Metallo β- lactamase producing pseudomonas aeruginosa: a worrisome situation to handle

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Pseudomonas aeruginosa is ubiquitous and very commonly found in hospital settings. In individuals with weakened immune systems, it can cause a number of ailments, inclusive of lung pneumoniae, gastrointestinal infections, keratitis, otitis media, and bacteremia. It is multi drug–resistant organism which is a worrisome situation. Multi-drug resistance is due to various factors like enzyme production, target mutation, expression of aminoglycoside-modifying enzymes (acetyltransferases, phosphotransferases) mediating aminoglycoside resistance, biofilm formation, but, among all of these, carbapenemases being one of most clinically significant. The most clinically significant carbapenemases are the Metallo β-lactamases (IMP, VIM, SPM, NDM, AIM and GIM genes). Understanding the epidemiology, resistance mechanism, molecular features, and for infection management and to prevent a potential global health crisis, techniques for identifying Carbapenem-Resistant-Pseudomonas aeruginosa (CRPA) are essential. For this review article, initial peer-review of publications from the various search engines ('Google search engine', 'Science direct', 'Pubmed', 'Google Scholar', 'Cross references' and 'Scopus') yielded a total of 97 papers. After reviewing the abstracts of the papers, 37 were eliminated and 60 were retained. Full text reading was undertaken to assess the quality of the articles, which resulted in the exclusion of 39 publications. After final peer-review screening, 17 publications were included in the study.

Keywords:
carbapenemases, metallo β-lactamases, *Pseudomonas aeruginosa*

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Introduction

Background and rationale

Pseudomonas aeruginosa is a gram negative, aerobic, motile, non-fermenting bacterium which is widely distributed. It is also frequently found in hospital settings and is a problem there because it is the second most frequent agent responsible for nosocomial infections [1,2]. It naturally resists to many antibiotics because of the outer membrane's limited permeability, the chromosomal β-lactamases it produces, and the efflux pumps [3]. Due to multitude of mechanisms for adaptability, survival, and resistance to many classes of antibiotics, infections produced by *P. aeruginosa* strains can be lethal and are increasingly posing a threat to public health on a global scale [4]. Numerous secretion systems that the bacterium possesses enable it to secrete a variety of proteins important to the pathogenesis of clinical strains. This bacterium can be found on and in medical equipment and thrives on most surfaces, leading to cross-infections in hospitals and clinics [2]. A prominent disease connected to healthcare is Carbapenem-resistant-Pseudomonas aeruginosa (CRPA) on a global scale [5]. Multiple mechanisms, such as target alteration, active efflux, decreased permeability, and enzyme degradation, can mediate it. Acquired resistance is brought on by mutations or the acquisition of exogenous resistance determinants [6].

The clinical usefulness of carbapenem as a reserve medication is in danger due to the rise of microorganisms that produce carbapenemase, particularly Metallo-β-lactamase [7]. Carbapenem-resistant pathogens, such as *Pseudomonas aeruginosa*, have recently emerged as a serious treatment problem. As acquired MBLs can hydrolyse any lactam antibiotic except monobactams, they are regarded to be more dangerous than other resistance mechanisms [8]. The likelihood of treatment failure in infections caused by CRPA is increasing as a result of the unrecognised existence of Carbapenemases. Combining phenotypic and genotypic approaches can speed up the onset of effective treatment and improve patient outcomes. Nevertheless, in many clinical laboratories, these

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tests are frequently not carried out on *P. aeruginosa* isolates. As a result, improving treatment for *P. aeruginosa* infections is still difficult [5]. MBLs seem to be present in isolates that are sensitive to imipenem and if these isolates are reported as carbapenem susceptible without being screened, these concealed MBLs might spread undetected in hospitals. Because they provide a significant treatment challenge, the laboratory identification of such isolates is essential [9].

Mortality rates in *P. aeruginosa* that produces MBL are significantly higher than those in non-MBL-producing strains [10]. Because acquired Metallo-beta-lactamase genes are typically carried by mobile genetic elements that spread quickly, there is a growing threat to public health from their global spread and the appearance of new variants. Early diagnosis and identification of MBL-producing organisms are essential for preventing nosocomial transmission through application of infection control measures [11].

