Short Communication

Distribution and antimicrobial sensitivity pattern of multidrug-resistant *Pseudomonas aeruginosa* from the clinical specimen in Aceh, Indonesia

Zinatul Hayati[1,2*], Yulia S. Ismail[3], Suhartono Suhartono[3], Miftahul Zikra[3], Teuku F. Karmil[4] and Ade Oktiviyari[5]

[1]Department of Microbiology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [2]Department of Microbiology, Faculty of Medicine, Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia; [3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia; [4]Department of Clinical Veterinary, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [5]Department of Microbiology, Faculty of Dentistry, Universitas Syiah Kuala, Banda Aceh, Indonesia.

*Corresponding author: hayatikarmil@usk.ac.id

Abstract

The development of *P. aeruginosa* resistance to antibiotics is increasing globally due to the inappropriate use of antibiotics. This retrospective descriptive study aimed to determine the distribution of multidrug resistant *P. aeruginosa* (MDRPA) and its antimicrobial sensitivity profiles. Data were taken from the bacterial identification and antibiotic sensitivity testing of clinical specimens in Aceh Provincial Referral Hospital from July 2017 to June 2019, where the VITEK® 2 Compact automatic device was used for the identification. In total, there were 307 *P. aeruginosa* isolates analyzed in this study. The results of this study revealed that 54.39% of *P. aeruginosa* isolates were multidrug resistant. The MDRPA were mostly found in sputum and pus cultures (41.91% and 23.95%, respectively). Based on the wardroom as the source, most samples came from the internal medicine unit, intensive care unit, and surgical unit with prevalence of 27.54%, 25.74%, and 20.35%, respectively. High proportion of infected patients were male (52.09%) and aged 56–65 years (23.95%). *P. aeruginosa* showed the highest sensitivity rate to amikacin (77.1%), followed by meropenem (74.4%), ceftazidime (70.1%), levofloxacin (65.1%), tobramycin (64.9%), piperacillin-tazobactam (64.3%), and gentamicin (61.4%). In conclusion, our data suggest that the MDRPA infection is prevalent in Aceh Province and Amikacin is recommended as the highest standard of therapy for MDRPA.

Keywords: Amikacin, antibiotic sensitivity, clinical specimens, multidrug resistant, *Pseudomonas aeruginosa*

Introduction

Infectious diseases caused by bacteria are one of the biggest health problems that occur not only in Indonesia but also throughout the world [1,2]. According to the results of a study conducted at the Dr. M. Djamil Hospital Padang, *Pseudomonas aeruginosa,* is a pathogenic bacterium that cause nosocomial infections from Gram-negative groups [3]. The *P. aeruginosa* bacterium is an opportunistic pathogen that causes nosocomial infection, invasive and toxigenic. These bacteria cause severe infection, especially in patients with impaired immune systems [4]. *P. aeruginosa* is the source of pneumonia, urinary tract infections, gastrointestinal infections, sepsis,

Received: May 30, 2023 - Accepted: August 23, 2023 - Published online: August 31, 2023

osteomyelitis, peritonitis, soft tissue infections, and skin infections, including infections of wounds and burns [5]. Nosocomial infection is transmitted during medical instrument use and practice of medical procedures in the hospital (such as catheter placement, infusion, and other operative procedures) [6,7].

P. aeruginosa can be treated with antibiotics. However, it is now challenged by the increase of antibiotic resistant *P. aeruginosa* [1,8,9]. Misuse of antibiotics facilitates the bacteria to be resistant to some classes of antibiotics by means of various mechanisms including altering the intracellular targets of the antibiotics or removing antibiotics from cells through efflux pumps [10]. *P. aeruginosa* that are resistant to various types of antibiotics, such as penicillin, cephalosporin, and carbapenem, are called as multidrug resistant *P. aeruginosa* (MDRPA) [11,12]. This consequently leads to difficulties to manage the infection worldwide, contributing to an increase in mortality [13]. Based on our primary data, *P. aeruginosa* is frequently found in the clinical specimens, especially sputum. Therefore, this study aimed to further evaluate the distribution and sensitivity profiles of MDRPA in Aceh Province, Indonesia.

