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Research Article

Carbapenem resistant strains of pseudomonas species at Minia university hospitals

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Abstract

Background: The incidence of Pseudomonas aeruginosa (P. aeruginosa), an important etiological cause of nosocomial infections, strains that produce beta-lactamases has been frequently recognized. Therefore, accurate and timely detection of isolates that produce carbapenemase is crucial. The purpose of this study was to determine the frequency of the bla-IMP gene in carbapenem-resistant P. aeruginosa strains recovered from hospitals affiliated with Minia University. **Methods:** From June 2018 to September 2018, 40 P. aeruginosa strains isolated from various specimens were used in this work. Carbapenem resistant strains were gathered and analyzed for bla-IMP gene expression using PCR. **Results**: Out of 40 P. aeruginosa strains, 26 isolates were carbapenem resistant detected by (Vitek-2, bioMérieux, France) expressing the bla-IMP gene was in 46% of isolates. **Conclusion**: Carbapenem resistance in our hospitals have developed into a serious issue that calls for accurate diagnosis and efficient treatment. In this study, colistin was the antibiotic that worked the best against isolates that were resistant to carbapenem. To determine the primary source of resistance, it is advised to conduct genetic analyses for the most prevalent genes responsible for carbapenem resistance on a significant number of clinical specimens. However, given its expensive price, this might be restricted.

Key Words: Carbapenem, bla-IMP, metallo-β-lactamases, P.aeruginosa.

Introduction

P. aeruginosa is a Gram negative aerobic, extensively spread bacteria that can live in hospitals and on a range of surfaces [1]. It is an opportunistic pathogen that accounts for 10–20% of nosocomial infections, including cystic fibrosis, urinary tract infections, pneumonia, burn infections, and wound infections[2]. It can also cause bacteremia and sepsis in intensive care units.

P. aeruginosa that is multidrug resistant (MDR) is resistant to at least three anti-pseudomonal antibiotics (quinolones, aminoglycosides, carbapenems, and penicillin/cephalosporin)[3].

Due to the organism's intrinsic resistance to numerous antibiotic classes and its ability to develop resistance to all potent antimicrobial medications, MDR P. aeruginosa is becoming a significant health concern [4].

P. aeruginosa produces metallo-lactamases (MβLs) enzymes, including IMP (imipenem active), which give rise to a variety of mechanisms of resistance against carbapenems. by creating the metallo-lactamases (MβLs) enzymes, particularly the IMP (imipenem active metallo-lactamase) and VIM (Verona integron-encoded metallo-lactamase) [5, 6].

Ambler classes A, B, and D are mentioned in relation to carbapenemase resistance [7,8], although MβL VIM and IMP are classified as Ambler B. Ethylene-diamine-tetra-acetic acid (EDTA) and sodium mercapto-acetic acid (SMA) inhibit MβLs, whereas β-lactase

inhibitors such clavulanic acid, sulbactam, and tazobactam had no effect on MLs [9].

The purpose of this study was to find out how frequently P. aeruginosa strains isolated from patients at Minia university hospitals included the bla-IMP gene.

Material and methods

Isolation and identification P. aeruginosa

The 40 pseudomonas species used in this study were obtained from clinical specimens sent to the Minia University hospitals' microbiology unit for culture and sensitivity testing between June 2018 and September 2018. The following procedures were applied to all isolates:

- Routine culture on blood and MacConkey agar media for 24-48 hours incubation at 37°C. Then identification and AST was done using (VITEK-2, bioMérieux - France).
- All Carbapenems (Imipenem and Meropenem) resistant pseudomonas isolates were preserved for evaluation of bla-IMP gene expression using PCR.

DNA extraction was done using (Promega Co., USA) kits following the manufacturer's protocol.

PCR detection: particular primer created from (bla-IMP): Reverse primer: (5'-AAC CAG TTT TGC CTT ACC AT-3'), forward primer: (5'-CTA CCG CAG CAG AGT CTT TG-3'). (Germany's Operant Co.) [10].