Methodology

For this review article, the key phrases utilised in the literature search were '*Pseudomonas aeruginosa*', 'Metallo β-lactamase producing *Pseudomonas aeruginosa*', 'Carbapenem Resistant *Pseudomonas aeruginosa*', 'Review', 'Prevalence of MBL in *Pseudomonas aeruginosa*', 'Classification of MBLs' and 'Mechanism of Action of MBL in *P. aeruginosa*' using 'Google search engine', 'Science direct', 'Pubmed', 'Google Scholar', 'Cross references' and 'Scopus'. Since 2005, all papers that contained the specified keywords have been included in this study.

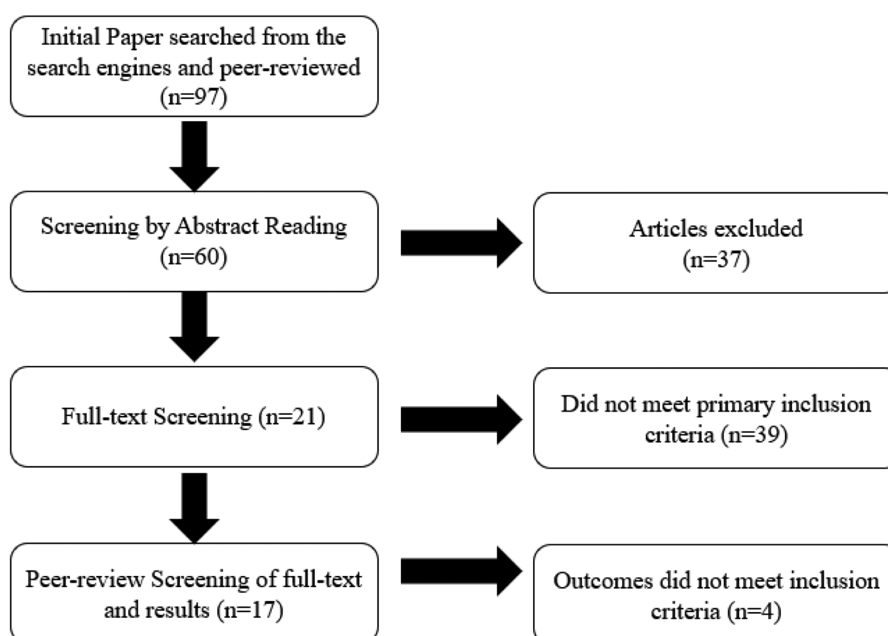
By initial Peer-review of the articles from the various search engines mentioned above gave a result of 97 papers. After reading the abstract of the papers, 37 articles were excluded and 60 were included. In order to evaluate the quality of the articles, full text reading was performed which led to the exclusion of 39 papers. Finally, 17 articles (Table 3) were included in the study (Fig. 1).

Virulence factors and pathogenesis

P. aeruginosa's complex genome and extensive and changeable collection of virulence factors are related to the pathogenic character of the organism. *P. aeruginosa* pathogenesis is aided by two key factors. The first factor is the host's overall health status. *P. aeruginosa* must first enter the host and colonise before it can cause infection. Although the organism is frequently acquired through inhalation into the respiratory system, it is challenging to point the exact mode of acquisition given the organism's extensive distribution [12].

Numerous *P. aeruginosa* virulence factors undermine the effects of the host's defences, and they can either directly harm the host's tissues or make the bacterium more competitive [13]. They can be divided into two groups based on how they function: those that aid in

Figure 1



Flowchart showing the article selection process.

the organism's connection to host cells (such as fimbriae and flagella) and those that aid in the organism's invasion of tissue and immune response. With the exception of pyocyanin, which is only produced by *P. aeruginosa*, all of the virulence factors that *P. aeruginosa* uses are also produced by other microbes [12]. The various factors which contribute to the pathogenicity of *Pseudomonas aeruginosa* is being described below in (Table 1).

