

Lab based surveillance of multidrug resistant *Pseudomonas aeruginosa* in Cairo university hospitals, Egypt

Abstract

Multidrug resistance is increasingly recorded among *P. aeruginosa* causing health care associated infections (HAI) due to the selective pressure by over-usage of antibiotics. The primary aim of the study was to perform a laboratory based surveillance to investigate the prevalence of β -lactamase and AmpC production and multi-drug resistance among clinical isolates of *P. aeruginosa* causing health care associated infections in Kasr Al Aini School of Medicine and National Cancer Institute, Cairo University, Egypt. β -lactamase and AmpC production was tested by the double disc test. Multidrug-resistant *P. aeruginosa* (MDRPA) were defined as demonstrating resistance to carbapenems, all anti-pseudomonal cephalosporins, amino glycosides and fluoroquinolones. In a six months period, out of 12,524 clinical samples, 2593 (20.7%) *P. aeruginosa* isolates were identified. Out of these isolates, 1138 (43.9%) were ceftazidime resistant, 138 (5.3%) were ESBL producers, and 661 (25.5%) isolates were confirmed positive for inducible AmpC beta-lactamase. The proportion of multidrug-resistant *P. aeruginosa* was 12.3%. In conclusion, high prevalence of AmpC indicates the necessity of performing routine tests to detect AmpC in ceftazidime resistant *P. aeruginosa* isolates. Lab based surveillance is important to identify the prevalence of MDRPA in order to guide empirical antibiotic therapy and prevent the spread of resistant strains.

Keywords: multidrug-resistant *P. aeruginosa*; extended spectrum beta-lactamase; inducible AmpC

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Mona wassef, Hadir El Mahallawy, Mai Mahmoud Zafer, Doaa Ghaith, Rasha Abdel Hamid

Department of Microbiology and Immunology, Ahrm Canadian University, Egypt

Correspondence: Mai Zafer, Department of Microbiology and Immunology, Faculty of Pharmacy, Ahrm Canadian University, 4th Industrial Zone, 6th of October, Giza, Egypt, Tel +2(02) 33806765, Email mai_zaffer@hotmail.com

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Abbreviations: MDRPA, multidrug-resistant *P. Aeruginosa*; ESBL, extended spectrum beta-lactamase; HAIs, health-care associated infections; NCI, national cancer institute; MHA, Muller-Hinton agar; CLSI, clinical laboratory standards institute; SSIs, surgical site infections; WHO, world health organization; CAZ, ceftazidime; FEP, cefepime; IMP, imipenem; MER, meropenem; PIP-TZ, piperacillin/tazobactam; ATM, aztreonam; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; CXM, cefuroxime; CEP, cefoperazone; CRO, ceftriaxone; PB, polymyxin B

Introduction

The increasing prevalence of health-care associated infections (HAIs) produced by multidrug-resistant (MDR) *P. aeruginosa* strains severely compromises the selection of appropriate treatments and is therefore associated with significant morbidity and mortality.¹ It has been identified as the 2nd most frequent organism causing ventilator-associated pneumonia, the 4th most common causing catheter-associated urinary tract infections, the 5th cause of surgical site infections and the 7th cause of central-line-associated bloodstream infections.²

The current increase in incidence of MDR isolates of *P. aeruginosa* raises serious concerns. Multi-drug resistance in *P. aeruginosa* is defined as the resistance to ≥ 3 of the following classes of antibiotics penicillin's/cephalosporin's/monobactams, carbapenems, amino glycosides, and fluoroquinolones. Antibiotic selection pressure represents the leading risk factor for MDRPA acquisition.³ *P. aeruginosa* strains produce different classes of extended spectrum β -lactamase (ESBL) that enable bacterium to stand against extended-

spectrum cephalosporins, such as cefotaxime, ceftriaxone and ceftazidime and have been reported with increasing frequency.⁴ One of the noteworthy mutation mediated resistance mechanisms are those leading to the repression or inactivation of the carbapenemporin OprD, the hyper production of the chromosomal cephalosporinase AmpC, or the up regulation of one of the several efflux pumps encoded in the *P. aeruginosa* genome.⁵ Resistance due to plasmid-mediated AmpC enzymes is less common than extended-spectrum beta-lactamase production in most parts of the world but may be both harder to detect and broader in spectrum.⁶ The aim of this study was to perform a laboratory based surveillance to investigate the prevalence of β -lactamase and AmpC production and multi-drug resistance among clinical isolates of *P. aeruginosa* causing health care associated infections in Kasr Al Aini School of Medicine and National Cancer Institute (NCI), Cairo University, Egypt.