Methods

Study design

This was a descriptive study aiming to determine the distribution and frequency profiles of MDRPA isolates from clinical specimen in Aceh Province, Indonesia. Retrospective collection was performed for data obtained from July 2017 to April 2019, while data from May - June 2019 were obtained prospectively. This experiment was divided into several stages namely, isolation, identification, and antibiotic sensitivity testing performed on clinical specimens collected from *P. aeruginosa*-infected patients. This study had priorly passed the ethical review from the Health Research Ethics Committee of the Faculty of Medicine, Dr. Zainoel Abidin Hospital, Banda Aceh (No. 66/EA/FK-RSUDZA/2019).

Bacterial isolation and identification

Bacterial isolation was carried out on Blood Agar (BA) and MacConkey Agar (MCA) media. Samples were inoculated onto a Petri dish filled with media, respectively, using an inoculation needle for 10 µL of each kind of specimen, except urine specimen which was carried using inoculation needle for 1 µl. Streaking was performed following the quadrant method. Urine specimens were streaked onto BA medium using inoculation needle, while the other specimens (pus, sputum, blood, fluid and mucous) were inoculated on MCA media. The media was incubated for 24 hours at 37°C in the incubator. Macroscopic observations were then carried out by observing the colony from the color, shape, edge, and surface structure of the bacterial colonies.

Gram staining

Around 1 to 10 µL of the bacterial culture was smeared on the slide with the help of an inoculation needle and cotton swab before air-dried for fixation. Crystal violet solution was drop-wised on the fixed culture, allowed to stand for 1 minute then the slide was washed with running water. Thereafter, Lugol drops were added onto the slide, covered using a thin layer of glass for 1 minute and then, washed with running water. Next, 96% ethanol was applied on the slide, allowed to stand for 30 seconds until the color of the violet crystals disappeared, and then the slide was rewashed using running water. Safranin solution was added to cover the smear. After 1 minute, the safranin solution was washed off using running water. The fixed culture was air-dried before added with a few drops of immersion oil and observed under the microscope (Olympus binocular microscope CX22) for the bacterial shape, color, and morphology. A few drops of immersion oil with main ingredient Benzyl benzoate (Merck, Germany) were dropped onto the slide, and microscope identification was performed. Purple and pink appearances indicate the Gram-positive and Gram-negative bacteria, respectively.

Antibiotic sensitivity tests using VITEK® 2 Compact

The identification process was carried out alongside the antibiotic sensitivity test using a set of VITEK® 2 Compact devices (bioMerieux, Jakarta, Indonesia). Firstly, the bacterial cells were

suspended in 3 mL NaCl 0.45% in a test tube and adjusted to McFarland's turbidity standard (bacteria 0.5–0.63 at 600 nm) using a densitometer (Densicheck, bioMerieux, Jakarta, Indonesia). The suspension (145 µL) was drawn, added onto a 64-well plastic ID-GNB cards, and inserted to the device before running the system.

Data analysis

Identification data and antibiotic sensitivity patterns were obtained from WHONET software (Boston, Massachusetts, United States) and then analyzed descriptively on Microsoft Excel (Redmond, Washington, United States).

Results

Distribution pattern of MDRPA based on patients' characteristics and care unit

Distribution of MDRPA according to the characteristics of patients recruited in this present study is presented in **Table 1**. By gender, higher MDRPA frequency was found in men (52.09%) as compared in women (47.90%). Those in the age range of 56–65 years old were the group with the highest number of MDRPA infections (*n*=40, 23.95%). As many as thirty MDRPA isolates (17.96%) were identified in >65 years old patients, and nine isolates (5.38%) – in 0–5 years old patients.

Most of MDRPA isolates were from the internal medicine unit (*n*=46, 27.54%). The number was followed by intensive care unit (*n*=43, 25.74%), the surgical unit (*n*=34, 20.35%), Otorhinolaryngology – head and neck unit (*n*=32, 19.16%), neonatal intensive care unit (*n*=8, 4.79%), and VIP room (*n*=4, 2.39%).

