Original Article

Antimicrobial susceptibility and metallo-β-lactamase production among *Pseudomonas aeruginosa* isolated from Makkah Hospitals

Atif H. Asghar

ABSTRACT

Objectives: *Pseudomonas aeruginosa* is the most prevalent pathogen in nosocomial infections—it produces metallo-β-lactamases (MBLs) that reduce antibiotic effectiveness. This study aimed to determine the frequency, antimicrobial-susceptibility patterns, and MBL types of *P. aeruginosa* infections in clinical specimens obtained from patients in Makkah hospitals.

Methodology: Clinical isolates (478) were collected during a 6-month period, from September 2009 (Ramadan 1430 AH), from various clinical wards of Al-Noor Specialist, Hera General, and King Abdul-Aziz Hospitals. All isolates were subjected to routine microbiological investigations and automated antibiotic-susceptibility testing. MBL production was assessed using double-disk synergy test by comparing the zone of inhibition given by disks containing imipenem with and without ethylene diaminetetraacetic acid (EDTA) and MBL types distinguished by polymerase chain reaction.

Results: Most *P. aeruginosa* strains (31%) were isolated from intensive care units (ICUs) and male medical wards (15.9%). *P. aeruginosa* mostly caused respiratory tract (52%), wound (26%), and urinary tract (12%) infections. *P. aeruginosa* was most susceptible to imipenem (65.9%), amikacin (62.7%), meropenem (58.7%), and piperacillin/tazobactam (57.2 %). MBL-producing *P. aeruginosa* were identified in 76 (15.9%) isolates. The rate of MBLs types were 21% and 18.4% for IMP and VIM, respectively.

Conclusions: These results can be used as guidelines for treatment of bacterial infections in Saudi Arabia. Multidrug-resistant and MBL-producing *P. aeruginosa* is a serious public health concern, which must be tackled.

KEY WORDS: Antibiotic resistance, Metallo-β-lactamases, Makkah hospitals, Nosocomial infections, *Pseudomonas aeruginosa*.

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INTRODUCTION

Pseudomonas aeruginosa is a non-fermentative, gram-negative bacterium that has minimal nutritional requirements and can survive on various surfaces as well as in aqueous environments. *P. aeruginosa* rarely cause serious infections in healthy individuals, but in critically ill and immunocompromised patients, particularly those in intensive care units (ICUs), this opportunistic pathogen can cause severe invasive infections.[1] Rate of infection with *P. aeruginosa* is high in patients who have been hospitalized long-term and/or have

received broad-spectrum antimicrobial therapy or cancer chemotherapy.[2] *P. aeruginosa* infections range from superficial skin infections to fulminant sepsis; it is the leading cause of nosocomial respiratory infections, and is thus of particular concern for intubated persons and patients with ventilator-associated pneumonia.[2]

P. aeruginosa is resistant to antimicrobials from several different structural classes, either intrinsically or through acquisition of genetic resistance determinants.[3] Increased multidrug resistance (MDR) of *P. aeruginosa* complicates the management of some infections, since therapeutic options are limited.[4] Carbapenems are currently the antibiotic of choice; however, increasing carbapenem resistance has become a major concern worldwide. Carbapenem resistance in *P. aeruginosa* may be mediated by loss of the OprD porin, up-regulation of multidrug efflux pumps, and interplay between impermeability and production of certain β-lactamases and carbapenemases.[5] Carbapenemases are classified into 2 molecular families: those with a serine at their active site, known as serine carbapenemases, and those with at least 1 zinc atom at their active site, known as metallo-carbapenemases, a subgroup of metallo-β-lactamases (MBLs).[6] The VIM (Verona integron-encoded MBL) and IMP (active in imipenem) types of MBLs, encoded by the *bla*VIM and *bla*IMP genes, are the most clinically significant carbapenemases.[7] The aim of this study was to determine the frequency of *P. aeruginosa* isolated from Makkah hospitals, to evaluate the pattern of antimicrobial resistance of this organism, and to identify the MBL types.

