Yemeni Journal of Medical and Health Research

A Comparative Study on the Antibiotics' Resistance in Nosocomial and Environmental Isolates of Pseudomonas Aeruginosa in Aden, Yemen, 2023

Wadhah M. Al-Qashbari[1] and Mohamed A. Al-Baghdadi[2]
DOI: https://doi.org/10.47372/yjmhr.2024(13).1.1

Abstract

Introduction: Pseudomonas aeruginosa is a common bacterial pathogen with wide spread distribution in health care settings. It is a multidrug resistant pathogen recognized for its ubiquity and intrinsically advanced antibiotic resistance mechanisms. This study aims to find out the isolation rate and to determine antimicrobial susceptibility patterns, and level of multidrug resistance from nosocomial and environmental isolates of P. aeruginosa.

Methods: A cross-sectional study was carried out over a 6-months period from September 2022 to February 2023 and a total of 217 samples were enrolled which included 130 different clinical specimens from patients with nosocomial infections and 87 samples of hospital environment at different departments in four hospitals in Aden governorate, Yemen. The isolates were identified by biochemical tests as well as the susceptibility patterns which were tested by 22 types of antibiotics. All data were analyzed using SPSS statistics version 22 with a significance level of $P<0.05$.

Results: The P. aeruginosa were isolated from 23.1% of clinical specimens and 32.2% from environmental samples. The statistical analysis showed no statistically significant difference between clinical and environmental samples in prevalence ($P>0.05$). The most effective antibiotic against clinical isolates was Pipracillin/tazobactam with only 6.7% resistance value. In contrast, the most effective antibiotic against environmental strains was ciprofloxacin without any resistance value. The resistance to other antibiotics was found to be high or completely resistant. Ninety percent of clinical P. aeruginosa isolates and 96.4% of environmental isolates were multidrug resistant.

Conclusion: The study concludes that environmental sources may play an important role in the spreading of MDR strains of P. aeruginosa.

Keywords: Pseudomonas aeruginosa, Antibiotics resistance, Multidrug resistance

[1]Department of Microbiology, Dr. Amin Nasher Higher Institute for Health Sciences
[2]Faculty of Medicine and Health Sciences, University of Aden
Corresponding Author: Wadhah Mohamed Hadi Al-Qashbari **Email:**.w734021616@gmail.com

مقارنة حول مقاومة المضادات الحيوية في العزلات المستشفياتية والبيئية للبكتيريا الزائفة الزنجارية في عدن، اليمن 2023

وضاح محمد القشبري و محمد علي البغدادي

ملخص الدراسة

المقدمة: تعتبر بكتيريا الزائفة الزنجارية من مسببات الأمراض البكتيرية الشائعة ذات الانتشار الواسع في مواقع الرعاية الصحية، وهو مُمْرِض مقاوم للأدوية المتعددة معروف بوجوده في كل مكان وآليات مقاومة المضادات الحيوية المتقدمة جوهريًا. تهدف هذه الدراسة إلى معرفة معدل العزل وتحديد أنماط الحساسية للمضادات الميكروبية ومستوى المقاومة للأدوية المتعددة من العزلات المستشفيات والبيئية لبكتيريا الزائفة الزنجارية.

المنهجية: تم إجراء دراسة مقطعية على مدى 6 أشهر من سبتمبر 2022 إلى فبراير 2023 حيث تم تسجيل ما مجموعه 217 عينة شملت 130 عينة سريرية مختلفة من المرضى الذين يعانون من عدوى المستشفيات و 87 عينة من بيئة المستشفى في أقسام مختلفة في أربعة مستشفيات في محافظة عدن - اليمن. تم التعرف على عزلات الزوائف الزنجارية من خلال الاختبارات الكيموحيوية كما خضعت جميع العزلات لاختبار الحساسية لـ 22 من المضادات الحيوية المختلفة. تم تحليل جميع البيانات باستخدام البرنامج الإحصائي SPSS الإصدار 22 بمستوى دلالة $P<0.05$.

النتائج: تم عزل بكتيريا الزائفة الزنجارية من 23.1٪ عينة سريرية و 32.2٪ من العينات البيئية. أظهر التحليل الإحصائي عدم وجود فروق ذات دلالة إحصائية بين العينات السريرية والبيئية في معدل الانتشار ($P>0.05$). كان المضاد الحيوي الأكثر فعالية ضد العزلات السريرية هو الببراسيلين-تازوباكتم بنسبة مقاومة 6.7٪ فقط. في المقابل، كان المضاد الحيوي الأكثر فعالية ضد السلالات البيئية هو السيبروفلوكساسين دون أي نسبة مقاومة. وتبين أن المقاومة للمضادات الحيوية الأخرى عالية أو مقاومة تمامًا. أظهرت النتائج الاحصائية وجود فروقات احصائية معنوية في مستوى مقاومة بعض المضادات الحيوية بين العزلات السريرية والبيئية للزوائف الزنجارية. كما أظهرت النتائج أن 90٪ من العزلات السريرية و 96.4٪ من العزلات البيئية كانت متعددة المقاومة للمضادات الحيوية المستخدمة.

