Phenotypic demonstration of β-lactamase (ESβLs, MβLs, and Amp-C) among MDR Pseudomonas aeruginosa isolates obtained from Burn wound infected in Yemen

Mahfuoz Nasser1*, Arun S. Kharat2
1Department of Biotechnology, Dr. Babasaheb Ambedkar Marathwada University, Osmanabad, India
2School of Life Science, Jawaharlal Nehru University, New Delhi, India

ARTICLE INFO
Article history:
Received on: August 08, 2019
Accepted on: August 23, 2019
Available online: November 12, 2019

Key words:
Pseudomonas aeruginosa, burn wound, β-lactamase (ESβLs, MβLs, and Amp-C), Yemen

ABSTRACT
In 2017, the World Health Organization published its first-ever list of antimicrobial-resistant bacteria “priority pathogens,” a catalog of 12 families of bacteria posing the greatest threat to human health. This list focuses on the risk of Gram-negative bacteria for multiple drug-resistant. Pseudomonas aeruginosa was at the top of the list and critical. A current study aiming to demonstrate the prevalence of β-lactamase among multidrug-resistant P. aeruginosa strains isolated from burn wound patients phenotypically. The isolates were identified then antibiotic susceptibility tested against 10 antipseudomonal agents, finally, phenotypically β-lactamase (ESβLs, MβLs, and Amp-C) production screened by combined disk diffusion test and Imipenem-ethylenediaminetetraacetic acid. Results in the current study identified 98 P. aeruginosa isolates from 200 clinical specimens obtained from burn wound patients. Our result showed 65 (66.3%) of the 98 P. aeruginosa isolates were multiple drug-resistant (MDR) strains. Out of 65 isolates, 37 (56.9%), 21 (32.3%), and 40 (61.5%) were ESβLs, MβLs, and Amp-C producing P. aeruginosa, respectively, according to phenotypic detection method. We found co-expression of various β-lactamases. In the present study, 16 isolates showed co-existence of AmpC + ESBL, 16 isolates were having ESBL + MBL + AmpC, and five isolates were having co-existence of ESBL + MBL. The occurrence of ESβLs, MβLs, and Amp-C producing P. aeruginosa was demonstrated, calling for phenotypical determination of antibiotic resistance mechanisms should be performed regularly to guide antibiotic selection during therapy.

Significant conclusions drawn from this work include a rise in the rate of β-lactamase (ESβLs, MβLs, and Amp-C) in MDR P. aeruginosa. Later research should, therefore, focus on the study of molecular characterization.

1. INTRODUCTION
Pseudomonas aeruginosa is the most common pathogens in infections with burns [1]. In the past years, there has been a growing interest in antimicrobial resistance, multiple drug-resistant (MDR) P. aeruginosa is the rising associate reason for mortality and morbidity in burn wound patients, which causes 4%–60% nosocomial infections in different parts of the globe [2]. Pseudomonas aeruginosa is one of the common pathogenic causes of severe burn wound infections worldwide [3]. Pseudomonas aeruginosa among hospitalized patients is one of the significant reasons for health-related diseases. Infections associated with healthcare predominantly lead to infections of the burn wound. This bacterium can develop resistance to all conventional anti-psudomonal antimicrobial through one of a kind intrinsic and acquired resistance mechanisms. This bacterium commonly demonstrates multiple resistant isolates, which leads to morbidity and mortality [4]. β-lactamase (ESβLs, MβLs, and Amp-C) are enzymes produced with various antibiotic-resistant isolates. Production of β-lactamases such as extended-spectrum β-lactamases (ESBLs), Metallo beta-lactamase (MBL), and AmpC β-lactamases is the dominant mechanism responsible for resistance to β-lactam agents among P. aeruginosa. β-lactamases are enzymes that hydrolyze β-lactam antibiotics, remain the greatest threat to make these antibiotic agents’ inactivity. Previous studies have shown that around the world, a wide variation in the prevalence of these mechanisms from region to region, also no data available.