METHODOLOGY

Study Design: This prospective study involved three main tertiary care hospitals in Makkah: Al-Noor Specialist Hospital (560 beds), Hera General Hospital (276 beds), and King Abdulaziz Hospital (400 beds) during a 6-month-period, from September 2009 (Ramadan 1430AH) to March 2010 (Rabi-al-awwal 1431AH).

Patients and Clinical Isolates: A total of 478 non-duplicated *P. aeruginosa* clinical isolates were obtained from 365 patients. Demographic data (age, gender, nationality, type of infection, ward of hospitalization) of patients with *P. aeruginosa* infection and laboratory results of the clinical specimens (antimicrobial susceptibilities) were collected from the medical and laboratory records of each patient using a standardized collection form. All clinical isolates were identified by routine microbiological methods, including morphology on culture media, Gram stain, and biochemical tests. All collected strains were stored at -86°C in brain-heart infusion, containing 15% glycerol, until used.

Detection of Antibiotic Susceptibility and MBL Production in Bacterial Isolates: Antimicrobial susceptibility testing was performed on all clinical isolates using automated instruments (Phoenix 100 BD, Maryland, USA and MicroScan Walkaway 96, Siemens, Germany). Minimum inhibitory concentration (MIC) for imipenim was determined using commercial E-test MIC strips. MDR was defined as resistance to ≥3 drugs of the following classes: β-lactams (ceftazidime, cefepime, piperacillin/tazobactam), aminoglycosides (gentamicin, amikacin), and fluoroquinolones (ciprofloxacin).[8] These results were statistically analysed using Statistical Package for Social Science software version 17 (SPSS Inc., Chicago, Il., USA). All *P. aeruginosa* clinical isolates were examined for MBL production using phenotypic double-disk synergy test as described prevously.[9,10] Briefly, discs containing 10μg of imipenem and a complementary disc containing 10μg imipenem and 10μl of 100 mM EDTA were placed with 20mm distance on MH agar plate containing pre-swabbed organism. An increase of 3mm or more in inhibition zone diameter in the presence of EDTA compared to those with IMP tested alone was considered to be a positive test for the presence of an MBL.

Molecular Detection of MBL types: VIM- and IMP-MBL types in *P. aeruginosa* were identified in suspected clinical isolates by PCR amplification. DNA template, primers, and PCR conditions used as have been described previously.[10] Agarose gel electrophoresis, followed by ethidium-bromide staining and gel documentation (BioDoct-It digital imaging system, UVP, Inc., Cambridge, UK) were used to identify the amplified fragments by size.

RESULTS

During a 6-month period, 478 *P. aeruginosa* pathogenic isolates were identified in various clinical specimens obtained from the sites of infection of patients. The majority of *P. aeruginosa* isolates were from Al-Noor Specialist Hospital (59%), followed by Hera General Hospital (24%) and King Abdul Aziz Hospital (17%). Most isolates were obtained from patients in ICUs, followed by the male medical ward, surgical ward, and female medical wards (Fig.1). The majority of patients

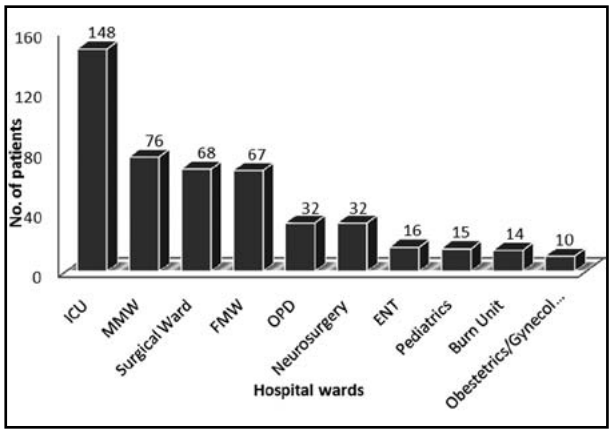

Fig.1: Frequency of *P. aeruginosa* isolates from different hospital wards (ICU: intensive care unit, MMW: male medical ward, FMW: female medical ward, OPD: outpatients department, ENT: Ear, Nose and Throat clinic)