The primer pair (5'-ATGGAAATGCTGAAATTCGGC-3') and (5'-CTTCTTCAGCTCGACGCGACG-3') was chosen as a reference for our research in order to amplify conserved portions of a target gene in P. aeruginosa and produce an identifiable PCR amplicon (500 bp) by gel electrophoresis.

Statistical analysis

The means and standard deviations of the numerical data were used to express them. Frequencies and percentages were used to express the qualitative data.

Results

40 Pseudomonas isolates from clinical specimens admitted to the microbiology unit at Minia university hospitals were used in this study, and they were dispersed based on the type of specimens as shown in the table (1).

Table (1): Pseudomonas distribution by specimen type:

Types of specimens	**No. (%)**
Wound swab	6(15%)
Sputum	2(5%)
Urine Culture	24(60%)
Blood culture	8(420%)
Total	**40**

Table (2): Antibiotic resistance pattern of isolated Pseudomonas spp.:

Antibiotic	**Resistant**	**Sensitive**
	No. (%)	**No. (%)**
Meropenem	26 (65%)	14 (35%)
Imipenem	25(62.5%)	15 (37.5%)
Cefazolin	30 (75%)	10 (25%)
Cefepime	24 (60%)	16 (40%)
Amikacin	24 (60%)	16 (40%)
Gentamicin	22 (55%)	18 (45%)
Ciprofloxacin	24 (60%)	16 (40%)
Levofloxacin	22 (55%)	18 (45%)
Ceftazidime	28 (70%)	12 (30%)
Colistin	0 (100%)	40 (0%)

Table (3): Distribution of carbapenem resistant Pseudomonas isolates according to the type of specimens

Types of specimens	No. (%)
Wound swab	4 (15.4%)
Urine	22 (84.6%)
Total	**26**

Table (4): Antibiotic resistance pattern of the carbapenem resistant isolates:

Antibiotic	Resistant No. (%)	Sensitive No. (%)
Cefepime	20 (76.9%)	6 (23.1%)
Amikacin	18 (69.2%)	8 (30.7%)
Ciprofloxacin	20 (76.9%)	6 (23.1%)
Levofloxacin	11 (42.3%)	15 (57.7%)
Cefotaxime	٢٤ (92.3%)	2 (6.7%)
Ceftazidime	26 (100%)	0 (0%)
Colistin	0 (0%)	26 (100%)

In this study, the resistance pattern of bla-IMP gene was expressed in 30% of all isolates representing about 46% of carbapenem resistant strains.

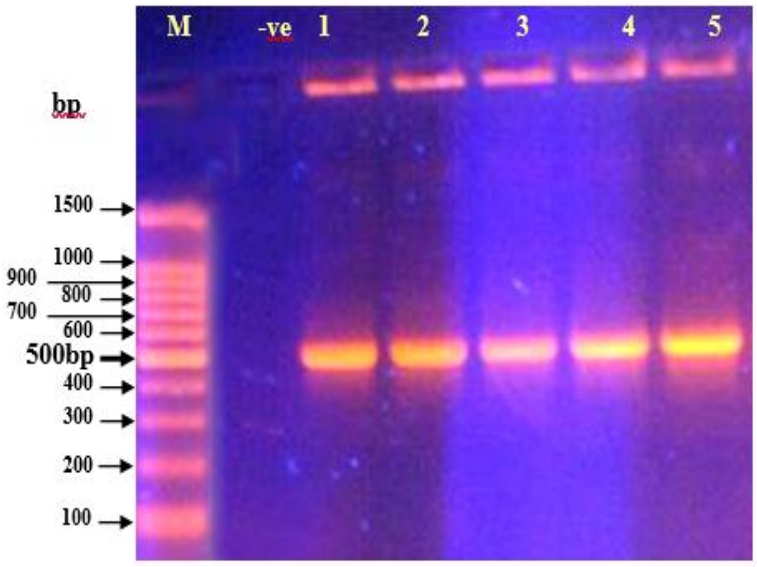

Figure (1): bla-IMP gene PCR detection on agarose gel electrophoresis. Samples in lanes (1, 2, 3, 4, and 5) tested positive for the bla-IMP gene.

