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RESEARCH ARTICLE

Characteristics of *Pseudomonas aeruginosa* Multidrug Resistance in Clinical Isolates to Selected Antibiotics in Dhamar, Yemen

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Abstract

A total of 93 clinical samples were collected. About 20% of the samples were collected from ENT infected patients. The other samples were obtained from Dhamar General Hospital Authority. There were 20 isolates of *P. aeruginosa* giving prevalence level (21.5%) most of them from wound and ear infections. Three isolates obtained from patients who had been admitted to a hospital for a casualty during the preceding weeks of isolation. Based on the inhibition zone diameter, the result shows that *P. aeruginosa* shows highest sensitivity toward piperacillin (73.7%), ciprofloxacin (57.9%) and amikacin (47.4%). On the other hand P. aeruginosa is completely resistant to the antimicrobial effect of ampicillin, tetracycline. All isolates produced beta-lactamase enzymes detected by iodometric method test. These results were in very good agreement with previously reported findings in international journals.

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Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the successful opportunistic Gram-negative bacteria that pose a major health problem due to failure of treatment with time. Although many antibiotic agents are being developed continuously, *P. aeruginosa* tend to resist many of these agents due to its genetic capacity to evolve a number of resistance mechanisms.

Materials and methods

Materials

Sample collection: Clinical samples from infected patients were collected at Dhamar General Hospital Authority and form out-patients by Dr. Nabeel Maiyas clinic. Isolates were sent for labeling and storage and analysis at the Microbiology Laboratory, College of Applied Science, Dhamar University.

Media, Reagents and Disks: All media (except the Pseudomonas Agar), antibiotic (disks, and powder), Petri dishes, glassware, and reagents were privately purchased from Medical Appliances suppliers in Sana'a.

Isolation media: The primary isolation media used in this study were blood agar and a highly-selective medium for Pseudomonas spp. Pseudomonas Agar for Pyocyanin (Sigma-Aldrich, Germany).

Blood agar is a nutrient agar- based medium with addition of 5% blood. The dehydrated powder of the nutrient agar processed for preparation and sterilization as recommended by the manufacturer (HiMedia, India), and poured in plastic Petri dishes. Storage in the refrigerator does not exceed 8 day as it recommended by American Society for Microbiology guidelines. The broth macrodilution method is applied in this project.

The Pseudomonas Agar for Pyocyanin dehydrated powder prepared by following the manufacturer instructions and stored in a refrigerator up to 5 - 8 days only. Formula of the medium:

Formula / Liter

Enzymatic Digest of Gelatin ..... 20 g
Magnesium Chloride..... 1.4 g
Potassium Sulfate ..... 10 g
Irgasan (Triclosan)..... 0.025 g
Agar ..... 13.6 g

Supplement / Liter

Glycerol…….. 20 mL

Enzymatic digest of gelatin provides nitrogen, vitamins, and carbon in Pseudomonas Isolation Agar. Magnesium chloride and potassium sulfate promote production of pyocyanin. Triclosan, an antimicrobial agent, selectively inhibits Gram-positive and Gram-negative bacteria other than Pseudomonas spp. by inhibiting an enzyme involved in lipid biosynthesis (Tortora et al., 2010). Glycerol serves as an energy source. Agar is the solidifying agent.

Selecting of antimicrobial agents for testing

Several principles should be followed in order to select the antibacterial agents to be tested: (i) the widely used antibiotics should be included; (ii) the species to be tested, and (iii) the availability of the antibacterial agent (Turnidge et al., 1999). Parameters of choosing a testing method and antibacterial agent can be found in the Clinical and Laboratory Standards Institute (CLSI).This system has been followed in this project. The selected antimicrobial agents have been used are given in table 1.

Table1. Antibiotics used in this project and their strength and abbreviation

Antibiotic	Strength (µg)	Abbreviation
Amikacin	30	Ak
Ampicillin	10	Amp
Ceftazidime	30	Caz
Ciprofloxacin	5	Cip
Erythromycin	15	E
Gentamicin	10	Ge
Piperacillin	100	Pl
Tetracycline	30	Te

Methods

Isolation and Identification of *P. aeruginosa*

Most of the samples plated directly on Pseudomonas Agar for Pyocyanin medium after a transport time lesser than 5 hours (technically *P. aeruginosa* withstands such harsh circumstances and that may provide a selection step).Some specimens plated on blood agar to recover injured cells of *P. aeruginosa* from samples and sub cultured on Pseudomonas Agar for Pyocyanin medium for further selection of P. aeruginosa.