with *P. aeruginosa* infection were male (57%) and >60 years old (174 patients; 36.4%; Fig.2). Most *P. aeruginosa* strains were isolated from sputum (177, 37%), followed by wound swabs (121, 25.3%), and urine (58, 12.1%; Fig.3). Respiratory tract infection (RTI; 52%), wound infection (26%), and urinary tract infection (UTI; 12%) were the most common infections caused by *P. aeruginosa*, while genital infections (3%) were the rarest (Fig.4). *P. aeruginosa* infection was distributed among 30 different nationalities; the majority were Saudi (306; 64%), followed by Pakistani (34; 7.1%), Egyptian (24; 5.0%), Yemeni (16; 3.3%), Indian (15; 3.1%), and Nigerian (9; 1.9%) individuals.

The antimicrobial susceptibilities of *P. aeruginosa* isolates are shown in Table-I. Results showed that 65.9%, 62.7%, 58.7%, 57.2%, 55.4%, 53.1%, and 52% of tested bacterial isolates were susceptible to imipenem, amikacin, meropenem, piperacillin/tazobactam, ciprofloxacin, piperacillin, and gentamycin, respectively. High resistance rates (>90%)

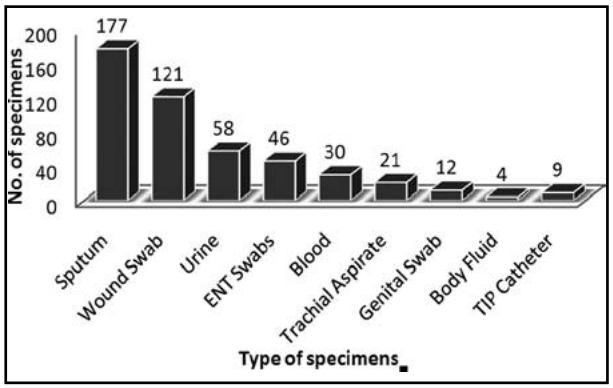

Fig.3: Distribution of clinical specimens for *P. aeruginosa* isolates.

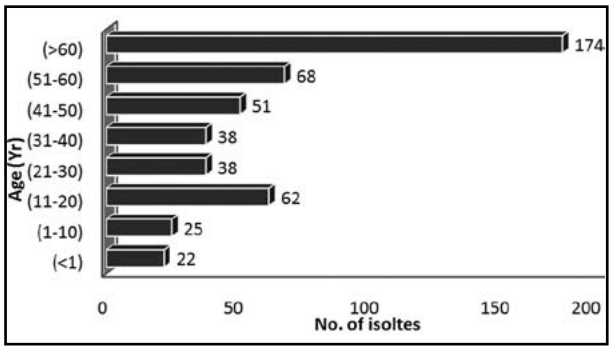

Fig.2: Frequency of *P. aeruginosa* isolates among patients of different age groups.

of *P. aeruginosa* were shown against ampicillin, amoxicillin/calvulanic acid, and trimethoprim/sulfamethoxazole.

According to the E-test results, the MIC_{50} and MIC_{90} of isolates against imipenem were 0.5 and 16, respectively. Using the double-disk synergy test, 76 (15.9%) isolates were identified as MBL-producing organisms; these were then tested for the presence of MBL genes (*bla*IMP and *bla*VIM) by PCR (Fig.5). Thirty-three isolates harboured MBL genes; *bla*IMP was present in 16 (21%) isolates, *bla*VIM in 14 (18.4%), and 3 (3.9%) isolates carried both genes.