Discussion

MDR P. aeruginosa is regarded as a serious issue, and a variety of antibiotics were used, including piperacillin-tazobactam, third-generation cephalosporin (ceftazidime), fourth-generation cephalosporin (cefepime), carbapenems (imipenem and meropenem), aminoglycosides (amikacin, gentamicin, and tobramycin), fluoroquinolones (ciprofloxacin, ofloxacin, and levofloxacin), monobactam (aztreonam), and colistin [11]. Particularly with imipenem, the antibiotic resistance pattern in pseudomonas slacks off quickly [12]. When treating MDR P. aeruginosa, carbapenems (such as imipenem and meropenem) are frequently utilized as a last option [13].

Antibiotic resistance to carbapenems is thought to be primarily caused by the production or acquisition of MβL genes (such as IMP and VIM) [14, 15].

The infection control practises and strategies followed in hospitals influence the MβLs that produce P.aeruginosa in Egypt [16]. According to the current study, Carbapenem resistance was observed in 65% of P. aeruginosa clinical isolates against (imipenem and meropenem). These outcomes were comparable to those reported by Rehab Mousa et al.,[17] who reported 69% of her isolates. In our study, P. aeruginosa had a 62.5% imipenem resistance rate and a 65% meropenem resistance rate. According on regional antibiotic policies, strain origin, and geographic location, carbapenem resistance rates can change.

In this work, bla-IMP gene was expressed in 30% of all pseudomonas isolates representing about 46% of carbapenem resistant strains.
Al-Agamy et al., discovered that 34% of his pseudomonas strains had carbapenem resistance, and roughly 22% of these isolates developed MBLs [18]. On the other hand, our findings contradict a research from Iran that claimed that only 8 (9.75%) of P. aeruginosa isolates that produced MBL were positive for bla-IMP [19]. And about 70 (70%) of the 100 imipenem-resistant pseudomonas isolates in a study conducted in Iran were identified to produce MBLs [20]. In order to provide suitable targeted therapy and prevent the nosocomial transmission of these resistant strains, it was crucial to discover MBL-producing isolates [21].

According to the results of the current work, several P. aeruginosa isolates showed varying degrees of resistance to aminoglycosides, including Amikacin and Gentamicin, with percentages of 60% and 55%, respectively. Similar to the current findings, earlier studies from Iran confirmed the high frequencies of P. aeruginosa resistance to aminoglycosides (gentamycin, amikacin) [22].

According to the current study, there was 60% and 55%, respectively, resistance to levofloxacin and ciprofloxacin. These findings were in contrast to those made by Al.Fahadawi et al., in their study on pseudomonas isolates that showed good efficacy against ciprofloxacin and norfloxacin, where the percentages of sensitivity and resistance were, respectively, (85.3%, 76.5%) and (11.8%, 17.6%) [23]. In contrast, El-Badawy et al., showed that 42.4% of his isolates had at least one resistance against one quinolone antibiotic[24]. While P. aeruginosa isolates were demonstrated to be responsive to ciprofloxacin by Corona-Nakamura et al., [25] .The global spread of P. aeruginosa MDR strains may be to blame for this variation.

One of the polymyxin class of antibiotics, colistin exhibits a broad spectrum of activity against the majority of Gram-negative bacteria. Colistin is increasingly often utilized in clinical settings, particularly for MDR P. aeruginosa. According to CLSI [26] colistin has a sensitive breakpoint of $\leq$ 2 mg/L and a resistant breakpoint of $\geq$ 4 mg/L against P. aeruginosa. Intriguingly, colistin proved to be the most effective antibiotic in our research because it was shown to be sensitive to all of the 26 isolates we tested for carbapenem resistance.