Materials and methods

Kasr El Aini School of Medicine and National Cancer Institute are tertiary hospitals belonging to Cairo University, Egypt. The study was approved by Ethics Committee of Cairo University and an informed consent was obtained from all patients receiving treatment and participating in the study. A total of 12,524 clinical samples were collected from patients who were diagnosed to have infections in ICUs and different hospital service units at Kasr El Aini School of Medicine and National Cancer Institute, Cairo University in the period from June 2012 till January 2013. Out of these specimens, 2593 (20.7%) *P. aeruginosa* isolates were identified and recovered using standard microbiological procedures. The type of specimen and numbers of *P.*

aeruginosa isolated from each one summarized in (Table 1). Resistance patterns in *P. aeruginosa* were classified according to recently published proposed interim definitions. An isolate was defined as MDR if it was resistant to ≥ 1 drug in ≥ 3 categories of drugs. The drugs on which our categorization was based included anti pseudomonal

cephalosporin's (ceftazidime [CAZ], cefepime [FEP]), carbapenems (imipenem [IMP], meropenem [MER]), piperacillin/tazobactam (PIP-TZ), Aztreonam (ATM), Fluroquinolone (ciprofloxacin [CIP]), and amino glycosides (gentamicin [GEN], tobramycin [TOB], amikacin [AMK]).

Table 1 Differential numbers of *P. aeruginosa* in relation to different sites of clinical specimens

Specimen	Total number of specimens (N)	<i>Pseudomonas</i> isolates N (%)
Urinary tract infections (UTIs)		
Urine	3551	823 (23)
Surgical site infections (SSIs)		
Burns and Skin infections	2412	905 (37.5)
Wound	1939	657(34)
Pus	473	248(52.4)
Blood Stream infections (BSIs)		
Peripheral Blood	4967	185 (3.7)
Central venous Line (CVL)	4835	172(3.5)
	132	13(10)
Lower respiratory tract infections		
Sputum& Endotracheal	1512	669 (44.2)
Pleural fluid	1320	597(45.2)
Pleural fluid	126	71(56.3)
Bronchoalveolar lavage	66	1 (1.5)
CSF		
	82	11 (13.4)
Total	12,524	2593 (20.7)

In-vitro antimicrobial susceptibility testing

Susceptibility of the isolates to the following antibacterial agents was tested by the Kirby-Bauer disc diffusion method using disks (Oxoid Ltd., Basin Stoke, Hants, England) on Mueller Hinton agar (Oxoid) and interpreted as recommended by Clinical Laboratory Standards Institute guidelines (CLSI,2010) guidelines: imipenem (IPM,10 μ g), meropenem (MEM,10 μ g), ceftazidime (CAZ,30 μ g), ceftazidime (30 μ g), cefotaxime (CTX,30 μ g), cefepime (FEP,30 μ g), ciprofloxacin (CIP,5 μ), piperacillin/tazobactam (TPZ,100/10 μ g), amikacin (AK,30 μ g), tobramycin (TOB,10 μ g), Aztreonam (ATM,30 μ g), Cefuroxime (CXM,30 μ g), Cefoperazone (CFP,30 μ g), ceftriaxone (CRO,30 μ g), and Polymyxin B (PB, 300 units).

Extended spectrum β -lactamase (ESBL) detection

All *P. aeruginosa* isolates which showed resistance to ceftazidime were evaluated for ESBL production by using the phenotypic confirmatory test.⁷ Briefly, a 0.5 McFarland's suspension of each isolate was spread on a Muller-Hinton agar (MHA) plate and ceftazidime (30 μ g) and ceftazidime / clavulanic acid (30 μ g/10 μ g) discs were placed aseptically on the agar plate. A distance of about 15mm was kept between the two discs (edge to edge) and the cultures were incubated at 37°C overnight. The observation of a ≥ 5 mm increase in the zone diameter for the antimicrobial agent which was tested in combination with clavulanic acid, versus its zone diameter when tested alone, confirmed the presence of ESBL production by the organism. The increase in the zone diameter was due to the inhibition of the β -lactamase by clavulanic acid.

Inducible AmpC β -lactamase detection

The ceftazidime-cloxacillin disk diffusion test was performed as described by Tan et al.⁸ The test is based on the inhibitory effect of cloxacillin on AmpC. In brief, 30 μ g ceftazidime disks (Becton Dickinson, Germany) were supplemented with 200 μ g cloxacillin. The test strain was inoculated on Mueller-Hinton agar. The diameters of the ceftazidime inhibition zones were compared with and without cloxacillin; if the difference in inhibition was ≥ 4 mm, the strain was considered positive for AmpC production.

