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Article

Detection of *bla-AIM* Metallo Beta Lactamase Gene among *Stenotrophomonas Maltophilia* and Carbapenem Resistant *Pseudomonas Aeruginosa* Isolated from Various Infections in AL- Najaf Province

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ABSTRACT

Stenotrophomonas maltophilia is a "rapidly evolving pathogen of concern" that is increasingly being identified. The World Health Organization also recognizes it as one of the hospitals' most significant multi-drug-resistant pathogens. Also, *Pseudomonas aeruginosa* is an opportunistic human pathogen that causes most healthcare-associated infections, and it is considered a paradigm of antibiotic resistance development. In many hospitals across the globe, carbapenem-resistant *Pseudomonas aeruginosa* has emerged as a significant source of infection. The present study aimed to study the isolation and diagnosis of *S. maltophilia* and *P. aeruginosa* from different clinical samples, Evaluate the occurrence of carbapenem resistance *of P. aeruginosa* isolated from clinical samples and investigate the dissemination of the bla-AIM genes between these isolates. A total of 850 specimens were collected from various clinical samples between 2022 and 2023. The specimens included 220 swabs (burn), 200 (urine), 140 (stool), and 130(wound). 90 (ear),50 (throat), 10 (Cerebrospinal fluid), and 10 (blood). Represented by 680 specimens contained bacterial growth, and 170 specimens had no bacterial growth. Out of the 680 bacterial growth isolates, 410 revealed growths of Gram-negative bacteria, and 270 were Gram-positive bacteria. On MacConkey ag, ar 180/410 bacteria were lactose ferment; other isolates, es 230/410 of the isolates were lactose non-fermented bacteria. In a cross-sectional manner, *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* isolates during this period were isolated and identified depending on the primary methods of diagnosis, then the use of the VITEK-2 compact system. The results showed 42 isolates of *S. maltophilia* and 80 isolates of *P. aeruginosa* from total Gram-negative bacteria. The results show that only five isolates contained the *AIM* gene, with a percentage of (10.4 %) of the 48 Carbapenem Resistant *Pseudomonas aeruginosa* isolates, five isolates from 42 *S. maltophilia* contain the *AIM* gene with a percentage (11.9%), based on the Polymerase chain reactions assay.

Keywords: *Stenotrophomonas maltophilia*, Carbapenem Resistance, *Pseudomonas aerginosa.*

INTRODUCTION

The opportunistic pathogen *Stenotrophomonas maltophilia* is becoming more critical. It has emerged as a hospital-acquired infection because of the widespread use of broad-spectrum antibiotics and the increasing number of invasive operations and immunocompromised patients. *S. maltophilia* exhibits a variety of antimicrobial resistance mechanisms, including the production of enzymes that hydrolyze or modify antibiotics, changes in membrane permeability, and a multi-drug efflux mechanism [1]. Infections caused by *P. aeruginosa*, an opportunistic bacterium, include pneumonia, urinary tract infection, soft-tissue infection, and septicemia [2]. Multidrug-resistant (MDR) *P. aeruginosa* infections have been linked to high rates of illness and death.

Antibiotic treatment options are limited by the high degree of inherent and acquired resistance shown by *P. aeruginosa*. Carbapenems have been generally acknowledged as the most effective -lactams and extensively employed as the cornerstone and empirical therapy of severe infections caused by MDR *P. aeruginosa*; as a last-resort antibiotic, the discovery of carbapenem resistance is concerning, Carbapenem-resistant *P. aeruginosa* has now been discovered and is spreading across the globe [3]. When it comes to the hydrolysis of carbapenem and other beta-lactamases (excluding monobactam), metallo beta-lactamases have a high degree of efficacy and are unaffected by any of the currently known clinically available beta lactamases-inhibitors, all but a few MBL genes are found in integron gene cassettes coupled to mobile elements, which makes it easier for them to be transferred across different bacterial genera and species through horizontal gene transfer [4]. In the early 1990s, the Impeneme and Vancomycin-type enzymes, the most prominent of the acquired Metalobeta-lactamase, were discovered, and since then, several more kinds of acquired Metalobeta-lactamase enzymes [5]. This study aims to determine the occurrence of *the bla-AIM* gene among multi-resistance *S. maltophilia* and *P. aeruginosa* isolated from hospitals in Najaf.