Incubation and obtaining of pure cultures

All the plates incubated aerobically for 24 hour at 37^{o}C then the colonies that match the description of *P. aeruginosa* colonies morphologies streaked on Pseudomonas Agar for Pyocyanin plates to obtain pure cultures and further incubation at 42^{o}C to eliminate the fluorescent group members, which are not capable of growth at 42^{o}C. Incubation in darkness up to 7 days was carried out to enhance pigmentation (Haleem et. al., 2011). The wrinkled colonies on Pseudomonas Agar excluded because they belong to other species of the genus that can grow on this medium (such as P. stutzeri, P. mendocina).

Identification

The identification of *P. aeruginosa* is not an easy work because of the biochemical and physiological similarities between species of this genus and varieties between strains of the same species also occur.

Non-pigmented strains cannot be identified properly in the college's poorly-equipped laboratory. However some non-pigmented isolates was identified by their colony morphology and some biochemical tests. These isolates can

only be identified by a reference laboratory where more extensive biochemical analysis and DNA-based methods are carried out. With such highly-selective media, Gram stain is not a valuable criterion in identification, however it was done according to the general method described by Forbes et al. (2007) and the slides showed a Gram-negative single bacilli and short chains. Blue-green color producing isolates subjected to oxidase test using Kovác's reagent (1% tetramethyl-p-phenylenediamine dihydrochloride) as an alternative of oxidase reagent (1% dimethyl-p-phenelynediamine hydrochloride) because it was unavailable as it cited by Murray et al., 2003. According to the Manual of Clinical Microbiology, catalase test have no valuable advantage in this case, because all pseudomonads are positive, however it was done as a way of double check. Growth on acetamide test, denitrification test, arginine dihydrolase test, and other tests could not be performed due to lack of materials in our laboratories. Attempts were done to get it from other laboratories belong to Dhamar University, but all efforts were unfortunately unsuccessful.
Pyocyanin production (blue-green pigment) on Pseudomonas agar and oxidase test was sufficient to identify *P. aeruginosa* grown on such selective medium (Holt et al., 1994). Growth on Kligler Iron Agar tubes showed no changed in color after 24 hours and medium color in tubes persisted red. Table 2 shows the biochemical tests panel used to identify *P. aeruginosa*.

Table 2: Identification characters of the isolates of *P. aeruginosa* on Pseudomonas Agar for Pyocyanin medium

Colonies diameter	Gram stain	Morphology	Catalase	Oxidase	KIA *	Pigments	Growth at 42ºC
1-5 mm	Negative	rod	+	+	R/R	B,G, BG or nil	+

*KIA= Kligler Iron Agar, R= red, B= blue, G= green, BG= Blue

Antibiotic sensitivity testing of the isolates

All the procedures in susceptibility testing were done according to the guidelines of Clinical Laboratory Standard Institute (CLSI) published in 2013. All the clinical isolates subjected to susceptibility test against the antibiotics shown in table 1. McFarland standard solution has been used to calibrate the turbidity of bacterial suspensions used in performing Kirby-Bauer method to achieve a turbidity equal to a suspension contains 1.5×10^8 CFU/ml (Cavalieri et al., 2005).

Performing the Kirby-Bauer method

Antibiotic disks have been used to perform the diffusion method on Mueller-Hinton (MH) agar medium (HiMedia Co., India) using 24-hour-old colonies grown on nutrient agar.
Bacterial suspension have been made in a grease-free cleaned glass tube containing 4ml distilled water and the turbidity had been adjusted to match McFarland's solution 0.5, comparison was done by comparing the two tubes at black background.
Cotton swab had been used to deliver a thin layer of bacteria on the surface of MH agar. Four disks applied to each 90 cm^2 plate and incubated immediately in ambient air at 35ºC. After 18 hours the inhibition, zones diameters had been measured by a scale and compared with breakpoint values of CLSI guidelines.

MIC determination

The MICs of tow antibiotics were determined by the broth macrodilution method, according to the CLSI guidelines (2013), on Antibiotic assay broth medium 3 (Becton Dickenson, USA). The breakpoint MIC of a drug is the highest concentration that can be safely attained in serum using the recommended dose. The MIC for a resistant organism surpasses the breakpoint MIC of the drug, and for that drug the risk of toxicity outweighs the potential benefits of therapy. Organisms are considered susceptible to a drug if the MIC is below the breakpoint MIC. Organisms characterized as intermediate if inhibited at concentrations that approach breakpoint. The isolate were

tested for only two antibiotics (Ciprofloxacin and Gentamicin) to determine their MICs because it is expensive and time-consuming to test MICs of all antibiotics included in the project.