Pseudomonas aeruginosa resistance – A rising threat

Several human disorders, particularly in immune-compromised patients, as well as numerous hospital-acquired infections are brought on by the pathogenic bacterium *P. aeruginosa*. Antimicrobial resistance makes it challenging to treat and remove due to its occurrence. Most antimicrobial medications, including 3rd generation cephalosporins and carbapenem, which are used to treat bacteria which are multidrug-resistant (MDR), are ineffective against some strains of *P. aeruginosa* [14]. One of the bacteria that is most dangerous to human health that is antimicrobial-resistant, according to the WHO, is *P. aeruginosa*. The emergence of so-called 'high-risk clones' of MDR or XDR *P. aeruginosa*, has recently become a global public health concern that requires urgent research and steadfast management [4]. Antimicrobial resistance in *P. aeruginosa* falls into three categories: (1) intrinsic, (2) acquired, and (3) adaptive [14]. Antibiotics from numerous classes, including beta-lactams, carbapenems, aminoglycosides, fluoroquinolones, and polymyxins, have been found to be ineffective against *P. aeruginosa*. Bacterial resistance to beta-lactam drugs is typically caused by beta-lactamases. Metalloβ-lactamases, whereby practically all beta-lactam drugs can be hydrolysed, are among the most vital resistance mechanisms in *P. aeruginosa*, as they severely limit therapeutic options [15–17]. MBLs such as 'Imipenemase (IMP)', 'Verona integron-encoded Metallo-lactamase (VIM)', 'Germany Imipenemase (GIM)', 'Adelaide Imipenemase (AIM)', 'Sao Paulo Metallo-lactamase (SPM)', 'Florence Imipenemase (FIM)', and 'New Delhi Metallo-lactamase (NDM)' have been linked to these resistance mechanisms in *P. aeruginosa*. It is well known that when patients are treated with antibiotics to which the organism is completely resistant, they have a poor prognosis for significant infections brought on by MBLs producing organisms [18].

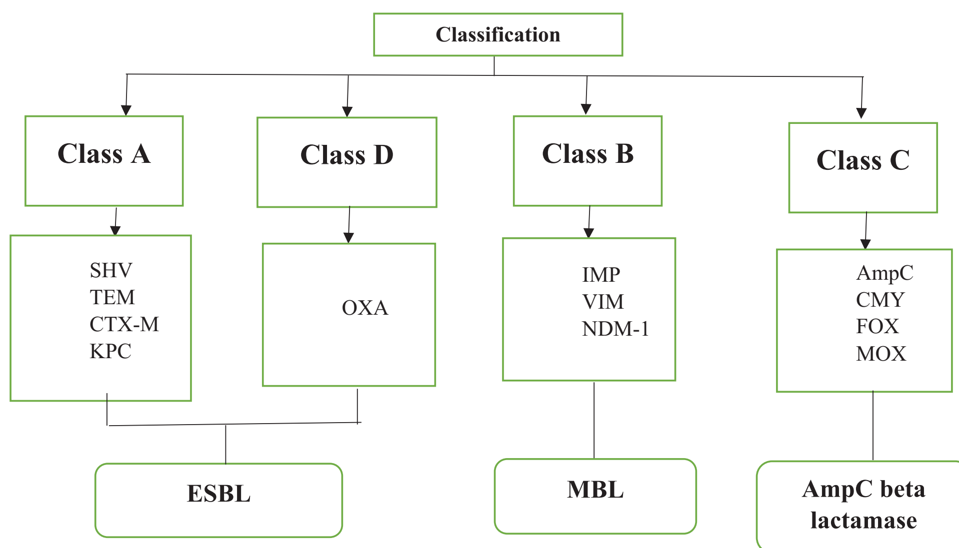
β –lactamase classification

The amide link within the β-lactam ring is hydrolysed by β-lactamases, which results in the production of by-products. Both in Gram-+ve and in Gram-ve bacteria, these products have frequently led to resistance [19]. β-lactamases are found in a wide range of environmental bacteria, but it is most widespread and effective in Gram-negative bacteria [20]. Two classification schemes:

(1) Ambler system based on sequence specificity and,
(2) Bush-Jacoby-Medeiros activity-dependent scheme; are used to categorise this group of enzymes [21].