Conclusion

Additional monitoring, robust preventative measures, and the implementation of infection-control procedures are required to stop creeping carbapenem resistance among pseudomonas strains. For all isolates, molecular validation by PCR of various carbapenemase manufacturers is necessary in addition to standard phenotypic methods for identifying carbapenemases production in order to identify the hidden genes. The most efficient treatment policies must also be determined for each area through a regular observation programs.

Conflict of interest

No conflicts of interest exist, according to the authors, with the publishing of this paper.

References

1. Diggle, Stephen P., and Marvin Whiteley. "Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat." Microbiology 166, no. 1 (2020): 30.
2. Reynolds, Dan, and Marin Kollef. "The epidemiology and pathogenesis and treatment of Pseudomonas aeruginosa infections: an update." Drugs 81, no. 18 (2021): 2117-2131.

3. Malhotra, Sankalp, Don Hayes Jr, and Daniel J. Wozniak. "Cystic fibrosis and Pseudomonas aeruginosa: the host-microbe interface." Clinical microbiology reviews 32, no. 3 (2019): e00138-18.
4. Pachori, Preeti, Ragini Gothalwal, and Puneet Gandhi. "Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review." Genes & diseases 6, no. 2 (2019): 109-119.
5. Lee, J.-Y. and K.S. Ko, OprD mutations and inactivation, expression of efflux pumps and AmpC, and metallo-β-lactamases in carbapenem-resistant Pseudomonas aeruginosa isolates from South Korea. International journal of antimicrobial agents, 2012. 40(2): p. 168-172.
6. Rameshkumar, Gunasekaran, Ranjithkumar Dhandapani, Prajna Lalitha, Siva Ganesa Karthikeyan Rajapandian, Velmurugan Palanivel, Sathiamoorthi Thangavelu, Abdullah A. Alyousef et al., "Prevalence and Molecular Characterization of Metallo β-Lactamase Producing Gram-Negative Pathogens Causing Eye Infections." Frontiers in public health 10 (2022).
7. Hammoudi Halat, Dalal, and Carole Ayoub Moubareck. "The current burden of carbapenemases: Review of significant properties and dissemination among gram-negative bacteria." Antibiotics 9, no.4 (2020): 186.
8. Golshani, B., J. Lamotte-Brasseur, and G.M. Rossolini, Standard numbering schemefor class (B)-lactamases. Antimicrob Agents Chemother, 2005. 45: p. 660-3.
9. Bahr, Guillermo, Lisandro J. Gonzalez, and Alejandro J. Vila. "Metallo-β-lactamases in the age of multidrug resistance: from structure and mechanism to evolution, dissemination, and inhibitor design." Chemical Reviews 121, no. 13 (2021): 7957-8094.
10. Mendes, R.E., et al., Rapid detection and identification of metallo-β-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. Journal of clinical microbiology,2007.45(2):p.544-547
11. Al-Orphaly M, Hadi HA, Eltayeb FK, et al., Epidemiology of multidrug-resistant Pseudomonas aeruginosa in the Middle East and North Africa Region. mSphere. 2021;6(3).
12. Shah, Dania Aijaz, Shehnaz Wasim, and Farhan Essa Abdullah. "Antibiotic resistance pattern of Pseudomonas aeruginosa isolated from urine samples of Urinary Tract Infections patients in Karachi, Pakistan." Pakistan journal of medical sciences 31, no. 2 (2015): 341.
13. Farzana, Aleya, and S. M. Shamsuzzaman. "In vitro efficacy of synergistic antibiotic combinations in imipenem resistant Pseudomonas aeruginosa strains." Bangladesh Journal of Medical Microbiology 9, no. 1 (2015): 3-8.
14. Mustafa, Muhammad-Hariri, Hussein Chalhoub, Olivier Denis, Ariane Deplano, Anne Vergison, Hector Rodriguez-Villalobos, Michael M. Tunney et al., "Antimicrobial susceptibility of Pseudomonas aeruginosa isolated from cystic fibrosis patients in Northern Europe." Antimicrobial agents and chemotherapy 60, no. 11 (2016): 6735-6741.
15. El-Far, A., Samir, S., El-Gebaly, E., Omar, M., Dahroug, H., El-Shenawy, A., ... & Gamal, D. High Rates of Aminoglycoside Methyltransferases Associated with Metallo-Beta-Lactamases in Multidrug-Resistant and Extensively Drug-Resistant Pseudomonas aeruginosa Clinical Isolates from a Tertiary Care Hospital in Egypt. Infection and Drug Resistance, (2021).:14, 4849.
16. El Maraghy, Nermine, Manal Said, Rasha Emad, Mona Salama, and Abdullah Hashish. "Detection of IMP and VIM genes in Pseudomonas aeruginosa isolated from Egyptian patients." Archives of Medical Science-Civilization Diseases 4, no.1 (2019): 58-63.
17. Mousa, R., et al., Occurrence of carbapenem multidrug-resistant Pseudomonas aeruginosa carrying blaVIM metallo-β-lactamases and their biofilm phenotypes in Al-Azhar University Hospital. Journal of Applied Pharmaceutical Science, 2022. 12(3): p. 073-081.
18. Al-Agamy, M.H.M., et al., High prevalence of metallo-β-lactamase-producing Pseudomonas aeruginosa from Saudi Arabia. Journal of Chemotherapy, 2009. 21(4): p. 461-462.
19. Sarhangi, M., M. Motamedifar, and J. Sarvari, Dissemination of Pseudomonas aeruginosa producing blaIMP1, blaVIM2,