MATERIALS AND METHODS

Patients Demography

Between 2022 and 2023, 850 clinical samples were taken from patients at the Al-Sadder Medical City, Al-Hakeem General Hospital, Al-Forat General Hospital, Al-Zahra Maternity and Children, Al-Sajad Hospital, and Burn Center in the province of Al-Najaf who were af-flicted with various infections. Al-Sadder Medical City 190 (22.3%) and Burn Center 220 (25.8%) produced the majority of the isolates, respectively, as shown in Table (1).

Table (1): Distribution of the number of clinical samples in different Najaf hospitals

Hospitals	Samples No.	Percentage
Burn Center	220	25.8%
Al-Sadder Medical City	190	22.3%
Al-Hakeem General Hospital	160	18.8%
Al-Forat General Hospital	150	17.6%
Al-Zahra for Maternity and Children	100	11.7%
Al-Sajaad Hospital	30	3.5 %
Total	850	100%

All samples were cultured on MacConkey medium and blood agar. After a 24-hour incubation period, the results showed 680 samples containing only bacterial growth. Two hundred two isolates represented the bacterial growth isolates were recovered from burn infections, 178 from urinary tract infections, 100 were from gastroenteritis, 103 bacterial isolates recovered from wounds, and 60, 30, and 3,4 were obtained from ear, throat, cerebrospinal fluid, blood respectively as show Table (2).

Table (2): Distribution of bacterial growth with infection site

Results

Specimens	Bacterial Growth	No Growth	Total
Burn	202	18	220
Urine	178	22	200
Stool	100	40	140
Wound	103	27	130
Ear	60	30	90
Throat	30	20	50
CSF	3	7	10
Blood	4	6	10
Total	680	170	850

Isolation and Identification of Stenotrophomonas maltophilia and Pseudomonas aerginosa Isolates.
The colony appearance, microscopic inspection, and biochemical characteristics were used to identify bacterial isolates. 410 of the 680 bacterial growth isolates showed Gram-negative bacteria growth, while the remaining 270 could not grow on the differential medium (Mac-Conkey Agar) utilized in this investigation. On MacConkey agar, 180/410 of the bacteria produced pink colonies since the bacteria lactose fermented grew on MaCconkey agar and produced pink colonies; other isolates 230/410 isolates produced yellow or colorless colonies since they were lactose non-fermented bacteria. In microscopic examination (Gram film), the organism appeared as a Gram-negative bacillus with a slightly smaller size. These are 230 samples, on which many of the biochemical tests available were conducted, including catalase, oxidase, motility, IMVICs, urease and TSI test to approximate the results for the diagnosis of Stenotrophomonas maltophilia and Pseudomonas aeruginosa using VITEK2 system. The re-sults showed the presence of 230 Gram-negative bacterial isolates that did not ferment to lactose after growing on MacConkey, including 42 isolates of S. maltophilia and 80 isolates of P. aeruginosa.
As a result of S. maltophilia having polar flagella, it became mobile and grew effectively on MacConkey, in addition to producing a distinctive pigment on the medium. The catalase test was positive, and the oxidase test was negative, distinguishing it from other members of the genus. In addition, the IMVC test was negative, except for the citrate test, the result of which was different between the isolat, es, but P. aeruginosa produces pyocyanin and gives off a strong oxidase positive7.
Antibiotic susceptibility test to detect carbapenem resistance P. aeruginosa.
The Eighty clinical isolates of P. aeruginosa were examined for their ability to develop in the presence of carbapenem drugs, Using the Kirby-Bauer disk diffusion technique and an-ti-pseudomonal drugs (imipenem and meropenem) from one antimicrobial category (be-ta-lactam medication), recommendations were followed to conduct preliminary susceptibility screening on the isolates. The findings of drug susceptibility testing include 48 isolate re-sistance to imipenem and 44 isolate resistances to meropenem 8.
DNA extraction
Based on the commercial kits used to extract the genome, the kit (Favorgen, Taiwan) was used in this study.
Molecular identification
The bla-AIM gene for S. maltophilia and P. aeruginosa was detected by PCR using a specific primer with the sequence mentioned in Table 3. Amplification conditions were performed as previously described by (Reference) and summarized in Table 4