Table 1 Factors affecting pathogenicity

Virulence Factor	Action
Pyocyanin	Generates reactive oxygen intermediates and inhibits the growth of lymphocytes and function of cilia.
Siderophores	Pyoverdine (formerly known as fluorescein) and pyochelin are the two siderophores that *P. aeruginosa* produces. In addition to meeting iron requirements, siderophores can aid in the formation of other virulence factors by transferring iron, such as biofilms and poisonous substances.
Elastase, Proteases, Hemolysins and leucocidin	Helps in tissue penetration and destroy host cells
Fimbriae	Embedding and induction of pro-inflammatory gene expression in host cells
Polar flagella	Interleukin-8 activation, host cell adhesion, and motility
Exotoxin A	Hinders the synthesis of proteins in host cells and promotes the spread of the organism
Lipopolysaccharide	It acts as Endo-toxin
Alginate	Scavenger of free radicals; prevents phagocytosis, neutrophil chemotaxis, and complement activation
Type III secretion system	Injecting toxins (ExoS, ExoT, ExoU, and ExoY) into host cells.
Quorum-sensing molecules	Supports the production of biofilms and coordinates gene expression in other pseudomonal cells.
Urease	Ammonia and carbon dioxide (CO_2) can be produced when urea is hydrolysed. Urinary tract infection is connected to it.
Outer membrane proteins	These protein constituents assist with the transportation of carbon sources, the absorption of antibiotics, and the transportation of amino acids and peptides. They are necessary for bacterial adhesion, the secretion of pathogenicity, and host recognition.



Flowchart showing Ambler classification, which separates beta-lactamases into four classes [21].

Ambler originally described two classes

(1) active-site serine β-lactamases (class A)
(2) Metallo- β-lactamases (class B), which involved a bivalent metal ion (Zn^{2+}) for action.

Eventually, a new class of Serine β-lactamases with no sequence resemblance to the previously identified class A enzymes was discovered. The 'AmpC' β-lactamases are another name for class C β-lactamases. Class C β-lactamases are also known as the 'AmpC' β-lactamases. Another class of serine b-lactamases, classified as OXA b-lactamases, was discovered to have little in common with either class A or class C and was given the label class D [22]. The Bush-Jacoby-Medeiros scheme, on the other hand, categorizes β-lactamases based on their functional features (substrates and inhibitors) [23].

Metallo β-lactamases

A diversified group of enzymes known as Metallo-β-lactamases catalyse the hydrolysis of a wide variety of β-lactam medications, including carbapenems [19] MBLs are classified as based on the similarity of their amino acid sequences, and Bush group 3 based on the composition of their substrate profiles 'IMP, VIM, SPM-1, GIM-1, and SIM-1' are the five distinct forms of acquired MBLs that have been so far identified [24] B1, B2, and B3 are subclasses of class B beta-lactamases based on structural and functional characteristics. The most common kind of carbapenemase generated by *P. aeruginosa* clinical isolates is metallo-beta-lactamase among which the most prevalent is VIM followed by imipenemases (IMPs). Additionally, NDMs (New Delhi MBLs) have also been recognised [25]. The Various type of MBLs as described below Table 2.

Metallo-β-lactamases' mechanism of action

βeta-lactamases are the enzymes that are designed to split the four membered ring of β-lactam antibiotics which is the principal resistance mechanism [26]. MBLs are zinc-binding metalloproteins in which a hydroxide ion acts as the attacking nucleophile and is activated and positioned by the Zn (II) cations and supporting residues from the active site [27].

The β-lactam binds to the metal centre of the enzyme, connecting with Zn^1 through the carbonyl oxygen and Zn2 through the carboxyl group on the 5- or 6-membered fused ring. Zn1 and Zn2 stabilise the hydroxide ion, which is present between the metal

Table 2 Types of MBLs and their year of detection and place where it was first isolated in *Pseudomonas aeruginosa*

	Type of MBL	Year of Detection	Place (1st Isolated)
Plasmid mediated	IMP	1998	Japan
	VIM	1999	Several parts of Countries
	SPM	1997	Brazil
	GIM	2002	Germany
	NDM	2011	New Delhi
	FIM	2012	Italy
	DIM	2007	Dutch
	DIM-1 (*Pseudomonas stuzeri*)	2014	India
	HMB-1	2012	Hamburg

Dutch imipenemases (**DIM**); Florence Imipenemase (**FIM**); German Imipenemase **(GIM**); Hamburg Metallo β-lactamase (**HMB**); Imipenemase **(IMP)**; **New** Delhi Metallo β-lactamase (**NDM**); Sao Paulo Metallo β-lactamase (**SPM**); Verona integron-encoded Metallo β-lactamase (**VIM)**.

