Brief Original Article

Multidrug resistance in *Pseudomonas aeruginosa* isolated from nosocomial respiratory and urinary infections in Aleppo, Syria

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Abstract

Introduction: *Pseudomonas aeruginosa* represents a serious clinical challenge due to its frequent involvement in nosocomial infections and its tendency towards multidrug resistance.
Methodology: This study uncovered antibiotic susceptibility patterns in 177 isolates from inpatients in three key hospitals in Aleppo, the largest city in Syria.
Results: Exceptionally low susceptibility to most routinely used antibiotics was uncovered; resistance to ciprofloxacin and gentamicin was 64.9% and 70.3%, respectively. Contrarily, susceptibility to colistin was the highest (89.1%).
Conclusions: Multidrug resistance was rife, found at a rate of 53.67% among studied *P. aeruginosa* isolates.

Key words: *Pseudomonas aeruginosa*; nosocomial infections; multidrug resistance; respiratory infections; urinary infections; Syria.

Introduction

Pseudomonas aeruginosa is a Gram-negative, obligate aerobic, and oxidase-positive bacillus. In healthy subjects, *P. aeruginosa* is part of skin flora; however, it can cause serious morbidity in individuals with predisposing factors such as considerable body injury or certain pulmonary conditions (e.g., cystic fibrosis) [1]. This opportunistic pathogen is a common cause of hospital-acquired infections and, to a lesser extent, community-acquired infections. Examples of infections include pneumonia, urinary tract infections (UTIs), bacteremia, and wound and burn infections. Nosocomial pneumonia due to *P. aeruginosa* is usually associated with poor survival rates [2,3].

Despite rapid advancement in healthcare provision, partially through the introduction of highly effective antibiotics into clinical practice, *P. aeruginosa* remains a pathogen to be reckoned with. This is certainly the case at intensive care units (ICUs), particularly in patients who are put on a mechanical ventilator [3]. Nosocomial bacteremia accounts for over 50% of ICU infections, and the causative pathogen is frequently found to be *P. aeruginosa* [4].

Multidrug resistance (MDR) displayed by *P. aeruginosa* is usually found during empirical therapy or following lengthy exposure to antibiotics. *P. aeruginosa* has myriad intrinsic and extrinsic factors that work together to cause resistance to structurally and functionally dissimilar antibiotics. Certain resistance mechanisms that have been found in other bacteria may also play a role in MDR in *P. aeruginosa* [5]. Importantly, *P. aeruginosa* can express chromosomally encoded multidrug efflux pump genes coupled with outer membrane impermeability. *P. aeruginosa* possesses a spectrum of virulence factors, including a type III secretion system, which contributes massively to its pathogenicity [2].

Additionally, *P. aeruginosa* is capable of developing acquired antimicrobial resistance. The latter emerges due to mutations arising in chromosomally-encoded genes and/or horizontal gene transfer of resistance genes, a variety of which can be carried together on transferable structures (integrons) [6]. Finally, *P. aeruginosa* is adept at forming biofilms on surfaces, so it can survive antibiotics and other disinfectant chemicals as well as the body's innate and adaptive inflammatory defenses [2,7].

Studies from different geographical localities worldwide highlight the dilemma of increased occurrence of infections with MDR *P. aeruginosa*. These pose a heavy human and economical burden

because antibiotics are costly and frequently ineffective, leading to high mortality rates [2-7]. Informative data about these types of infections are lacking from Syria; therefore, this study was conducted to probe for prevalence rates of MDR among *P. aeruginosa* isolates in northern Syria. This study focused on susceptibility towards antimicrobial agents that are routinely administered in local hospitals.

Methodology

This cross-sectional study was conducted between September 2011 and September 2012 at three major hospitals in Aleppo, Syria: Ibn Rushd Hospital, Aleppo University Hospital, and Al-Basel Center for Heart Disease and Cardiac Surgery. The relevant approval was obtained from the ethics committee of Aleppo University. Samples were taken from ICU patients with lower respiratory infections (LRIs) and from patients with nosocomial UTIs. The mean age of the patients was 52.7 years. All lower respiratory and urinary samples came from inpatients who had been hospitalized for $\geq$ 48 hours. A total of 177 non-repetitive isolates of *P. aeruginosa* were found, and the guidelines of the Clinical and Laboratory Standards Institute (CLSI) were followed for pathogen

identification and testing [8]. Female patients contributed 59 of the isolates, while the rest came from male patients. *P. aeruginosa* was isolated and identified using standard biochemical reactions, and the results were confirmed using the Phoenix Automated Microbiology System by BD (Becton, Dickinson and Company, Franklin Lakes, USA). Afterwards, bacterial isolates were tested for susceptibility to antibiotics by the standard Kirby-Bauer disk diffusion method. Twenty-three antimicrobial susceptibility testing disks (Oxoid, Basingstoke, UK; codes are listed in Table 1) were used, and results were determined and interpreted based on CLSI guidelines. Multidrug resistance was defined as resistance to three or more unrelated antibacterial agents.

