

Research Article

Prevalence and Phenotypic Antimicrobial Resistance Profiles of Community- and Hospital-Acquired Metallo- Beta-Lactamases-Producing *Pseudomonas aeruginosa* from Wound Infections in Maiduguri, Nigeria

Tijani Isa*, Adam Mustapha, Ibrahim Yusuf Ngoshe, Hauwa Musa, Musa Markus Zafi, Usman Ali Bukar, and Aminatu Umar Faruk

Department of Microbiology, Faculty of Science, University of Maiduguri, Borno State, Nigeria

*Corresponding author: tjelyakub@gmail.com, doi.org/10.55639/607.121110

ARTICLE INFO:

Keyword:

Metallo-beta-lactamases,
P. aeruginosa,
Antibiotic resistance,
Wound infection,
Hospital- acquired
infection

ABSTRACT

Pseudomonas aeruginosa is among the most common bacterial pathogens isolated from wounds worldwide, both in number of cases per year and in terms of the high prevalence of antibiotic resistance. The resistance is attributed to the production of metallo- β -lactamases (M β LS). In this study, *P. aeruginosa* was isolated and identified from wound samples using Bergey's Manual of Systemic Bacteriology. Wound swab samples were inoculated in to MacConkey agar and Cefrimide agar. The EDTA disk testing method and modified Kirby-Bauer disc diffusion method was conducted to determine the phenotypic characterization and antimicrobial profile of M β LS-producing *P. aeruginosa*. The results of this study shows that Out of the ninety eight (98) samples examined, 17 yielded positive growths for *P. aeruginosa*, giving a prevalence rate of 17.3%. The prevalence of M β LS- producing strains of *P. aeruginosa* was 9/17(52.9%) which was highest among female 5/17 (29.41%), compared to 4/17 (23.52%) observed among male. Patients who are 61 years or older recorded the highest prevalence rate of 17.65% (3/17) compared to those less than 61 years old 11.76% (2/17). The prevalence of M β LS positive isolates showed that most of the isolates were recovered from community-based submission 6/17 (35.29%) compared to 3/17 (17.65%) observed from the hospital samples. The antimicrobial profile of M β LS-producing *P. aeruginosa* isolates indicated a 100% resistance against imipenem 10 μ g, cefepime 30 μ g and ceftazidime 30 μ g, while 56% of the isolates were susceptible to meropenem 10 μ g and 45% were susceptible to ciprofloxacin 5 μ g. Therefore, this study has illustrated that M β LS- producing *P. aeruginosa* strains exhibited distinct prevalence among community- and hospital- based submission. Phenotypic antibiotic resistance was increasingly high and appears to be linked to the presence of M β L enzymes.

Corresponding author: Tijani Isa, Email: tjelyakub@gmail.com

Department of Microbiology, Faculty of Science, University of Maiduguri, Borno State, Nigeria

INTRODUCTION

Wound infections are one of the health problems, generally estimated to affect 1–2% of the world's population (Hemant, 2021). It is caused and aggravated by the invasion of pathogenic organisms in different parts of the body leading to the death of large numbers of people mostly in developing countries (Akingbade *et al.*, 2012). The proliferation of multiple bacterial species in wounds contributes to delayed healing, in addition to the cost in terms of quality of life to the patient and high healthcare costs (Caldwell *et al.*, 2020; Prakash *et al.*, 2022).

Pseudomonas aeruginosa (*P. aeruginosa*) is among the most common bacterial pathogens isolated from wounds, often forming antimicrobial-tolerant biofilms that are difficult to eradicate (Nazia *et al.*, 2020). It is one of the most important pathogens worldwide, both in number of cases per year and in terms of the high prevalence of antibiotic resistance (Josef *et al.*, 2015; Preeti *et al.*, 2019). Presence of *P. aeruginosa* in wound is associated with more severe wound outcomes and is often larger in size and experience prolonged duration (Turner *et al.*, 2011; Nazia *et al.*, 2020).

