Journal of Global Antimicrobial Resistance 16 (2019) 274–277

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Short Communication

Changing drug resistance profile in *Pseudomonas aeruginosa* infection among HIV patients from 2010–2017: A retrospective study

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ARTICLE INFO

Article history:
Received 10 April 2018
Received in revised form 14 September 2018
Accepted 22 October 2018
Available online 30 October 2018

Keywords:
HIV
Pseudomonas aeruginosa
Antibacterial resistance
Imipenem

ABSTRACT

Objectives: Pseudomonas aeruginosa is an important aetiological agent causing pneumonia, urinary tract infections and bacteraemia. High antibiotic use in nosocomial settings and for immunocompromised conditions results in increasing multidrug resistance. This study analysed the antimicrobial resistance profile of *P. aeruginosa* isolates in an HIV setting.
Methods: A total of 7386 clinical specimens were collected from HIV patients attending YRG CARE from 2010–2017. *P. aeruginosa* isolated from clinical specimens were identified conventionally, and antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method.
Results: A total of 260 *P. aeruginosa* strains were isolated, with 165 *P. aeruginosa* (63.5%) being isolated from hospitalised patients. A higher incidence of *P. aeruginosa* infection (25.8%) was observed in 2017, and most of the *P. aeruginosa* were isolated from sputum specimens (57.3%). A high level of resistance was noted to ceftazidime (49.6%), followed by ticarcillin (41.5%). Imipenem and meropenem resistance was observed in 15.0% and 16.9% of *P. aeruginosa* isolates, respectively. A high rate of imipenem resistance was noted in 2016 (46.2%) and a high rate of meropenem resistance was noted in 2017 (20.5%). An increasing resistance rate of *P. aeruginosa* was observed against aztreonam, cefepime, levofloxacin, meropenem, piperacillin, piperacillin/tazobactam, ticarcillin and tobramycin from 2010 to 2017.
Conclusion: A constant increase in drug-resistant *P. aeruginosa* isolates from HIV patients was observed from 2010 to 2017. Findings from this study urge the need for periodical monitoring and surveillance of the *P. aeruginosa* resistance profile, especially in hospitalised and immunocompromised patients in resource-limited settings.

1. Introduction

Pseudomonas aeruginosa is a rod-shaped, aerobic, non-lactose-fermenting, Gram-negative bacterium belonging to the family Pseudomonadaceae. It is a common bacterium observed in nosocomial settings, responsible for 10% of hospital-acquired infections [1]. Expression of various virulence factors in *P. aeruginosa* aids colonisation and invasion of the human host to cause serious infections [2]. *P. aeruginosa* causes life-threatening nosocomial infections such as pneumonia, urinary tract infections and chronic lung infections in patients with cystic fibrosis [3]. In general, the human population is refractory to *Pseudomonas* infection, however the bacterium has the ability to act as an opportunistic pathogen in immunocompromised individuals [4].

Pseudomonas is intrinsically resistant to multiple antibacterial agents and may acquire new resistance determinants even when an infected person is under antimicrobial therapy, making it difficult to treat. Antimicrobial resistance among *P. aeruginosa* has increased globally through the dissemination of several international multidrug-resistant (MDR) 'epidemic' clones [5]. *P. aeruginosa* currently shows resistance to antibiotics such as aminopenicillins, including those combined with β-lactamase inhibitors, first- and second-generation cephalosporins, piperacillin, piperacillin/tazobactam (TZP), cefepime, ceftazidime, aminoglycosides, quinolones and carbapenems as well as colistin and fosfomycin [6]. Carbapenem-resistant *P. aeruginosa* is categorised

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https://doi.org/10.1016/j.jgar.2018.10.019

by the World Health Organization (WHO) as a critical priority drug-resistant bacterium for the development of new antibiotics and also for effective treatment [7]. Increasing resistance of *P. aeruginosa* to multiple antibiotics occurs as a result of excessive antibiotic administration as well as cross-resistance between antibiotics, thus resulting in the evolution of MDR *P. aeruginosa* strains [8]. Hence, it has become immensely important to monitor antimicrobial resistance trends of *P. aeruginosa* strains periodically, which in turn has major clinical significance in choosing appropriate antibiotics to treat *P. aeruginosa* infections. In this study, a retrospective analysis was performed on the antimicrobial susceptibility profile of *P. aeruginosa* isolated from 2010–2017 in a human immunodeficiency virus (HIV) setting.