Results

This study sought to characterize the pattern of antibiotic resistance among *P. aeruginosa* isolates obtained from three major hospitals in the largest urban center in Aleppo, northern Syria. A total of 138 isolates were obtained from ICU patients with LRIs, and the rest (39 isolates) came from patients with UTIs. Around a quarter (26%) of the LRIs isolates and over half (59%) of the UTIs isolates came from

Table 1. Antibiogram depicting antimicrobial susceptibility of *Pseudomonas aeruginosa* isolates collected between September 2011 and September 2012 in Aleppo, Syria

Pseudomonas aeruginosa Maximum no. tested = 177 Antibiotic (Oxoid disc code)	Percent susceptible
Amikacin (code CT0107B)	42.8
Amoxicillin/clavulanic acid (code CT0223B)	2.3
Azithromycin (code CT0906B)	5.7
Cefotaxime (code CT0166B)	6
Cefepime (code CT0771B)	21.7
Ceftazidime (code CT0412B)	28.6
Ceftriaxone (code CT0417B)	18.8
Clarithromycin (code CT0693B)	3.8
Ciprofloxacin (code CT0425B)	29.2
Colistin (code CT0017B)	89.1
Doxycycline (code CT0018B)	9.1
Gentamicin (code CT0024B)	27
Imipenem (code CT0455B)	56.1
Levofloxacin (code CT1587B)	34.7
Nitrofurantoin (code CT0036B)	3.2
Norfloxacin (code CT0434B)	34.7
Tetracycline (code CT0054B)	5.1
Tobramycin (code CT0056B)	29.9
Trimethoprim/sulfamethoxazole (code CT0052B)	11.7
Aztreonam (code CT0264B)	17
Meropenem (code CT0774B)	59.1
Piperacillin (code CT0199B)	36.4
Piperacillin/tazobactam (code CT0725B)	54.5

women.

Generally, tested *P. aeruginosa* isolates displayed high levels of antibiotic resistance to the majority of antibiotics that are routinely used in local clinical settings (Table 1). Levels of susceptibility to amoxicillin/clavulanic acid and nitrofurantoin were the lowest: 2.3% and 3.2%, respectively. Susceptibility to each of clarithromycin, tetracycline, azithromycin, cefotaxime, and doxycycline never exceeded 10%. Considerably higher susceptibility was obtained with imipenem (56.1%) and meropenem (59.1%); however, the most optimal response *in vitro* was obtained with colistin (89.1%).

Discussion

To put our results in the right context, a comprehensive multicenter study by Sader *et al.* [9] was used as a major referential point. The latter study covered 31 medical centers located in 13 European countries plus Turkey and Israel, and it reported on antibiotic resistance and MDR of *P. aeruginosa*. Generally, susceptibility rates uncovered in this study were alarmingly lower than these reported by Sader *et al.* [9]. Examples include the fourth-generation cephalosporin, cefepime (21.7% in Syria versus 71.4% in the 15 countries covered in [9]), the fluoroquinolone, levofloxacin (34.7% in Syria versus 64.1% in the 15 countries covered in [9]), and piperacillin/tazobactam. Additionally, resistance to ciprofloxacin was as high as 64.9%. The latter antibiotics are misused in Syria widely and frequently due to their low prices and the ease with which the general public can obtain them without a prescription.

Resistance to gentamicin (70.3%) was much higher than to amikacin (40%). It is worth noting that the latter antibiotic is used less frequently in local healthcare establishments, mainly due to its overly publicized ototoxicity and nephrotoxicity. Also, amikacin is far less vulnerable to the destructive effects of aminoglycoside-modifying enzymes produced by *P. aeruginosa*. Nevertheless, the Syrian figures are much higher than their counterparts from Europe, Turkey, and Israel [9], where resistance rates to gentamicin and amikacin were found to be 22% and 11.4%, respectively.

Nearly 54% (95 isolates) of tested *P. aeruginosa* isolates were MDR. Reported rates of MDR among *P. aeruginosa* varied widely based on sample size, source of samples, and geographical locality. For instance, numerous studies from Iran reported on multidrug-resistant *P. aeruginosa*. One of the larger studies [10] reported an extremely low rate (5.46%). A higher rate

(31.9%) was reported by Sader *et al.* [9] from two Middle Eastern countries and 13 European countries. Still, the latter rate is significantly lower than the figure uncovered in this study.

There have been many reports worldwide describing the trend of increasing antibiotic resistance in general and MDR in particular among *P. aeruginosa* [11,12]. For example, a nine-year American study showed a considerable increase in multidrug-resistant *P. aeruginosa*, from 1% in 1994 to 16% in 2002. This is a good indicator of the degree of increase in MDR in the largest industrialized country in the world [13]. On the bright side, reviving some old antibiotics might offer an alternative way to fight MDR [14]. Our results are necessary for guiding local health authorities in Syria; however, on a global scale, our findings help to fill a significant information gap by demonstrating such a high rate of antibacterial resistance. Our data is expected to encourage correct use of antibiotics and to persuade practitioners to rely more on antibiotic susceptibility tests in making treatment decisions in individual cases.

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