Table 3 Various studies since 2012 with regards to production of Metallo β-lactamase in *Pseudomonas aeruginosa*

Author	Year	Place of Study	Total Sample Size	MBL Producers in *P. aeruginosa*	Methods
Nutan B. *et al*. [35]	2012	Nagpur	310	27=DDST 29=MHT	DDST, MHT
Silpi Basak[36]	2012	Wardha	250	28	DDST, DPT, MHT, MBL E-test
Arunava K. *et al.* [37]	2013	Pondicherry	49	8	DDST
Smita Sood[28]	2014	Jaipur	95	69	DDST, DPT, Thiol-based Compound Test
Abhishek M. e.al [11]	2016	Uttar Pradesh	150	12	CDT, DDST
Bandana B. *et al.* [7]	2017	Nepal	85	14	CDT
Narinder K. *et al.* [38]	2017	Bathinda	276 (GNB) 49 (*P.aeruginosa*)	17	DDT, MHT
Raouf *et al.* [39]	2018	Minia	70	17	MBL, E-test, PCR
Abbas *et al.* [40]	2018	Zagazig	50	2	Imipenem/EDTA, CDT, PCR
Vijeta B. *et al.* [41]	2019	New Delhi	126	105	MBL E-test, MHT
Vinita C. *et al.* [42]	2019	Jaipur	180	36	MHT
Soma S. *et al.* [1]	2020	Kolkata	394	49	CDST, PCR
Yang M. *et al.* [43]	2020	Nepal	79	8	E-test, CDT
K.Mahesh *et al.*[44]	2021	Chennai	100	18=MHT 17=CDT 14=E-test	MHT, CDT, E-test and PCR
Muddassir*et al.* [45]	2021	Pakistan	1159 255 (*P. aeruginosa*)	83=CDT 87=E-test 110=MHT	CDT, E-test, MHT and PCR
Karabi S. *et al.* [10]	2022	Dhaka	82	18	CDT, DDST, MBL E-test, PCR
Radhika *et al.* [46]	2022	Ghanpur, Medhal	60	9	CDT, DDST, E-test MBL

Combined Disc Synergic Test (**CDST)**; Combined Disc Test **(CDT**); Disc Potentiation Test (**DPT)**; Double Disc Synergy Test (**DDST)**; Epsilometer test(**E-test)**; Ethylenediamine tetraacetic acid (**EDTA**); Metallo-beta-Lactamase (**MBL);** Modified Hodge Test (**MHT)**; Polymerase Chain Reaction (**PCR)**.

ions and is ready to attack carbonyl carbon. Tetrahedral intermediate is produced as a result of the hydroxide's nucleophilic attack on the carbonyl carbon. The C-N link may be broken, and the tetrahedral intermediate may disintegrate in one of two ways: First, bond breaking, and nitrogen protonation may take place simultaneously, or secondly, cleavage may occur without nitrogen protonation, leading to the accumulation of an anionic nitrogen intermediate [19].

Methods for Metallo β-lactamase detection

Early detection of the production of these β-lactamases is crucial for epidemiological research and efficient infection control measures to prevent the spread of infection. This is because the resistance mechanisms of the MDR strains, which are the basis for designing appropriate medications, must be identified. There are several phenotypic approaches for detecting MBL generating bacteria [28]. These tests include the MBL Epsilometer-test (E-test), a combined disc test (CDT) using EDTA with imipenem (IPM) or ceftazidime (CAZ), double-disk synergy test (DDST) using EDTA and IPM or CAZ, EDTA disc potentiation test (PT) using CAZ, ceftizoxime, cefepime, and cefotaxime, the modified Hodge test (MHT) and 2-mercaptopropionic acid with CAZ or IPM [29].

For carbapenemase detection in *P. aeruginosa*, the Clinical and Laboratory Standards Institute (CLSI) currently recommends the CarbaNP and modified carbapenem inactivation technique (mCIM). As a combination phenotypic test to identify and distinguish between serine and metallo-based carbapenemases for Enterobacterales, EDTA-modified carbapenem inactivation method (eCIM) has evolved. They are advantageous for clinical laboratories with restricted resources because they use inexpensive products and produce findings that are simple to interpret.A simplified carbapenem inactivation method (sCIM) was used in a research by Xiaopeng Jing to identify carbapenemase in gram-negative organisms. It is a straightforward procedure that uses the traditional paper diffusion method and doesn't call for any specialised tools or reagents [30–32].