DISCUSSION

In the present study, 478 *P. aeruginosa* pathogenic isolates were identified in clinical specimens, most of which originated from Al-Noor Specialist Hospital, possibly due to the high number of patients referred to this hospital, the largest hospital in Makkah. In this study, most *P. aeruginosa* strains were isolated from ICUs, followed by male medical ward, surgical ward, and female medical wards, similar findings were find in Saudi Arabia.[11,12] However, a study in Pakistan showed that, the majority of

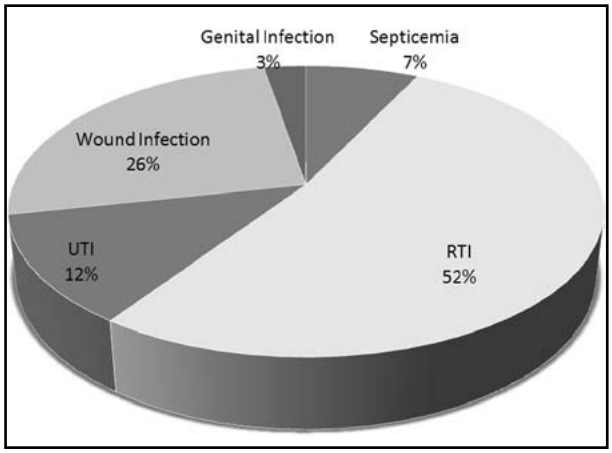

Fig.4: Types of infection for *P. aeruginosa* isolates.

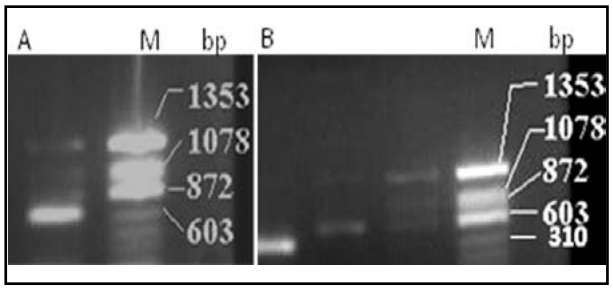

Fig.5: Gel electrophoresis pattern of PCR products.
(A) 587 bp amplified product from blaIMP gene.
(B) 382 bp amplified product from blaVIM gene.
(M) Φχ174-HaeIII marker

P. aeruginosa isolates were from an orthopaedic ward (24.6%), followed by OPD (20%), medical ward (13%), gynaecology/obstetrics (7.69%), and ICU (6.15%).[13] It has been mentioned that ICU patients are particularly susceptible to nosocomial infections, because the normal skin and mucosal barriers to infection are commonly compromised by the use of invasive devices.[14]

In this study, 57% of patients with *P. aeruginosa* infection were male, similar to a previous study in this locality.[15] This may be linked to the higher prevalence of diabetes in males; diabetes can cause a decrease in tissue blood flow, which ultimately increases the possibility of opportunistic infections mainly by *P. aeruginosa*.[15] This may also explain the high rate of *P. aeruginosa* isolates from wound and tissue samples: RTI, wound infection, and UTI were the most common infections caused by *P. aeruginosa*. Local and international studies showed a similar preference for infection sites.[11,16,17]

The majority of patients with *P. aeruginosa* infection in this study were >60 years old. According to Bennett, very young and very old patients had overall higher rates of infection than did other age groups; however, the risk of infection in different age groups differed between sites.[18]

This study demonstrated that *P. aeruginosa* infection was distributed among 30 different nationalities; this is likely due to crowding in the two Arabic months; Ramadan and Dhu-Al-Hijja, in which many Muslims visited Makkah to perform Umrah and Hajj rituals.[19]

A high-to-moderate susceptibility to imipenem, amikacin, meropenem, piperacillin/tazobactam, ciprofloxacin, piperacillin, and gentamycin was found. In 2005, a study in the same area found similar, or higher, susceptibility for most anti-pseudomonal agents; however, the rate of resistance to meropenem and piperacillin/tazobactam had increased slightly compared to the previous study.[11] A study by the National Nosocomial Infections Surveillance, comparing the resistance rates of bacterial isolates collected in 2003 with those collected in 1998–2002, showed a continuous increase in the incidence of antibiotic resistance.[20]

Table-I: Antibiotic susceptibility of P. aeruginosa.