P. aeruginosa is predominantly known to produce beta- lactamase which has the ability to hydrolyze the amide bond which leads to the opening of the beta- lactam ring and confer resistance to carbapenems (imipenem, meropenem, ertapenem) and other β - lactam antibiotics by inactivation of the antibiotics (Drawz and Bonomo, 2010). The organism was reported to be isolated from different parts of the world including Asia, Europe, Australia, South America, and North America (Johann *et al.*, 2005). In Nigeria, the occurrence of metallo-beta-lactamases (M β LS)- producing *P. aeruginosa* has being increasingly reported not only in human but in other animals including poultry (Ejikeugwu *et al.*, 2014; Ejikeugwu *et*

al., 2016; Zubair and Iregbu 2018; Israel *et al.*, 2020; Chika *et al.*, 2021; Oluwatoyin *et al.*, 2021). Human infections caused by M β LS-producing *P. aeruginosa* strains have been shown to be associated with higher mortality rates than infections with M β LS- negative *P. aeruginosa* isolates (Zavascki *et al.*, 2008). The mortality rate is however estimated to range from 70% to 90% (Cornaglia *et al.*, 2011; Zavascki *et al.*, 2008).

The prevalence of M β LS- producing *P. aeruginosa* in North-Eastern region of Nigeria is not broadly known due to so many factors including limited surveillance data in both the community and hospital environments. Therefore, this study was conducted to determine the phenotypic prevalence and resistant pattern of M β LS- producing *P. aeruginosa* in both community and hospital settings in Maiduguri metropolis, Borno State, Nigeria as a way of generating baseline data on the burden of M β LS- producing *P. aeruginosa* infections in our community and dissemination of the results for use by health-care professionals.

MATERIALS AND METHODS

Study area

The study was conducted in Maiduguri, Borno State, Nigeria from February, 2022 to July, 2022. Maiduguri is located in the North-Eastern part of Nigeria which lies within latitude 11.15°N and longitude 30.05°E in the Sudano-Sahelian savanna zone with a dense population who are mostly crop farmers, fishermen, herdsmen and traders (Udo, 1978). The state has an area of 71.210sq km with the population of 4,151,193 according to the National census conducted in 2006 (NPC, 2006).

Samples population, collection and processing

A total of ninety eight (98) wound swabs were aseptically collected using convenience purposive sampling from community-associated (male: 28 and female: 21= 49) and hospital

infection (including in- and out- patients) (male: 27 and female: 22= 49). The samples were preserved on ice and transported to Microbiology laboratory for investigation at the Department of Microbiology Laboratory, University of Maiduguri. All individuals in the community with wound infection were prospectively identified during the surveillance period. Informed consent for each sample collected was obtained, and included the age of the patient, sex, wound location and community- or hospital-based submission were recorded.

Isolation and identification of *Pseudomonas aeruginosa*

Isolation and identification of the bacteria was conducted as described in Bergey's Manual of Systemic Bacteriology (Michael *et al.*, 2012). 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 (Cowan and Steel, 2002).

MβLs- Phenotypic detection

The phenotypic detection of MβLs- 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 10⁸ CFU/ml). The bacterial suspension was streaked evenly onto a 150-mm-diameter plate containing Muller Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, England) plates. 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 MβLs- producing *P.*

aeruginosa (Pitout *et al.*, 2005; Sachdeva *et al.*, 2017).

MβLs isolates-antibiotic susceptibility testing

The antibiotic susceptibility profile of the MβLs-producing *P. aeruginosa* isolates was evaluated using the Kirby-Bauer disk diffusion method as recommended by the Clinical Laboratory Standard Institute (CLSI) guidelines (Bauer *et al.*, 1966; CLSI, 2021). Bacterial suspensions were prepared in 2 mL of Mueller-Hinton broth (MHB), with turbidity 0.5 McFarland Standard (which is approximately 10⁸ CFU/ml). The bacterial suspension was streaked evenly onto a 150-mm-diameter plate containing Muller Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, England) plates. The anti-biogram was determined by comparing the zone of inhibition with the CLSI interpretative chart using meropenem 10 μg, imipenem 10 μg, ceftazidime 30 μg, cefepime 30 μg, gentamicin 10 μg and ciprofloxacin 5 μg.

Statistical analysis

The data obtained were analyzed using Microsoft Excel 2010 and were presented as percentage prevalence. Association between variables was assessed using the One-Way ANOVA test and the students "T" test to compare the prevalence of MβLs- producing *P. aeruginosa* isolates from community-associated and hospital infection with respect to age, sex, wound location. $p < 0.05$ (95% confidence interval) was considered statistically significant.

RESULTS

The prevalence of *P. aeruginosa* and MβLs production in the isolates of *P. aeruginosa* was determined. Out of the ninety eight (98) samples: superficial lesion (43), wound bite (25), burn (28) and diabetic ulcer (2) were examined, 17 yielded positive growth of *P. aeruginosa*, giving a prevalence rate of 17.3%, which was highest among females 9/17 (9.2%), compared to 8/17 (8.1%) observed among males (Table 1) ($p > 0.05$). The age distribution of positive samples revealed that subjects that are

21-40 years old recorded the highest prevalence rate of 7.1% (7/17) and least was observed among 0-20 years 2/17 (2.0%) (Table 2). However, the variation in sex and the age group

of the subjects in relation to the prevalence of *P. aeruginosa* infection was statistically significant ($p < 0.05$).