2. Methods

Clinical specimens such as sputum, urine, pus, fine needle aspiration, body fluid, vaginal swab, eye swab and skin swab were collected from HIV patients attending Y.R. Gaitonde Centre for AIDS Research and Education (YRG CARE) for routine clinical care. Bacterial aetiology was identified by inoculating specimens immediately on MacConkey and blood agar (5% sheep blood) plates followed by incubation at 37 °C for 24 h. Suspected *P. aeruginosa* strains were isolated and were identified by a series of microbiological tests such as Gram staining, motility, catalase test, oxidase test and standard biochemical tests such as indole, methyl red, Voges–Proskauer, citrate, urease, nitrate reduction and triple sugar ion tests. Antimicrobial susceptibility testing of the identified *P. aeruginosa* strains was performed by the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [9]. Briefly, isolated bacterial colonies were suspended in sterile saline to achieve a culture suspension adjusted to a 0.5 McFarland standard. Lawn cultures were made on Mueller–Hinton agar plates from culture suspensions using a sterile swab. Antibiotic disks of amikacin, aztreonam, cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, piperacillin, TZP, ticarcillin and tobramycin (HiMedia, Mumbai, India) were then placed on the agar surface and the plates were incubated at 37 °C for 18–24 h. Following incubation, the diameter of the zone of inhibition of each antibiotic disk was measured and the results were interpreted as susceptible, intermediate or resistant based on CLSI standards [9]. Antimicrobial susceptibility data of *P. aeruginosa* infections in HIV patients from 2010–2017 were then analysed to study the antibacterial drug resistance profile.

3. Results

During the study period, a total of 7386 clinical specimens from HIV patients were processed, from which 260 *P. aeruginosa* strains were isolated. Of the 260 *P. aeruginosa* strains, 165 (63.5%) were isolated from hospitalised HIV patients and the remaining 95 (36.5%) were isolated from outpatients (Fig. 1). Year-wise positivity data showed a higher frequency of *P. aeruginosa* infections in 2017 (25.8%; 67/260) and a lower frequency in 2010 (6.5%; 17/260) (Table 1). The sex-wise distribution showed that *P. aeruginosa* positivity was higher in males (161/260; 61.9%) compared with females (99/260; 38.1%), with an elevated prevalence of *P. aeruginosa* infections in the age group 31–45 years (136/260; 52.3%). The infection rates for other age groups are shown in Table 1. Most *P. aeruginosa* strains were isolated from sputum (57.3%; 149/260), followed by urine (31.2%; 81/260), pus (10.4%; 27/260), vaginal swab (0.4%; 1/260), eye swab (0.4%; 1/260) and skin swab (0.4%; 2/260) (Fig. 1).

P. aeruginosa showed a high level of resistance to ceftazidime (49.6%; 129/260) followed by ticarcillin (41.5%; 108/260), whilst the lowest resistance rate was observed to amikacin (5.8%; 15/260).

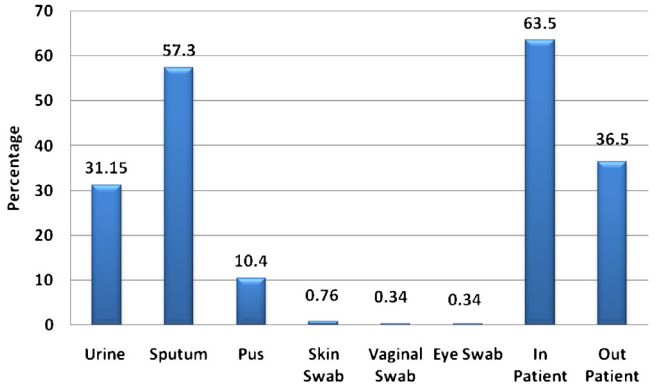

Fig. 1. Demographic data of *Pseudomonas aeruginosa* isolates from human immunodeficiency virus (HIV) patients, 2010–2017.

When carbapenem resistance among *P. aeruginosa* isolates was analysed, it was noted that 15.0% (39/260) of strains were imipenem-resistant and 16.9% (44/260) were meropenem-resistant. A high rate of imipenem resistance was observed in 2016 (46.2%; 18/39), with meropenem showing its peak resistance rate in 2017 (20.5%; 9/44). Year-wise analysis of antimicrobial resistance among *P. aeruginosa* isolates revealed that there was a frequent increase in the percentage of resistance towards antibiotics such as aztreonam, cefepime, levofloxacin, meropenem, piperacillin, TZP, ticarcillin and tobramycin from 2010 to 2017 (Fig. 2).

4. Discussion

P. aeruginosa infections in HIV patients are usually life-threatening and share many risk factors with patients. The US Centers for Disease Control and Prevention (CDC) has recognised the increasing importance of recurrent bacterial infections in HIV patients with immunodeficiency [10]. Changing antimicrobial resistance patterns owing to continuous and overexposure to antibiotics has led *P. aeruginosa* to emerge as a multidrug-resistant and extensively drug-resistant organism. Hence, it has become a daunting task to treat drug-resistant opportunistic *P. aeruginosa* infections especially in an HIV care setting.

