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# Prevalence and Antibigram Patterns of *Pseudomonas aeruginosa* Isolated from Two Wastewater Treatment Plants in Aden Governorate-Yemen

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## ABSTRACT

**Introduction:** *Pseudomonas aeruginosa* is a gram-negative rod-shaped obligatorily aerobic bacterium. This bacterium is most frequently associated with human infections and is regarded as an opportunistic pathogen, primarily causing nosocomial infections in immunocompromised patients. *P. aeruginosa* as a waterborne pathogen is a growing concern to public health sectors. Many sources of environmental water as well as in other sources such as sewage treatment plants could potentially be acting as a reservoir for potentially pathogenic strains of *P. aeruginosa*. Therefore, the aim of this study was to determine the prevalence, antibiogram pattern and the multiple antibiotics resistant (MAR) of *P. aeruginosa*.

**Methods:** The study was designed as a cross-sectional study, conducted between September 2022 and February 2023. A total of 193 wastewater samples, (97/193) & (96/193) were obtained from two region AL-Magari and Kaputa wastewater treatment plants (WWTPs), respectively. The wastewater samples were serially diluted and cultured on enrichment medium, then cultured on cetrimide agar as a selective medium for *P. aeruginosa*. The isolates were identified primarily based on cultural characteristics and then by biochemical tests. A panel of 32 antibiotics were used to determine the susceptibility patterns and the MAR.

**Results:** The prevalence of *P. aeruginosa* was 33.7 % (65/193), while the statistical data revealed no a statistically significant difference between the prevalence of *P. aeruginosa* isolates from AL-Magari and Kaputa ( $P>0.05$ ). The study revealed the presence of antipseudomonal agents among the wastewater isolates of *P. aeruginosa*. Ciprofloxacin antibiotic has the highest activity against *P. aeruginosa* strains with the lowest resistant rate (7.7%), followed by Levofloxacin, Ofloxacin and Piperacillin/Tazobactam, (12.3%), (18.5%), and (20%), respectively. The MAR index ranged between 0.562 to 1.

**Conclusion:** The study demonstrated that MAR *P. aeruginosa* were quite prevalent in the wastewater effluents of WWTPs in Aden governorate-Yemen with no statistically significant difference in resistant pattern between wastewater isolates from AL-Magari and Kaputa region to the antibiotics used in this study ( $P>0.05$ ); and this can lead to serious health risk for community, and natural vegetation where plants grow.

**Keywords:** *Pseudomonas*, multiple-antibiotic-resistance, wastewater treatment plants, AL-Magari and Kaputa regions.

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## Introduction

*Pseudomonas* species are adaptable and colonized a wide range of ecological habitats worldwide, including water, sewage, soil, plants, and animals [1]. *P. aeruginosa* is opportunistic pathogen that are frequently linked to infections of the urinary tract, respiratory system, soft tissue, bone and joints, gastrointestinal infections, dermatitis, bacteremia, and a variety of systemic infections, especially in people who have had severe burns, cancer, or AIDS [2].

Although Pseudomonads are not typically thought of as waterborne pathogens, recent data indicate that water systems are increasingly becoming the pathogens' preferred interface. *Pseudomonas* species have been linked to a number of waterborne epidemics, particularly those connected to the usage of recreational waters [3]. Wastewater is defined as any water that has been used such as for domestic (sewage wastewater) or industrial (non-sewage wastewater) use and contains waste products of potential contaminants and concentrations [4]. The principal microbiological species present in wastewater include bacteria, protozoa, viruses, fungus, algae, and helminthes. Diseases spread as a result of the majority of these organisms being present in water [5].

In context, wastewater generated after domestic use is a large source of pathogenic organisms which can give rise to potential hazard to the health of humans, animals and plants. The health risk associated with wastewater pose the greatest threat to public health; especially when the untreated wastewater is used for agricultural purpose [6]. Farmers in Yemen living near the large cities such as Sana'a, Aden, Taiz, and Ibb are already reuse of untreated or partially treated wastewater for agriculture purposes which may increase the risk of transfer of the pathogenic organisms to plants, animal and human and make various infectious diseases [7].