Molecular techniques have been presented as useful tools for screening antibiotic resistance genes in various isolates throughout the last two decades. In comparison to traditional PCR methods, real-timePCR is a molecular tool that can deliver data more quickly and accurately. Using group-specific primers, PCR is performed on the isolates to perform a genotypic study of the various MBL genes [33]. Multiplex real-

timePCR is now used as a reliable assay for the quick and simultaneous detection of numerous MBL genes in bacterial clinical isolates [34]. Various Studies Since 2012 as shown in (Table 3).

Conclusion

MBL-producing *Pseudomonas aeruginosa* is becoming more common across the globe as a result of the prevalence of multidrug-resistant Pseudomonas aeruginosa infections, which is alarming. An increasing hazard to public health is posed by the global spread of acquired Metallo-beta-lactamase genes and the emergence of novel variants since these genes are often carried by mobile genetic components that disperse swiftly. In order for clinicians to choose an appropriate antibiotic for the prompt treatment of infectious diseases while aiding in the prevention of the spread of resistance, phenotypic and genotypic detection of AMR must be combined. This will allow for better characterization regarding the resistance patterns of *Pseudomonas species*. Regardless of the bacterial host, it emphasises the necessity to manage carbapenemase-producing species. Control approaches include surveillance studies and antibiotic stewardship. The appearance and dissemination of *P. aeruginosa* clones with a high risk obviously require more attention. Early diagnosis and identification of MBL-producing organisms are essential in order to stop nosocomial transmission through proper treatment and the adoption of infection control procedures.

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Conflicts of interest

There are no conflicts of interest.

References

1 Sarkar S, Dutta S, Namhata A, Banerjee C, Sengupta M, Sengupta M. Beta-lactamase profile and biofilm production of pseudomonas aeruginosa isolated from a tertiary care hospital in Kolkata, India. J Clin Diagn Res 2020; 14:22–27.

2 Bunyan IA, Hadi OM, Al-Mansoori HAK. Molecular detection of Metallo-beta lactamase producing Pseudomonas aeruginosa isolated from different sites of infection. J Pharm Sci Res. 2018; 10:1072–1078.

3 Ahmadi N, Salimizand H, Zomorodi AR, Abbas JE, Ramazanzadeh R, Haghi F, *et al.* Genomic diversity of β-lactamase producing pseudomonas aeruginosa in Iran; the impact of Global High-risk clones. Res Sq. 2022; 1–14.

4 Spagnolo AM, Sartini M, Cristina ML. Pseudomonas aeruginosa in the healthcare facility setting. Rev Med Microbiol. 2021; 32:169–175.

5 Tenover FC, Nicolau DP, Gill CM. Carbapenemase-producing *pseudomonas aeruginosa* –an emerging challenge. Emerg Microbes Infect. 2022; 11:811–814.

6 Rossolini GM, Mantengoli E. Treatment and control of severe infections caused by multiresistant pseudomonas aeruginosa. Clin Microbiol Infect 2005; 11:17–32.

7 Baniya B, Pant ND, Neupane S, Khatiwada S, Yadav UN, Bhandari N, *et al.* Biofilm and metallo beta-lactamase production among the strains of pseudomonas aeruginosa and Acinetobacter spp. at a tertiary care hospital in Kathmandu, Nepal. Ann Clin Microbiol Antimicrob 2017; 16:1–4.

8 Yousefi S, Farajnia S, Nahaei MR, Akhi MT, Ghotaslou R, Soroush MH, *et al.* Detection of metallo-β-lactamase-encoding genes among clinical isolates of pseudomonas aeruginosa in northwest of Iran. Diagn Microbiol Infect Dis 2010; 68:322–325.

9 Renu G, Rajeev T, Smita S. The existence of Metallo Beta Lactamases in Carbapenem susceptible gram negative Baclli: A cause for concern. J Clin Diagnostic Res 2010; 4:2679–2684.

10 Saha K, Kabir ND, Islam MR, Amin MB, Hoque KI, Halder K, *et al.* Isolation and characterisation of carbapenem-resistant pseudomonas aeruginosa from hospital environments in tertiary care hospitals in Dhaka, Bangladesh. J Glob Antimicrob Resist. 2022; 30:31–37.

11 Mehta A, Prabhu T. Detection and characterization of metallo-beta-lactamases producing pseudomonas aeruginosa clinical isolates at a tertiary care hospital of Bhopal, India. Int J Rese Med Sci. 2016; 4 (9):4084–4088.

