Available online at *www.ujpronline.com*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

PREVALENCE OF *PSEUDOMONAS AERUGINOSA* (*P. AERUGINOSA*) AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AT A PRIVATE HOSPITAL IN SANA'A, YEMEN

Ali Alyahawi[1*], Abdul Monem Alhomidi[2], Nawal Al-Henhena[3]
[1]*Department of Pharmacy, Faculty of Medical Sciences, Al-Razi University, Republic of Yemen.*
[2]*Faculty of Medicine, Sana'a University, Republic of Yemen.*
[3]*Faculty of Laboratory Medicine, Sana'aUniversity, Republic of Yemen.*

Article Info:

Article History:
Received: 24 May 2018
Reviewed: 4 July 2018
Accepted: 24 August 2018
Published: 15 September 2018

Cite this article:
Ali A, Alhomidi AM, Al-Henhena N. Prevalence of *Pseudomonas aeruginosa* (*P. aeruginosa*) and antimicrobial susceptibility patterns at a private hospital in Sana'a, Yemen. Universal Journal of Pharmaceutical Research 2018; 3(4): 12-18.
https://doi.org/10.22270/ujpr.v3i4.177

***Address for Correspondence:**
Dr. Ali Alyahawi, Department of Pharmacy, Faculty of Medical Sciences, Al-Razi University, Republic of Yemen.
E-mail: *alyahawipharm@yahoo.com.*

Abstract

Objective: *Pseudomonas aeruginosa* is clinically significant and opportunistic pathogen that causes infections in hospitalized patients. Antibiotic resistance is a major concern in clinical practice. The ongoing emergence of resistant strains that cause nosocomial infections contributes substantially to the morbidity and mortality of hospitalized patients. Objective of present study was to estimate the prevalence of *Pseudomonas aeruginosa* and the antimicrobial resistance patterns of *P. aeruginosa* isolates from hospitalized patients.
Methods: The study was performed at microbiology department of a local hospital in Sana'a, Yemen. All the patients' samples of hospital departments from January, 2017 to December, 2017 were included. A Total of 2079 samples were collected during the study period. Among them, 193 strains of *Pseudomonas spp*. were isolated. Antimicrobial susceptibility pattern of each isolates was carried out by the Kirby-Bauer disk diffusion method as per CLSI guidelines. Majority of *P. aeruginosa* were isolated from Sputum, followed by urine specimens.
Results: The isolate pathogen showed the highest sensitive to Meropenem (85.5%), followed by Amikacin (80.5%), Imipenem (80.0%), and Piperacillin/tazobactam (77.2). The highest frequency of resistance (96.2%) was observed with amoxicillin /clavulinic Acid followed by cefuroxime 94.6%, ampicillin/sulbactam 94.5%, Co-Trimoxzole 80.5%, and norfloxacin 54%.
Conclusion: The result confirmed the occurrence of drug resistance strains of *P. aeruginosa*. Meropenem, imipenem, and amikacin, were found to be the most effective antimicrobial drugs. It therefore calls for a very judicious, appropriate treatment regimens selection by the physicians to limit the further spread of antimicrobial resistance *P. aeruginosa*.
Keywords: Antimicrobial susceptibility, Imipenem, multi drug-resistant, *Pseudomonas aeruginosa*.