*Corresponding Author
Mahfuoz Nasser, Department of Biotechnology, Dr Babasaheb Ambedkar Marathwada University, Aurangabad, India.
E-mail: Mahfuonzasser@yahoo.com

© 2019 Nasser, et al. This is an open access article distributed under the terms of the Creative Commons Attribution License -NonCommercial-ShareAlike Unported License (http://creativecommons.org/licenses/by-nc-sa/3.0/).
from western Yemen. The isolates can be conveniently classified into several resistant phenotypes, based on their resistance to β-lactam/β-lactamase inhibitors antibiotic. β-lactamase phenotype determination not only can help for patients treatment select but it also can be a principal for bla gene screening. This is the first study that determined the phenotype of beta-lactamase among MDR *P. aeruginosa* isolates obtained from burn wound infected in West Yemen. This study contributes to the understanding of antimicrobial resistance, phenotypic characterization of the causes and mechanisms of resistance to help within the management of burn wound infections as a result of *P. aeruginosa*. The current study aimed to determine the β-lactamase phenotypes among MDR *P. aeruginosa* isolates obtained from burn wound infected in West Yemen.

2. MATERIALS AND METHODS

2.1. Sample collected/ bacteria isolation

In the current study during the period from July 2018 to December 2018, we identified 98 *P. aeruginosa* isolates from 200 clinical specimens obtained from burn wound patients admitted at the General Al-THawrah Hospital in Hodeidah City Western Yemen. The identification was based on colony characteristics, Gram’s staining, and biochemical tests.

2.2. Antimicrobial susceptibility test

Antimicrobial susceptibility test was done by the Kirby–Bauer disk diffusion method on Muller–Hinton agar according to CLSI guidance [5]. Antibiogram disks containing Ceftazidime (30 μg), Gentamicin (10 μg), Amikacin (30 μg), Ciprofloxacin (5 μg), Meropenem (10 μg), Imipenem (10 μg), Tobramycin (10 μg), Piperacillin/Tazobactam (100/10 μg), Cefepime (10 μg), and aztreonam (30 μg).

2.3. Detection of MDR bacteria strains

Isolates are showing resistance to one antimicrobial agent in three different categories of antimicrobials described as multiple drug-resistant (MDR) strains [6,7].

2.4. Phenotypic identification of β-lactamase (ESβLs, MβLs, and Amp-C) producing isolates

Screening for ESβLs, MβLs, and Amp-C production, according to [5,8,9].

ESβL producing isolates were phenotypically identified using combination disk test (CDT). All MDR isolates have been assessed using a Mueller–Hinton agar (MHA) plate and a Ceftazidime (30 μg) and Ceftazidime/Clavulanic acid (30 μg/10 μg) disks to evaluate the production of ESβL. The observation of a rise of 5 mm in the zone diameter for the incorporation of ceftazidime with clavulanic acid compared to its zone diameter when testing ceftazidime alone [5].

Imipenem-ethylenediaminetetraacetic acid (EDTA) synergy test was recommended based on the phenotypic identification of MβL producing isolates. MβLs can be inhibited by metal chelators like EDTA or 2-mercaptopyrroplionic acid experimentally. As outlined in Lee et al. [8], used 750 μg EDTA, MHA media was accomplished. When the zone variations between Imipenem + EDTA disks and Imipenem disks exceeded 7 mm, the combined MβL disk test was regarded as positive.

Detection of the production of Amp C β-lactamases, the production of Amp-C was evaluated by an inhibitor-based strategy using boronic acid as an inhibitor and cefoxitin. Inhibitor-based test: a 30 μg cefoxitin disk and an additional 30 μg cefoxitin with 400 μg boronic acid contained the laundered culture of test *P. aeruginosa* on the MHA and incubated at 37°C overnight. Besides cefoxitin alone, in the presence of boronic acid, the rise in the zone diameter of 5 mm or more was regarded as positive for amp C production [9].

