

Multi Drug Resistant *Pseudomonas aeruginosa* in a Health Care Setting in Alexandria

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Abstract: The emergence of Multi drug resistant *Pseudomonas aeruginosa* (MDRPA) among intensive care unit (ICU) patients is increasingly recognized as a public health threat worldwide. This work aimed to study the occurrence of MDRPA among critically ill patients in a health care setting in Alexandria. During a 12 months period, different clinical samples (sputum, endotracheal aspirates, blood, urine, and pus) obtained from ICU patients were tested for the isolation and identification of *Pseudomonas aeruginosa* (*P. aeruginosa*) strains; that were screened for their antimicrobial susceptibility patterns using single disc diffusion method. Identified MDRPA strains were further tested for their susceptibility to polymyxin E (colistin), polymyxin B, and tigecycline. Polymerase chain reaction (PCR) assay was performed to detect VIM and IMP MBL genes. Of the 105 *P. aeruginosa* strains isolated from various clinical samples, 20 (19%) were found to be MDRPA, of which 16 (80%) were sensitive to each of colistin and polymyxin B, while only 5 (25%) strains were sensitive to tigecycline. PCR assay revealed that 9 (45%) strains possessed VIM MBL gene and none (0%) harbored IMP MBL gene. The occurrence of MDRPA strains among critically ill patients in this study was noticeable; with colistin and polymyxin B being effective upon the majority of identified MDRPA strains, and VIM MBL gene was found to be significantly harbored.

Keywords: Multidrug resistance *Pseudomonas aeruginosa*, metallo beta lactamases, polymyxins, colistin, tigecycline, VIM & IMP MBL genes.

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a major hospital-associated pathogen; that can cause a wide spectrum of severe infections, most notably in immunocompromised patients or those hospitalized in intensive care units (ICUs).⁽¹⁾

It is responsible for 10–15% of the nosocomial infections worldwide. Often these infections are hard to treat due to the natural resistance of the species, as well as its remarkable ability of acquiring further mechanisms of resistance to multiple

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groups of antimicrobial agents. (2, 3)

The multi drug resistant *P. aeruginosa* (MDRPA) is on the rise and the increase in its incidence raises serious concerns. (4) A review of studies on MDR and 'pan-drug resistant' *P. aeruginosa* infections revealed considerably different definitions used in the literature, ranging from resistance to a single antibiotic agent/class to resistance to all tested antibiotics. In the majority of the published studies, multidrug resistance was defined as resistance to at least three drugs from a variety of antibiotic classes, mainly antipseudomonal penicillins, aminoglycosides, cephalosporins, carbapenems and fluoroquinolones. (5, 6)

A national surveillance of 13,999 nonduplicate *P. aeruginosa* isolates from ICU patients showed that multidrug resistance increased significantly, from 4% in 1993 to 14% in 2002. (7)

P. aeruginosa is intrinsically resistant to many drugs and is able to become resistant to virtually any antimicrobial agent. Acquired

mechanisms contributing to resistance in *P. aeruginosa* include β -lactamases, notably the extended-spectrum β -lactamases and the carbapenemases that hydrolyze most β -lactams, aminoglycoside-modifying enzymes, and 16S rRNA methylases that provide high-level pan-aminoglycoside resistance. Resistance to carbapenems is often mediated by production of Metallo-Beta-Lactamases (MBL); a class B type of beta-lactamases that is the most worrisome and require bivalent metal ions, usually zinc for their activity. (8) Five MBL types, namely, the IMP, VIM, SPM, GIM, and SIM types of MBLs, have been identified; however, the IMP and VIM types are the most commonly detected MBLs worldwide. (9, 10)

P. aeruginosa, producing MBLs, was first reported from Japan in 1991 and since then has been described from various parts of the world, including Asia, Europe, Australia, South America, and North America. (11,12) In some countries, *P. aeruginosa* possessing MBLs constitute nearly 20% of all nosocomial isolates. (10) In India, published reports

indicated that the prevalence of MBLs ranged from 7-65 %, with a study reporting 34% occurrence. ^(13, 14) MDRPA isolates have been responsible for several outbreaks in tertiary centers in different parts of the world, and have been associated with failure of therapy with carbapenems, illustrating the need for proper infection control practices. ^(15, 16) In Egypt, an outbreak in an ICU of a university hospital over 14weeks period has been reported; where 25 ICU patients developed infections by MDRPA. ⁽¹⁷⁾