INTRODUCTION

Pseudomonas aeruginosa is clinically significant and opportunistic pathogen that causes infections in hospitalized patients. In addition, most *Pseudomonas* species have intrinsic resistance to many antibiotics and ongoing emergence of new resistance can be developed after commonly prescribed antimicrobial agents[1]. *P. aeruginosa* has naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane lipopolysaccharide (LPS). Only few antibiotics are effective against *Pseudomonas* and even these antibiotics are not effective against all strains[2]. Antibiotic resistance is a major concern in clinical practice. The resistant strains of *P. aeruginosa* that cause nosocomial infections contributes substantially to the morbidity and mortality of hospitalized patients[3]. Despite the availability of a variety of effective antimicrobial agents, treatment of *P. aeruginosa* is often challenging[4] antimicrobial resistance is a growing problem worldwide, especially in hospitals, where resistant organisms are often first detected in ICUs[5]. The organism had been isolated from various infections like respiratory tract infections, cystic fibrosis, ear infections, orthopaedic infections, urinary tract infections, surgical infections, severe burns, etc. It was also reported frequently from patients undergoing chemotherapy for neoplastic diseases[6]. The variations of antibiotic protocols in clinics or in regions result in the different resistance profiles[4]. It is,

therefore, the goal of this study to determine the prevalence of *P. aeruginosa* isolates in a private hospital in Sana'a, Yemen also to evaluate its susceptibility against certain antibiotics, as limited work has been previously conducted on this subject.

METHODS

The study was performed at university of science and technology hospital in Sana'a, Yemen. It is one of the major private hospitals in Yemen. All the patients' samples from January, 2017 to December, 2017 were included. A Total of 2079 samples were gathered during the study period. Among them, 193 strains of *P. aeruginosa* were isolated. The medical records of these patients were retrieved and reviewed. All information regarding patients' gender and age as well as origin of clinical samples were collected.

Antimicrobial susceptibility testing of all the *P. aeruginosa* isolates was performed by Kirby-Bauer disk diffusion method and the result were interpreted by the Clinical Laboratory Standard Institute (CLSI) guidelines[7]. The antimicrobial susceptibility patterns of all the *P. aeruginosa* strains were determined against the following antibiotics of standard strength: ceftazidime, amikacin, gentamicin, imipenem, meropenem, ciprofloxacin, cefoperazone, piperacillin /tazobactam, amoxicillin/clavulinic acid, moxifloxacin, cefepime, ceftizoxime, ampicillin/ sulbactam, cefuroxime, ceftriaxone, co-trimoxzole, and levofloxacin. Full ethical clearance was obtained from the qualified authorities who approved the study design. All data were analyzed using SPSS Statistics 21. Data was presented in tables and graphs.

RESULTS

According to result findings, there were more than half of *P. aeruginosa* isolates in age group of 60 years and greater with 55(28%), followed by the age between 46 to 60 years in second rank about 38(20%), and finally the age between 31 to 45 years only about 20(10%). In this study, overall *P. aeruginosa* prevalence was 9.3 % (n=193/2079). The Figure 2 showed that there were about 154(80%) of *Pseudomonas* isolates form male, whereas the female had only about 39(20%). According to the study results, the medical department had the highest prevalence of *P. aeruginosa* isolates about 48(25%), followed by the intensive care unit in second rank about 41 (21%), the surgical department in third rank about 37(19%) and finally the pediatric and gynecology departments had only about 16(8%).

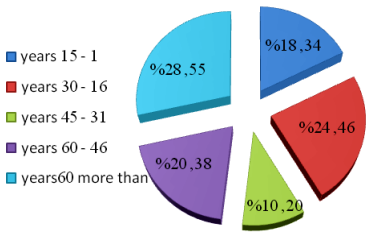

Figure 1: Distribution of *P. aeruginosa* isolates according to age.

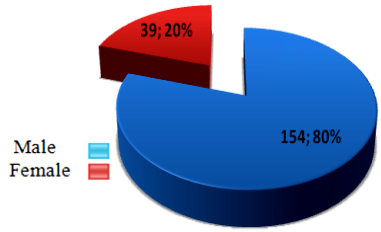

Figure 2: Distribution of *P. aeruginosa* according to gender.

The Figure 4 showed that the most of sample tests from sputum culture about 82(42.5%), followed by the sample from urine culture in second rank about 34(17.6%), and finally the sample test from other rout only about 6(3.1%).