3. RESULT

In the current study during the period from July 1, 2018 to December 31, 2018, 98 (49%) out of 200 samples collected from the patients who attended at the burn and wound ward, general Al-THawrah hospital, Hodiedah city, West Yemen were *P. aeruginosa*. Preliminary identification tests performed on all the isolates (Gram stain, oxidase, and catalase tests), and the isolates were identified using a variety of techniques; These included morphological characteristics, biochemical testing, and pigment production. Based on these results, the isolates were identified as *Pseudomonas aeruginosa*. The antimicrobial susceptibility testing carried out on Mueller Hinton agar as described by [10]. Table 1 showed that the highest level of antibiotic resistance was 85.7% of isolates exhibited resistance to gentamycin (10 μg), then 74.5% to Tobramycin (10 μg) and 83.7% to Amikacin (10 μg), while 77.5 to Ceftazidime (30 μg), 72.0% to Cefepime (10 μg), and 26.5% were to Aztreonam (30 μg). 54.1% were resistant to piperacillin-tazobactam (100/10 μg), while 66.3% were resistant to a fluoroquinolone antibiotic Ciprofloxacin (5 μg). Resistance to carbapenems was 21.4% and 20.4%, respectively, to Imipenem (10 μg) and Meropenem (10 μg). Our result showed 65 (66.3%) of the 98 *P. aeruginosa* isolates resistance to at least one antimicrobial agent in three antimicrobial groups and are considered MDR strains. Out of 65 isolates, 37 (56.9%), 21 (32.3%), and 40 (61.5%) were ESβLs, MβLs, and Amp-C producing *P. aeruginosa*, respectively, according to phenotypic detection method (Fig. 1). We found co-expression of various β-lactamases

| Sr. No | Categories | Antimicrobial | Resistant No. (%) | Sensitive No. (%) |
|--------|------------|---------------|------------------|------------------|
| 1      | Aminoglycosides | Gentamicin | 84 (85.7) | 14 (14.3) |
| 2      | Tobramycin | 73 (74.5) | 25 (25.5) |
| 3      | Amikacin | 82 (83.7) | 16 (16.3) |
| 4      | Carbapenems | Imipenem | 21 (21.4) | 77 (78.6) |
| 5      | Meropenem | 20 (20.4) | 78 (79.6) |
| 6      | Cephalosporins | Ceftazidime | 76 (77.5) | 22 (22.5) |
| 7      | Cefepime | 71 (72.4) | 27 (27.6) |
| 8      | Penicillins | Piperacillin-tazobactam | 53 (54.1) | 45 (45.9) |
| 9      | Monobactams | Aztreonam | 26 (26.5) | 72 (73.5) |
in multiple drug-resistant *P. aeruginosa*. In the present study, 16 isolates showed co-existence of AmpC + ESβL, 16 isolates were having ESβLs + MβLs + Amp-C, and five isolates were having co-existence of ESβLs + MβLs. Expression of AmpC and MβL simultaneously found the increasing frequency of the co-existence of ESβLs, MβLs, and Amp C-β-lactamases in bacteria that common mechanism of drug resistance in the present study (Table 2).

4. DISCUSSION
Recent studies indicate that resistance to multiple antibiotic classes, especially fluoroquinolones and beta-lactam antibiotics, is rising, thus limiting the treatment regimens. Also, this study revealed that the prevalence of β-lactamase producing *P. aeruginosa* isolates obtained from burn wound infection in western Yemen is high and it was 37 (56.9%), 21 (32.3%), and 40 (61.5%) for ESβLs, MβLs, Amp-C, respectively. This work has shown that Amp-C β-lactamase was the most prevalent β-lactamase in *P. aeruginosa* isolates. Abbas et al. [11], Kumar et al. [12] have also found Amp-C to be the most common β-lactamase. In this study, ESβLs, MβLs, Amp-C were 56.9%, 32.3%, and 61.5%. The current study results are the highest among the studies by Vinita et al. [13], and Gupta et al. [14] who had reported that (ESβLs, MβLs, and Amp-C) prevalence as (27.7%, 12%, and 21.6%), and (21.4%, 21.4%, and 51.1%) respectively. Also, isolates that co-produce all an ESβLs, MβLs, and Amp-C β-lactamases are becoming more common, increasing frequency of the co-existence of ESβLs, MβLs, and Amp C-β-lactamases in bacteria is a severe threat for treating bacterial infections. To detect these resistant bacteria, a simple disk method can be used regularly. Disk diffusion test would screen all beta-lactamase enzymes producing Gram-negative bacilli in the diagnostic laboratory.

5. CONCLUSION
The occurrence of ESβLs, MβLs, and Amp-C producing *P. aeruginosa* was demonstrated, calling for phenotypical determination of antibiotic resistance mechanisms should be performed regularly to guide antibiotic selection during therapy. Significant conclusions drawn from this work include a rise in the rate of β-lactamase (ESβLs, MβLs, and Amp-C) in MDR *P. aeruginosa*. Later research should, therefore, focus on the study of molecular characterization of MDR *P. aeruginosa*.