الاستنتاج: خلصت الدراسة إلى أن المصادر البيئية قد تلعب دورًا مهمًا في انتشار سلالات الزوائف الزنجارية ذات المقاومة المتعددة للمضادات الحيوية.

الكلمات المفتاحية: الزائفة الزنجارية، مقاومة المضادات الحيوية، مقاومة الأدوية المتعددة

[1]قسم الاحياء الدقيقة معهد د. أمين ناشر العالي للعلوم الصحية ، الجمهورية اليمنية .
[2]كلية الطب والعلوم الصحية، جامعة عدن، الجمهورية اليمنية .

Introduction

Pseudomonas aeruginosa is ubiquitous, opportunistic, saprophytic Gram-negative bacilli, obligate aerobic, and motile with single polar flagellum. Infections caused by P. aeruginosa are environmentally acquired and rarely spread from person to person. It is termed as 'opportunistic' pathogen because it rarely infects healthy individuals, The main targets are immunocompromised individuals [1].

It can colonize and grow fast in various environmental niches, including soil, wastewater, distilled water, abattoir effluent, marine habitats, plant and animals, this wide distribution is probably because P. aeruginosa seems to have specific functions to interact with other microorganisms, limited nutritional requirements, withstand adverse environmental conditions, able to utilize over 100 different organic compounds for growth [2]. This bacterium is a major cause of mortality and morbidity in people with the immunosuppressive condition and a leading cause of nosocomial infections and also known to cause human infections of wounds, ear, eyes, skin, burns, urethra and respiratory tract, also it frequently colonizes the medical devices [3].

P. aeruginosa resist many antimicrobial agents, this multidrug resistance has increased dramatically in recent years and is due to using two mechanisms of resistance that is intrinsic (low outer membrane permeability, expression of efflux pumps and the production inactivating enzymes against antibiotics) and acquired resistance mechanisms (horizontal transfer of resistance gene or mutational changes), this make it a great public health issue and difficult to treat [4]. Another reasons that contribute to make P. aeruginosa resistant to many antimicrobial agents is a consequence of uncontrolled acquisition and misuse of antibiotics especially in developing countries as many studies reported a relevancy between increasing use of antimicrobial agents and increasing the rate of antimicrobial resistance (AMR) [5–7].

Presumably, strains of P. aeruginosa found in environment are less pathogenic as compared to clinical one, but unfortunately, environment may contaminated through discharge of waste materials from the healthcare centers, untreated wastewater or domestic waste disposal that introduce clinical strain to it [8].

As many environmental sources containing P. aeruginosa, they play an important role as a source of infection. However, very few previous studies in Yemen denote the prevalence of *P. aeruginosa* and conducted on its antibiotics susceptibility pattern in clinical samples [9,10]. Therefore, the aim of this study is to find out the isolation rate and to determine antimicrobial susceptibility patterns, and level of multidrug resistance from nosocomial and environmental isolates of P. aeruginosa.

Methods

Study design

A cross sectional study was carried out over a 6-month period from September 2022 to February 2023 for patients and hospital environment in four major general hospitals in Aden city (AL-Gamhuria Teaching Hospital, 22 MAY Hospital, AL-Sadaka Hospital and Basuhaib Military Hospital).

Collection of samples

A total of 217 specimens comprising 130 from clinical and 87 from environmental sources were collected from hospitalized patients at the four hospitals. The clinical samples were collected from hospitalized patients at different departments of hospitals and made up of 47 burns samples, 17 ear swab samples, 32 urine samples, 7 sputa, 3 eye swabs, and 24 swab samples from wounds. The environmental samples were collected from the four hospitals environment (14 floor swabs, walls, 20 beds swabs, 12 disinfectant solutions, 17 ventilator swabs, 10 swabs samples from sinks).

Collection of clinical samples

Urine samples were collected from mid-stream urine 15-20 ml early morning in sterilized universal wide mouth container and preserved at 2-8 C until further use.

Burns, eye and ear discharge were collected in sterile cotton swabs whereas wounds' samples were collected either in sterile cotton swab or sterile syringes. On the other hand, sputum was collected by requesting the patient to cough deeply (to produce a sputum specimen) into sterile screw-cap cup [11]. All samples were transported to the lab for further analysis. All clinical specimens were properly labeled with patient number, date and type of specimen (i.e. urine, ear and burn).

Processing of clinical samples and isolation of P. aeruginosa

Urine specimens were inoculated on CLED medium by streaking technique, sputum, ear, wound and burns specimens were inoculated on blood and MaCconkey agar plate. All the inoculated plates were incubated for 18 – 24 hours aerobically.

Collection of hospitals environmental Samples

Samples from disinfectant solutions were collected in sterile container, samples from floors, walls, beds, sinks and ventilators were collected by sterile swabs.