Table 1. Distribution of MDRPA based on patients' characteristics and wardroom

Characteristics	*n* (%)
Gender	
Men	87 (52.09)
Women	80 (47.90)
Age (years)	
0–5	9 (5.38)
6–11	4 (2.39)
12–16	4 (2.39)
17–25	20 (11.97)
26–35	13 (7.78)
36–45	19 (11.37)
46–55	28 (16.76)
56–65	40 (23.95)
>65	30 (17.96)
Wardroom	
Internal medicine unit	46 (27.54)
Neonatal intensive care unit	8 (4.79)
Intensive care unit	43 (25.74)
Surgical unit	34 (20.35)
VIP room	4 (2.39)
Otorhinolaryngology – head and neck unit	32 (19.16)

Macroscopic and microscopic characteristics of *P. aeruginosa* isolate

Macroscopic and microscopic images of the clinical isolates of *P. aeruginosa* are presented in **Figure 1**. The colony appeared smooth, round, with uneven edges, and with a creamy color. Under 1000x magnification, *P. aeruginosa* cells were observed having straight rod-shaped and are seen as a single, clustered, and pink (Gram-negative) bacterium.

The distribution pattern of MDRPA per semester during the 2017–2019 period is presented in **Figure 2**. MDRPA infections reached the highest incidence in January–June 2018 (*n*=71, 97.26%), with a substantial increase from the previous semester (*n*=37, 82.22%). A drastic decrease was observed in July–December 2018 (*n*=34, 31.48%), and remained in the decreasing trend in January–June 2019 (*n*=25, 30.86).

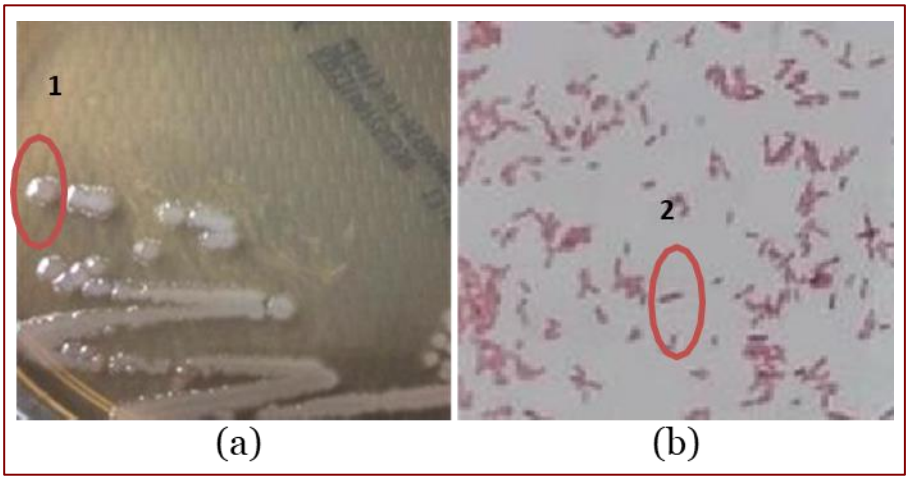

Figure 1. Macroscopic and microscopic images of *P. aeruginosa*. (a) Colonies of *P. aeruginosa* isolates grown on MacConkey media. (b) Stained *P. aeruginosa* cells observed with 1000x magnification. (1) Bacterial colonies. (2) Bacterial cells.

Table 2. MDRPA distribution based on the type of clinical specimens from patients (*n* total=167)

Specimen type	*n* (%)
Sputum	70 (41.91)
Pus	40 (23.95)
Blood	12 (7.18)
Urine	18 (10.77)
Fluid	5 (2.99)
Mucous	22 (13.17)

Antibiotic sensitivity

Antibiotic sensitivity profile or antibiogram of the *P. aeruginosa* isolates collected from patients and hospital equipment is presented in **Table 3**. The highest sensitivity of *P. aeruginosa* to cephalosporin antibiotics was 70.1% and 45.6%, observed in ceftazidime and cefepime, respectively. *P. aeruginosa* was found to be resistant to several groups of cephalosporins such as cefotaxime, cefoxitin, and ceftriaxone. The sensitivity of *P. aeruginosa* to aminoglycoside antibiotics such as amikacin, gentamicin, and tobramycin was relatively high (77.1%, 61.4%, and 64.9%, respectively). The sensitivity of *P. aeruginosa* to fluoroquinolone antibiotics (levofloxacin and ciprofloxacin) was 65.1% and 42.5%, respectively. In more detail, *P. aeruginosa* was found to be resistant to penicillin – ampicillin (1.0%), carbapenems–imipenem (0.2%), tetracyclines – tetracycline (2.6 %), cephalosporins – ceftriaxone, cefotaxime, and cefoxitin (0.8%, 2.2%, and 1.2%, respectively), miscellaneous antibiotics – fosfomycin (6.0%), and folate inhibitors – trimethoprim-sulfamethoxazole (3.3%).