The emergence of Gram-negative bacteria resistant to most available antibiotics has led to the readministration of polymyxin B and polymyxin E (colistin) as "salvage" therapy in critically ill patients. Recent studies demonstrated acceptable effectiveness and considerably less toxicity than reported in older studies of polymyxins. ⁽¹⁸⁾

Colistin, an old antibiotic also known as polymyxin E, has attracted more interest because of its significant activity against multi-resistant *P. aeruginosa*, *Acinetobacter*

baumannii and *Klebsiella pneumoniae*, and the low resistance rates to it. Because its use as an anti-pseudomonal agent was displaced by the potentially less toxic aminoglycosides in 1970s, our knowledge of this drug is limited. It is likely that colistin would be an important antimicrobial option against multi-resistant Gram-negative bacteria, for some years to come. ⁽¹⁹⁾

This work aimed at studying the occurrence of MDRPA among critically ill patients in a health care setting in Alexandria, Egypt. It included the isolation and identification of *P. aeruginosa* strains, testing their antimicrobial susceptibility patterns and identifying MDRPA strains, studying the susceptibility patterns of MDRPA strains to polymyxin E (colistin), polymyxin B, and tigecycline and detecting VIM and IMP MBL genes using PCR assay.

MATERIAL AND METHODS:

This study was carried out during a 12 months period from January 2009 till the end of December 2009. Different clinical samples

obtained from patients admitted to the 3 ICUs (General, Coronary and Intermediate) of the Alexandria University Students' Hospital were tested for the isolation and identification of MDRPA.

1] Isolation and Identification of *P. aeruginosa* strains: ⁽²⁰⁾

All collected clinical samples (blood-endotracheal aspirates-sputum-pus-urine) were cultured on Blood and MacConkey's agar plates, and incubated at 37°C aerobically for 24 hours. The strains were identified as *P. aeruginosa* by conventional methods. The isolates that were Gram negative bacilli, oxidase positive with large flat pigmented colonies and a sweet or grape like odour, feathered edge, opaque, β hemolytic on blood agar plates, and non lactose fermenting on MacConkey's agar plates were suspected of being *Pseudomonas* spp. Those that were positive for: citrate utilization, motility, and growth at 42° C, negative for: Indole, MR and VP tests, and on TSI they showed

alkaline slant /unchanged butt with no H₂S production or gas formation, were considered as *P. aeruginosa*.

2] Antimicrobial Susceptibility Testing:

Identified *P. aeruginosa* strains were screened for their antimicrobial susceptibility using single disc diffusion method described by Bauer et al. ⁽²¹⁾ The test was done on Mueller Hinton agar plates, using the selected antibiotic discs with various concentrations including piperacillin, piperacillin /tazobactam, ceftazidime, cefotaxime, ceftriaxone, ceftazolin, cefalothin, ceftiofur, aztreonam, meropenem, imipenem, gentamicin, tobramycin, amikacin, amoxicillin/clavulanic acid, ampicillin/sulbactam, ciprofloxacin, and levofloxacin (Oxoid). Inhibition zones were measured and susceptibility was interpreted as susceptible (S), Intermediate (I) and resistant (R) according to standard tables published by CLSI. ⁽²²⁾

Isolated *P. aeruginosa* strains were

considered multi drug resistant when they were found to be resistant to at least 3 of the following antibiotic classes: anti pseudomonal penicillins, cephalosporins, monobactams, carbapenems, aminoglycosides, and fluoroquinolones.