Table 1: Percentage rate of isolation of *Pseudomonas aeruginosa* infection in relation to gender of wound patients.

Test organism (<i>Pseudomonas aeruginosa</i>)	Sex		Total (%)
	Male (%)	Female (%)	
Positive	8(8.1)	9(9.2)	17(17.3)
Negative	48(48.10)	35(35.7)	83(84.7)
Total	56(57.1)	44(44.9)	98(100)

Table 2: Percentage rate of isolation of *Pseudomonas aeruginosa* infection in relation to age of wound patients

Test organism (<i>Pseudomonas aeruginosa</i>)	Age group (years)				Total (%)
	0 -20	21- 40	41- 60	>61	
Positive	2(2.0)	7(7.1)	4(4.0)	4(4.0)	17(17.3)
Negative	21(21.4)	31(31.6)	25(25.1)	6(6.1)	83(84.7)
Total	23(23.4)	38(38.8)	29(29.6)	0(10.2)	98(100)

The prevalence of MβLs- producing strains of *P. aeruginosa* (positive MβL producers) was 9/17 (52.9%) while 8/17 (47.1%) were non- MBL-producing strains (negative for MβL producers) by imipenem- impregnated EDTA synergy test (Table 3, Figure 1). However, this result showed no statistical significant difference in the prevalence ($p > 0.05$). The prevalence rate of MβLs- producing *P. aeruginosa* among female patients was 5/17 (29.41%), which is higher compared to 4/17 (23.52%) observed among Male patients. Patients who are 61 years or older recorded the highest incidence rate of 17.65%

(3/17) compared to those less than 61 years old 2/17 (11.76%). A direct relationship was found between patient age and acquisition of an infection caused by MβLs- producing strains (Table 4). There was no significant overall difference in prevalent observed between male and female patients. The percentage prevalence of the MβLs positive isolates obtained from community and hospital-acquired infections shows that most of the isolates were recovered from community-based submission 6 (35.29%) compared to 3 (17.65%) observed from the hospital (Table 5) ($p > 0.05$).

Table 3: Characterization of metallo- beta lactamase producing *Pseudomonas aeruginosa*

Isolates	Type of specimen	IET
AM 07	Burn	-
AM 08	Burn	+
AM 14	Superficial lesion	+
AM 28	Superficial lesion	-
AM 33	Superficial lesion	-
AM 37	Superficial lesion	-
AM 43	Superficial lesion	+
AM 54	Superficial lesion	+
AM 67	Wound bite	-
AM 6	Burn	-
AM 70	Superficial lesion	+
AM 71	Superficial lesion	+
AM 73	Superficial lesion	-
AM 77	Burn	+
AM 78	Superficial lesion	+
AM 81	Wound bite	+
AM 82	Superficial lesion	-