Antimicrobial agents	*Resistant*		*Susceptible*		*Intermediate*	
(No. of tested isolates)	*No.*	*%*	*No.*	*%*	*No.*	*%*
Amikacin (445)	141	31.7	279	62.7	25	5.6
Amoxicillin/clavulanic acid (89)	84	94.4	4	4.5	1	1.1
Ampicillin (103)	100	97.1	3	2.9	0	0
Aztreonam (228)	114	50	88	38.6	26	11.4
Cefepime (418)	219	52.4	173	41.4	26	6.2
Cefotaxime (183)	142	77.6	14	7.7	27	14.8
Gentamycin (443)	188	42.4	231	52.1	24	5.4
Cefotriaxone (90)	63	70	9	10	18	20
Cefoxitin (96)	90	93.8	5	5.2	1	1
Ceftazidime (357)	183	51.3	157	44	17	4.8
Cefuroxime (97)	91	93.8	6	6.2	0	0
Cephalothin (97)	94	96.9	3	3.1	0	0
Ciprofloxacin (327)	140	42.8	181	55.4	6	1.8
Imipenem (464)	136	29.3	306	65.9	22	4.7
Meropenem (184)	67	36.4	108	58.7	9	4.9
Piperacillin (262)	123	46.9	139	53.1	0	0
Piperacillin/tazobactam (325)	134	41.2	186	57.2	5	1.5
Tetracyclin (186)	153	32	33	17.7	0	0
Trimethoprim/sulfamethoxazole (105)	97	92.4	8	7.6	0	0

MDR in *P. aeruginosa* was common in the present study. The results indicated that >50% of resistant isolates were MDR, and about 50% of these isolates originated from ICU patients. A lower rate of MDR was found in previous studies in Saudi Arabia.[11,16] Emerging resistance in *P. aeruginosa* is critically reducing the number of effective antimicrobials, due to the high intrinsic resistance in this organism, caused by outer membrane permeability mutations, the presence of efflux pumps, and inducible β-lactamase production.[21,22] This complexity increases the need for continuous surveillance of currently available agents. Careful isolation and identification, and accurate studies of susceptibility to antibiotics, are critical for predicting the spread of strains, improving therapeutic measures, and facilitating understanding of the epidemiology of this pathogen.

This study found that 15.9% of *P. aeruginosa* strains isolated from Makkah hospitals were MBL-positive. It has been reported that imipenem resistance is increasing; MBLs are considered to be responsible for 20.57% of this resistance, involving the *bla*VIM-2 gene, in Saudi Arabia.[23] The clinically important MBL gene families reside in horizontally transferrable gene cassettes and can be spread among gram-negative bacteria. Many families of these enzymes have been reported from several geographical regions. The most commonly reported families are: IMP, VIM, GIM, SPM and SIM.[6] IMP- and VIM-producing *Pseudomonas* strains have been reported worldwide.[24] Previous studies have shown that VIM exceeds IMP, and in some reports IMP could not be detected in the MBL-producing *Pseudomonas* strains isolated.[25]

Although the rate of MBL production is not particularly high among clinical isolates in Makkah hospitals,[26,27] the mortality rate is reportedly increased with respect to VIM-producing *P. aeruginosa*, which emphasizes the importance of establishing rapid detection methods to report MBL-positive isolates, and identify the common types in Makkah and other cities in Saudi Arabia.[25] Rapid identification and a controlled isolation policy can reduce the spread of the resistance genes to other gram-negative bacteria, which could otherwise potentially increase the mortality rate.

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Conflicts of Interest: Nothing.

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