All swabs were moistened with nutrient broth and transported to the laboratory for cultured.

Processing of hospitals environmental samples and isolation of P. aeruginosa

All specimens were cultured on blood agar and MacConkey agar. All suspected isolates were subcultured on Cetrimide agar as a selective medium to obtain pure culture. All cultured plates were incubated at 37° C aerobically for 18-24 hours.

Identification of P. aeruginosa

P. Aeruginosa colonies were identified by their cultural characteristics (shape, size and colour of colonies), morphologically (gram staining and motility) and biochemical test such as Oxidase, Catalase, Methyl red, Voges proskauer, urease, Sulphide indole motility (SIM), Kligler's iron agar (KIA) and Citrate utilization test [12]. All isolates were examined for growth at 42° C.

Antimicrobial susceptibility test:

The susceptibility patterns of P. aeruginosa was tested using agar disc diffusion method of modified Kirby-Bauer method based on criteria of Clinical and Laboratory Standards Institute [13].

The discs utilized were panel of 22 different antibiotic discs belong to Aminoglycosides (Amikacin AK 30µg, gentamicin GEN 10 µg), 1st generation of cephalosporin (Cefradine CH 25 µg), 2nd generation of cephalosporin (Cefuroxime CXM 30µg), 3rd generation of cephalosporin (Ceftazidime CAZ 30 µg, cefoperazone CPZ 75 µg,), Fluoroquinolones (Ciprofloxacillin CIP 5 µg, ofloxacin OF 5 µg, levofloxacin LE 5µg),
Macrolids (Erythromycin E 15 µg), Carbpenems (Impanem IPM 10µg, Meropenem MRP 10 µg), Combination drug (augmentin AMC 30 µg, Co-trimoxazole COT 25 µg, Ticarcillin/clavulanic acid TCC 75/10 µg, Piperacillin/tazobactam PIT 100/10 µg), Tetracycline (tetracycline TET 30µg), Penicillin (Penicillin G P 10µg, Ampicillin AMP 10µg, Azlocillin AZ 75 µg), Monobactams (aztreonam AT 30 µg), and Chloramphenicol C 30 µg.

Multiple antibiotic resistances (MAR) index

MAR index was determined for each isolate from the following formula: MAR index = a/b Where a is the number of antibiotics to which the isolate is resistant, b the total number of antibiotics tested [14]. MAR index > 0.2 is indication of wide use of the antibiotics in the originating environment of the isolate.

Quality control

A standard bacteriological technique was applied to maintain accurate laboratory test results. American Type Culture Collection (ATCC) standard reference strain P. aeruginosa ATCC 27853 was used to control the quality of culture and drug susceptibility testing [13].

Data analysis

Differences in frequencies of antibiotics susceptibility pattern and prevalence among groups were evaluated using Chi-squared tests (χ^2) with a significance level of $P<0.05$. All data were analyzed using SPSS statistics version 22.

Ethical consideration

The study protocol was approved by the Ethics Research Committee of the Faculty of Medicine and Health Sciences-Aden University (Research code approval REC- 151-2023).

Results

Isolation of P. aeruginosa

Fifty-eight isolates suspected as *P. aeruginosa* were collected from different clinical and environmental sources. The prevalence of P. aeruginosa in nosocomial infections was 23.1% (30/130). There was significant difference between urine and sputum samples (P=0.008) in prevalence. The highest isolation rate of P. aeruginosa among nosocomial infections was wound infections 45.8% followed by burns 21.3%, urine (18.8%), sputum 14.3% and ear infection 11.8% as shown in Table 1.

Table 1: Prevalence of *P. aeruginosa* from Nosocomial Samples

Clinical site	No. of specimens	No. of positive isolates (%)[a]
Burns	47	10 (21.3)
Ear swab	17	2 (11.8)
Urine	32	6 (18.8)
Sputum	7	1 (14.3)
Eye swab	3	0 (00)
Wounds	24	11 (45.8)
Total	**130**	**30**

[a] Percentage of the number of positive isolates with respect to the total number of specimens. (P<0.05) between urine and sputum samples in prevalence (Chi-squared tests)

The prevalence of *P. aeruginosa* in environmental samples was 32.2%. The statistical analysis showed a significant difference between walls and floors samples (P=0.016) in prevalence, Table 2.

Table 2: Prevalence of P. aeruginosa from Environmental Sources

Site of collection	No. of sample examined	No. of positive isolates (%)[a]
Hospital walls	14	5 (35.7%)
Floors	14	6 (42.9%)
Beds	20	5 (25.0%)
Disinfectants	12	6 (50.0%)
Ventilator	17	3 (17.6%)
Sinks	10	3 (30.0%)
Total	**87**	**28**

[a] Percentage of the number of positive isolates with respect to the total number of specimens. (P<0.05) between walls and floors samples in prevalence (Chi-squared tests)

The highest isolation rate of P. aeruginosa in hospital environment was found in disinfectant solution 50.0% followed by floor 42.9%, walls (35.7%), sinks (30.0%), beds 25.0% and ventilator equipment 17.6%. From the environmental samples, the prevalence rate of P. aeruginosa was 32.2% (28/87) and not significantly different (P>0.05) from that of clinical samples 23.1 % (30/130).