MDRPA strains were further tested for their susceptibility to polymyxin E (colistin), polymyxin B, and tigecycline using disc diffusion method. Inhibition zones were interpreted according to CLSI guidelines.⁽²²⁾

3] MBL genes identification:

All 20 identified MDRPA strains were tested for VIM and IMP MBL genes using PCR assay as follows:

a) DNA Extraction:

Fresh Culture of the tested strains of *P. aeruginosa* and control strains were suspended in 500 µl of saline and vortexed to get a uniform suspension. The cells were lysed by heating them at 100°C for ten minutes, and cellular debris was removed by centrifugation at 8000 rpm for five minutes. The

supernatant was used as a source of template for amplification.

b) Amplification Reaction:⁽²³⁾

Duplex PCR amplification for the simultaneous detection of IMP and VIM MBL genes were carried out using primers supplied by Bioneer Corporation (Table 1). Aliquots of 2µl DNA extracts were used for amplification in a 25-µl PCR reaction mixture containing (DreamTaq™ Green PCR Master mix, Fermentas). Amplification was performed in a Biocycler Tc-S Thermocycler (Boeco Germany). The temperature profile used for the amplification was; 94°C for 2 min (initial denaturation), then 30 cycles of 94°C for 1 min (DNA denaturation), 54°C for 1 min (primer annealing) and 72°C for 1.5 min (primer extension) and a final extension step of 5 min at 72°C. The amplification products were analyzed by agarose gel electrophoresis and

ethidium bromide staining. The amplification was performed in duplicates and controls were included in each assay.

4] Statistical analysis:

- The results of the present study were tabulated and statistical analyses were conducted using PC with the software: Statistical Package for the Social Sciences (SPSS) version 15 and Excel.
- Statistical significance was set at 5% ($P < 0.05$). The **Z- test** was done.

RESULTS

In a 12 months period, a total of 105 *P. aeruginosa* strains were isolated from different clinical samples collected from patients admitted to the ICUs of the Alexandria University Students' Hospital. Twenty *P. aeruginosa* strains (19%) were found to be multi drug resistant to tested antimicrobial agents, of which 16 isolates (80%) and 4 (20%) were reported from males and females respectively. They

were distributed as 7(35%) from sputum samples, 5(25%) from pus, 3(15%) from each of urine and endotracheal aspirates, and 2(10%) from blood samples (Table 2).

All identified MDRPA strains in this study were resistant to all tested antimicrobial agents except for only 2 strains (10%) that were susceptible to aztreonam and one strain (5%) to meropenem.

Of the 20 tested MDRPA strains, 9 (45%) strains were found to harbor VIM MBL gene, while none of the tested strains (0%) was positive for IMP MBL gene by PCR assay. The 9 VIM positive strains were recovered from 5 (55%) females and 4 (45%) males, where 5 out of 9 (55%) strains were isolated from sputum samples and 4 (45%) were from pus samples.

As regards the susceptibility patterns of the 20 MDRPA strains to colistin, polymyxin B and tigecycline; 16 (80%) were sensitive to colistin and polymyxin B,

while only 5 (25%) strains were sensitive to tigecycline (Table 3).

DISCUSSION

The emergence of MDRPA in hospital acquired infections among ICU patients is increasingly recognized and is related to high morbidity and mortality.^(4,23) Its prevalence has increased over the past decade and has become a major public health concern.^(7,24) The phenomenon of multi drug resistant pathogens had emerged not only in Egypt but also worldwide due to the excessive antibiotic misuse.^(25,26)

The present work was conducted to study the occurrence of MDRPA among critically ill patients in ICUs. Intensive care patients create an environment for infection because of the debilitating effect of prolonged hospitalization, serious underlying disease, and compromised membrane and skin barriers following the application of invasive medical equipment (as airway, catheters, etc)⁽²⁷⁾

Reported rates of MDRPA infection

varied from 0.6-32% depending on geographic location and type of surveillance study.⁽²⁸⁾ In the present study, of the 105 isolated *P. aeruginosa* strains; 20 (19 %) were found to be MDRPA. Nearly similar results were reported in a university hospital in Italy ⁽²⁹⁾, where MDRPA recorded a rate of 17%, while in the United States (U.S.), Flamm et al recorded a rate of 23 to 26% of MDRPA isolates collected from 1999 to 2002. ⁽³⁰⁾ In 2005-2007, Tam et al documented a prevalence rate of 10–17 %.⁽³¹⁾