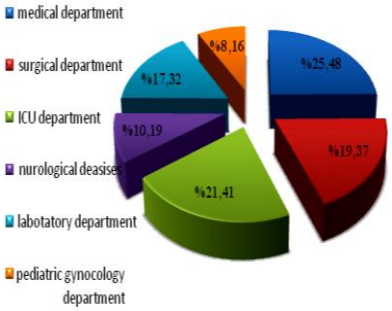

Figure 3: Distribution of *P. aeruginosa* isolates according to hospital departments.

According to the current study findings (Table 1),more than half of medication was sensitive to *P. aeruginosa* test about 12 drugs (54.5%), whereas the medication that resistance to *pseudomonas* tests about 10 drugs (45.5%).

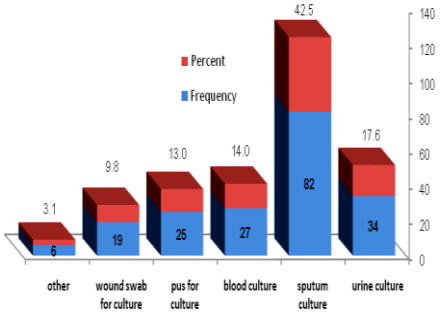

Figure 4: Distribution of P. *aeruginosa* isolates according to sample types.

P. aeruginosa strains showed resistance to ciprofloxacin 50.89%, ceftazidime 31.5%, ceftriaxone 78%, amoxicillin /clavulinic Acid 96.2%, ampicillin/ sulbactam 94.5%, cefuroxime 94.6%, nalidixic acid 83%, nitrofurantoin 88%, doxycycline 82.6%, norfloxacin 54%, and Co-Trimoxzole 80.5%. The highest frequency of sensitivity (85.5%) was observed with meropenem followed by amikacin 80.5%, imipenem 80%, piperacilline/tazobactam 77.2%, ceftizoxime 75%, ciprofloxacin 71.5%, levofloxacin 66%, cefoperazone 64%, gentamicin 56%, ceftazidime 54.5%, moxifloxacin 49%, and cefepime 44.5%. According to Figure 5 below, the highest resistance rate of anti-pseudomonal agent was with cefepime about 43.5% and the lowest resistance rate with

imipenem. Resistance to antipseudomonal drugs in current study was found to be cefepime (43.5%), ceftazidime (31.5%), ciprofloxacin (24%), piperacillin /tazobactam (16.5%), imipenim (15.4%). In the present study, multi drug resistance (MDR) rate (resistance to three or more of anti-*Pseudomonal* antimicrobials (i.e. piperacillin+tazobactam, imipenem, ceftazidime and amikacin) was determined to be 4.2% (8/193). Also MDR rate for only three anti Pseudomonal antimicrobials without imipenem was 4.2% (n=8/193) (i.e. piperacillin+tazobactam, ceftazidime and amikacin).

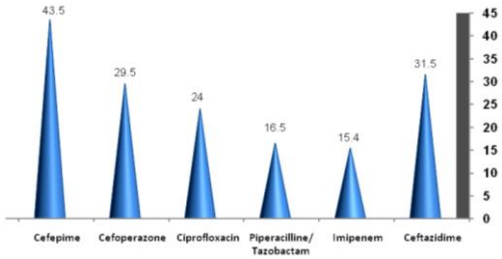

Figure 5: Resistance rates of anti-*pseudomonal* agent.