Preparation of antimicrobial agents' solutions

Stock solutions CLSI' recommendations for solvent and diluent have been followed, and stock solutions of the antibiotics (Multiples of 1000µg/ml) were made according to the following formula:

$$\text{Weight of powder(mg)} = \frac{\text{Volume of solvent (ml)} \times \text{Concentation (µg/ml)}}{\text{Potency of powder(µg/g)}}$$

Working solutions: Preparation of working solutions of the tested antibiotics was made twofold dilutions according to the following formula and serial dilutions for small volumes of stock solutions to reach the desired concentration:

$$C_2(\mu\,g/ml) = \frac{C_1(\mu\,g/ml) \times V_1(ml)}{V_2(ml)}$$

Where C_1 is the concentration of stock solution, C_2 is the desired concentration, V_1 is volume taken from stock solution, and V_2 is final volume with the desired concentration. The range of concentrations included in the test was matched to those recommended by CLSI publications of 2013

Preparation of the broth medium

Antibiotic assay broth (Becton Dickenson, USA) has been used to determine the MICs values of the isolates. Medium prepared as recommended by the manufacturer. Each tube contained 1ml of the broth and specific amount of the antibacterial agent including the recommended ranges to be tested. Tubes were labeled with the concentration of antibiotic in each.

Inoculum and inoculation:

From agar plate, 24-hours-old colonies used to make the bacterial suspension containing approximately 1.5×10^8 CFU/ml (compared to McFarland standard solution 0.5) and 1ml of the bacterial suspension dispensed to each tube including the positive control tube (antibiotic-free) and another tube left free of bacteria as negative control. Then all tubes incubated at 35^{o}C (ambient air) for 20 hours as recommended by CLSI guidelines (2013).

Reading results:

All tubes checked for growth judged by unaided eye and the tubes contain the lowest concentration that prevented growth after 20 hours considered the MIC of the isolate. Based on the zone diameter and MIC, bacteria were classified resistant, intermediate or sensitive according to the American CLSI guidelines (2013) as given in table 3.

Table3. The American CLSI guidelines (2013) of *P. aeruginosa* Breakpoints*

Antibiotic	zone (mm)			MIC		
	sensitive	intermediate	resistant	sensitive	intermediate	resistant
Ampicillin	≥ 17	14-16	≤ 13	≤ 8	16	≥ 32
Amikacin	≥ 17	15-16	≤ 14	≤ 16	32	≥ 64
Ceftazidime	≥ 18	15-17	≤ 14	≤ 8	16	≥ 32
Ciprofloxacin	≥ 21	16-20	≤ 15	≤ 1	2	≥ 4
Erythromycin	≥ 23	14-22	≤ 13	≤ 0.5	1-4	≥ 8
Gentamicin	≥ 15	13-14	≤ 12	≤ 4	8	≥ 16
Piperacillin	≥ 21	15-20	≤ 14	≤ 16	32-64	≥ 128
Tetracycline	≥ 15	12-14	≤ 11	≤ 4	8	≥ 16

* The breakpoints values of some antibiotics which are absent from *P. aeruginosa* table in the guideline document is reported from Enterobacteriaceae tables.

Detection of β-lactamase production

The iodometric method was employed to detect β-lactamase production. This method is cited in (Miles et al., 1994) and to be carried out according to the following steps:

- From an overnight culture, a heavy suspension (approximately 10^9CFU/ml) is made in distilled water containing 6g/L of penicillin.
- Place 0.1 ml of the suspension into a well of microtiter plate. After incubation for 1 hour at 37^{o}C, tow drops of the freshly prepared 1% soluble starch solution (prepared by dissolving the starch at 100^{o}C) to be added to each well.
- Add a drop of iodine reagent (consisting of 2.03g iodine and 5.32g potassium iodine in 100 ml distilled water).

If the blue colour is lost within 10 minutes, the presence of β-lactamase is inferred.

Results

A total of 93 clinical samples were collected. About 20% of the samples were provided by Dr. Nabeel Maiyas from infected patients at his ENT clinic. The other samples were obtained from Dhamar General Hospital Authority. Samples were obtained between November 2012 and April 2013. Details of collected samples are given in Table 4. There were 20 isolates of *P. aeruginosa* giving prevalence level (21.5%) most of them from wound and ear infections. Three isolates obtained from patients who had been admitted to a hospital for a casualty during the preceding weeks of isolation.

