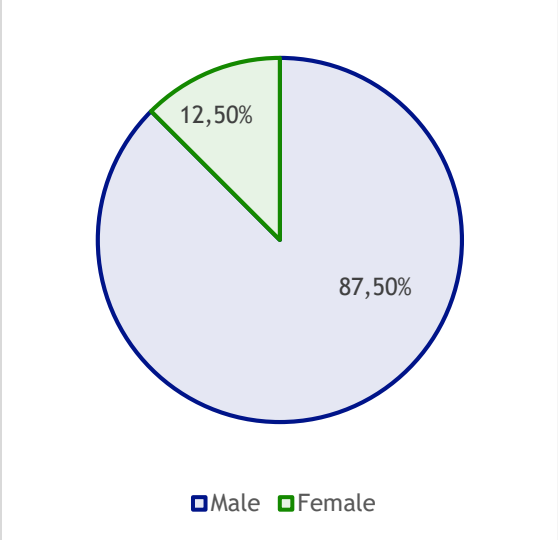

Figure 4: Percentage prevalence of metallo-ß lactamase producing *P. aeruginosa* based on gender of patients

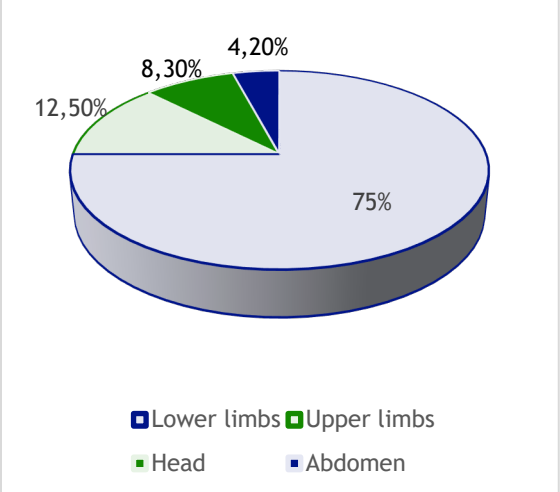

Figure 5: Percentage prevalence of *P. aeruginosa* based on wound locations.

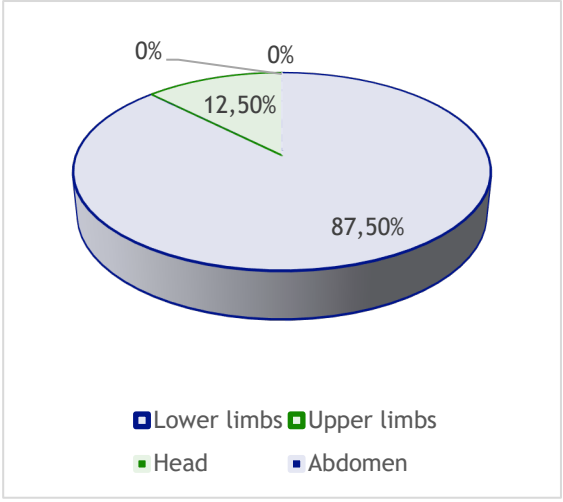

Figure 6: Percentage prevalence of metallo-ß lactamase producing *P. aruginosa* based on wound location.

Though, the prevalence rates in this study are low, MßLs among *P. aeruginosa* have been increasingly recognized from clinical isolates worldwide. Ettu *et al.* (2021) in Nigeria reported 81.5%; 37.6% in West of Iran, Hamadan by Arash *et al.*, (2022); 35.1% in Egypt by Rehab *et al.*, (2021); 34.2% in Ghana by Hayford *et al.*, (2021); 18.95% in India by Kunachgi *et al.* (2015); 24.2% in Korea by Nam *et al.*, (2010) and 15% in Nepal by Reshma *et al.* (2020).[30,31,32,33,34,35,36] The findings in this study, however are substantially higher than the reports from Europe and other developed nations where infection rates of 2.3% (23/996) in 2004 and 2.1% (21/992) in 2006 across clinical specimens were reported in Japan.[37] In Italy and Spain, the MßL prevalence in *P. aeruginosa* was reported as 1.3% and 0.1% respectively.[38,39]

The incidence of MßL-producing *P. aeruginosa* is due to intrinsic resistance of the organism,[40] and its associated risk factors, both of which led to spread of the bacteria. It has been emphasized that the detection of MßLs among *P. aeruginosa* is crucial for optimal treatment of patients due to the increase in ß-lactam usage and emergence of resistant bacteria under antibiotic pressure.[41]

Figure 7 and 8 show the antimicrobial susceptibility profile of *P. aeruginosa* isolates and MßL-producing *P. aeruginosa* isolates denoting the resistance, intermediate and

susceptibility patterns. The *P. aeruginosa* isolates demonstrated a 100% resistance to cefepime 30 µg and ceftazidime 30 µg, while the highest susceptibility was 20.8% each for meropenem 10 µg, imipenem 10 µg, and gentamicin 10 µg. This result indicates that the resistance levels were high across all the antibiotics tested. Such resistance has been previously reported and are due to the coexistence of genes encoding drug resistance to other antibiotics on the plasmids the isolates are harboring.[42.] However, because of the very low permeability of the cell wall, *P. aeruginosa* is naturally resistant to ß-lactams including broad spectrum cephalosporins and quinolones.[43,44,45]