One of the most frequent effect of war disasters was their impact on health, which is directly tied to the water and sanitation crises that spread after such catastrophes [8]. In recent years and due to war of 2015 in Yemen, the WWTPs in Aden governorate act as pools to collect the wastewater without treatment (Figure 1). In Yemen very few investigations have been conducted on the *P. aeruginosa* and distribution of such bacteria and their antibiogram profile. Thus, this study was therefore designed to investigate the prevalence, and antibiogram profiles of *P. aeruginosa* isolated from untreated wastewater effluents of two wastewater treatment plants in the Aden governorate of Yemen.



(A) AL-Magari at Al-Alam area.



(B) Kaputa at Kaputa area.

**Figure 1:** (A) AL-Magari and Kaputa wastewater treatment plants.

## Methods

### Study design and area

A cross sectional study was carried out over a 6-months period from September 2022 up to February 2023 from two WWTPs (AL-Magari and Kaputa areas) in Aden governorate.

### Collection of samples

A total of 193 Wastewater samples (97 from AL-Magari area and 96 from Kaputa area) were collected on a weekly based, from WWTPs in Al-Magari region that locate near Al-Alam area in the east of Aden and Kaputa area in Northwest of the Aden governorate. They discharge untreated effluent into the environment which contains various toxins, poisons, chemical and industrial effluent of factories. Wastewater sample was collected in sterile glass bottles of 250 ml volume and then transported to the laboratory in a cooler box with ice for analysis within 6 hours of collection.

### Processing of samples

Untreated wastewater samples were serially diluted with distilled water ( $10^{-1}$  -  $10^{-3}$ ) to reduce the load of organisms in wastewater sample. One ml from each dilution was dispensed into glass bottle containing 5 ml of tryptone soy broth (Himedia, India) as enrichment medium and incubated at 37° C for 24 h. In addition, aliquot of 100 µl from the enrichment broth was spread on cetrimide agar (Himedia, India) as a selective medium for *Pseudomonas* and incubated for 18 – 24 h in an inverted position.

### Identification of *P. aeruginosa*

The primary identification of *P. aeruginosa* isolates was performed depending on growth

in selective media with colony morphology (shape of colony, texture, color, odor and edges), gram staining and biochemical tests such as; oxidase, catalase, Methyl Red-Voges Proskauer (MR-VP), urease, Sulfide-Indole-Motility-test (SIM), Kligler's Iron Agar (KIA) and citrate utilization test were done and interpreted according to Bergey's manual of determinative bacteriology [9]. All isolates were examined for growth at 42° C.

### Antimicrobial susceptibility test

The antibiotic sensitivity tests of isolates were carried out following procedures of modified Kirby-Bauer disc diffusion as recommended by the Clinical Laboratory Standard Institute (CLSI 2015) [10]. After the incubation period, the zones of inhibition were measured and compared with zone diameter interpretative chart to determine susceptibility of the isolates to antibiotics.

### Antibiotics used in the Study

The antibiotics used in this study were panels of 32 antibiotics belonging to different groups obtained from (Himedia); Chloramphenicol **C** 30µg, Tetracycline **TET** 30µg, Doxycycline **DO** 30µg, Erythromycin **E** 15µg, Moxifloxacin **MO** 5µg, Ciprofloxacin **CIP** 5µg, Levofloxacin **LE** 5µg, Ofloxacin **OF** 5µg, Norfloxacin **NX** 10µg, Ampicillin/cloxacillin **AX** 10µg, Co-trimoxazole **COT** 25 µg, Augmentin **AMC** 30µg, Piperacillin/tazobactam **PIT** 100/10 µg, Ticarcillin/Clavulanic acid **TCC** 75/10 µg, Cefpirome/Clavulanic (CPC), Amoxicillin **AMX** 10 µg, Ampicillin **AMP** 10 µg, Azlocillin **AZ** 75µg, Penicillin G **P** 10µg, Cefradine **CH** 25µg, Cefadroxil **CFR** 30µg, Cefuroxime **CXM** 20 µg, Ceftizoxime **CZX** 30µg, Cefoperazone **CPZ** 75



µg, Ceftazidime **CAZ** 30µg, Ceftriaxone **CTR** 30µg, Meropenem **MRP** 10µg, Imipenem **IPM** 10µg, Aztreonam **AT** 30µg, Tobramycin **TOB** 10µg, Gentamicin **GEN** 10µg, and Amikacin **AK** 30µg.