Results

P. aeruginosa were isolated from 20.7% of samples. The specimens showing the highest isolation of *P. aeruginosa* were from lower respiratory tract infections (44.2%) followed by surgical site infections (SSIs), burns & skin infections (37.5%) and urinary tract infections (23.2%). Table 1 demonstrates the isolation of *P. aeruginosa* from different sites in relation to total numbers of clinical specimens. The patterns of antibiotic sensitivity of isolated strains of *P. aeruginosa* are shown in Table 2.

Figure 1 illustrates positive case of AmpC β -lactamase by the ceftazidime-cloxacillin disk diffusion test. Figure 2 demonstrates the number of ESBL, AmpC and MDR among *P. aeruginosa* isolates investigated. Of the total *P. aeruginosa* isolates, 1138 showed ceftazidime resistance (43.9%). A total of 661 (25.5%) isolates were confirmed to be positive for inducible AmpC beta-lactamase. Three hundred and eighteen *P. aeruginosa* isolates (12.3%) demonstrated

resistance to imipenem, meropenem, to all anti pseudomonal cephalosporin's, amino glycosides, fluoroquinolones, and thus, were considered multidrug resistant isolates. Among the 2593 *P.aeruginosa* isolates, 138 (5.3%) were ESBL producers.

Table 2 Patterns of *P. aeruginosa* resistance within different types of clinical specimen

Specimen	Total	Number of susceptible <i>P. aeruginosa</i> N (%)	Number of ESBL producing <i>P. aeruginosa</i> N (%)	Number of Amp C producing <i>P. aeruginosa</i> N (%)	Number of MDR <i>P. aeruginosa</i> N (%)
Urinary tract Infections (UTIs) Urine	823	451 (54.8)	94(11.42)	211(25.6)	67(8.1)
Surgical site infections(SSIs)	905	552 (61)	18(2.0)	222(24.5)	113(12.5)
Burns and Skin infections					
Wound	657	467(71.1)	18(2.7)	106(16)	66(10.04)
Pus	248	85 (34.3)		116(46.7)	47(19)
Lower respiratory tract infections	669	341(51)	25(3.7)	190(28.4)	113(17)
Sputum & endotracheal	597	318(53.2)		166(27.8)	113(19)
Pleural fluid	71	23(32.4)	25(35.2)	23(32.4)	
BAL	1			1(100)	
Blood stream infections (BSIs)	185	127(69)	22(12)	34(18.3)	23(12.4)
Peripheral blood	172	125(73)		24(14)	23(13.4)
CVL	13	8(61.5)	3(23.1)	2(15.4)	
CSF	11	5 (45.45)		4 (36.4)	2 (18.2)



Figure 1 A positive case of AmpC β -lactamases producer performed by the ceftazidime-disk diffusion test.

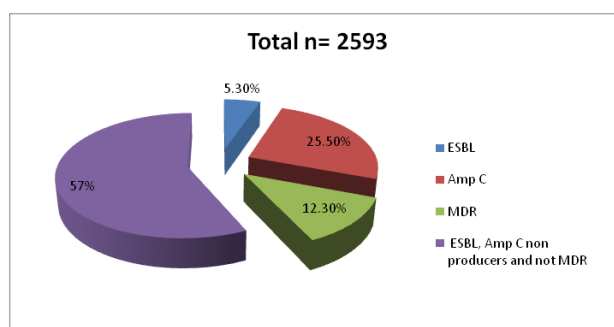


Figure 2 Of the total *P. aeruginosa* tested, 661 were Amp C, 318 were MDR and 138 were ESBL.

Discussion

P. aeruginosa is a leading cause of health-care associated infections⁹ and is extremely difficult to treat due to its high intrinsic and adaptive resistance.¹⁰ Despite the discovery of ESBL, Amp C β -lactamase and MBL at least a decade ago, there remains

a low level of awareness of their importance and many clinical laboratories do not undergo routine detection of ESBL & Amp C β -lactamase. Failure to detect these enzymes has contributed to their spread and commonly to therapeutic failures.