Gene	Name of gene	Primer sequence (5 -3)	Bp	References
AIM	Adelaide imipenemase	CTGAAGGTGTACGGAAACAC		
		GTTCGGCCACCTCGAATTG	322	9

Table (3) primer used in this study

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Cycle
AIM	95˚C / 5 min	95˚C/ 30 sec	58˚C/30sec	72˚C/40sec	72˚C/7min	35

Table (4) PCR program to (bla-AIM gene).

RESULTS AND DISCUSSION

Molecular screening of *blah-AIM* producers to *Stenotrophomonas maltophilia* and Molecular screening of *AIM* producers to carbapenem resistance *Pseudomonas aeruginosa*

Molecular screening of *bla-AIM* producers *S. maltophilia*.

Using specialized Ambler class B MB (AIM) primers, all *S. maltophilia* isolates underwent conventional PCR screening for possible MBL gene determinants. With only five isolated positive findings and a proportion of (11.9%) %) as shown in Figure (1).

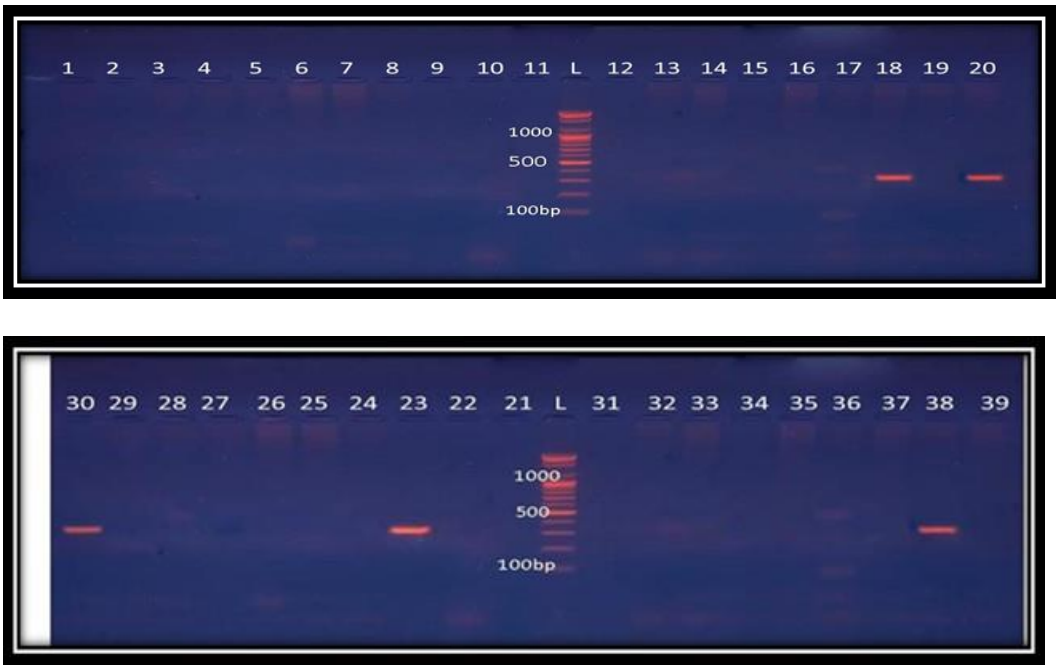

Figure 1: Amplification of *bla-AIM* gene by PCR from *S. maltophilia* isolates. Lane (18, 20, 23, 30, 38) shows positive results of *the bla-AIM* gene with 322 bp.