IET= imipenem-EDTA synergy test

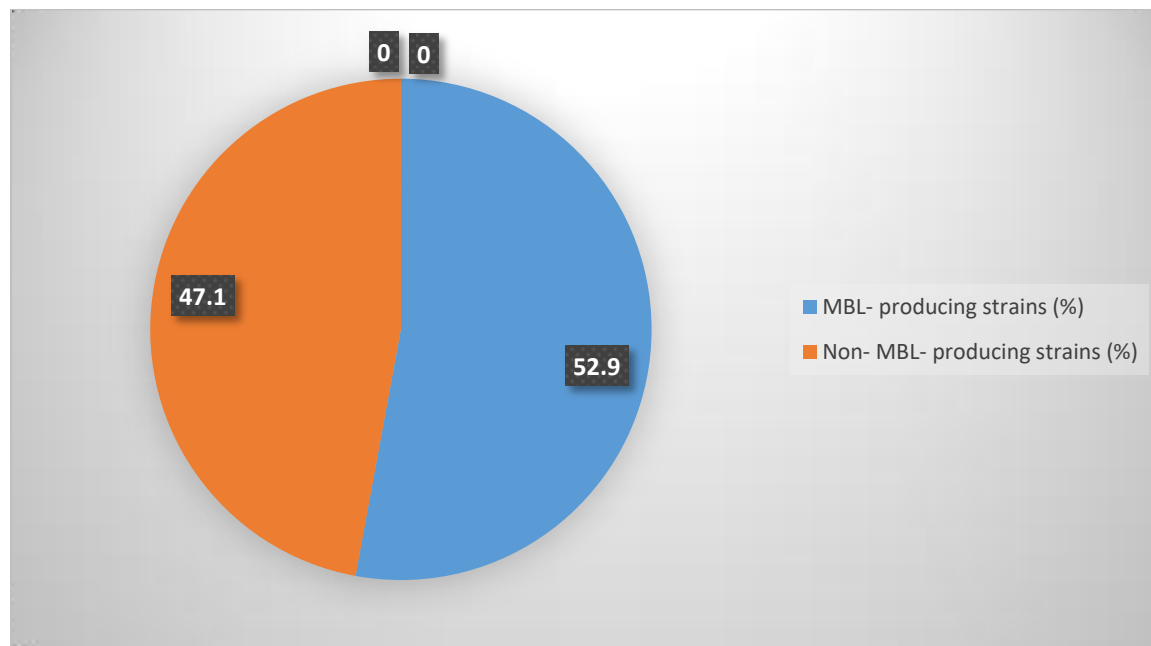


Figure 1: Prevalence of metallo- beta lactamases producing *Pseudomonas aeruginosa*

Table 4: Prevalence of metallo- beta lactamases producing *Pseudomonas aeruginosa* in relation to age and gender of wound patients

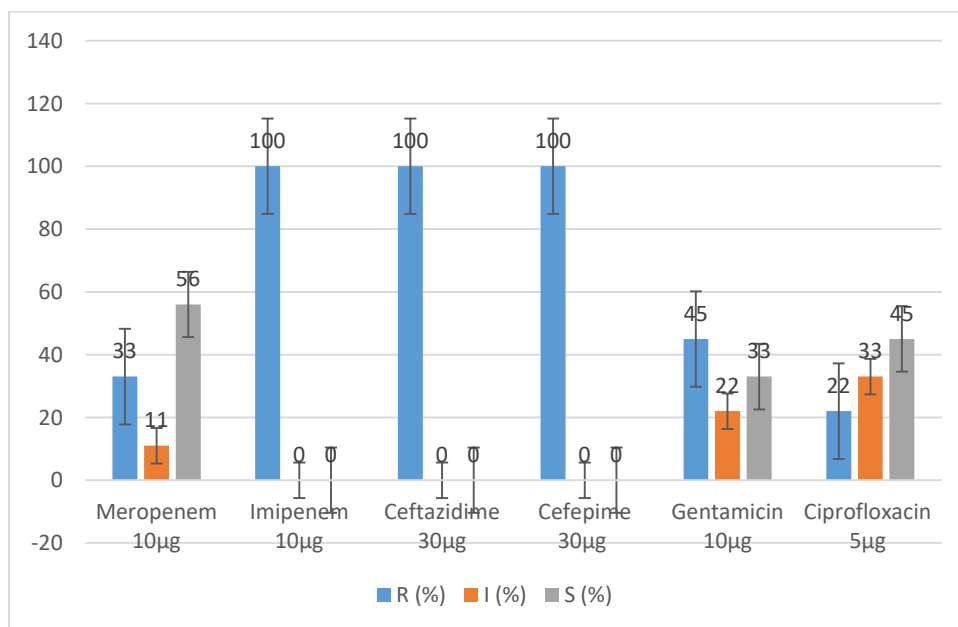
Test organism (<i>P. aeruginosa</i>) (%)	Age group (years)				Total (%)	Sex		Total
	0 -20	21- 40	41- 60	>61		Male (%)	Female (%)	
Positive	2(11.76)	2(11.76)	2(11.76)	3(17.65)	9(52.94)	4(23.52)	5(29.41)	9(52.94)
Negative	0(0.00)	5(29.41)	2(11.76)	1(5.88)	8(47.06)	4(23.52)	4(23.52)	8(47.06)
Total	2(11.76)	7(41.17)	4(23.52)	4(23.52)	17(100)	8(47.06)	9(52.94)	17(100)

Table 5: Prevalence and distribution of metallo- beta lactamases producing *Pseudomonas aeruginosa* based on location of wound patients.

Location	No of samples screened	No of isolates recovered	MβLs negative (%)	MβLs positive (%)	Total (%)
Community	49	10	4(23.53)	6(35.29)	10(58.82)
Hospital	49	7	4(23.53)	3(17.65)	7(41.18)
Total	98	17	8(47.06)	9(52.94)	17(100)

Figure 2 shows the antimicrobial susceptibility profile of MβLs- producing *P. aeruginosa* isolates (n = 9) denoting their resistance, intermediate and susceptibility qualities. Hundred percent (100%) of the isolates

exhibited resistance to imipenem 10 µg, cefepime 30 µg and ceftazidime 30 µg. While 56% of the isolates were susceptible to meropenem 10 µg and 45% were susceptible to ciprofloxacin 5 µg.