12 Moore NM, Flaws ML. Epidemiology and pathogenesis of Pseudomonas aeruginosa Infections. Am Soc Clin Lab Sci. 2011; 24:43–46. https://doi.org/10.29074/ascls.24.1.43

13 Gellatly SL, Hancock REW. *pseudomonas aeruginosa*: New insights into pathogenesis and host defenses. Pathog Dis 2013; 67:159–173.

14 Azam MW, Khan AU. Updates on the pathogenicity status of pseudomonas aeruginosa. Drug Discov Today 2019; 24:350–359.

15 Hong DJ, Bae IK, Jang I-H., Jeong SH, Kang H-K., Lee K. Epidemiology and characteristics of Metallo-β-lactamase-producing *pseudomonas aeruginosa*. Infect Chemother. 2015; 47:81.

16 Bebrone C. Metallo-β-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. Biochem Pharmacol 2007; 74:1686–1701.

17 Matos EC, Andriolo RB, Rodrigues YC, Lima PD, Carneiro IC, Lima KV. Mortality in patients with multidrug-resistant pseudomonas aeruginosa infections: A meta-analysis. Rev Soc Bras Med Trop 2018; 51:415–420.

18 Dawadi P, Khadka C, Shyaula M, Syangtan G, Joshi TP, Pepper SH, *et al.* Prevalence of Metallo-β-lactamases as a correlate of multidrug resistance among clinical pseudomonas aeruginosa isolates in Nepal. Sci Total Environ. 2022; 850:157975.

19 Palzkill T. Metallo-β-lactamase structure and function. Ann N Y Acad Sci 2012; 1277:91–104.

20 Dr Taoufik Djerboua Follow. Beta lactamases: Structure, classification and investigations [Internet]. Share and Discover Knowledge on SlideShare. [cited 2023 Jan12]. Available from: https://www.slideshare.net/TaoufikDjerboua/beta-lactamases-structure-classification-and-investigations

21 Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, Spencer J. β-lactamases and β-lactamase inhibitors in the 21st Century. J Mol Biol 2019; 431:3472–3500.

22 Hall BG, Barlow M. Revised Ambler classification of β-lactamases. J Antimicrob Chemother 2005; 55:1050–1051.

23 da Silva K, Knöbl T, Moreno A. Antimicrobial resistance in veterinary medicine: mechanisms and bacterial agents with the greatest impact on human health. Braz J Vet Res Anim Sci. 2013; 50:171–183.

24 Maltezou HC. Metallo-β-lactamases in gram-negative bacteria: Introducing the era of pan-resistance? Int J Antimicrob Agents 2009; 33:405.e1–405.e4057.

25 Yoon E-J., Jeong SH. Mobile carbapenemase genes in pseudomonas aeruginosa. Front Microbiol 2021; 12:1–21.

26 Bahr G, González LJ, Vila AJ. Metallo-β-lactamases in the age of multidrug resistance: From structure and mechanism to evolution, dissemination, and inhibitor design. Chem Rev 2021; 121:7957–8094.

27 Wang Z, Fast W, Valentine AM, Benkovic SJ. Metallo-β-lactamase: structure and mechanism. Curr Opin Chem Biol 1999; 3:614–622. https://doi.org/10.1016/s1367-5931(99) 00017-4

28 Sood S. Phenotypic tests for detecting incidence of Metallo-Î -Lactamase (Mbl) producing Pseudomonas aeruginosa in Jaipur. Natl J Lab Med. 2014; 3:22–27.

29 Ranjan S, Banashankari GS, Babu PRS. Evaluation of phenotypic tests and screening markers for detection of Metallo-β-lactamases in clinical isolates

of pseudomonas aeruginosa: A prospective study. Med J Dr DY Patil Univ. 2015; 8:599–605.