Table 1: Antimicrobial susceptibility patterns for *P. aeruginosa* isolates

Antibiotics	Expected options	Response F	Response %	Antibiotics	Expected options	Response F	Response %
Ceftriaxone	S	11	18.5	Ceftazidime	S	103	54.5
	R	46	78		R	60	31.5
	I	2	3.5		I	26	14
Cefoperazone sulbactam	S	58	64	Ciprofloxacin	S	118	71.5
	R	27	29.5		R	40	24
	I	6	6.5		I	7	4.5
Levofloxacin	S	108	66	Co-Trimoxzole	S	37	19.5
	R	44	26.8		R	152	80.5
	I	12	7.2		I	0	0.0
Ampicillin/ sulbactam	S	2	3.7	Imipenem	S	150	80
	R	51	94.5		R	29	15.4
	I	1	1.8		I	9	4.6
Amoxicillin / Clavulinic Acid	S	4	2.1	Norfloxacin	S	10	38.5
	R	179	96.2		R	14	54
	I	3	1.7		I	2	8
Amikacin	S	152	80.5	Cefepime	S	83	44.5
	R	28	14.8		R	81	43.5
	I	9	4.7		I	22	12
Gentamicin	S	105	56	Meropenem	S	89	85.5
	R	65	35		R	10	9.5
	I	17	9		I	5	5
Moxifloxacin	S	77	49	Piperacillin/ tazobactam	S	146	77.2
	R	69	44		R	31	16.5
	I	11	7		I	12	6.3
Cefuroxime	S	8	4.2	Ceftizoxime	S	1	4
	R	178	94.6		R	18	75
	I	2	1.2		I	5	21

DISCUSSION

P. aeruginosa has defined as one of the most common nosocomial pathogens. Hence we have undertaken this study to analyze the prevalence and antimicrobial susceptibility pattern of *P. aeruginosa* from various clinical samples of a private hospital. Periodic antimicrobial resistance monitoring in *P. aeruginosa* is fundamental to updating the current activity level of commonly used antipseudomonal drugs. The present study measures the rate of isolation of *P. aeruginosa* (n=193/2079; 9.3%) as which is lower than previous studies as by Tadvi *et al.*,[8] (22.67%) and Viren *et al*[9]. The occurrence of *P. aeruginosa* was found to be higher in males, inpatients in age group >60 years and in surgery department, which is same as reported by Marzoqi *et al.*,[10]. This might be due to prolonged hospitalization and other associated co-morbidities in these age groups. The distribution of *P. aeruginosa* isolates specimens may vary with each hospital as each hospital and each health facility has a different environment associated with it. According to the study results, more than 42.5% of the *P. aeruginosa* isolates were obtained from sputum samples. The distribution rank of the isolates according to the types of specimens was (respiratory sputum > urine > blood > pus >wound swap > others). Respiratory isolates (42.5%) were the most frequently encountered. *P. aeruginosa* isolates from respiratory tract as observed in a similar study of inpatient isolates done in a Saudi Arabian hospital[11]. In the present study, the maximum clinical isolates of *P. aeruginosa* were isolated from medical department (25%), followed by ICU (21%) and surgical department (19%). This was similar to study of Pathmanathan SG[12]. The distribution of specimens of *P. aeruginosa* might vary with each hospital as each hospital facility has a different environment associated with it. The correlation between specimen type and multidrug resistance would have been more noteworthy if supported by data on patients' clinical conditions. Prevalence of infection was higher in medical ward

followed by ICU as maximum isolates were isolated from sputum samples.