S= Susceptible, I= Intermediate, R= Resistance

Figure 2: Antimicrobial profile pattern of metallo-beta-lactamases producing *Pseudomonas aeruginosa*

DISCUSSION

The global spread of β -lactamases poses significant therapeutic challenges in clinical and non-clinical settings (Noor- ul *et al.*, 2020). More than 28 species of M β L- producing Gram negative bacteria have been reported from more than forty countries and are associated with higher rates of mortality, morbidity, and health care costs (Jombo *et al.*, 2008; Kaleem *et al.*, 2010). Therefore, early detection of these mobile broad-spectrum organisms is necessary to prevent its propagation mainly in the healthcare and community-setting. In this study, we reported a total prevalence of 52.9% M β L-producing *P. aeruginosa* among the *P. aeruginosa* isolates recovered, which was higher in females than in males. The prevalence rate was found to be higher among the age group of 61 years and above. In relation to the demographic properties and the associated risk factors of the organisms in the population, various reports have mentioned an increasing number of M β L production from clinical samples worldwide (Nigeria inclusive) and this heralds an alarming deteriorating situation (Ettu *et al.*, 2021; Oluwatoyin *et al.*, 2021). However, M β L production in our isolates was higher compared to reports from other countries (Arash *et al.*, 2022; Rehab *et al.*, 2021; Hayford *et al.*, 2021; Nam *et al.*, 2010; Kunachgi *et al.*, 2015; Reshma *et al.*, 2020).

M β Ls are important sources of hospital- and community- acquired infections. Whereas *P. aeruginosa* is among the most prominent bacteria causing wound infections in both the hospital and community settings (Iregbu *et al.*, 2013; Kavita *et al.*, 2021). In this study, higher prevalence of M β Ls- producing *P. aeruginosa* from wound infection was found in the community- based submission (35.29%) compared to hospital- based submission. Our findings are an alarming indication that an outbreak of M β Ls- producing strains can occur not only in hospitals that provide various services to

patients but also in the community which could be attributed partly to uncontrolled usage and administration of extended spectrum antibiotics among the populace and lack of effective infection control. This explains the increased prevalence rate of infections caused by MBLs-producing strains in the community- setting. This scenario might in no doubt allow bacteria to develop resistance through selective pressure mounted on them over time as a result of irrational use of these drugs and possible poor infection control practices (Ejikeugwu *et al.*, 2014). The severity of infection caused by M β L-positive isolates in community setting and particularly in health care settings poses a therapeutic problem, as well as a serious concern for infection control management (Breidenstein *et al.*, 2011; Oliver *et al.*, 2015).

The presence of M β L enzymes in clinically important organisms like *P. aeruginosa* leads to variable range of resistance to all β - lactam antibiotics with the exception of monobactams (Ejikeugwu *et al.*, 2014; Zhao *et al.*, 2011). In this study, we observed higher resistance rate of M β L- producing *P. aeruginosa* to cepheims, a sub-group of β -lactam antibiotics including cephalosporins and cephamycins (Vitaku *et al.*, 2014), with percentage resistance of 100% to both cefepime and ceftazidime. This is in harmony to a resistance rate of 100% in a study by Reshma *et al.* (2020). For over two decades, ceftazidime and other cephem antibiotics have been the foremost drug for the treatment of severe infections caused by *P. aeruginosa* in many tertiary healthcare institutions in Nigeria, and this may be responsible for its high resistance (Zubair and Iregbu, 2018). The increasing resistance to cephalosporins, most especially the fourth generation cephalosporin, necessitates the need for proper guideline of antibiotic prescriptions (Oluwatoyin *et al.*, 2021).

The antibiotic susceptibility testing also noted high resistance to carbapenems, another class of

β -lactam antibiotics. Among the carbapenems, a resistance 100% to imipenem was observed compared to meropenem (33%). Carbapenems are described as a potent antibiotics which are usually reserved for last resort use with efficacy against M β L-producing Gram-negative bacterial infections. On the other hand, resistance to carbapenems are on the rise (Nabarro *et al.*, 2017). Carbapenem resistance is defined as non-susceptibility to at least one anti-pseudomonal carbapenem (Oluwatoyin *et al.*, 2021). As in the present study, other researchers have reported a decreased susceptibility of M β L producers to carbapenems antibiotics, a drug that was once considered and reported in literature to be effective against *P. aeruginosa* (Chaudhary *et al.*, 2019). However, the most susceptible antibiotics against our isolates were meropenem and ciprofloxacin 56% and 45% respectively. These findings highlight the importance of considering meropenem and ciprofloxacin in the treatment of M β Ls-producing *P. aeruginosa* causing community and hospital-acquired infections.

