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-psenomonal 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

Abbreviations: MDRPA: Multidrug-Resistant P. aeruginosa; ESBL: Extended Spectrum Beta-Lactamase; HAI: 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/TOZ: 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 bacteria 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 carbapenem porin 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. 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-TAZ), Aztreonam (ATM), Fluroquinolone (ciprofloxacin [CIP]), and amino glycosides (gentamicin [GÉN], tobramycin [TOB], amikacin [AMK]).

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

| Specimen                              | Total Number of Specimens | Pseudomonas Isolates N (%) |
|---------------------------------------|---------------------------|---------------------------|
| Urinary Tract Infections (UTIs)       |                           |                           |
| Urine                                | 3551                      | 823 (23)                  |
| Surgical Site Infections (SSIs)       | 2412                      | 905 (37.5)                |
| Burns and Skin infections             |                           |                           |
| Wound                                | 1939                      | 657(34)                   |
| Pus                                   | 473                       | 248(52.4)                 |
| Blood Stream Infections (BSIs)        | 4967                      | 185 (3.7)                 |
| Peripheral Blood                      | 4835                      | 172(3.5)                  |
| Central venous Line (CVL)             | 132                       | 13(10)                    |
| Lower Respiratory Tract Infections    | 1512                      | 669 (44.2)                |
| Sputum & Endotracheal                 | 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., Basen Stoke, Hants, England) on Mueller Hinton agar (Oxoid) and interpreted as recommended by Clinical Laboratory Standards Institute guidelines (CLSI,2010) guidelines: imipenem (IPM,10µg), meropenem (MEM,10µg), ceftazidime (CAZ,30µg), cefotaxime (CTX,30µg), cefepime (FEP,30µg), ciprofloxacin (CIP,5µ), piperacillin/tazobactam (PIP-TAZ,100/10µg), amikacin (AK,30µg), tobramycin (TOB,10µg), Aztreonam (ATM,30µg), Cefuroxime (CXM,30µg), Cefoperazone (CIP,30µg), ceftriaxone (CRO,30µg), 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 µg) and ceftazidime / clavulanic acid (30 µg/ 10 µg) 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 ≥ 5mm 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 cefoxitin-doxacillin 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 µg cefoxitin disks (Becton Dickinson, Germany) were supplemented with 200 µg cloxacillin. The test strain was inoculated on Mueller-Hinton agar. The diameters of the cefoxitin 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 (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 cefoxitin-doxacillin 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 *P. aeruginosa* N (%) | Number of ESBL Producing *P. aeruginosa* N (%) | Number of Amp C Producing *P. aeruginosa* N (%) | Number of MDR *P. aeruginosa* 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-lactamasessproducer performed by the cefoxitin-cloxacillin 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 infections [9] and is extremely difficult to treat due to its high intrinsic and adaptive antibiotic 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 HAI 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 HAI [13].

In the current study, 1138 of P. aeruginosa isolated showed response 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 AmpC and ESBL production, we found a low prevalence of ESBL producer strains (5.3%); higher rates of AmpC 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 (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 B-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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References

1. Mesaros N, Nordmann P, Plesiat P, Roussel-Delvallez M, Van Eldere J, et al. (2007) Pseudomonas aeruginosa: resistance and therapeutics options in the turn of the new millennium. Clin Microbiol Infect 13(6): 560-578.
2. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, et al. (2008)
NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol 29(11): 996-1011.

3. Barbier F, Wolf M (2010) Multi-drug resistant Pseudomonas aeruginosa: towards a therapeutic dead end? Med Sci (Paris) 26(11): 960-968.

4. Mirsalehian A, Feizabadi M, Nakhjavani FA, Jabalameli E, Goli H, et al. (2010) Detection of VEB-1, OXA-10 and PER-1 genotypes in extended-spectrum beta-lactamase-producing Pseudomonas aeruginosa strains isolated from burn patients. Burns 36(1): 70-74.

5. Moya B, Dotsch A, Juan C, Blazquez J, Zamorano L, et al. (2009) Beta-lactam resistance response triggered by inactivation of a nonessential penicillin-binding protein. PLoS Pathog 5(3): e1000353.

6. Jacoby GA (2009) AmpC beta-lactamases. Clin Microbiol Rev 22(1): 161-182.

7. Clinical and Laboratory Standards Institute (2012) Performance standards for antimicrobial disk tests. Approved Standards, (9th edn), CLSI Document M2-A9, Wayne PA. 2006, 26: 1.

8. Tan TY, Ng LS, He J, Koh T H, Hsu LY (2009) Evaluation of screening methods to detect plasmid mediated AmpC in Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis. Antimicrob. Agents Chemother 53(1): 146-149.

9. Picco RC, Poirel L, Gales AC, Nordmann P (2009) Diversity of β-Lactamases produced by ceftazidime-resistant Pseudomonas aeruginosa isolates causing bloodstream infections in Brazil. Antimicrob Agents Chemother 53(9): 3908-3913.

10. Nekid A, Yeung AT, Rosay T, Tettmann B, Strempel N, et al. (2013) TypA is involved in virulence, antimicrobial resistance and biofilm formation in Pseudomonas aeruginosa. BMC Microbiol 13: 77.

11. Mahmoud BA, Zahrn AW, Hindawi RG, Labib ZA, Galal R (2013) Prevalence of multidrug-resistant Pseudomonas aeruginosa in patients with nosocomial infections at a University Hospital in Egypt, with special reference to typing methods. Journal of Virology & Microbiology 2013: 13.

12. Gad GF, El-Domany RA, Zaki S, Ashour HM (2007) Characterization of Pseudomonas aeruginosa isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. J Antimicrob Chemother 60(5): 1010-1017.

13. Ali AM, Abbasi SA, Ahmed M (2009) Frequency of ESβLs producing nosocomial isolates in a tertiary care hospital in Rawalpindi. Pakistan Armed Forces Medical Journal 12(3): 23-28.

14. Al-Agamy MH, Shibli MA, Zaki AS, Tawfik Abdulkader F (2011) Antimicrobial resistance pattern and prevalence of metallo-β-lactamases in Pseudomonas aeruginosa from Saudi Arabia. African Journal of Microbiology Research 5(30): 5528-5533.

15. Prashan DP, Peerapur BV (2011) ESBL and MBL mediated resistance in Pseudomonas aeruginosa: An emerging threat to clinical therapeutics. Journal of Clinical and Diagnostic Research 5(8): 1552-1554.

16. Pathmanathan SG, Samat NA, Mohamed R (2009) Antimicrobial susceptibility of clinical isolates of Pseudomonas aeruginosa from a Malaysian Hospital. Malays J Med Sci 16(2): 27-32.

17. Zahra T, Moniri R (2011) Detection of ESβLs and MDR in Pseudomonas aeruginosa in a tertiary-care teaching hospital. Iranian Journal of Clinical Infectious Diseases 6(1): 18-23.

18. Ulleh F, Malik SA, Ahmed J (2009) Antimicrobial susceptibility and ESBL prevalence in Pseudomonas aeruginosa isolated from burn patients in the north west of Pakistan. Burns 35(7): 1020-1025.

19. Kumar V, Sen MR, Nigam C, Gahlot R, Kumari S (2012) 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 16(3): 136-140.

20. Upadhyay S, Sen MR, Bhattacharjee A (2010) Presence of different beta-lactamase classes among clinical isolates of Pseudomonas aeruginosa expressing AmpC beta-lactamase enzyme. J Infect Dev Ctries 4(4): 239-242.

21. World Health Organization (2011) Antimicrobial resistance. Fact sheet number 194.