### Determination of Multiple Antibiotic resistances (MAR) Index

MAR index was calculated as described by Blasco et al. (2008) [11] as follows:  $MAR = a/b$  where a = number of antibiotics to which the isolate was resistant; b = total number of antibiotics against which individual isolate was tested.

### Quality Control

A standard bacteriological technique was applied to maintain accurate laboratory test results.

### Statistical Analysis of Data

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

## Results

### Isolation of *P. aeruginosa*

The present study shows that the prevalence of *P. aeruginosa* in wastewater was 33.7 % (65/193). The prevalence of *P. aeruginosa* in wastewater collected from AL-Magari region & Kaputa region was 40.2% & 27.1%, respectively, as seen in Table (1). However, there was no statistically significant difference between wastewater isolates from both regions ( $P > 0.05$ ).

**Table 1: Prevalence of *P. aeruginosa* from wastewater**

Site of Collection	No. of Sample Examined	No. of Positive Isolate
Wastewater	AL-Magari	97 (39/97) 40.2%
	Kaputa	96 (26/96) 27.1%
<b>Total</b>	<b>193</b>	<b>(65/193) 33.7%</b>

### Antimicrobial Susceptibility of *P. aeruginosa* Isolates

In the present study, data revealed a high resistant to the most groups of antibiotics under study. All isolates obtained from both regions were completely resistant 65(100%) to Tetracycline, Doxycycline, Chloramphenicol, Penicillin G, Amoxicillin, Ampicillin, Cefuroxime, Cefadroxil, Cefradine, Ampicillin/cloxacillin, Augmentin, and Erythromycin, as shown in Table (2). The total resistance of Ceftriaxone was (95.4%), followed by Ceftizoxime & Aztreonam which was (90.8%). The total resistance of Ticarcillin/ Clavulanic acid, Co-trimoxazole, Meropenem & Imipenem was same (87.7%), while the total resistance of Azlocillin, Ceftazidime and Cefpirome/ Clavulanic acid was (81.5%), (80%) and (67.7%), respectively.

The total resistance of Aminoglycosides groups such as Tobramycin, Gentamicin and Amikacin was; (52.3%), (46.2%), & (36.9%), respectively. The lowest total resistance in descending manner for Moxifloxacin, Norfloxacin, Pipracillin/ Tazobactam, Ofloxacin, Levofloxacin and Ciprofloxacin was



as following; (32.3%), (23.1%), (20%), (18.5%), (12.3%) & (7.7%), respectively. The isolates were most sensitive to Ciprofloxacin, Levofloxacin, Ofloxacin, Pipracillin/tazobactam, and Norfloxacin; (92.3%), (87.7%), (81.5%), (80%), (76.9%), respectively.