30 Gill CM, Lasko MJ, Asempa TE, Nicolau DP. Evaluation of the EDTA-modified Carbapenem inactivation method for detecting Metallo-β-Lactamase-producing Pseudomonas aeruginosa. J Clin Microbiol 2020; 58:6. https://doi.org/10.1128/JCM.02015-19

31 Lasko MJ, Gill CM, Asempa TE, *et al.* EDTA-modified carbapenem inactivation method (eCIM) for detecting IMP Metallo-β-lactamase-producing Pseudomonas aeruginosa: an assessment of increasing EDTA concentrations. BMC Microbiol 2020; 20:220. https://doi.org/10.1186/s12866-020-01902-8

32 Jing X, Zhou H, Min X, Zhang X, Yang Q, Du S, *et al.* The Simplified Carbapenem Inactivation Method (sCIM) for simple and accurate detection of carbapenemase-producing gram-negative Bacilli. Front Microbiol 2018; 9:2391. doi: 10.3389/fmicb.2018.02391

33 Goudarzi H, Mirsamadi ES, Ghalavand Z, Hakemi Vala M, Mirjalali H, Hashemi A. Rapid detection and molecular survey of BLAVIM, BLAIMP and BLANDM genes among clinical isolates of Acinetobacter baumannii using new multiplex real-time PCR and melting curve analysis. BMC Microbiol 2019; 19:1–7.

34 Siddiqui SA, Noorjahan CM, Arunagirinathan N. Phenotypic and molecular detection of carbapenemase new delhi metallo beta lactamase-1 (NDM-1) gene among pseudomonas aeruginosa from various clinical isolates. Int J Pharm Sci Res. 2020; 11:5856–5863.

35 Bhongle NN, Nagdeo NV, Thombare VR. The prevalence of Metallo β-Lactamases in the clinical isolates of pseudomonas aeruginosa in a tertiary care hospital: an alarming threat. J Clin Diagnostic Res. 2012; 6:1200–1202.

36 Basak S O.R, Rajurkar M. Pseudomonas aeruginosa and newer β-lactamases: An emerging resistance threat. Infect Control – Updates. 2012; 181–198.

37 Kali A. Detection of metallo-beta-lactamase producing pseudomonas aeruginosa in intensive care units. Aust Med J. 2013; 6:686–693.

38 Amandeep Kaur NK, Singh S. Prevalence of ESBL and MBL producing gram negative isolates from various clinical samples in a tertiary care hospital. Int J Curr Microbiol Appl Sci. 2017; 6:1423–1430.

39 Raouf MR, Sayed M, Rizk HA, Hassuna NA. High incidence of MBL-mediated imipenem resistance among pseudomonas aeruginosa from surgical site infections in Egypt. J Infect Dev Ctries. 2018; 12:520–525.

40 Abbas HA, El-Ganiny AM, Kamel HA. Phenotypic and genotypic detection of antibiotic resistance of pseudomonas aeruginosa isolated from urinary tract infections. Afr Health Sci 2018; 18:11–21.

41 Mathur P, Bajpai V, Govindaswamy A, Khurana S, Batra P, Aravinda A, *et al.* Phenotypic and genotypic profile of antimicrobial resistance in pseudomonas species in hospitalized patients. Ind J Med Res. 2019; 149:216–221.

42 Pal N, Choudhary V, Hooja S. Prevalence and antibiotic resistance pattern of metallo-β-lactamase-producing pseudomonas aeruginosa isolates from clinical specimens in a tertiary care hospital. J Mahatma Gandhi Inst Med Sci. 2019; 24:19–22.

43 Metok Y, Hosuru Subramanya S, Thapa Shrestha U, Reus Rodrigues Perez L, Adhikari N, Nayak N, *et al.* 2151. biofilm and metallo-β-lactamase (MBL) production among imipenem-resistant pseudomonas aeruginosa and Acinetobacter spp. MicroMedicine. 2020; 8:63–73.

44 MaheshKumar K, Illamani Saikumar C. Study the antimicrobial susceptibility pattern of pseudomonas aeruginosa and detection of metallo beta lactamase producing strain with special reference to blaNDM-1 and blaVIM. Ann RSCB. 2021; 25:2998–3013.

45 Muddassir M, Munir S, Raza A, Basirat A, Ahmed M, Farooq U, *et al.* Epidemiology and high incidence of Metallo-β-lactamase and AmpC-β-lactamases in nosocomial Pseudomonas aeruginosa. Iran J Basic Med Sci 2021; 24:1373–1379.

46 A R, Lakhsmi JT, Ariyanachi K, Sakthivadive V. Detection of Metallo Beta-Lactamase (MBL) producing pseudomonas aeruginosa in a tertiary care hospital, Ghanpur, Medchal, India. Mædica – J Clin Med 2022; 17:134–142.