LIMITATIONS

Deficiency in a proper comparison group is a definite limitation in our study since the great proportion of the study population was male subjects/patients. Molecular analysis such as PCR and sequencing to determine the M β L types and resistant genes were not carried out due to funding constraint.

CONCLUSION

Our study has illustrated an alarming increase of antibiotic resistance in M β Ls-producing *P. aeruginosa* that cause either community- or

hospital-acquired infections which constitutes major threat to human health. Consequently, detection of M β Ls-producing organisms in an early stage is paramount to prevent or limit their spread and establish appropriate antimicrobial stewardship.

ACKNOWLEDGMENTS

The authors would like to thank the management and staff more especially the Hospital Ethics Committee of State Specialist Hospital Maiduguri, Nigeria. The authors also wish to thank the Head of Department, Head of Laboratory and staff Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria for their technical support during this research study.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit organizations.

ETHICS DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Patients' consent were sought and obtained with emphasis on the significance of this investigation and ethical approval was granted by ethics and research committee of the State Specialist Hospital, Maiduguri Nigeria with the approval number: SSH/GEN/641/Vol.1 before data and sample collection from patients.

CONSENT FOR PUBLICATION

Individual's consent was sought for the publication of the study as approved by the institutions.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

REFERENCES

Akingbade OA, Balogun SA, Ojo DA, et al. (2012). Plasmid Profile Analysis of Multidrug Resistant *Pseudomonas aeruginosa* isolated from Wound Infections in South West, Nigeria.

World Applied Sciences Journal, DOI: 10.5829/idosi.wasj.2012.20.06.667.

Arash S, Sanaz D, Hamed T, et al. (2022). Detection of blaOXA-145, blaOXA-224, blaOXA-539, and blaOXA-675 Genes and Carbapenem-Hydrolyzing Class D-Lactamases (CHDLs) in

- Clinical Isolates of *Pseudomonas aeruginosa* Collected from West of Iran, Hamadan. *International Journal of Microbiology*, 3841161: 10 doi.org/10.1155/2022/3841161.
- Bauer AW, Kirby, WM, Sherris JC and Turck M. (1966). Antibiotic susceptibility testing by a standard single disc method. *American Journal Clinical Pathology*, 45: 493-496.
- Breidenstein EBM, de la Fuente-Núñez C, Hancock R. (2011). *Pseudomonas aeruginosa*: all roads lead to resistance. *Trends Microbiol*, 19:419–426. DOI: 10.1016/j.tim.2011.04.005.
- Chaudhary NA, Munawa MD, Khan MT, et al. (2019). Epidemiology, Bacteriological Profile and Antibiotic Sensitivity Pattern of Burn Wounds in the Burn Unit of a Tertiary Care Hospital. *Cureus*, 11:6. e4794.
- Chika E, Okoro N, Morteza S, et al. (2021). Metallo- β -lactamases and AmpC genes in *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolates from abattoir and poultry origin in Nigeria. *BMC Microbiology*, 21:124. doi.org/10.1186/s12866-021-02179-1.
- Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Approved standard-31st edition, CLSI supplement M100, Clinical and Laboratory Standards Institute, 940 West Valley Road, Wayne Pennsylvania, USA. 2021; 2B-1.
- Cornaglia G, Giamarellou H, Rossolini GM. (2011). Metallo- β -lactamases s: A last frontier for β -lactams? *Lancet Infect Dis*, 11:381-93.
- Cowon ST, Steel KJ. Manual for the identification of medical bacteria (2nd Edition). Cambridge University Press 2002; P. 51-60.
- Drawz SM, Bonomo RA. (2010). Three decades of β -lactamases inhibitors. *Clin. Microbiol. Rev.* 23:1, 160–201.
- Ejikeugwu C, Iroha I, Duru C, et al. (2016). Occurrence of Metallo-Beta-Lactamases -Producing Enterobacteriaceae from a Local Poultry Farm in Abakaliki, Nigeria. *International Journal of Applied Pharmaceutical Sciences and Research*, 1:2, 70-75 DOI.org/10.21477/ijapsr.v1i2.10178.
- Ejikeugwu C, Ugwu M, Iroha I, et al. (2014). Phenotypic Detection of Metallo- β -Lactamases (MBL) Enzyme in Enugu, Southeast Nigeria. *American Journal of Biological, Chemical and Pharmaceutical Sciences*, 2:2, 1 - 6, ISSN: 2328 - 6814 (Online) Available online at <http://www.ajbcps.com/1>.
- Ettu AO, Oladapo BA, Oduyebo O. (2021). Prevalence of carbapenemase production in *Pseudomonas aeruginosa* isolates causing clinical infections in Lagos University Teaching Hospital, Nigeria. *Afr. J. Clin. Exper. Microbiol*, 22: 498–503, doi.org/10.4314/ajcem.v22i4.10.
- Hayford O, Vivian EB, Yaw Duah B, et al. Prevalence and Phenotypic and Genotypic Resistance Mechanisms of Multidrug-Resistant *Pseudomonas aeruginosa* Strains Isolated from Clinical, Environmental, and Poultry Litter Samples from the Ashanti Region of Ghana. *Hindawi Journal of Environmental and Public Health*, Article ID 9976064, 12. doi.org/10.1155/2021/9976064.
- Hemant Singhal. (2021). Wound Infection. .medscape, Updated: Jul 01, 2021.
- Iregbu KC1, Uwaezuoke NS, Nwajiobi-Princewill IP, et al. (2013). A Profile of Wound Infections in National Hospital Abuja. *Afr. J. Clin. Exper. Microbiol.* 14:3, 160-163. doi.org/10.4314/ajcem.v14i3.7.
- Johann DD, Pitout DB, Gregson LP, et al. (2005). Detection of *Pseudomonas aeruginosa* Producing Metallo- beta-Lactamases in a Large Centralized Laboratory. *Journal of Clinical Microbiology*, 43:7, 3129–3135, doi:10.1128/JCM.43.7.3129–3135.2005.