Antimicrobial susceptibility of P. aeruginosa isolates

The antibiotic susceptibility patterns of clinical and environmental isolates are shown in Table 3. The study revealed presence of resistance to various antimicrobial including antipseudomonal agents among the

nosocomial and environmental isolates of P. aeruginosa. In the present study, Pipracillin/tazobactam antibiotic has the highest activity against clinical isolates of P. aeruginosa strains with only 6.7% resistance value followed by ciprofloxacin 10%, levofloxacin and ofloxacin 13.3%. In contrast, the most effective antibiotic against environmental strains was ciprofloxacin without any resistance value followed by levofloxacin, Pipracillin/tazobactam (7.1%) and ofloxacin (10.7%).

There was a significant difference in resistant pattern between nosocomial and environmental isolates to the gentamicin, Meropenem, Pipracillin / tazobactam, Ticarcillin/clavulanic acid, Aztreonam (P<0.05), and highly significant difference to levofloxacin, ofloxacin, Amikacin, and Cefoperazone (P<0.001). There was no significant difference in resistant pattern between nosocomial and environmental isolates to the majority of antibiotics: ciprofloxacin, Impanem, azlocillin, chloramphenicol, tetracycline, penicillin G, Ampicillin, Erythromycin, Co-trimoxazole, Cefuroxime, Cefradine, Augmentin, and Ceftazidime (P>0.05).

Table 3: Antibiotic Sensitivity Pattern of *P. aeruginosa* Isolates from Nosocomial and Environment Sources

Antibiotics name	**Antibiotics symbol**	**Sources of bacterial isolates and their resistance**	
		Patients No. (30)	**Hospitals No. (28)**
Chloramphenicol	C	30 (100%)	28 (100%)
Tetracycline	TET	30 (100%)	28 (100%)
Erythromycin	E	30 (100%)	28 (100%)
Ciprofloxacillin	CIP	3 (10%)	-
Levofloxacin	LE**	4 (13.3%)	2 (7.1%)
Ofloxacin	OF**	4 (13.3%)	3 (10.7%)
Co_trimoxazole	COT	19(63.3%)	24(85.7%)
Augmentin	AMC	29(96.7%)	28 (100%)
Piperacillin/tazobactam	PIT*	2 (6.7%)	2 (7.1%)
Ticarcillin/clavulanic acid	TCC*	28(93.3%)	24(85.7%)
Ampicillin	AMP	30 (100%)	28 (100%)
Azlocillin	AZ	24 (80%)	26 (92.9%)
Penicillin G	P	30 (100%)	28 (100%)
Cefradine	CH	29(96.7%)	28 (100%)
Cefuroxime	CXM	30 (100%)	28 (100%)
Cefoperazone	CPZ**	9 (30%)	8 (28.6%)
Ceftazidime	CAZ	25(83.3%)	28 (100%)
Meropenem	MRP*	25(83.3%)	22(78.6%)
Impanem	IPM	21 (70%)	17(60.7%)
Aztreonam	AT*	25(83.3%)	24(85.7%)
Gentamicin	GEN*	9 (30%)	9 (32.1%)
Amikacin	AK**	7 (23.3%)	8 (28.6%)

***** (p<0.05); ****** (p<0.001)

MAR

All isolates were resistant to at least 11 Antibiotics and have MAR index greater than 0.2 indicating high contaminated sources and possible transmission of infection as shown in Table 4.

Table 4: Multi Antibiotic Resistance (MAR) Index of *P. aeruginosa* Isolates

MAR index	No. of antibiotic resistance	No. and sources of isolates
0.954	21	1 nosocomial
0.863	19	2 nosocomial 1 hospital
0.818	18	1 nosocomial 2 hospital
0.772	17	3 nosocomial 5 hospital
0.727	16	4 nosocomial 3 hospital
0.681	15	7 nosocomial 5 hospital
0.636	14	3 nosocomial 3 hospital
0.590	13	4 nosocomial 6 hospital
0.545	12	3 nosocomial 2 hospital
0.50	11	2 nosocomial 1 hospital

Discussion

Previous studies showed varying isolation rate of P. aeruginosa from clinical samples. These include a Yemeni study (2020) which reported 8.7% for urine, 13.6% for ear, 7.84% for wound, 5.79% for pus swab and 25% for sputum [7], a study in Nigeria (2018) which reported a high prevalence rate in wound infection 27.7% among hospital clinical samples [15], and a study in Iraq (2020) which reported 26.6% from burns [16]. In the present study, the highest isolation rate was observed in wound and burns samples. This may be explained by the ability of P. aeruginosa to establish and colonize the damaged tissues [17]. Furthermore, this represents a major public health hazard for acquired infection for both hospital and community especially for contamination of surgical wound.