Table 3. Antibiogram of the *P. aeruginosa* isolated from various sources in the hospital (*n* total=482)

Antibiotics	Sensitive isolate (%)	Antibiotics	Sensitive isolate (%)
Ceftazidime	70.1	Meropenem	74.4
Gentamycin	61.4	Ampicillin	1.0*
Tobramycin	64.9	Ceftriaxone	0.8*
Piperacillin tazobactam	64.3	Cefotaxime	2.2*
Amikacin	77.1	Fosfomycin	6.0*
Cefepime	45.6	Tetracycline	2.6*
Ciprofloxacin	42.5	Ampicillin/sulbactam	2.6*
Levofloxacin	65.1	Cefoxitin	1.2*
Imipenem	0.2*	Trimethoprim-Sulfamethoxazole	3.3*

*Sensitive isolate < 60%.

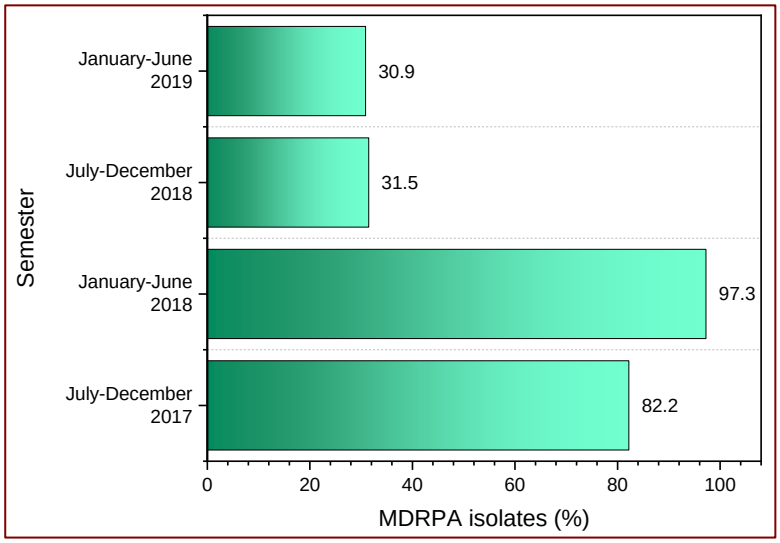

Figure 2. Graph of MDRPA distribution in Dr. Zainoel Abidin Hospital based on clinical specimens per semester during the 2017–2019 period.

Discussion

Our data suggests a concerning prevalence of MDRPA infection (more than 50% of total *P. aeruginosa* isolates) from all type of clinical specimens collected from July 2017 to April 2019. Based on a previous report, the prevalence of MDRPA reaching more than 70% is considered as very high category [14]. For comparison, the MDRPA rate at the Dr. M. Djamil Padang reached 34.17% [15]. According to a published report, MDR can be caused due to several things, including the wrong dose of antibiotics, misdiagnosis of the cause of infectious disease, and mistargeted bacteria [16]. The cause of *P. aeruginosa* infection is probably linked with the colonization of these bacteria in the hospital (such as medical equipment, air, and water). Further, several medical procedures might pose higher risk to the patient including but not limited to nerve surgery and installation of cerebrospinal fluid aids or other assistive devices [7]. Other than that, endogenous infection could occur by the bacterial translocation or transfer from the gastrointestinal tract through the epithelium and subsequently into the systemic circulation [17].

Herein, of the identified MDRPA isolates, we found that they were from sputum samples. This could be an indication of *P. aeruginosa* in the respiratory tract [17]. Similarly, in a previous report, *P. aeruginosa* isolates were commonly found in sputum specimens [7]. *P. aeruginosa* infects the respiratory tract cause various pathological problems such as acute pneumonia and other respiratory infections [7, 17]. A previous study reported that men are more at risk of contracting to respiratory infections than women [18]. It is worth noting that our present study also found that men had higher prevalence of MDRPA as compared to women. As suggested by a published report, higher prevalence in men is associated in with smoking habits and lower protective hormonal enzymes (such as 17 beta-estradiol) [19]. Other than gender, in this present study, our data suggest that older individuals are more likely to be infected with MDRPA. This is in line with a previous report from the United States, suggesting that those aged over 50 years were susceptible to MDRPA infections [20]. Older population is more at risk to contract the infection since they experience immunosenescence which consequently causes a decline in the immune response to bacterial infection [21].