**Table 2:** The Antibiotic Resistance Pattern of *P. aeruginosa* Isolated from Wastewater

Antibiotics	Symbol	(1) N (39)	(2) n (26)	Total Resistance n (%)
Tetracycline	TET	39(100%)	26(100%)	65(100%)
Doxycycline	DO	39(100%)	26(100%)	65 (100%)
Chloramphenicol	C	39(100%)	26(100%)	65(100%)
Penicillin G	P	39(100%)	26(100%)	65(100%)
Amoxicillin	AMX	39(100%)	26(100%)	65(100%)
Ampicillin	AMP	39(100%)	26(100%)	65(100%)
Cefuroxime	CXM	39(100%)	26(100%)	65(100%)
Cefadroxil	CFR	39(100%)	26(100%)	65(100%)
Cefradine	CH	39(100%)	26(100%)	65(100%)
Ampicillin/Cloxacillin	AX	39(100%)	26(100%)	65(100%)
Augmentin	AMC	39(100%)	26(100%)	65(100%)
Erythromycin	E	39(100%)	26(100%)	65(100%)
Ceftriaxone	CTR	36(92.3%)	26(100%)	62(95.4%)
Ceftizoxime	CZX	35(89.7%)	24(92.3%)	59(90.8%)
Aztreonam	AT	36(92.3%)	23(88.5%)	59(90.8%)
Ticarcillin/Clavulanic acid	TCC	35(89.7%)	22(84.6%)	57(87.7%)
Co-trimoxazole	COT	31(79.5%)	26(100%)	57(87.7%)
Meropenem	MRP	31(79.5%)	26(100%)	57(87.7%)
Imipenem	IPM	31(79.5%)	26(100%)	57(87.7%)
Azlocillin	AZ	31(79.5%)	22(84.6%)	53(81.5%)
Ceftazidime	CAZ	31(79.5%)	21(80.8%)	52(80%)
Cefpirome/Clavulanic acid.	CPC	26(66.7%)	18(69.2%)	44 (67.7%)
Tobramycin	TOB	21(53.8%)	13(50%)	34(52.3%)
Gentamicin	GEN	20(51.3%)	10(38.5%)	30(46.2%)
Amikacin	AK	14(35.5%)	10(38.5%)	24(36.9%)
Cefoperazone	CPZ	12(30.8%)	11(42.3%)	23(35.4%)
Moxifloxacin	MO	11(28.2%)	10(38.5%)	21(32.3%)
Norfloxacin	NX	6(15.4%)	9(34.6%)	15(23.1%)
Pipracillin/Tazobactam.	PIT	5(12.8%)	8(30.8%)	13(20%)
Ofloxacin	OF	7(17.9%)	5(19.2%)	12(18.5%)
Levofloxacin	LE	4(10.3%)	4(15.4%)	8(12.3%)
Ciprofloxacin	CIP	2(5.1%)	3(11.5%)	5(7.7%)

(1); Wastewater from AL-Magari, (2); Wastewater from Kaputa.

**Multiple Antibiotic Resistances (MAR)**

All isolates were resistant to at least 18 Antibiotics and have MAR index ranged between 0.562 to 1 that is greater than (0.2) indicating high contaminated sources and possible transmission of infection Table (3).

**Table 3:** Multiple Antibiotic Resistance (MAR)-Index of *P. aeruginosa* Isolated.

MAR Index	No. of Antibiotic Resistance	No. of Isolates %
1	32	1
0.968	31	2
0.906	29	2
0.875	28	1
0.843	27	3
0.812	26	2
0.781	25	8
0.75	24	8
0.718	23	11
0.687	22	8
0.656	21	14
0.625	20	1
0.593	19	3
0.562	18	1
		65

**Discussion**

The present study shows that the prevalence of *P. aeruginosa* in wastewater was 33.7%, this agree with a local study in Ibb-Yemen who reported 36.6% from raw sewage [7] and disagree with a study in South Africa who reported 6.25%. [12]. In Yemen, According to an unpublished report by parliament’s water and environment committee 2006, 75% of population at high risk of waterborne disease [13].

In our province-Aden, the poor sanitation and the poor quality of sewage effluent from sewage treatment plants further to the stop of these plants due to the war are the main



causes of polluted water. As these stations do not treat wastewater after the war of 2015 and act only as pools to collect wastewater, further to their near locations from houses where they are only a few meters from human activities. Moreover, the 65 isolates represent only the number of *P. aeruginosa* isolate, the remaining specimens also revealed a high content of various bacteria, so they represent a risk source for dissemination of pathogens and represent a challenge for health care. As water is essential for life, over 100 different types of enteric pathogens including fungi, viruses, bacteria and parasites may be found in wastewater and sludge and can cause waterborne diseases [14]. A study was conducted in Colombia showed that *P. aeruginosa* isolated from environmental sources have highly conserved genome, that enable this organism to inhabit various environments and confers the capacity to cause human infection. In addition, the presence of *P. aeruginosa* in water is not affected by changes in temperature or levels of water [15].