In the current study, it was noted that the infection rate of *P. aeruginosa* among HIV patients has been constantly increasing from 2010 to 2017. In 2010, the infection rate was only 6.5% whilst in 2017 it increased to 25.8%. This clearly denotes the increasing opportunistic dominance of *P. aeruginosa* infections in HIV-infected individuals. Epidemiological studies revealed that infections caused by drug-resistant *P. aeruginosa* are associated with significant increases in morbidity, mortality, surgical interference, long hospital stay and chronic care [8,11]. *P. aeruginosa* isolates have been reported to show resistance to aminopenicillins, first- and second-generation cephalosporins, piperacillin, TZP, cefepime, ceftazidime, aminoglycosides, quinolones and carbapenems [12]. Bacterial transmission between patients and the environment is mainly contributing to the spread of these MDR *P. aeruginosa* clones [13]. Similarly, observations from this study show that >10% of *P. aeruginosa* strains isolated from 2010–2017 were resistant to ceftazidime, ticarcillin, piperacillin, cefepime, meropenem, gentamicin, imipenem, levofloxacin, TZP, ciprofloxacin and aztreonam.

Cefepime is one of the few antibiotics described to have constant antipseudomonal activity over the years, yet increasing resistance to cefepime has been reported [14]. In agreement, this study also showed increasing resistance of *P. aeruginosa* to cefepime over the study period, reaching its peak in the year 2017 (42.3%). Ceftazidime was considered as an effective antibiotic among third-generation cephalosporins for the treatment of

Table 1
Age-wise distribution of *Pseudomonas aeruginosa* isolates from human immunodeficiency virus (HIV) patients.

Age group	2010	2011	2012	2013	2014	2015	2016	2017	Total
<15 years	0	0.4%	0.4%	0.4%	0	0	0	0.4%	1.5% (*n* = 4)
16–30 years	0.8%	0.4%	1.1%	3.8%	2.3%	0.8%	1.5%	1.5%	12.3% (*n* = 32)
31–45 years	4.2%	4.2%	4.6%	4.6%	4.6%	4.2%	10%	15.8%	52.3% (*n* = 136)
46–60 years	1.1%	1.1%	1.5%	1.9%	2.3%	5%	6.9%	6.9%	26.9% (*n* = 70)
>60 years	0.4%	0.8%	0.8%	0.8%	0.8%	1.5%	0.8%	1.1%	6.9% (*n* = 18)