Literature showed varying isolation rate of P. aeruginosa from hospital environment. These include a study in Nigeria (2017) which reported 13.6% from sink, 4.5% form beds. 4.5% form floor, 4.5% form disinfectant, 9.1% from mops [14]; a study in Libya (2017) which reported 33% form disinfectant, 11% form liquid soaps, 17% from beds, 5.5%for

walls and floors, 11% from ventilator equipment [18], and a study in Iraq (2011) which reported 1.97% for disinfectant, 2.6% form beds, 4.6% form catheter [19], and a study in Nigeria (2018) which reported 10.9% form walls, 9.1 % form beds, 14.5% for sinks, 20% from catheter [15].

The use of diluted disinfectant solutions contaminated with P. aeruginosa to disinfect various wards of hospitals including operation theaters and ICU rooms contributes in the dissemination of this pathogen and represent one of the most source of infection in our hospitals. The absence of strict restrictions for the entry of escorts into the intensive care units and absence of personnel hygienic role may contribute in the contamination [20]. In hospital environment, P. aeruginosa can thrives and survives in wide range of reservoirs which include disinfectants, sinks, endoscopes, mop heads, cleaning equipment, tap water, respiratory equipment [21]. Many inherited properties of P. aeruginosa make it survive in hospital environment. This organism inherently resistant to disinfectant through the multidrug efflux pumps mechanism. The formation of biofilm on inanimate surfaces by this organism increase its ability to resist the action of disinfectants and prevent its easy physical removal. Additionally, the ability of P. aeruginosa to colonize and kill free living amoeba associated biofilm by type III secretion system (T3SS) also favors its persistence in moist environments [22].

Resistance level within the classes of antibiotics

In this study, all P. aeruginosa isolate showed high resistant to beta lactams antibiotics where resistant 100% to Penicillin G and Ampicillin, and 80%-92.9% to Azlocillin. These results are corresponding to a study in Iraq (2011) which reported 100% resistance for penicillin, ampicillin in clinical and environmental isolates of P. aeruginosa [19], and is in agreement with a study in Bulgaria (2007) which reported 91.6% resistant to Azlocillin [23]. The high resistance of P. aeruginosa to β-lactam antibiotics can be explained by many mechanisms such as formation of β- lactamases with its important hydrolytic effect on β-lactam ring of penicillins [24].

In the present work, most P. aeruginosa isolates exhibit high resistant rates to cephalosporin with the exception of Cefoperazone which reveal moderate resistance rate 30% and 28.6%. Similar results about Cefoperazone against P. aeruginosa were obtained by a study in Egypt (2018) which reported 34% resistance rate [25], and a study in Nigeria (2015) which reported 90.5% resistance rate for Ceftazidime [26]. Another study in Pakistan (2015) found that resistance rate for Cefradine was 99.2% which is in agreement with our results [27]. In contrast, another study in Nigeria showed two-thirds activity of Ceftazidime and Cefuroxime against P. aeruginosa isolates [28]. This high resistant to cephalosporins may be due to partially the high production of ESβLs in our isolates.

Analysis of results indicate that both clinical and environmental isolates exhibit high resistant to most combination antibiotics used in this study. However, Pipracillin/ tazobactam revealed the lowest resistant 6.7% and 7.1% respectively

which make it still a choice for treatment of infections caused by P. aeruginosa. In this regards, a study carried out over 10 year period from 1993 to 2002 suggests increasing resistance of P. aeruginosa against combination antibiotics [29]. The high resistance present in the present study can be explained as a result of high production of Metallo-β-Lactamase (MβLs) in which a study conducted by Montero *et al* (2010) associating MβLs producing organisms and high resistant to β-lactam and extend spectrum cephalosporins and β-lactamase inhibitor combination [30].

Regarding class Carbpenems, the resistant of Imipenem towards clinical and environmental isolates of P. aeruginosa in the present study was 70% and 79.9% respectively. Likewise, Meropenem show 83.3% and 78.6% respectively. This result disagrees with a local study in Yemen which reported that 5.4% of isolate from clinical and non-clinical sources were resistant to Imipenem [31], and a study in Egypt which reported that 22% of clinical isolates and 46% of environmental P. aeruginosa isolates were resistant to Meropenem [32]. In contrast, a study in Nigeria carried out on abattoir isolates revealed a full resistant 100% to Imipenem [33]. However, in agreement to our finding, a study in Lithuania revealed that resistant to Carbpenems has increased dramatically for Imipenem from 53.3% in 2003 to 87.8% in 2005, which is in line with our finding [34]. The high resistance rate observed in this study toward Meropenem is consistent with the increasing resistance occurrence worldwide in P. aeruginosa strains [35].