There was statistical significant relationship between the piperacilline/tazobactam susceptibility and sample types (*p* value=0.04). On other hand, there was no statistical significant relationship between the other antibiotics susceptibility (ceftazidime, imipenem, cefipeme) and sample types. As with this study, *P. aeruginosa* infection was primarily noted among older adults (n =55, 28%) particularly respiratory infection (n = 82, 42.5%). There are a number of reasons why older adults are burdened by this type of infection. These include age-associated impairments in immunity that lead to reduced response to vaccination, a constellation of chronic and comorbid diseases, and functional limitations associated with advanced age. Additionally, older adults are at risk for aspiration pneumonia, outbreaks of gastroenteritis, recurrent urinary tract infection, and prosthetic device infections[13]. In the European Prevalence of Infection in Intensive Care (EPIC), *P. aeruginosa* was predominant gram-negative bacteria isolated from broncho pulmonary infections and accounts for 17% of health care-associated pneumonia and late–onset ventilate associated pneumonia[14] and accounts for significant cases of cystic fibriosis[15].The distribution of isolates differs with studies and clinical specimens[16]. Intensive care patients especially create an environment for infection because of the debilitating effect of a prolonged hospitalisation and the application of medical equipment (airways, catheters *etc*)[17]. ICUs are generally considered epicenters of antibiotic resistance and the principal sources of outbreaks of multi-resistant bacteria. The most important risk factors are excessive consumption of antibiotics exerting selective pressure on bacteria, the frequent use of invasive devices and relative density of a susceptible patient population with severe compelling diseases[18]. Thus, in ICUs, empirical antibiotic treatments should be avoided and treatment should be carried out using antibiotic susceptibility tests. ICUs should be regularly monitored resistance pattern against the various antibiotics. *P. aeruginosa* was responsible for pneumonia and septicaemia with deaths rate about 30% in immune compromised patients[19]. In the current study results, *P. aeruginosa* showed resistance to amoxicillin/ clavulinic Acid 96.2%, ampicillin/sulbactam 94.5%, cefuroxime 94.6%, nalidixic acid 83%, nitrofurantoin 88%, doxycycline 82.6%, ciprofloxacin 50.89%, ceftazidime 31.5%, ceftriaxone 78%, norfloxacin 54%, and co-trimoxzole 80.5%. However, the highest frequency of sensitivity (85.5%) was observed with meropenem followed by amikacin 80.5%, imipenem 80%, piperacilline/tazobactam 77.2%, ceftizoxime 75%, ciprofloxacin 71.5%, Levofloxacin 66%, Cefoperazone 64%, Gentamicin 56%, ceftazidime 54.5%, moxifloxacin 49%, and cefepime 44.5%. This may be explained by the fact that routine use of these antibiotics can lead to clinically significant resistance. One remarkable finding in the present study was the highest frequency of sensitivity (85.5%) was observed with meropenem, 85.5%, amikacin (80.5%), and piperacillin/ tazobactem (77.2%). These drugs were the most effective drugs against *P. aeruginosa* infections. This similar to study finding by Taranasarwat *et al.*,[20], who reported highest sensitivity to imipenam. Also it was quite similar to the findings of Shaikh *et al.*, (100%)[21] and Mohan *et al.*, (94.3%)[22]. One striking feature in this study was that all the *P. aeruginosa* isolates were found to be sensitive to imipenem. This may be due to the restricted use of imipenem in this hospital. This is consistent with a report published in 2002 in Mangalore, India[23]. The emergence of carbapenem resistance is a serious concern[24]. In various studies across the world, varying rates of resistance from 4-60% have been reported for imipenem and meropenem[25]. Another survey found that resistance to imipenem was 19%, while other studies have reported low rates (5.8% and 9%) and high rates (38.6%) of resistance to imipenem[26]. Piperacillin+ tazobactam showed a sensitive rate of 77.2 % in this study and cefoperazone-sulbactum showed a lower resistance of 29.5% only, indicating beta-lactamase inhibitor markedly expands the spectrum of activity of beta-lactams, which makes the combination drug the preferred choice against *P. aeruginosa* infections. Thus, emphasis should be given towards use of combined antibiotics in the treatment of *pseudomonal* infections[27]. Bayani *et al.*, found that the resistance rate of *P. aeruginosa* to amikacin, ceftazidime, cefepime, imipenem, and ciprofloxacin was 53.3%, 43.3%, 40%, 40%, and 33.3%, respectively, and the prevalence of *P. aeruginosa* resistant isolates has increased[28]. According to previous evidence, the rate of susceptibility was most productive for antimicrobial agent of class carbapenem against *P. aeruginosa*[29]**.** Supported current results as 85.5% of strains were susceptible to Meronem and 80% to imipenem of class carbapenems. Although the resistance to carbapenems that include imipenem (16%) and meropenem (17.1) was low in this study, quite alarming should take into account that carbapenems are the last line of antibiotics for treating Gram-negative bacilli infections. Resistance to carbapenems may be due to a result of complex interactions of several mechanisms including production of carbapenemase, overproduction of efflux system and loss of outer membrane porins. *P. aeruginosa* isolates that are carbapenem resistant, specifically carbapenemase producing, are the worst, for the reason that they are associated with a higher mortality rate[24]. Amikacin in this study was noted to be the most effective drug (80.5% sensitive). However, it is not commonly prescribed drug, because of its numerous side effects including renal toxicity, blurred vision, hearing loss, Bartter-like syndromes[30], neuromuscular blockade, arthralgia, and apnoea. In addition, ciprofloxacin (71.5% sensitive) proved to be within the most effective drugs for routine use among the *P. aeruginosa* strains investigated in this study. The result finding in this study was similar in a previous study finding that reported that amikacin had the highest sensitivity against *P. aeruginosa*[9]**.** Also **i**n France, a higher susceptibility rate of 86% of amikacin was reported by Cavallo *et al.*,[31]. An earlier study reported from Kathmandu, Nepal[32] shown amikacin (81.4% sensitive) and ciprofloxacin (70.3% sensitive)