In the present study a total of 2593 *P. aeruginosa* strains were isolated in the period from June 2012 till January 2013, accounting for 20.7 % of organisms responsible for health-care associated infections in different hospitals of Kasr Al Aini and National Cancer Institute, Cairo University with 12% MDR phenotype. These hospitals are tertiary hospitals with expected high rates of complicated cases due to receiving difficult cases from all over the country. Similarly 19% *P. aeruginosa* were reported as causes of HAIs in Menofia Hospital University, Egypt with 9.5% of the isolated *P. aeruginosa* are corded as MDR.¹¹ A figure of 18% was recorded by Gad in Minia hospitals in Egypt.¹² It was concluded that MDR *P. aeruginosa* has emerged in Egypt in recent years and is mainly seen in HAIs.¹³

In the current study, 1138 of *P. aeruginosa* isolated showed resistance to ceftazidime accounting for 43.8% of the isolates. A high rate of ceftazidime resistance was also reported in a similar Egyptian study in which 45 % of *P. aeruginosa* isolates showed resistance to ceftazidime.¹⁴ In India, the resistance rate of *P. aeruginosa* to ceftazidime was 53%.¹⁵ A much higher rate of ceftazidime resistant *P. aeruginosa*, (91%) was detected in another part of Egypt.¹¹ Thus, it is evident that high rates of ceftazidime resistance in *P. aeruginosa* are detected in different areas of Egypt.

P. aeruginosa were most commonly isolated from lower respiratory tract infections (44.2%), followed by SSIs (37.5%), and urinary tract infections (23%) in the present study. This may be due to the prolonged hospital stay of patients in the ICU with the need of mechanical ventilation and to the increasing numbers of heart and lung operative procedures done in Kasr Al Aini and NCI hospitals, Cairo University. This is in contrast with the previous studies concluding that *P. aeruginosa* is a nosocomial pathogen often isolated from burn infections.¹⁶ In the study done by Aly and his colleagues, *P. aeruginosa* accounted for 45% of infections in the burn unit.

On investigating the rates of *P. aeruginosa* MDR phenotype, inducible Amp C and ESBL production, we found a low prevalence of ESBL producer strains (5.3%), higher rates of Amp C producer strains (25.5%); whereas, 12.3% of isolates were showing an MDR phenotype. In a study done by Zahra and associates (2011) in Iran, low levels of ESBL *P. aeruginosa* producers (9.2%) was recorded, but with higher levels of the MDR phenotype (30%).¹⁷ A high prevalence of ESBL producer strains were reported in a study done in Pakistan accounting for 35.85% of isolates with 29% showing an MDR phenotype.¹⁸ An alarming high prevalence of MDR *P. aeruginosa* (52%) and ESBLs producer strains (45.6%) was recently detected in another study done by an Egyptian group of investigators.¹¹ A high rate of MDR was reported in an Egyptian study, Gad et al.,¹² observed that 36% of isolated *P. aeruginosa* in their institution were MDR and that β -lactamase production was the main mechanism of resistance, as 95% were ESBL producers.

AmpC producing *P. aeruginosa* were remarkably higher than ESBL producers in our study. Different rates of inducible AmpC producer *P. aeruginosa* strains were recorded in previous studies, varying from 30%¹⁹ and up to 60%.²⁰ Being difficult to detect without being alert to its presence, inducible AmpC producers have to be searched for in routine laboratories whenever *P. aeruginosa* is isolated, especially with the increasing rates of ceftazidime resistance. The high rate of

inducible AmpC production necessitates exerting efforts to control these types of infections.

The problem of antimicrobial resistance is highlighted by the world health organization (WHO) and combating antimicrobial resistance has been selected as the theme for World Health Day 2011. As the prevalence rates of resistance in gram negative pathogens increase, the treatment options available become limited. Thus, WHO is calling all parties involved in this problem to act and take responsibility for combating antimicrobial resistance.²¹ The rate of MDR *P. aeruginosa* at our hospitals are not alarming, still awareness, continuous surveillance and antibiotic stewardship are necessary to control and prevent the spread of resistant strains.

In Conclusion, *P. aeruginosa* represented a significant cause of health care associated infections (HAI) in Kasr Al Aini and NCI, Cairo University hospitals. High percent of ceftazidime resistance could be an important cause of treatment failure. AmpC producing *P. aeruginosa* were remarkably higher than ESBL producers detected by double diffusion testing signifying the importance of performing routine tests to detect AmpC in ceftazidime resistant *P. aeruginosa* isolates. Implementing simple and rapid lab screening tests aiming for routine detection of the β -lactamase including the AmpC is needed to monitor the panel of resistance among *P. aeruginosa*. Although MDRPA figures in our hospitals are not alarming, still it is necessary to implement policies to prevent antimicrobial resistance. It is recommended to establish a national program setting on strategies to gather data centrally using sensitive and specific tests to detect mechanisms of resistance and keep a record of the rates of MDR all over Egypt. The optimal contribution of microbiology laboratories is essential to combat the spread of multiply antibiotic-resistant pathogens, which is vital for patient care and safety.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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