In this present study, our findings indicate that MDRPA is more likely to infect patients in the internal medicine unit. We suspect that contamination of medical devices (*i.e.* intravenous catheters) as the transmission route owing to the frequent use in this unit. Of course, other possibilities such as frequent contact between visitors and patients should be considered. It is also worth noting that excessive use of empirical antibiotics may contribute to the increase in developing MDR bacteria, especially for those with immunocompromised conditions [22]. Inappropriate use of antibiotics leads to the mutation and resistance of the bacteria [23]. Collectively, these findings stress the importance of enforcing strict preventive measures against nosocomial infection and appropriate use of antibiotics therapies, especially in units with high

prevalence of MDRPA. Based on the frequency of MDRPA cases in each semester in this present study, a significant decline was observed in the second semester of 2018. This could be attributed to the success of proper antibiotic use following the suggestion from the MDR bacteria monitoring with antibiogram. MDRPA detection is very helpful in controlling infections in the hospital. It is challenging to treat *P. aeruginosa* infection because the bacteria are often resistant to many antibiotics. Alternatively, MDR bacteria are treated with combinatorial antibiotic therapies. Based on the antibiotic resistance profile herein (built by using 18 types of Gram-negative antibiotics), it was suggested that amikacin (one of aminoglycosides) is the highest standard therapy for MDRPA infection in Aceh, Indonesia.

As limitation of this study, we did not perform analytical statistics which limited the interpretation of risk factors (such as age and gender) in this present study. Moreover, we did not conduct a systematic investigation to the source of infection, including risky behaviors that could cause cross-infection. Future studies using cross-sectional and exploratory designs are therefore encouraged. Further, it is important to conduct genomic surveillance on the MDRPA to prevent the outbreak.

Conclusion

Out of 307 isolates obtained from the patients, more than 50% of which were identified as MDRPA suggesting its high prevalence in Aceh Province, Indonesia. Most of the MDRPA isolates were derived from sputum and pus specimens. The highest prevalence of MDRPA was found among male and individuals aged 56–65 years. MDRPA cases were found prominently in internal medicine unit, intensive care unit and surgical unit suggesting the necessity to conduct stronger preventive measures in the foregoing units. Based on the antibiotic sensitivity profile, we recommend amikacin as the highest standard of therapy for MDRPA.

Ethics approval

Ethical clearance was granted by the Health Research Ethics Committee of Medical School of Universitas Syiah Kuala, Dr. Zainoel Abidin Hospital, Banda Aceh (No. 66/EA/FK-RSUDZA/2019).

Acknowledgments

This work was facilitated by the Clinical Microbiology Laboratory of Dr. Zainoel Abidin General Hospital, Banda Aceh, Indonesia.

Competing interests

All authors declare that they have no conflicts of interest.

Funding

This study received no external funding.

Underlying data

All underlying data underlying have been presented in this article

How to cite

Hayati Z, Ismail YS, Suhartono S, *et al*. Distribution and antimicrobial sensitivity pattern of multidrug-resistant *Pseudomonas aeruginosa* from the clinical specimen in Aceh, Indonesia. Narra X 2023; 1 (2): e87 - http://doi.org/10.52225/narrax.v1i2.87.

References

1. Murray CJ, Ikuta KS, Sharara F, *et al*. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. Lancet 2022;399(10325):629–655.