For Monobactam class, the present study shows that 83.3% and 87.7% are the resistance rate of P. aeruginosa isolates from clinical and environmental sources respectively for antibiotic Aztreonam. These findings are agreed with a study in Egypt conducted on nosocomial isolates where the resistance rate was 82.5% [36]. However, these findings are different from those of a previous study in Yemen on clinical and non-clinical isolates in which the resistance rate was 23.2% for Aztreonam [31]. For Aminoglycosides, the resistance to these agents in both clinical and environmental isolates were respectively as follow; Amikacin 23.3%-29.2%**,** and Gentamicin 30%-39.6%. The results of the present study are nearly in accordance with a study in Pakistan where 25.3%, and 35.3% of P. aeruginosa isolates were resistant to Amikacin and Gentamicin respectively [27]. Another study in Nigeria conducted on clinical and environmental isolates of this pathogen exhibit different resistant rate in clinical and environmental samples where the resistance were 20%-25% for Amikacin, and 71%-44% for Gentamicin [15].

Aminoglycosides has vital role as antipseudomonal chemotherapy, though, the resistant antipseudomonal aminoglycosides is present throughout the world. The resistant mechanisms typically results from inactivation of aminoglycosides by modifying enzymes encoded on plasmid or chromosome harbored by resistant strains as well as by other mechanisms including impermeability resistance (reduced aminoglycoside uptake and accumulation) [37].

For Fluoroquinolone class, the following resistivity were registered; 10%, 5.2% for ciprofloxacin, 13.3%, 12.3% for levofloxacin, and 13.3%-13% for ofloxacin, against clinical and environmental isolates respectively. The present study shows high efficacy of this class against P. aeruginosa isolates. However, these findings are different from those of a previous local study in Yemen, in which the resistant rate were 24% for ciprofloxacin, and 26.8% for levofloxacin [38]. On the other hand, in agreement with this study, a study in Australia showed that the resistant among clinical isolates were 9.1%for ciprofloxacin, 5.4% for levofloxacin, and 13% for ofloxacin [39].

Bacterial resistance toward fluoroquinolones can happen through various mechanisms, most important one are mutations in the gyrase (gyrA) and topoisomerase IV (parC) encoding DNA genes [40]. Generally, quinolones are considered a best choice for empirical therapy due to their reasonable cost and easy intake as oral forms [41]. Among quinolones, ciprofloxacin is preferred as the best antibiotic for treatment of P. aeruginosa infections.

The present study showed high resistant 100% in both clinical and environmental isolates to tetracycline, chloramphenicol and erythromycin.

This result is in agreement with a study in Iraq which detected 100% resistance rate [19]. This resistance is mainly due to high misuse because of constant and indiscriminate usage in environment [15].

In the present study, environmental isolates exhibited high resistance 67.1% when compared to the clinical isolates 41.3%. This may be related to the insufficient elimination of chemicals and antibiotics in the environment which may cause a selective pressure on bacteria and lead to transferring resistance genes from clinical to environmental strains [42].

MAR :

MDR defined as resistance to at least one antibiotic in three groups or more antimicrobial classes [43]. All isolates have MAR index greater than 0.2. This result indicates that the isolates obtained in this study were originated from high contaminated sources as illustrated from Table 4.

The results explained the link between nosocomial and environmental strains of P. aeruginosa, but failed to show a direct relationship between patients and environmental sources. For nosocomial isolates, the highest multi antibiotic resistance index was 0.954 and the lowest MAR index was 0.5. In the same context, the highest MARI for environmental isolates was 0.863 and the lowest MARI was 0.5 which differs from that of another work in South Africa (2021) that reported an index ranging from 0.08 and 0.69 from non-clinical environment [44].

In the current study, 90% of the nosocomial isolates and 96.4% of hospital environmental isolates were MDR. Similar results were observed in a study in Nepal (2012) where 89.4% MDR from clinical samples was registered [45], and another study in Colombia (2020) which reported 66.7% and 90.7% for clinical and environmental isolates respectively [42].

These results show that the problem of MDR is not only limited to the clinical isolates of P. aeruginosa, as environmental isolates also present with high MDR.

Conclusion

This study confirmed the presence of P. aeruginosa in various clinical and environmental sources. In addition, high resistance to various antimicrobial agents was encountered among the nosocomial and environmental isolates of P. aeruginosa and the sources were originated from high-risk sources of contamination. This study highlighted that environmental sources may have a significant role in the transmission of P. aeruginosa and spread of MDR P. aeruginosa among hospitalized patients.