Table4. Distribution of *P. aeruginosa* in various clinical specimens

Source / Site	No. of samples	No. of *P. aeruginosa* Isolates	%
Burn	12	2	16.7
Ear	19	6	31.6
Hospital environment	13	2	15.4
ICU room	8	1	12.5
Solutions*	8	1	12.5
Urinary tract	3	1	33.3
Total	**93**	**20**	**21.5**

*Solutions (disinfectants and distilled water)

Results obtained for disk diffusion method (Inhibition zone diameters) for the different antibiotics used in this experiment are shown in table 5. Statistical calculations had been done using Microsoft Excel 2007.

Table5. Measurements of inhibition zone diameters (mm) of 20 isolates of *P. aeruginosa*

Isolate No	Amk_{30}	Amp_{10}	Caz_{30}	Cip_5	E_{15}	Gen_{10}	Pl_{100}	Te_{30}
1	21	0	15	27	9	19	31	10
2	24	0	21	23	12	16	19	9
3	14	0	17	25	12	10	18	7
4	19	0	15	18	0	13	23	10
5	16	0	13	22	0	11	22	0
6	12	0	16	20	10	9	15	0
7	10	0	16	14	11	12	25	8
8	19	0	14	20	10	13	24	12
9	16	0	19	29	13	17	19	7
10	15	0	17	13	12	14	27	9
11	20	0	15	27	14	19	22	8
12	16	0	20	23	14	19	23	11
13	18	0	19	25	9	18	21	0
14	14	0	21	19	13	13	17	10
15	17	0	16	26	11	20	21	0
16	22	0	22	24	15	14	30	7
17	15	0	11	15	8	21	25	0
18*	0	0	0	0	0	0	0	0
19	20	0	24	29	15	13	27	9

20	16	0	15	20	12	10	29	0
Mean	17.1	0	17.2	22.1	10.5	14.8	23.1	6.2
Median	16.0	0	16.0	23.0	12.0	14.0	23.0	8.0
Range	10-24	0	11-24	13-29	0-15	9-21	15-31	0-12
Standard Deviation	3.5	0	3.4	4.8	4.2	3.7	4.5	4.5

Amk_{30}=Amikacin 30 µg, **Amp_{10}**=Ampicillin 10 µg, **Caz_{30}**=Ceftazidime 30 µg, **Cip_5**=Ciprofloxacin 5 µg, **E_{15}**=Erythromycin 15 µg, **Gen_{10}**=Gentamicin 10 µg, **Pl_{100}**=Piperacillin 100 µg, **Te_{30}**=Tetracycline 30 µg.
* Isolate no. 18 was excluded from the statistical calculation (see discussion)

The inhibition zone diameters (mean ± standard deviation) were 23.1±4.5, 22.1± 4.8, 17.2 ± 3.4, 17.1 ± 3.5, 10.5 ± 4.2 and 6.2 ± 4.5 mm for piperacillin, ciprofloxacin, amikacin, erythromycin, gentamicin, and tetracycline, respectively. While there the sensitivity to ampicillin is completely absent.
Classification of *P. aeruginosa* susceptibility results obtained for each individual isolate based on American CLSI guideline (2013) classification system is given in table 6. This table shows that *P. aeruginosa* is completely resistant to ampicillin, erythromycin and tetracycline, and the least resistance observed for ciprofloxacin and ceftazidime, while it shows no resistance for piperacillin, but intermediately resistant isolates are reported. The percentage of resistant isolates in the sample studied for each antibiotic is given in table 7 and represented graphically in **figure 1**.

Table 6: Antibiotic resistance and sensitivity pattern of 20 isolates of *P. aeruginosa*

Isolate Number	Amk_{30}	Amp_{10}	Caz_{30}	Cip_5	E_{15}	Gen_{10}	Pl_{100}	Te_{30}
1	S	R	I	S	R	S	S	R
2	S	R	S	S	R	S	I	R
3	R	R	I	S	R	R	I	R
4	S	R	I	I	R	I	S	R
5	I	R	R	S	R	R	S	R
6	R	R	I	I	R	R	I	R
7	R	R	I	R	R	R	S	R
8	S	R	R	I	R	I	S	R
9	I	R	S	S	R	S	I	R
10	I	R	I	R	R	I	S	R
11	S	R	I	S	R	S	S	R
12	I	R	S	S	R	S	S	R
13	S	R	S	S	R	S	S	R
14	R	R	S	I	R	I	I	R
15	S	R	I	S	R	S	S	R
16	S	R	S	S	R	I	S	R
17	I	R	R	R	R	S	S	R
18	R	R	R	R	R	R	R	R
19	S	R	S	S	R	I	S	R
20	I	R	I	I	R	R	S	R