among *P. aeruginosa* strains examined. Amikacin seems to be a promising therapy for *pseudomonal* infection. Hence, its use should be restricted to severe nosocomial infections, in order to avoid rapid emergence of resistant strains[33]. However, high resistance to aminoglycosides had been reported in studies done in Bangladesh[34], Turkey[4] and Malaysia[35]. Similarly, higher rates of resistance to fluoroquinolones such as ciprofloxacin resistance (92%) were shown in a study from Malaysia[36]. Also study findings by Zhanel *et al.,* reported moxifloxacin 58% and ciprofloxacin 46.7%[37]. Recently, ceftazidime and cefepime are the most frequently prescribed third and fourth generation cephalosporins respectively. Ceftadizime is known antipseudomonal drug that has demonstrated high susceptibility pattern with *P. aeruginosa* isolates. The increased prevalence of ceftazidime resistant *P. aeruginosa* is related to the increased use of beta lactam antibiotics such as amoxicillin and ceftazidime. However, the resistance to cefadizime was reported as 31.5% in this study. This value of resistance was less than reported from Gujarat, with a resistance value of 75%[9]. *P. aeruginosa* strains in this study exhibited a high rate of resistance to the third generation cephalosporin drug such as ceftriaxone (78%). A much higher resistance to ceftriaxone of 75%, 86% and 93.9% had been reported in studies done in India[38] Bangladesh[34] and Nepal[27]. Several studies have confirmed that *P. aeruginosa* was mostly resistant against ceftriaxone. However, this high level of resistance is not quite surprising as some suggest that ceftriaxone has considerably low activity against *P. aeruginosa*[39,40]. Another study reported the following rates of resistance to cefepime 64.8%, piperacilline/tazobatctam 45%, ciprofloxacin 38.9%, levofloxacin 36.1%, gentamicin 37.3% and amikacin 30%[41]. Relatively low piperacilline/tazobatctam resistance (11.5%) had been reported in a hospital isolates of *P. aeruginosa* in a study from Saudi Arabia[11]. In a study done in Kathmandu, Nepal[27], *P. aeruginosa* isolates obtained from intensive care unit of a national heart centre showed a high cefoperazone-sulbactum sensitivity rate of 84.8%. A previous study discovered an increased mortality rate associated with empiric piperacillin-tazobactam therapy given to patients with *P. aeruginosa* bacteraemia; the isolates had reduced piperacillin-tazobactam susceptibility[42]. In this study, amoxicillin /clavulinicacid had established 96.2 % resistance. Similarly, in a study conducted in Pakistan reported by Khan *et al.*,[43] had a high resistance rate of penicillin that is 98%; current findings are also in agreement with other studies as reported by Sasirekha *et al.*,[44] and Ullah *et al.*,[45] with respect to penicillin's. Also the same findings were obtained with amoxicillin/clavulinic acid (1.88%) and showed increasing resistance. Multi drug efflux pumps in the inner and outer membrane of *Ps. Aeruginosa* may protect the bacterium from β-lactam agents[46]. Similar pattern had been reported in study in Nigeria[47]. In addition, susceptibility to fourth-generation such as cefepime reported in India 32%[48] and in Bulgaria 42%[49] against *P. aeruginosa* isolates. The high resistance to cephalosporins may be due to production of extended spectrum β-lactamases by the bacteria involved[50]. Cefuroxime was one of the cephalosporin drugs tested in this study, with resistance value of 94.6%. This high