2. Ikuta KS, Swetschinski LR, Aguilar GR, *et al*. Global mortality associated with 33 bacterial pathogens in 2019: A systematic analysis for the Global Burden of Disease Study 2019. Lancet 2022;400(10369):2221–2248.
3. Sjahjadi NR, Rasyid R, Rustam E, *et al*. Prevalensi kuman multi drug resistance (MDR) di laboratorium mikrobiologi RSUP Dr. M. Djamil Padang periode Januari 2010-Desember 2012. J Kesehat Andalas 2014;3(3):440–443.
4. Reynolds D, Kollef M. The epidemiology and pathogenesis and treatment of *Pseudomonas aeruginosa* infections: An update. Drugs 2021;81(18):2117–2131.
5. Capatina D, Feier B, Hosu O, *et al*. Analytical methods for the characterization and diagnosis of infection with *Pseudomonas aeruginosa*: A critical review. Anal Chim Acta 2022;1204:339696.
6. Farfán-Cano G, Silva-Rojas GA. Achromobacter xylosoxidans as emerging pathogen in healthcare settings: A mini review. Narra X 2023;1(1):e74.
7. Hopman J, Meijer C, Kenters N, *et al*. Risk assessment after a severe hospital-acquired infection associated with carbapenemase-producing *Pseudomonas aeruginosa*. JAMA Netw Open 2019;2(2):e187665.
8. Palavutitotai N, Jitmuang A, Tongsai S, *et al*. Epidemiology and risk factors of extensively drug-resistant *Pseudomonas aeruginosa* infections. PloS One 2018;13(2):e0193431.
9. Horcajada JP, Montero M, Oliver A, *et al*. Epidemiology and treatment of multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa* infections. Clin Microbiol Reviews 2019;32(4):31–19.
10. Hou A-m, Yang D, Miao J, *et al*. Chlorine injury enhances antibiotic resistance in *Pseudomonas aeruginosa* through over expression of drug efflux pumps. Water Res 2019;156:366–371.
11. Pushparaj Selvadoss P, Nellore J, Balaraman Ravindrran M, *et al*. Enhancement of antimicrobial activity by liposomal oleic acid-loaded antibiotics for the treatment of multidrug-resistant *Pseudomonas aeruginosa*. Artif Cells Nanomed Biotechnol 2018;46(2):268–273.
12. Karampatakis T, Antachopoulos C, Tsakris A, *et al*. Molecular epidemiology of carbapenem-resistant *Pseudomonas aeruginosa* in an endemic area: Comparison with global data. Eur J Clin Microbiol Infect Dis 2018;37:1211–1220.
13. Matos ECOd, Andriolo RB, Rodrigues YC, *et al*. Mortality in patients with multidrug-resistant *Pseudomonas aeruginosa* infections: A meta-analysis. Rev Soc Bras Med Trop 2018;51:415–420.
14. Nathwani D, Raman G, Sulham K, *et al*. Clinical and economic consequences of hospital-acquired resistant and multidrug-resistant *Pseudomonas aeruginosa* infections: A systematic review and meta-analysis. Antimicrob Resist Infect Control 2014;3(1):1–16.
15. Rustini IS, Armin F. Penentuan multidrug resisten *Pseudomonas aeruginosa* (MDRPA) yang berasal dari sampel klinis pasien RSUP Dr. M. Djamil Padang. Prosiding Rakernas dan Pertemuan Ilmiah Tahunan Ikatan Apoteker Indonesia 2016;2016:87–91.
16. Magiorakos A, Srinivasan A, Carey R, *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria. Clin Microbiol Infect 2021;1(18):3.
17. Wheatley RM, Caballero JD, van der Schalk TE, *et al*. Gut to lung translocation and antibiotic mediated selection shape the dynamics of *Pseudomonas aeruginosa* in an ICU patient. Nat Commun 2022;13(1):6523.
18. Chamekh M, Deny M, Romano M, *et al*. Differential susceptibility to infectious respiratory diseases between males and females linked to sex-specific innate immune inflammatory response. Front Immunol 2017;8:1806.
19. Abid S, Xie S, Bose M, *et al*. 17β-estradiol dysregulates innate immune responses to *Pseudomonas aeruginosa* respiratory infection and is modulated by estrogen receptor antagonism. Infect Immun 2017;85(10):417–422.
20. Tabak YP, Merchant S, Ye G, *et al*. Incremental clinical and economic burden of suspected respiratory infections due to multi-drug-resistant *Pseudomonas aeruginosa* in the United States. J Hosp Infect 2019;103(2):134–141.
21. Cillóniz C, Dominedò C, Pericàs JM, *et al*. Community-acquired pneumonia in critically ill very old patients: A growing problem. Eur Respir Rev 2020;29(155):190126.
22. Mohamed A, Abdelhamid F. Antibiotic susceptibility of *Pseudomonas aeruginosa* isolated from different clinical sources. Zagazig J Pharm Sci 2020;28(2):10–17.
23. Botelho J, Grosso F, Peixe L. Antibiotic resistance in *Pseudomonas aeruginosa*–Mechanisms, epidemiology and evolution. Drug Resist Updat 2019;44:100640.