Amk_{30}=Amikacin 30 µg, **Amp_{10}**=Ampicillin 10 µg, **Caz_{30}**=Ceftazidime 30 µg, **Cip_5**=Ciprofloxacin 5 µg, **E_{15}**=Erythromycin 15 µg, **Gen_{10}**=Gentamicin 10 µg, **Pl_{100}**=Piperacillin 100 µg, **T_{30}**=Tetracycline 30 µg, **S**= sensitive, **I**= Intermediate, **R**= resistant

Table7. Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolates

Antibiotic	susceptibility pattern			Susceptible (%)	Intermediate susceptibility (%)	Resistant (%)
	S	I	R			
Amikacin	9	6	4	47.4	31.6	21.1
Ampicillin	0	0	19	0.0	0.0	100.0
Ceftazidime	7	9	3	36.8	47.4	15.8
Ciprofloxacin	11	5	3	57.9	26.3	15.8

Erythromycin	0	0	19	0.0	0.0	100.0
Gentamicin	7	7	5	36.8	36.8	26.3
Pipracillin	14	5	0	73.7	26.3	0.0
Tetracycline	0	0	19	0.0	0.0	100.0

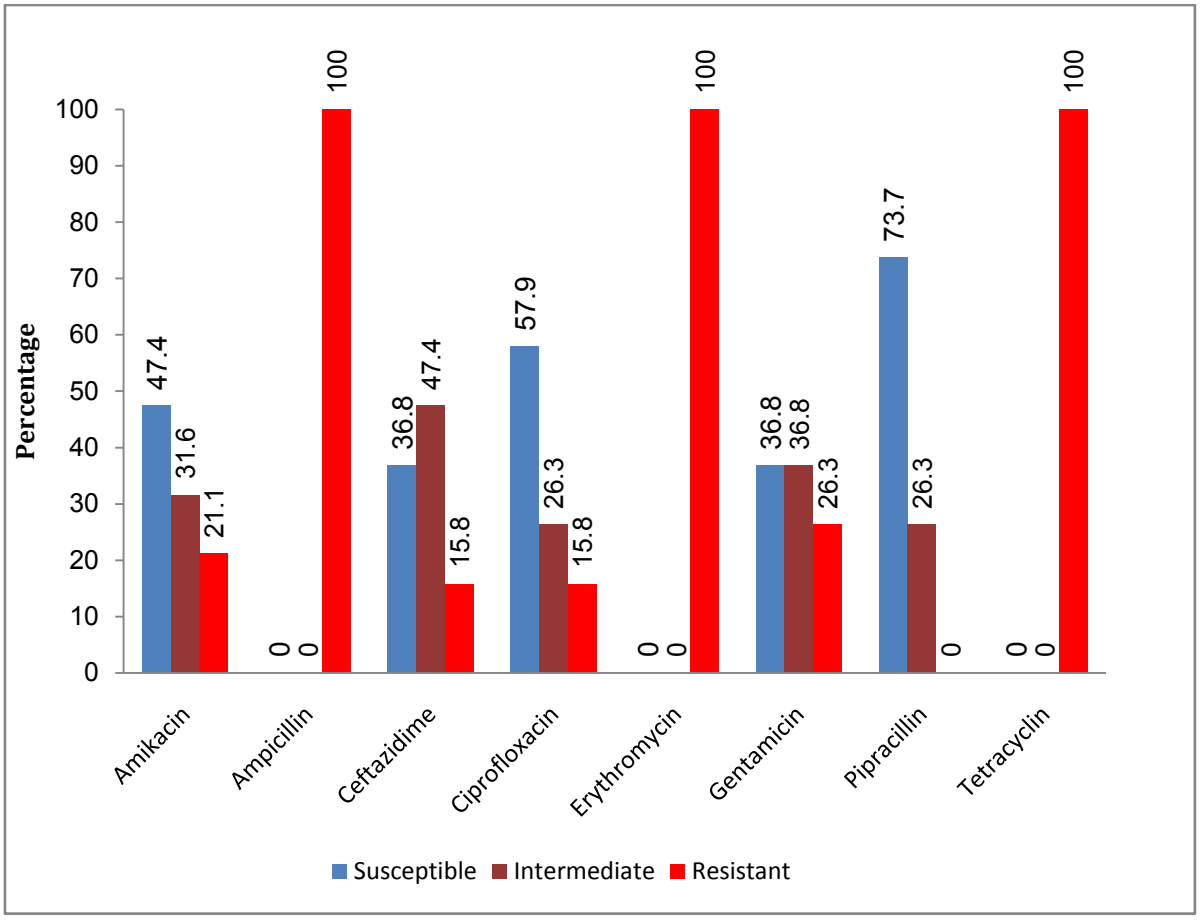


Figure1. Percentage of resistant isolates in the studied sample (sample size=19)