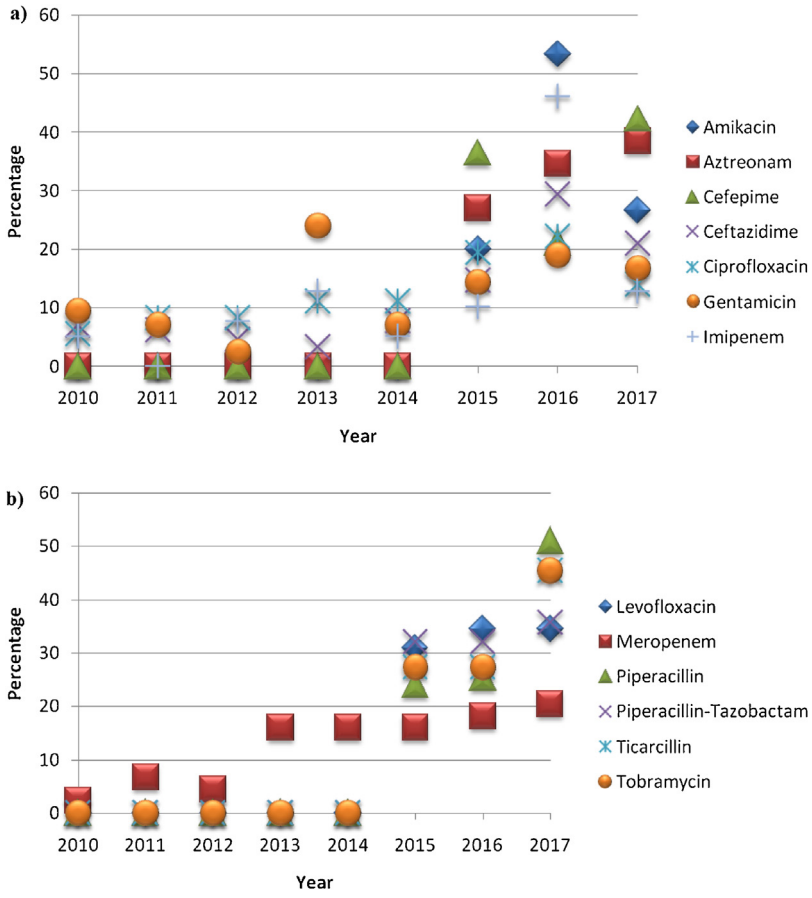

Fig. 2. Year-wise resistance profile of *Pseudomonas aeruginosa* isolated from clinical specimens from human immunodeficiency virus (HIV) patients from 2012–2017 for: (a) amikacin, aztreonam, cefepime, ceftazidime, ciprofloxacin, gentamicin and imipenem; and (b) levofloxacin, meropenem, piperacillin, piperacillin/tazobactam, ticarcillin and tobramycin.

pneumonia caused by *P. aeruginosa* [15]. However, in the current study ceftazidime exhibited low activity with a resistance rate of 49.6% of *P. aeruginosa* isolates, which turned out to be the antibiotic with the highest resistance rate. Piperacillin has broad-spectrum activity against *Pseudomonas*, and piperacillin combined with the β-lactamase inhibitor tazobactam has been used as combination therapy for effective treatment of severe pneumonia caused by *P. aeruginosa*, yet the resistance rate to TZP has also been reported to be increasing [16]. In this study, overall resistance rates to piperacillin and TZP were 24.2% and 11.5%, respectively. Year-wise monitoring of the piperacillin resistance profile showed that the resistance rate was alarmingly more than doubled in 2017 (51%) compared with 2015 (24%).

Carbapenems are high-potential β-lactam antibiotics showing broad-spectrum activity against many Gram-negative bacteria. Imipenem and meropenem are beneficial carbapenems used for the treatment of pneumonia caused by *P. aeruginosa*. Metallo-β-lactamases synthesised by the bacterium hydrolyse the carbapenem class of antibiotics in an efficient manner and are responsible for carbapenem resistance [17], which might have been responsible for the increased level of resistance to carbapenem antibiotics of *P. aeruginosa* in this study. The resistance rate of *P. aeruginosa* to imipenem was 5.1% in 2010 but had increased to 46.2% in 2016. The resistance rate to meropenem increased from 2.3% in 2010 to 20.5% in 2017. These findings indicate a frequent increase in carbapenem-resistant *P. aeruginosa* among the HIV-infected population. Development of resistance to various antibiotics based on their exposure was studied and it was reported that none of the antibiotics investigated developed resistance within 1–3 days [18]. Meropenem resistance developed early in the period 8–15 days, whereas resistance to other antibiotics developed by exposure of >15 days. This denotes that continuous exposure might increase the chance of developing resistance to meropenem compared with other antibiotics.

Tobramycin is an aminoglycoside antibiotic possessing broad-spectrum antibacterial activity and is often used for the treatment of infections caused by *P. aeruginosa* in cystic fibrosis patients and also for the treatment of different Gram-negative bacterial infections.

Higher usage of this antibiotic is enhancing the resistance rate significantly [19]. Here it was observed that tobramycin resistance also increased from 27.3% in 2015 to 45.4% in 2017. Despite the antipseudomonal effectiveness of tobramycin, increased resistance of *P. aeruginosa* to tobramycin was evident in this study, making it difficult to use it as a drug of choice. Resistance to gentamicin was also found to be increasing in this study. Gentamicin is often used in combination with β-lactam antibiotics or cephalosporins for the clinical management of severe pneumonia [20]. In this study, amikacin was found to show the highest susceptibility rate against *P. aeruginosa* isolated from HIV patients. The overall resistance rate of the study isolates to amikacin was 5.8%. Amikacin possess activity against *P. aeruginosa* but the combination of amikacin with cefepime was reported to be more effective in the treatment of nosocomial *P. aeruginosa* causing pneumonia [14].

This study revealed that the antimicrobial resistance of *P. aeruginosa* is constantly on the rise and certain life-threatening resistant bacterial strains such as carbapenem-resistant *P. aeruginosa* require immediate attention and priority. These resistant strains might have severe public health consequences and impacts on infected patient, as per the WHO statement [7]. This retrospective study revealed that the drug resistance profile of *P. aeruginosa* is under constant variation. Accurate antimicrobial susceptibility patterns determined by microbiologists and appropriate antibiotic regimen selection by clinicians could play a major role in the clinical management of *P. aeruginosa* infections. Future studies are needed on better administration of the existing antibiotic armamentarium, along with antimicrobial stewardship programmes as well as the development of new antibiotics to overcome increasing antimicrobial resistance.

Funding

None.

Competing interests

None declared.

Ethical approval

Not required.

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