References

1. Streeter K, Katouli M. Pseudomonas aeruginosa: A review of their pathogenesis and prevalence in clinical settings and the environment. Infect Epidemiol Med. 2016;2(1):25–32.
2. Khan NH, Ahsan M, Taylor WD and, Kogure K. Culturability and survival of marine, freshwater and clinical Pseudomonas aeruginosa. Microbes Environ. 2010;25(4):266–74.
3. Mielko KA, Jabłoński SJ, Milczewska J, Sands D, Łukaszewicz M, Młynarz P. Metabolomic studies of Pseudomonas aeruginosa. World J Microbiol Biotechnol. 2019;35(11):1–11.
4. Panga Z, Raudonisb R, Glickc B, Lina T-J, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnology Advances. 2019;37(1):177–92.
5. Turnidge J, Christiansen K. Antibiotic use and resistance–proving the obvious. The Lancet. 2005;365(9459):548–9.
6. Ferech M, Coenen S, Malhotra-Kumar S, Dvorakova K, Hendrickx E, Suetens C, *et al*. European surveillance of antimicrobial consumption (ESAC): outpatient antibiotic use in Europe. J Antimicrob Chemother. 2006;58:401–7.
7. Badulla WFS, Alshakka M, and Ibrahim MIM. Antimicrobial resistance profiles for different isolates in Aden , Yemen : A cross-sectional study in a resource-poor setting. Biomed Res Int. 2020;2020.
8. Bhuiya M, Sarkar MKI, Sohag MH, Ali H, Roy CK, Akther L et al. Enumerating antibiotic susceptibility patterns of Pseudomonas aeruginosa isolated from different sources in Dhaka City. Open Microbiol J. 2018;12:172–80.
9. EL-Shouny WA, Magaam S. Sensitivity of multi-drug resistant Pseudomonas aeruginosa isolated from surgical wounds infections to essential oils and plant extract. World J Med Sci. 2009;4(2):104–11.
10. Yacin N. 2009.Studies on Pseudomonas aeruginosa prevalent in the main hospitals in Sana'a City-Yemen. A thesis of M.Sc. Department of Biology, Faculty of Sciences, Sana'a University.
11. Brandon M. Procedure : microbiology specimen collection from Different Anatomical Sites. Dayt Child Hosp One Child Plaza Dayton, Ohio 45404. 2017;1–13.
12. Bhatia R, Ichhpujani RL. Essentials of medical microbiology, (4th ed.). New Delhi Jaypee Brother Med Publ.

2008;46–7.
13. Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing: twenty-fifth informational supplement. CLSI document M 100-S25. USA: clinical and laboratory standards institute, 950 west valley road, suite 2500, Wayne, Pennsylvania 19087 USA; 2015. 1–240 p.
14. Chika EO, Nneka AR, Dorothy ON, Chika E. Multi-drug resistant *Pseudomonas aeruginosa* isolated from hospitals in Onitsha , south-eastern Nigeria. Int Arch Biomed Clin Res. 2017;3(3):7–12.
15. Isichei-Ukah O, Enabulele O. Prevalence and antimicrobial resistance of Pseudomonas aeruginosa recovered from environmental and clinical sources in Benin city, Nigeria. Life J Sci. 2018;20(3):547–55.
16. Ali MDY, Abdulrahman ZFA. Molecular identification, susceptibility pattern, and detection of some virulence genes in Pseudomonas aeruginosa isolated from burn patients. Plant Arch. 2020;20(1):2573–80.
17. Marquart M, Caballero A, Chomnawang M et al. Identification of a novel secreted protease from Pseudomonas aeruginosa that causes corneal erosions. Investig Ophthalmol Vis Sci. 2005;46(10):3761–8.
18. ALshaiki JMM, Toweir AA. Prevalance Pseudomonas aeruginosa among Libyan patients and its association with hospital's environment in Benghazi. J Med Microbiol Diagnosis J. 2017;6(2):1–8.
19. Haleem H, Kadhim Tarrad J, Abbas Banyan I. Isolation of Pseudomonas aeruginosa from clinical cases and environmental samples, and analysis of its antibiotic resistant spectrum at Hilla teaching hospital. Med J Babylon. 2011;8(4):618–24.
20. Tajeddin E, Rashidan M, Razaghi M, Javadi SSS, Sherafat SJ, Alebouyeh M et al. The role of the intensive care unit environment and health-care workers in the transmission of bacteria associated with hospital acquired infections. J Infect Public Health. 2016;9(1):13–23.
21. Ayliffe GAJ, Collins BJ, Babb JR, Lowbury EJL, Newsom SWB. Pseudomonas aeruginosa in hospital sinks. The Lancet. 1974;304(7880): 578-581.
22. Matz C, Moreno A, Alhede M, Manefield M, Hauser A, Givskov M, *et al*. Pseudomonas aeruginosa uses type III secretion system to kill biofilm-associated amoebae. ISME J. 2008;2:843–52.
23. Strateva T, Ouzounova-Raykova V, Markova B, Todorova A, Marteva-Proevska Y, Mitov I. Problematic clinical isolates of Pseudomonas aeruginosa from the university hospitals in Sofia, Bulgaria: Current status of antimicrobial resistance and prevailing resistance mechanisms. J Med Microbiol. 2007;56(7):956–63.
24. Strateva T, Yordanov D. Pseudomonas aeruginosa - A phenomenon of bacterial resistance. J Med Microbiol. 2009;58(9):1133–48.
25. Abbas HA, El-Ganiny AM, Kamel HA. Phenotypic and genotypic detection of antibiotic resistance of Pseudomonas aeruginosa isolated from urinary tract infections. Afr Health Sci. 2018;18(1):11–21.
26. Thomas B, Adebayo O, Makanjuola S, Davies A.