Rates of MDRPA in individual institutions are even higher than those reported in large surveillance studies. Earlier in a university hospital in Brazil, 10.2% of *P. aeruginosa* isolates (48/472) were reported as MDRPA in 1992. ⁽³²⁾ An institution in Greece declared that 24.3% (25/103) of non-duplicate *P. aeruginosa* isolates from 1996-1997 were resistant to all antimicrobials tested. ⁽³³⁾ A higher isolation rate of 32% (13/41) was reported in another academic medical center in the U.S. in 2002. ⁽³⁴⁾ While in 2007, Lodise

et al found that among 351 *P. aeruginosa* infected patients, the proportion of MDRPA was 35%.⁽²⁴⁾

In this work, different clinical samples were collected and tested for MDRPA including blood, endotracheal aspirates, sputum, urine, and pus from wounds. The most common site of isolation of MDRPA was sputum 7 (35%), followed by pus from wounds 5(25%), endotracheal aspirates and urine samples revealed 3(15%) each, while blood yielded only 2 (10%) of MDRPA isolates (Table 2). In concordance to the present findings, in a study conducted in Egypt (2004) to identify the causative agent of an outbreak in an ICU of a university hospital, sputum yielded the highest rate of MDRPA isolation 14 (38.8%).⁽¹⁷⁾ This could be attributed to the fact that most of the patients admitted to ICUs are mechanically ventilated and have respiratory infections. This agreed with Gales et al (2001) who documented that the respiratory tract was the most frequent source of *P. aeruginosa* isolates, followed by

wounds, urine, and bloodstream.⁽⁶⁾

The increasing prevalence of MDRPA in ICU patients has rekindled interest in polymyxins, as a salvage therapy to these patients; where limited therapeutic options have forced infectious disease clinicians and microbiologists to reappraise the clinical application of polymyxin antibiotics discovered more than 50 years ago.⁽³⁵⁾ In the current study, the susceptibility pattern for polymyxin E (colistin), polymyxin B and tigecycline were tested. It was found that 16 (80%) of isolated MDRPA were sensitive to colistin and polymyxin B (Table 3). In agreement with our findings, multiple studies have suggested that polymyxins are effective and safe therapy for infections caused by MDR strains. However, the role of these antibiotics has not been definitively established. Most studies have included only a small number of patients and the polymyxin was administered in combination with several other different antibiotics.^(36, 37)

Colistin (polymyxin E) remains active on

virtually all MDRPA isolates, and increasingly appears as the last available option to treat infections caused by these strains. However, the emergence of colistin resistance has been reported in *P. aeruginosa*, which may announce the spread of pan-resistant strains in a close future. ⁽³⁸⁾

On the other hand, tigecycline the new glycylicycline with an expanded broad-spectrum antibiotic, including inhibition of Gram-positive, Gram-negative, atypical, anaerobic, and antibiotic-resistant organisms; **has less activity against *P. aeruginosa* than it does** against some of the other non-Enterobacteriaceae;^(39,40) this is in accordance with the results of this study, where only 5 (25%) strains were sensitive to tigecycline.

Carbapenemases are beta-lactamases with versatile hydrolytic capacities. They have the ability to hydrolyze penicillins, cephalosporins, monobactams, and carbapenems. Carbapenemases are members of the molecular class A, B, and D beta-lactamases. Class A and D enzymes

have a serine-based hydrolytic mechanism, while class B enzymes are MBLs that are the most worrisome of all beta-lactamases.⁽⁹⁾

MBL have been reported for *P. aeruginosa* isolates from nearly all regions of the globe. ⁽⁴¹⁾ VIM-type MBLs are predominant in Europe, particularly in the Mediterranean region. Recently, 18 VIM-type variants have been described and another 4 have been assigned to the database. ⁽⁴²⁾

In the present study, all the 20 identified MDRPA strains were tested for VIM and IMP MBL genes using PCR assay. Nine strains (45%) were positive for VIM MBL gene, while none of the isolates (0%) was positive for IPM MBL type, and they were found to be statistically significant (Table 1 & figure1). This agrees with what was previously published by Manoharan et al (2010), where PCR testing detected VIM type of MBL among 15 of the 48 tested isolates (31%); and all were negative for IMP MBL gene. ⁽²³⁾ In Greece, Siarkou et al (2009) recorded that of

29 non replicate *P. aeruginosa* isolates resistant to carbapenems and ceftazidime, 14 (48%) were positive for VIM MBL production.