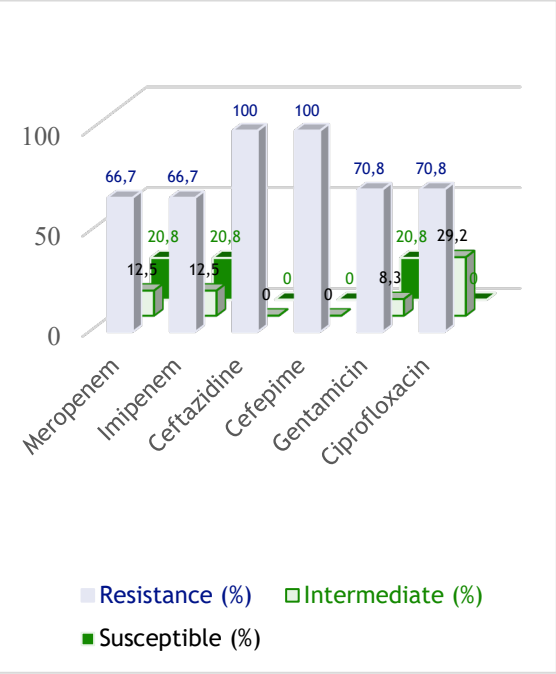

Figure 7: Antimicrobial profile *P. aeruginosa* isolate to ß-lactam antibiotics and other non-ß-lactam antibiotics

All eight (8) of the MßL-producing *P. aeruginosa* isolates recorded a 100% resistance against all the antibiotics examined in this study. A particularly important feature is that the MBL producers were resistant to all the ß-lactam antibiotics and the non-ß-lactam antibiotics. This however indicates the multi-drug resistant attribute of MßL-producing *P. aeruginosa* isolates and supports the claim that these 'superbugs' are minimally susceptible to empirical therapy.[41] This was comparable to reports from other parts of the world, which

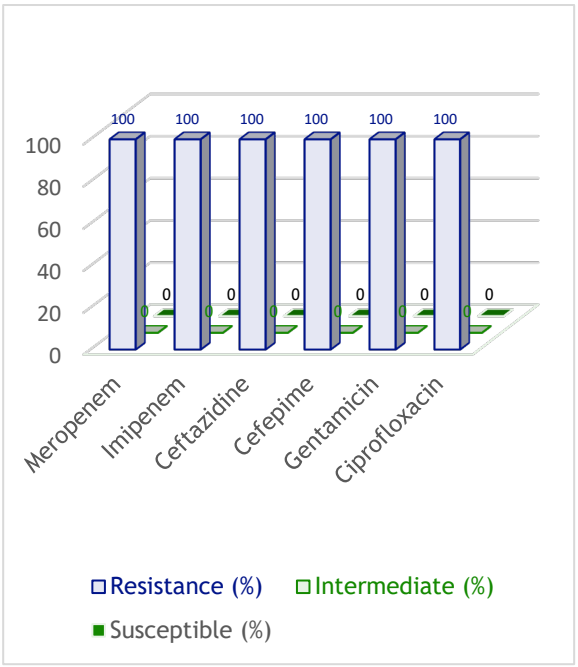

Figure 8: Antimicrobial profile of the metallo-ß lactamase producing *P. aeruginosa* isolates to ß-lactam antibiotics and other non-ß-lactam antibiotics

also revealed multiple drug resistance among *P. aeruginosa* and the limited treatment options for wounds.[14] Moreover, there are reports of growing concern of the MßL-producing *P. aeruginosa* showing cross resistance to non-ß-lactam antibiotics.[46] Kateete *et al.* and Horieh *et al.* reported that resistance in *P. aeruginosa* was mainly due to the production of MßLs and other factors such as genetic mutations in over expression of the ampC gene, increased expression of the efflux pumps, decreased expression of proteins, impermeability through alteration or loss of the porin OprD, reduced outer membrane permeability, alteration in the target sites of antibiotics and less possibly involved are the increased activity of chromosomal cephalosporinases.[15,41]

This study also demonstrated significant differences in the susceptibility profiles between MßL-producing and non MßL-producing *P. aeruginosa* for meropenem, imipenem and gentamicin, except for ceftazidime, cefepime and ciprofloxacin where no significant difference was observed in the susceptibility patterns of both organisms. This indicates that MßL-producing strains of *P. aeruginosa* are

more likely to have low susceptibility to non ß-lactam antibiotics compared with non MßL-producing isolates of *P. aeruginosa*. In this regard, the hospitals should formulate an effective antibiotic policy.

Limitations

In this study, the great proportion of the population are male in- patients. All patients were determined to have wound infections resulting from the gunshot injuries during counter-insurgency/counter-terrorism operations in Bono State, Nigeria. Hence, lack of a comparison group is a definite limitation of the study. Clinical details of patients such as history of antibiotic use are not included due to insufficient information. Also, molecular epidemiologic analysis and characterization to determine the MßL types and resistant genes were not carried out due to funding constraint.

Conclusion

This study revealed the presence and prevalence of MßLs in *P. aeruginosa* of gunshot wound patients. The isolates showed >60% resistance to ß-lactam antibiotics including carbapenems and cephems and other non-ß-lactam antibiotics, including gentamicin and ciprofloxacin. This high level of resistance may put the afflicted patient at an increased risk of developing a multidrug resistant infection. However, accurate identification and reporting of MBL-producing *P. aeruginosa* will aid in limiting the spread of the organism.

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Ethical Approval

The study was approved by the Research Ethics Committee of the University of Maiduguri Teaching Hospital Maiduguri, Nigeria and Nigeria Army 7 Division Medical Services and Hospital, Maiduguri Nigeria (decision number: UMTH/REC/21/949 and 7 DMSH/G1/300/26 respectively).

Informed Consent

The written informed consent was obtained from the study participants.

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