resistance value observed were comparable with the report from Gujarat, India with resistance value of 73.2%[9], but higher than reports from Malaysia of 40%[51]. In similar to previous study done in Bangladesh[34] showed rate of resistance for co-trimoxazole to be 93.5% in wound swab and pus isolates of *P. aeruginosa* while a Nigerian study[52] showed *P. aeruginosa* isolates 100% resistant to co-trimoxazole. So, imipenem which is both an anti-pseudomonal drug and carbapenem was the best drug. According to the study findings, MDR rate (resistance to three or more of anti Pseudomonal antimicrobials (*i.e.* piperacillin+ tazobactam, imipenem, ceftazidime and amikacin) was determined to be 4.2% (8/193). Also MDR rate for only three anti Pseudomonal antimicrobials without imipenem was 4.2% (8/193) (i.e. piperacillin+ tazobactam, ceftazidime and amikacin). A study done by Unan *et al.*,[53]. in Turkey reported rates of MDR, which were as high as 60%, whereas study done by Sabir *et al.*, in Pakistan detected lower rates of MDR (22.08%)[54]. Moreover, the rate of current study was lower than a study done in Egypt, where Gad *et al.*,[55] observed 36% MDR *P. aeruginosa*. On comparing the sensitivity patterns of these antimicrobials, it was found that there was a considerable difference in the sensitivity pattern among these studies. This indicates that the sensitivity pattern changes from hospital to hospital and population to population. This has been possibly resulted from indiscriminate use of antibiotic, lack of awareness, patient non-compliance and unhygienic conditions[56]. According to Berglund[57] one of the reasons for resistance among bacteria is a result of either overuse and misuse of antibiotics. The current study results indicated that *P. aeruginosa* was becoming resistant to commonly used antibiotics due to excessive consumption. The empirical antibiotic treatment should be limited and treatment should be carried out using antibiotic susceptibility test and efforts should be made to prevent spread of resistant bacteria[56].

CONCLUSIONS

In conclusion, results of the present study clearly demonstrated the occurrence of resistance to various antipseudomonal agents among the *P. aeruginosa* isolates. The statistics in this study showed low rates of antibiotic resistance to meropenem, amikacin, and meropenem, and piperacillin/ tazobactam and maximum sensitivity against *P. aeruginosa* strains. We suggest a more restricted and a more rational use of these drugs in hospital setting in order to avoid rapid emergence of resistant strains. Regular anti-microbial susceptibility monitoring is essential of local, regional and national level isolates. Every effort should be made to prevent spread of resistant organisms. The solution can be planned by continuous efforts of microbiologist, clinician, pharmacist and community to promote greater understanding of this problem. Frequent hand

washing to prevent spread of organism should be encouraged.

ACKNOWLEDGEMENTS

The authors extend their thanks and appreciation to the Al-Razi University, Republic of Yemen to provide necessary facilities for this work.

AUTHOR'S CONTRIBUTION

Ali A: writing original draft, conceptualization. **Alhomidi AM:** methodology, investigation. **Al-Henhena N:** writing, review, and editing, supervision, resources. All authors read and approved the final manuscript for publication.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

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