Even though multiple stable medications and inhibitor combinations are in different phases of development, they elude all recently approved -lactam—lactamase inhibitor combinations.[9] in Iraq reported that *S. maltophilia* is an emerging opportunistic nosocomial pathogen causing various infections. Ceftazidime and chloramphenicol did not affect any of the specimens. Moreover, 100 percent and 43 percent of them developed extended spectrum β-lactamases (ESBLs) and carbapenemases, respectively.

Molecular screening of *bla-AIM* producers to carbapenem resistance *Pseudomonas aeruginosa.*

All 48 carbapenem-resistant *P. aeruginosa* isolates were screened by conventional PCR for potential gene determinants encoding MBL using specific Ambler class B MBL(*AIM*) primers. Only five isolates had positive results for this gene with a percentage (10.4%), as shown in Figure (2).

Figure 2. Amplification of *bla-AIM* gene by PCR from *Pseudomonas aeruginosa* isolates. lanes (4, 9, 24, 38, 39) show positive results of the *bla-AIM* gene with 322 bp.

S. maltophilia has shown high resistance to many antibiotics, including beta-lactams, aminoglycosides, and chloramphenicol because it contains genes carried on the chromosome such as *blaL1* metallo β-lactamase and *blaL2* β-lactamase genes, this was consistent with the isolate's resistance profile[10]. This result agrees with another study in Iraq showing that *S. maltophilia* possesses many resistance genes, the most important of which is the metallo beta-lactamase gene[11].

Contrary to the other B3 MBLs (such as *Stenotrophomonas maltophilia L1 and Janthina bacteria lividum THIN-B, Chryseobacterium meningosepticum GOB, Legionella gormanii FEZ-1, and Caulobacter crescentus CAU-1*), this one can survive in the presence of antibiotics. Despite having the same MBL fold, B3 enzymes have a significantly different active site architecture than other subclasses [12].

An earlier investigation found that Carbapenemase synthesis in *P. aeruginosa* is particularly critical because of the fast spread of CRPA due to the acquisition of carbapenemase genes through mobile genetic elements. *P. aeruginosa* has been shown to generate carbapenemases of classes A, B, and D so far, with the most common being the Verona Integron-encoded Metallo—lactamase (*VIM*), imipenemases (*IMP*), and New Delhi Metallo—lactamase (*NDM*) all belonging to class B Metallo—lactamase (MBL) enzymes [13]

This result also agrees with another research *study, P. aeruginosa*, a prominent human pathogen, which produced *AIM-1*. This subclass B3 enzyme was the first to be discovered on a mobile genetic element; *AIM-1* hydrolyzes most lactams, except aztreonam and ceftazidime, to a lesser extent. However, it has much higher activity for cefepime and carbapenems than most other MBLs[14].

CONCLUSIONS

The presence of isolates of *S. maltophilia*, causing several infections, containing a gene (*bla-AIM*) with a percentage (11.9%). Also, the presence of Carbapenem Resistant *Pseudomonas aeruginosa* isolates caused several infections, containing a gene (*bla-AIM*) with a percentage (10.4%).