⁽⁴²⁾ On the contrary, Ramos et al (2008) in Mexico declared that of the 40 tested carbapenem-resistant isolates with PCR for VIM and IMP MBL specific primers, only 13(32.5%) isolates displayed a MBL phenotype and yielded positive amplicons with the IMP MBL specific primers.⁽⁴³⁾

Multiple antibiotic resistance in bacterial populations is a pervasive and growing clinical problem, which is recognized as a threat to public health. Hence, there is a need to conduct area-specific monitoring studies to profile different pathogens responsible for specific infections and their resistance patterns.

Conclusions:

- 1) The occurrence of MDRPA strains among critically ill patients was noticeable, where they represented 19% of the isolated strains.
- 2) Colistin and polymyxin B were found to

be active upon the majority of identified MDRPA strains, where 80% of the strains were sensitive to both of them, while tigecycline was found to be less effective as only 25% of strains were sensitive to it.

- 3) VIM MBL gene was significantly present among the tested MDRPA strains, where 45% were positive by PCR assay.

RECOMMENDATION

MDRPA health care associated infections are on the rise; hence rigorous studying and monitoring of these clinically significant strains especially among critically ill patients should be emphasized, so as to generate data that would help clinicians to choose the correct treatment.

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Table 1: Oligonucleotides used as primers for testing 20 MDRPA strains by Duplex PCR assay.

Primers	Sequence (5' to 3') ⁽²³⁾	Amplicon (nucleotide)	Positive strains		Z test
			No	%	
IMP-A	GAA GGY GTT TAT GTT CAT AC	587-bp amplicon	0/20	0	3.41*
IMP-B	GTA MGT TTC AAG AGT GAT GC				
VIM2004A	GTT TGG TCG CAT ATC GCA AC	382-bp amplicon	9/20	45	
VIM2004B	AAT GCG CAG CAC CAG GAT AG				

Table 2: Distribution of the 20 MDRPA strains isolated from different clinical samples, Alexandria.

Type of Samples	Frequency	
	No	%
Sputum	7	35
Pus (purulent discharge)	5	25
Urine	3	15
Endotracheal aspirates	3	15
Blood	2	10
Total	20	100

Table 3: Susceptibility patterns of the 20 MDRPA to colistin, polymyxin B and tigecycline.

Susceptibility patterns	Susceptible		Resistant		Total	
	No	%	No	%	No	%
Tested antimicrobials						
Colistin	16	80	4	20	20	100
Polymyxin B	16	80	4	20	20	100
Tigecycline	5	25	15	75	20	100

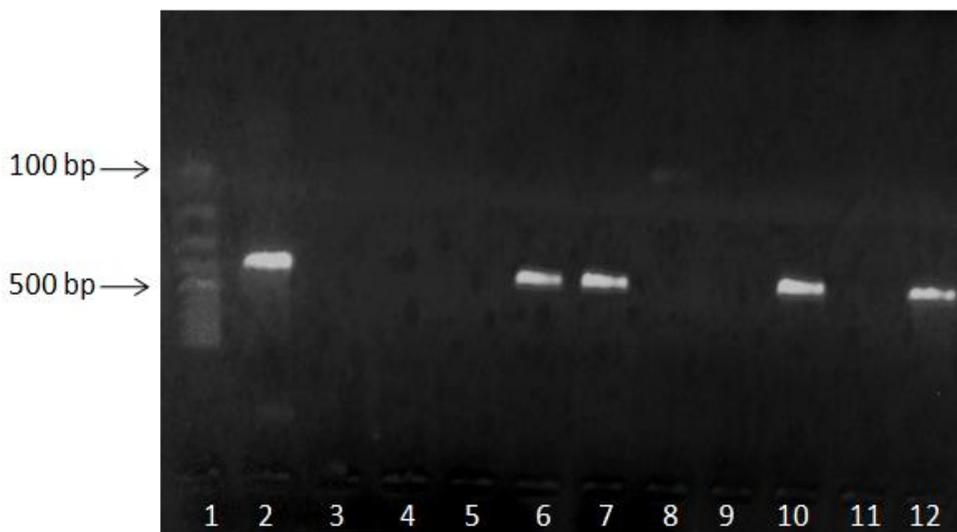


Figure 1: Duplex PCR of MDRPA strains with positive VIM MBL gene at 382-bp.

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