Extended spectrum beta lactamases producing Pseudomonas aeruginosa expressing Amp. Int J Microbiol Res. 2015;6(2):90–4.
27. Shah D, Wasim S, Abdullah F. Antibiotic resistance pattern of Pseudomonas aeruginosa isolated from urine samples of urinary tract infections patients in Karachi, Pakistan. Pakistan J Med Sci. 2015;31(2):341–5.
28. Oni A, Nwaorgu O, Bakare R, Ogunkunle M, Toki R. The discharging ears in adults in Ibadan, Nigeria causative agents and antimicrobial sensitivity pattern. African Journal of Clinical & Experimental Microbiology. 2002;3(1).
29. Obritsch M, Fish D, MacLaren R, Jung R. National surveillance of antimicrobial resistance in Pseudomonas aeruginosa isolates obtained from intensive care unit patients from 1993 to 2002. Antimicrob Agents Chemother. 2004;48(12):4606–10.
30. Montero M, Sala M, Riu M, Belvis F, Salvado M, Grau S *et al*. Risk factors for multidrug-resistant Pseudomonas aeruginosa acquisition. Impact of antibiotic use in a double case-control study. Eur J Clin Microbiol Infect Dis. 2010;29(3):335–9.
31. Al-haik WM, Almahdi A, Al-haddad AM. Genotypic characteristics of clinical and non-clinical isolates. Jordan J Biol Sci. 2016;9(3):185–94.
32. Gad GF, El-domany RA, Zaki S, Ashour HM. Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia , Egypt : prevalence , antibiogram and resistance mechanisms. J Antimicrob Chemother. 2007;60:1010–7.
33. Onuoha SC, Okafor CO, Aduo BC, Nwaka FC. Distribution of antibiotic resistant bacteria from abattoir wastes and its receiving waters at nkwo-ezzamgbo, Ebonyi state, Nigeria. World J Med Sci. 2016;13(4):242–50.
34. Vitkauskienė A, Skrodenienė E, Dambrauskienė A, Bakšytė G, Macas A, Sakalauskas R. Characteristics of carbapenem-resistant Pseudomonas aeruginosa strains in patients with ventilator-associated pneumonia in Intensive Care Units. Med. 2011;47(12):652–6.
35. El-Mahdy R, El-Kannishy G. Virulence factors of carbapenem-resistant Pseudomonas aeruginosa in hospital-acquired infections in Mansoura, Egypt. Infect Drug Resist. 2019;12:3455–61.
36. Mahmoud A, Zahran W, Hindawi G, Labib A, Galal R. Prevalence of multidrug-resistant Pseudomonas aeruginosa in patients with nosocomial infections at a university hospital in Egypt, with special reference to typing methods. J Virol Microbiol. 2013;2013:1–13.
37. Dowling A, Dwyer J, Adley C. Antibiotics : mode of action and mechanisms of resistance . Antimicrob Res Nov bioknowledge Educ programs. 2017;536–45.
38. Alyahawi A, Alhomidi A, Al-Henhena N. Prevalence of Pseudomonas aeruginosa and antimicrobial susceptibility patterns at a private hospital in Sana'a, Yemen. Univers J Pharm Res. 2018;3(4):12–8.
39. Farvo F. Clinical and experimental overview of mechanisms of antibiotic resistance in Pseudomonas

aeruginosa : an ocular perspective. Clin Exp Optom. 2018;101(2):162–71.
40. Jalal S, Ciofu O, Hoiby N, Gotoh N, Wretlind B. Erratum: Molecular mechanisms of fluoroquinolone resistance in Pseudomonas aeruginosa isolates from cystic fibrosis patients. Antimicrob Agents Chemother. 2000;44(5):1410.
41. Drago L, Vecchi E De, Mombelli B, Nicola L, Valli M, Gismondo MR. Activity of levofloxacin and ciprofloxacin against urinary pathogens. J Antimicrob Chemother. 2001;48(1):37–45.
42. Chávez M, Cabezas AF, Ferauds M, Castillo JE, Caicedo LD. Antimicrobial resistance patterns and genotypic diversity between clinical and water systems isolates of Pseudomonas aeruginosa in cali, Colombia. Trop Biomed. 2020;37(3):650–62.
43. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.
44. Hosu MC, Vasaikar S, Okuthe GE, Apalata T. Molecular detection of antibiotic-resistant genes in Pseudomonas aeruginosa from non-clinical environment: public health implications in Mthatha, eastern cape province, South Africa. Int J Microbiol. 2021;2021:1–9.
45. Bhandari S, Banjara MR, Lekhak B, Regmi SR. Multi-drug and pan-drug resistant Pseudomonas aeruginosa : A challenge in post-antibiotic era. Nepal J Sci Technol. 2012;13(2):197–202.