### Resistance Level within the Classes of Antibiotics

In the present study, all *P. aeruginosa* isolates showed high resistant to  $\beta$ -lactams antibiotics where Penicillin G (100%), Amoxicillin (100%), Ampicillin (100%), and Azlocillin (81.5%). These results were corresponded to a study done in Iraq [16] who reported (100%) resistance for *P. aeruginosa* Penicillin and Ampicillin in environmental isolates of *P. aeruginosa*.

The current result was agreed with a study conducted in Minia, Egypt [17] where resistance to Ampicillin and Amoxicillin was (100%), and a study was conducted in Sofia,

Bulgaria; reported (91.6%) resistant rate to Azlocillin somewhat greater than our result [18].

The high resistance of *P. aeruginosa* to  $\beta$ -lactam antibiotics can be explained by many mechanisms. The most important mechanism of Penicillin resistance is the formation of bacterial enzymes such as  $\beta$ -lactamases which is the most important and catalyze the hydrolytic activity of  $\beta$ -lactam ring of Penicillins to produce inactive penicilloic acids [19]. Another mechanisms to resist Penicillins include decrease permeability to penicillins and alteration the number or nature of porins in cell envelope and efflux pump system [20].

### Regarding Carbapenems

The two antibiotics which are used in this study were Imipenem and Meropenem. The resistant of *P. aeruginosa* isolates towards Imipenem and Meropenem was same (87.7%). This result is disagree with a local study in Sana'a-Yemen that reported (5.4%) of isolate from non-clinical sources were resistant to Imipenem [21], and disagree with a study conducted in Egypt that reported (46%) of environmental *P. aeruginosa* isolates were resistant to Meropenem [17].

Furthermore, in agreement to our finding, a study in Lithuania revealed that resistant to Carbapenems has increased dramatically for Imipenem from (53.3%) in 2003 to (87.8%) in 2005, which is online with our finding [22].

### For Monobactam class

Our results showed (90.8%) resistant rate of *P. aeruginosa* isolates for antibiotic Aztreonam. This finding was greater than a study in Egypt where the resistant rate was (82.5%) for Aztreonam [23]. However, this finding is



different from those of a previous study in Yemen in which the resistant rate was (23.2%) for Aztreonam [21].

### Cephalosporins

Eight antibiotics belonging to different generations of cephalosporins were used in this study (Cefpirome is discussed in the combination therapy). The first generation include: Cefradine and Cefadroxil; Second generation: Cefuroxime, and the third generation which comprises Cefoperazone, Ceftizoxime, Ceftazidime and Ceftriaxone.

In present study, most *P. aeruginosa* isolates exhibit high resistant rates to these antibiotics with the exception of Cefoperazone which reveal moderate resistance rate (35.4%) in contrast to (90.8%) for Ceftizoxime, (80%) for Ceftazidime, (95.4%) for Ceftriaxone, and (100%) for Cefuroxime, Cefradine and Cefadroxil.

Similar results about Cefoperazone against *P. aeruginosa* were obtained by a study in Egypt [24]. A study in Ethiopia about Ceftriaxone who detected (100%) resistance rate [25], and a study in Nigeria who obtained (91.8%) and (90.5%) for Ceftizoxime and Ceftazidime respectively [26]. High resistance rate present in the current study against first generation of cephalosporins, likewise previous study in Sudan showed that (80%) of isolates were resistant and the others were moderately sensitive to Cefradine [27]. Another study in Pakistan found that resistance rate for Cefradine was (99.2%) which is in agree with our results [28]. For Cefadroxil, the result was similar to that obtained by a study in Egypt who found (100%) resistance rate for this

antibiotic [29]. This high resistant value to Cephalosporins may be partially due to the high production of ESβLs in the isolates.