- Jombo GT, Jonah P, Ayeni JA. (2008). Multiple resistant *Pseudomonas aeruginosa* in contemporary medical practice: Findings from urinary isolates at a Nigerian University Teaching Hospital. *Niger J Physiol Sci*, 23:105-9.
- Josef Y, Beniam G and Kurt R. (2015). Antibiotic Resistance of *Pseudomonas aeruginosa* in Pneumonia at a Single University Hospital Center in Germany over a 10-Year Period. *PLoS One*, 10:10, e0139836, doi: 10.1371/journal.pone.0139836.
- Kaleem F, et al. Frequency and Susceptibility Pattern of Metallo-beta-lactamase producers in a hospital in Pakistan. *J Infect Dev. Ctries* 2010; 4: 1050.
- Kavita N, Shilpa BM, Shripad A, et al. (2021). An Overview of Extended Spectrum Beta Lactamases and Metallo Beta Lactamases. *Advances in Microbiology*, 11, 37-62, doi: 10.4236/aim.2021.111004.
- Kunachgi PN, Syed SR, Hima BM, et al. Antibiotic sensitivity pattern and imipenem-EDTA double disk synergy test for the detection of Metallo-beta-lactamase producing *Pseudomonas aeruginosa* from clinical samples in a teaching hospital. *Int. J. Curr. Microbiol. App. Sci*, 4: 866-871.
- Laupland KB, Parkins MD, Church DL, et al. (2005). Population-based epidemiological study of infections caused by carbapenem-resistant *Pseudomonas aeruginosa* in the Calgary Health Region: importance of metallo- lactamases (MBL)-producing strains. *J. Infect. Dis*, 192:1606–1612.
- Michael DC. (2020). Bacteria and Antibiotics in Wound Healing. *Surgical Clinics of North America*, 100:4, 757-776, doi:10.1016/j.suc.2020.05.007.
- Michael G, Peter K, Hans-Jürgen B, et al. Bergey's manual of systematic bacteriology. Second edition, Volume five. Springer New York Dordrecht Heidelberg London 2012; DOI 10.1007/978-0-387-68233-4, ISBN 978-0-387-95043-3, ISBN 978-0-387-68233-4 (eBook).
- Nabarro LE, Shankar C, Pragasam A, et al. (2017) Clinical and bacterial risk factors for mortality in children with carbapenem resistant Enterobacteriaceae bloodstream infections in India. *Paed Infect Dis*, 36:6, e161–e166, doi. org/ 10. 1097/ INF. 00000 00000 00149.
- Nam HR, Jung SH, Dong SJ, et al. Prevalence of Metallo-β-lactamases in Imipenem-nonsusceptible *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Korean J Clin Microbiol*, 13: 169.
- Nazia A, Zeeshan N, Muhammad AZ, et al. (2020). Association of biofilm formation with multi drug resistance in clinical isolates of *Pseudomonas aeruginosa*. *Excli J*, 19: 201–208, doi: 10.17179/excli2019-2049.
- Noor ul A, Samyyia A, Rehan AKS, Abdul H, et al. (2020). Systematic surveillance and meta-analysis on the prevalence of metallo-β-lactamase producers among carbapenem resistant clinical isolates in Pakistan. *Journal of Global Antimicrobial Resistance*, 23: 2020, 55–63.
- Olutayo IF and Emmanuel OI. (2020). Extended Spectrum β-Lactamases and Metallo β-Lactamases Producing *Pseudomonas* Species Isolated From Fish Pond Water in Ibadan, Nigeria. *International journal of environmental studies*, 77:5, 865-875.
- Oliver A, Mulet X, López-Causapé C, et al. (2015). The increasing threat of *Pseudomonas aeruginosa* high-risk clones. *Drug Resist Updat*, 21:22, 41–59, doi: 10.1016/j.drup.2015.08.002.
- Oluwatoyin B. O, Olufemi E. A, Ahmed D, et al. (2021). Incidence and Molecular Characterization of Carbapenemase Genes in Association with Multidrug-Resistant Clinical Isolates of *Pseudomonas aeruginosa* from Tertiary Healthcare Facilities in Southwest Nigeria. *Current*