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REFERENCES

1. Huang Y.-W., Liou R.-S., Lin Y.-T., Huang H.-H., and Yang T.-C.A linkage between SmeIJK efflux pump, cell envelope integrity, and σEmediated envelope stress response in Stenotrophomonas maltophilia.2014.
2. Rosenthal V.D., Bijie H. Maki D.G., Mehta, Y. Apisarnthanarak A. and Medeiros E.A. International Nosocomial Infection Control Consortium (INICC) report, data summary of 36 countries, for2004–2009. Am J Infect Control .2012;40:396–407.
3. Vural E., Delialioglu N., Ulger S. T., Emekdas G. and Serin M. S. Phenotypic and molecular detection of the metallo-beta-lactamases in carbapenem-resistant Pseudomonas aeruginosa isolates from clinical samples. Jundishapur, J. Microbiol. 2020 e90034: 1-8. 10.5812/jjm.90034.
4. Edelstein M.V., Skleenova E.N., Shevchenko O.V., D'Souza J.W., Tapalski D.V., Azizov I.S., Sukhorukova M.V., Pavlukov R.A., Kozlov R.S., Toleman M.A. and Walsh T.R. Spread of extensively resistant VIM-2-positive ST235 Pseudomonas aeruginosa in Belarus, Kazakhstan, and Russia: a longitudinal epidemiological and clinical study. Lancet Infect Dis. 2013; 13:867-76.
5. Wachino J., Yoshida H., Yamane K., Suzuki S., Matsui M., Yamagishi T., Tsutsui A., Konda T., Shibayama K. and Arakawa Y. SMB-1, a novel subclass B3 metallo-beta-lactamase, associated with ISCR1 and a class 1 integron, from a carbapenem- resistant Serratia marcescens clinical isolate. Antimicrob Agents Chemother.2011 ;55:5143-9.
6. Miller, R. D., Iinishi, A., Modaresi, S. M., Yoo, B. K., Curtis, T. D., Lariviere, P. J., ... & Lewis, K. (2022). Computational identification of a systemic antibiotic for gram-negative bacteria. *Nature Microbiology*, *7*(10), 1661-1672.
7. Koneman E. W., Allen S. D., Janda W.M., Winn W.C., Procop G.W., Schreckenberger P.C. and Woods,G.L.The non fermentive gram negative bacilli, Color Atlas And Textbook of Diagnostic Microbiology, 6 th edition, (J.B.Lippincott Co., Philadelphia,).2006;316-328.
8. Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage Pseudomonas aeruginosa infections. Drugs in context. 2018;7.
9. Poirel L., Walsh T. R., Cuvillier V. and Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagnostic microbiology and infectious disease.2011; 70(1), 119-123.
10. Kumwenda, G. P.; Kasambara, W.; Phiri, A.; Chizani, K.; Banda, A.; Choonara, F. and Lichapa, B. (2021). A multidrug-resistant Stenotrophomonas maltophilia clinical isolate from Kamuzu Central Hospital, Malawi. Malawi Medical Journal, 33(2), 82-84.
11. Saleh, R. O.; Hussen, B. M.; Mubarak, S. M. and Mostafavi, S. K. (2021). High diversity of virulent and multidrug-resistant Stenotrophomonas maltophilia in Iraq. Gene Reports, 23.
12. Khorvash F., Yazdani M., Shabani S. and Soudi A. Pseudomonas aeruginosa-producing metallo-β-lactamases (VIM, IMP, SME, and AIM) in the clinical isolates of intensive care units, a university hospital in Isfahan, Iran. Advanced biomedical research.2017;6.
13. Bording-Jorgensen M., Tyrrell H., Lloyd C., & Chui, L. Comparison of Common Enrichment Broths Used in Diagnostic Laboratories for Shiga Toxin—Producing Escherichia coli. Microorganisms.2021; 9(3), 503.
14. Yong, D.; Toleman, M. A.; Bell, J.; Ritchie, B.; Pratt, R.; Ryley, H. and Walsh, T. R. (2012). Genetic and biochemical characterization of an acquired subgroup B3 metallo-β-lactamase gene, bla AIM-1, and its unique genetic context in Pseudomonas aeruginosa from Australia. Antimicrobial agents and chemotherapy, 56(12), 6154-6159.

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