### Antibiotic Combination therapy

The combined use of two antibiotics has been a popular method for controlling infectious diseases. All combination therapy used in this study did not show high efficacy against *P. aeruginosa* isolates except for Pipracillin/Tazobactam where the resistance rate was (20%), in contrast to Augmentin, and Ampicillin/Cloxacillin was; (100%), Co-trimoxazole, Ticarcillin/Clavulanic acid having the same rate of resistance (87.7%), Cefpirome/Clavulanic acid was (67.7%). This results did not corroborate with previous study in Egypt who found high resistance (94.7%) for Pipracillin/Tazobactam [23]. In agree with the present result, a study in Nigeria reported (23%) resistance rate to Pipracillin/tazobactam [26].

For Amoxicillin/Clavulanic acid, the findings showed full resistance (100%), which is in agree with a study done in Sana'-Yemen who detected (96.2%) [30], also our data was in agree with a study in Erbil-Iraq who detected (100%) resistance rate for Amoxicillin/Clavulanic acid [31]. For Ticarcillin/Clavulanic, we revealed high resistance rate (87.7%) which was approach to a study [32] who reported (55%) resistance rate, while they differed from a study in Ghana that reported (14%) resistant rate [33]. The resistant of Ampicillin/cloxacillin towards environmental isolates in our present study was (100%) which is agree with a study in Nigeria that is (100%) resistant to



Ampicillin/cloxacillin in clinical isolates [33]. For Cefpirome/Clavulanic, the results showed (67.7%) from environmental isolates was resist the Cefpirome/Clavulanic. This result is disagree with a study in Philippines who found 5% resistance rate for Cefpirome [34].

In case of Co-trimoxazole, the resistance rate of isolates was (87.7%). This result was similar to our study [35] in which the resistance rate was (87%). Analysis of results indicated that environmental isolates exhibit high resistant to most combination antibiotics used in this study, however, Pipracillin/Tazobactam revealed the lowest resistance (20%) which make it still a choice for treatment of infections caused by *P. aeruginosa*. A study carried out over 10 year period from 1993 to 2002 suggests about increasing the resistance of *P. aeruginosa* against combination antibiotics [36].

The high resistance present in the present study can be explained as a result of high producing of Metallo- $\beta$ -Lactamases (MBLs) in which many studies associated between MBLs producing organisms and high resistant to  $\beta$ -lactam, extend spectrum cephalosporins and  $\beta$ -lactamase inhibitor combination [37].

### For Aminoglycosides

In the current study, three antibiotics were used in this class; Amikacin, Gentamicin and Tobramycin. The resistant to these agents was (36.9%), (46.2%) and (52.3%), respectively. There were variable data about Aminoglycoside action. In the present study, the results were nearly incorporated with a study in Pakistan where the authors concluded that (25.3%), (35.3%) and (58.4%) of *P.*

*aeruginosa* isolates were resistant to Amikacin, Gentamicin and Tobramycin respectively [28], and a study in Nigeria conducted on environmental isolates of this pathogen exhibit different resistant rate where the resistant was (25%) for Amikacin, (44%) for Gentamicin, and (64%) for Tobramycin [35].

Aminoglycosides has vital role as Antipseudomonal chemotherapy. The resistant of antipseudomonal aminoglycosides such as Amikacin, gentamicin and tobramycin is present throughout the world. The resistant mechanisms typically resulted from inactivation of aminoglycosides by modifying enzymes encoded on plasmid or chromosome harbored by resistant strains [38], as like as by other mechanisms including impermeability resistance (reduced aminoglycoside uptake and accumulation) [39]. The geographical differences in resistant pattern of Aminoglycosides as like as with any antimicrobial agents has been reflect the differences in Aminoglycoside prescription patterns and/or the quality of infection control practices [40].

### For Fluoroquinolone class

Five antibiotics were included in our study with the following resistivity were registered; (7.7%) for Ciprofloxacin, (12.3%) for Levofloxacin, (18.5%) for Ofloxacin, (32.1%) for Norfloxacin, and (32.3%) for Moxifloxacin against environmental isolates. These findings show high efficacy of this class against *P. aeruginosa* isolates, however, these findings are differ from those of a previous local study in Yemen, in which the resistant rate were (24%) for Ciprofloxacin, (26.8%) for Levofloxacin, (44%) for Moxifloxacin, and (54%) for Norfloxacin [30]. On the other hand,





in agreement with current study, a study in Australia showed that the resistant among clinical isolates were (9.1%) for Ciprofloxacin, (5.4%) for Levofloxacin, (27.1%) for Moxifloxacin, and (13%) for Ofloxacin [41].