- Microbiology*,
doi.org/10.1007/s00284-021-02706-3.
- Pitout JDD, Gregson DB, Poirel L, et al. (2005). Detection of *Pseudomonas aeruginosa* producing metallo-beta-lactamases in a large centralized laboratory. *J Clin Microbiol*, 43: 3129-3135.
- Prakash M, Mathikere NC, Annapoorni R, et al. (2022). Challenges in Healing Wound: Role of Complementary and Alternative Medicine. *Front. Nutr.*, 8:2021 | doi.org/10.3389/fnut.2021.791899.
- Preeti P, Ragini G, and Puneet G. (2019). Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; a critical review. *Genes Dis*, 6:2, 109–119, doi: 10.1016/j.gendis.2019.04.001.
- Rehab M, Magdy FE, Asmaa A, et al. Identification and Characterization of Metallo-B- Lactamases Producing *Pseudomonas aeruginosa* Clinical Isolates in Al-Azher University Hospital, Assiut. *Bull. Pharm. Sci., Assiut University*, 44: 161-172.
- Report of Nigeria's National Population Commission (NPC) on the 2006 Census Population and Development Review. Published By: Population Council 2017; 33:206-210 <https://www.jstor.org/stable/25434601/>
- Reshma M, Basudha S, Sanjit S, Khadga BA, et al. (2020). Detection of Metallo-β-Lactamases and Carbapenemase Producing *Pseudomonas aeruginosa* Isolates from Burn Wound Infection. *TUJM*, 7: 1, doi: <https://doi.org/10.3126/tujm.v7i0.33800>.
- Sachdeva R, Sharma B, Sharma R. (2017). Evaluation of different phenotypic tests for detection of metallo-β-lactamases in imipenem-resistant *Pseudomonas aeruginosa*. *Journal of laboratory physicians*, 9:249.
- Udo RK. "A Comprehensive Geography of West Africa". (1st edition). Heinemann Educational Books Nig. Ltd., 1978.
- Vitaku E, Smith DT and Njardarson JT. (2014). "Analysis of the Structural Diversity, Substitution Patterns, and Frequency of Nitrogen Heterocycles among U.S. FDA Approved Pharmaceuticals". *Journal of Medicinal Chemistry*, 57: 10257–10274.
- Zavascki AP, Barth AL and Goldani LZ. (2008). Hospital bloodstream infections due to metallo-β-lactamases -producing *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* 61:1183–1185.
- Zhao WH and Hu ZQ. (2011). Epidemiology and Genetics of VIM-type Metallo-β-Lactamases in Gram-Negative Bacilli; 2011. Available from: http://www.medscape.com/viewarticle/740371_1.
- Zubair KO and Iregbu KC. (2018). Resistance Pattern and Detection of Metallo-beta-lactamases Genes in Clinical Isolates of *Pseudomonas aeruginosa* in a Central Nigeria Tertiary Hospital. *Journal of Clinical Practice*, 21:176-82.