ACKNOWLEDGMENTS
The authors gratefully acknowledge Dr. Khaled Suhai, hospital manager, and the authors gratefully acknowledge all the colleagues who help via samples collected.

CONFLICT OF INTEREST
The authors declared that they have no conflict of interest.

REFERENCES
1. Ibrahim SONBOL F, Abd El Fattah KHALIL M, Badr MOHAMED A, Samir ALI S. Correlation between antibiotic resistance and virulence of *Pseudomonas aeruginosa* clinical isolates. Turk J Med Sci 2015;45:568–77; doi:10.3906/sag-1406-58
2. Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant *Pseudomonas aeruginosa*: comparison of risks associated with different antipseudomonal agents. Antimicrob Agents Chemother 1999:43:1379–82.
3. Jabalameili F, Mirsalehian A, Khoramian B, Aligholi M, Khoramrooz SS, Asadollahi P, et al. Evaluation of biofilm production and characterization of genes encoding type III secretion system among *Pseudomonas aeruginosa* isolated from burn patients. Burns 2012;38:1192–7; doi:10.1016/j.burns.2012.07.030
4. Paramythiotou E, Routsi C. Association between infections caused by multidrug-resistant gram-negative bacteria and mortality in critically ill patients. World J Crit Care Med 2016;5:111–20; doi:10.5492/wjccm.v5.i2.111
5. CLSI. Performance standards for antimicrobial susceptibility testing; Twenty-fourth informational supplement. CLSI document M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA, 2014.
6. Falagas ME, Koletsi PK, Bliziotis, IA. The diversity of definitions of multidrug-resistant (MDR) and pan drug-resistant (PDR) Acinetobacter baumannii and *Pseudomonas aeruginosa*. J Med Microbiol 2006; doi:10.1099/jmm.0.46747-0
7. Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81; doi:10.1111/j.1469-0691.2011.03570.x
8. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating Metallobeta-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J Clin Microbiol 2003;41:4623–9; doi:10.1128/JCM.41.10.4623-4629.2003
9. Thomson, KS. Controversies about extended-spectrum and AmpC beta-lactamases. Emerg Infect Dis 2001;7:333–6; doi:10.3201/ eid0702.000333
10. Jorgensen JH, Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods*. Man Clin Microbiol. 11th edition, American Society of Microbiology, Washington, DC, pp 1253–7, 32015; doi:10.1128/9781555817381.ch71.
11. Abbas HA, El-Ganiny AM, Kamel HA. Phenotypic and genotypic detection of antibiotic resistance of Pseudomonas aeruginosa isolated from urinary tract infections. Afri Heal Sci 2018;18:11–213; doi:10.4314/ahs.v18i1.3

12. Kumar V, Sen MR, Nigam C, Gahlot R, Kumari S. Burden of different beta-lactamase classes among clinical isolates of AmpC-producing Pseudomonas aeruginosa in burn patients: a prospective study. Indian J Crit Care Med 2012;16:136–40; doi:10.4103/0972-5229.102077

13. Vinita C, Nita P, Saroj H. Phenotypic detection of ESBL, AMPC and MBL beta-lactamases among clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital of north India. Int J Curr Med Pharm Res 2018;4:3902–6. doi:10.24327/23956429.ijcmprr201812584.

14. Gupta R, Malik A, Rizvi M, Ahmed M. Presence of Metallobeta-lactamases (MBLs), extended-spectrum beta-lactamase (ESBL) and AmpC positive non-fermenting Gram-negative bacilli among Intensive Care Unit patients with special reference to molecular detection of blaCTX-M &amp; blaAmpC genes. Indian J Med Res 2016;144:271–5; doi:10.4103/0971-5916.195043

How to cite this article:
Nasser M Kharat AS. Phenotypic demonstration of beta-lactamase (ESβLs, MβLs, and Amp-C) among MDR Pseudomonas aeruginosa isolates obtained from Burn wound infected in Yemen. J Appl Biol Biotech 2019;7(06):31–34. DOI: 10.7324/JABB.2019.70605