Bacterial Resistance toward fluoroquinolones can be occurred through various mechanisms, most important one was mutations in the gyrase (*gyrA*) and topoisomerase IV (*parC*) encoding DNA genes [42]. Generally, quinolones are considered a best choice for empirical therapy this is due to their reasonable cost and easy intake as oral forms [43]. Furthermore, among quinolones, Ciprofloxacin is preferred as a best antibiotic for treatment of *P. aeruginosa* infections. Our study showed high resistant (100%) in the environmental isolates to Tetracycline, Doxycycline, Chloramphenicol and Erythromycin. This result is agree with a study in Egypt who obtained (100%) resistance isolates for Tetracycline [23], and is disagree with a study in Bangladesh who found (28.57%) resistant to Tetracycline [44], and disagree with a study in Erbil-Iraq who found (60%) resistance for Doxycycline [45].

This resistance is mainly due to highly misused, constant and indiscriminate usage in the environment [35], and intrinsic and acquired resistance mechanism by an active efflux pump system which lead to remove the antibiotic out the cell as a result of expression of the *mexR* efflux gene [46]. For Chloramphenicol, all clinical and environmental isolates of *P. aeruginosa* showed complete resistance (100%). This result agree with a study in Egypt who found (100%) for environmental isolates

[17], but disagree with a study in Nigeria who reported (83%) resistance rate from environmental isolates [47]. The resistance of *P. aeruginosa* to chloramphenicol is usually intrinsically, in part due to the MexAB-OprM efflux system [48]. In addition, all isolates exhibit a full resistance (100%) to erythromycin, this results agree with a study in India who detect (100%) resistance from clinical isolate [49], and disagree with result obtained by a study [50] who found (60.50%) of isolates were sensitive to erythromycin.

The results of the present study revealed the presence of resistance to various antimicrobial agents including antipseudomonal agents among the environmental isolates of *P. aeruginosa*. In the present study, Ciprofloxacin antibiotic has the highest activity against *P. aeruginosa* strains with the lowest resistant rate (7.7%) followed by Levofloxacin (12.3%), Ofloxacin (18.5%) and Piperacillin/Tazobactam (20%), respectively. In this study, environmental isolates were highly resistance to the antibiotics. 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 transfer resistance genes from clinical to environmental strains [15].

### Multiple Antibiotic Resistance (MAR)

All the tested isolates in this study showed MARs ranging from 18 to 32 antibiotics. In this study, MARs-indices varied from 0.562 to 1 which is higher than the 0.2 limit in all tested isolates. This correlate with study in Nigeria that reported an index ranging from 0.5 to 1 [51]. Bacterial resistance to multiple



antimicrobial drug classes has been linked to two main intrinsic mechanisms: (i) overexpression of multidrug efflux pumps, which tend to pump out antibiotics before the antibiotics have a chance to act on their target, and (ii) mutations in outer membrane porins that reduce their permeability to antimicrobials. MARs bacterial strains may also develop as a result of unrelated mechanisms building up in an organism over time [52]. MAR index ranges from 0.562 to 1.0 which is very high as shown in Table (3), This finding suggests a higher risk of contamination and plasmid-mediated resistance in areas where antibiotic usage was common (abuse of antibiotics).

## Conclusion

This study demonstrated that MARs *P. aeruginosa* were prevalent in wastewater stations in Aden governorate-Yemen. Since the emergence of MAR *P. aeruginosa* is a public health issue, the current results are supporting the need for regular and consistent monitoring of municipal sewage effluents with a view to preventing the dissemination of these pathogens into the environment.

## Conflict of Interest

The authors declare that no conflict of interest.

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