blaSIM1, blaSPM1 in Shiraz, Iran. Jundi-shapur Journal of Microbiology, 2013. 6(7).
20. Aghamiri, S., et al., Antibiotic resistance pattern and evaluation of metallo-beta lactamase genes including bla-IMP and bla-VIM types in Pseudomonas aeruginosa isolated from patients in Tehran hospitals. International Scholarly Research Notices, 2014. 2014.
21. Wei, Wen-Juan, Hai-Fei Yang, Ying Ye, and Jia-Bin Li. "New Delhi metallo-β-lactamase-mediated carbapenem resistance: origin, diagnosis, treatment and public health concern." Chinese medical journal 128, no. 14 (2015): 1969-1976.
22. Tarafdar, F., B. Jafari, and T. Azimi, Evaluating the antimicrobial resistance patterns and molecular frequency of blaoxa-48 and blaGES-2 genes in Pseudomonas aeruginosa and Acinetobacter baumannii strains isolated from burn wound infection in Tehran, Iran. New Microbes and New Infections, 2020. 37: p. 100686.
23. Al.Fahadawi, M.A., W.I. Al.Obadi, and A.S. Hasan, Antibiogram of Pseudomonas aeruginosa Isolated from Burn& Wound Infections Among Inpatients and Outpatients Attending to Ramadi Teaching Hospital in Ramadi, Iraq. Egyptian Academic Journal of Biological Sciences, G. Microbiology, 2019. 11(1): p. 13-22.
24. El-Badawy, Mohamed F., Majed M. Alrobaian, Mohamed M. Shohayeb, and Sayed F. Abdelwahab. "Investigation of six plasmid-mediated quinolone resistance genes among clinical isolates of pseudomonas: a genotypic study in Saudi Arabia." Infection and Drug Resistance 12 (2019): 915.
25. Corona-Nakamura, A.L., et al., Epidemiologic study of Pseudomonas aeruginosa in critical patients and reservoirs. Archives of Medical Research, 2001. 32(3): p. 238-242.
26. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. 2014, Clinical and Laboratory Standards Institute.