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

Mahfuoz Nasser[1*], Arun S. Kharat[2]

[1]Department of Biotechnology, Dr. Babasaheb Ambedkar Marathwada University, Osmanabad, India
[2]School of Life Science, Jawaharlal Nehru University, New Delhi, India

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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-pseudomonal 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 b-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: Mahfouznasser@yahoo.com

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-mercaptopropionic 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

Table 1: Antibiotic susceptibility results of 98 clinical isolates of *P. aeruginosa* from Burn wound infections patients.

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)
9	Penicillins	piperacillin-tazobactam	53 (54.1)	45 (45.9)
10	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 most common β-lactamase. In this study, the 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.

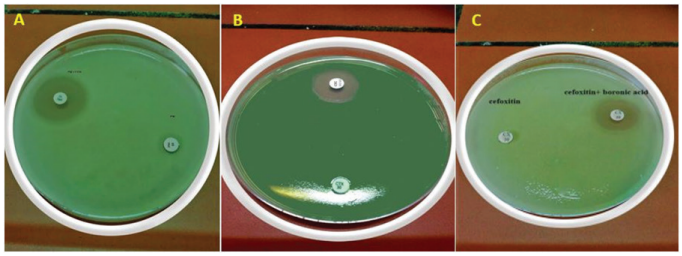

Figure 1: (A) ESβL production by a CDT. (B) MβL production by combination diffusion disk test. (C) Detection for Amp-C production by combination diffusion disk test.

Table 2: Summarize co-expression β-lactamases (ESβLs, MβLs, and Amp-C) phenotypically.

Beta-lactamase	Single genotype	Co-occurring	Total phenotypic
ESβLs	0	-	37
MβLs	0	-	21
Amp-C	8	-	40
EsβLs + MβLs	-	5	-
EsβLs + Amp-C	-	16	-
EsβLs + MβLs + Amp-C	-	16	-

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*.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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