It is clear that the spectrum rank order of the antimicrobial agents against *P. aeruginosa* in terms of percentage of susceptibility was: piperacillin (73.7%)> ciprofloxacin (57.9%)> amikacin (47.4)> ceftazidime (36.8%) = gentamicin (36.8%)> tetracycline (0%) = erythromycin (0%) = ampicillin (0%).
Significant proportion of the sample were intermediately susceptible to few antibiotic in the following order: ceftazidime (47.4%) >gentamicin (36.8%)> amikacin (31.6%) >ciprofloxacin (26.3%)> piperacillin (26.3%) =tetracycline (0%) = erythromycin (0%) = ampicillin (0%).

MICs results: Results obtained for MICs values determination at a specific concentration of ciprofloxacin 5 µg and gentamicin 10 µg for the individual isolates is shown in table 7.

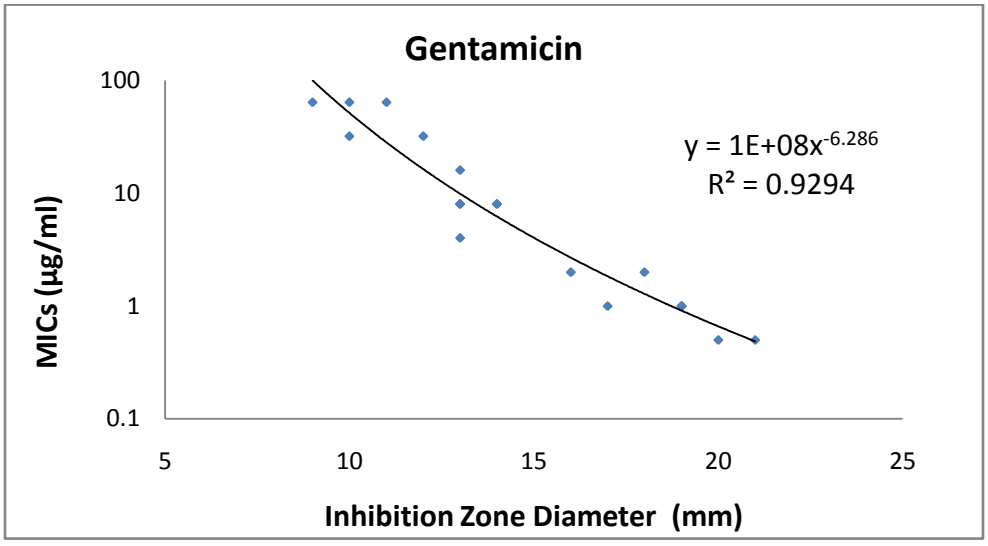
Table 7: MICs values of Ciprofloxacin and Gentamicin

Isolate Number	Ciprofloxacin		Gentamicin	
	Zone (mm)	MICs (µg/ml)	Zone (mm)	MICs (µg/ml)
1	27	0.5	19	1
2	23	0.5	16	2
3	25	0.5	10	64

4	18	2	13	8
5	22	1	11	64
6	20	2	9	64
7	14	8	12	32
8	20	2	13	4
9	29	0.5	17	1
10	13	16	14	8
11	27	0.5	19	1
12	23	0.5	19	1
13	25	0.5	18	2
14	19	4	13	16
15	26	0.5	20	0.5
16	24	0.5	14	8
17	15	8	21	0.5
18*	0	128	0	512
19	29	0.5	13	8
20	20	1	10	32
Mean	22.1	2.6	14.8	16.7
Median	23.0	0.5	14.0	8.0
Range	13 - 29	0.5 - 8	9 - 21	1 - 64
Standard Deviation	4.8	4.0	3.7	23.1

* Isolate no. 18 was excluded from the statistical calculation (see discussion)

Figure 2 shows the correlation between zone diameters and MICs for ciprofloxacin and gentamicin. It shows a high correlation between the two variables (R^2>0.8).



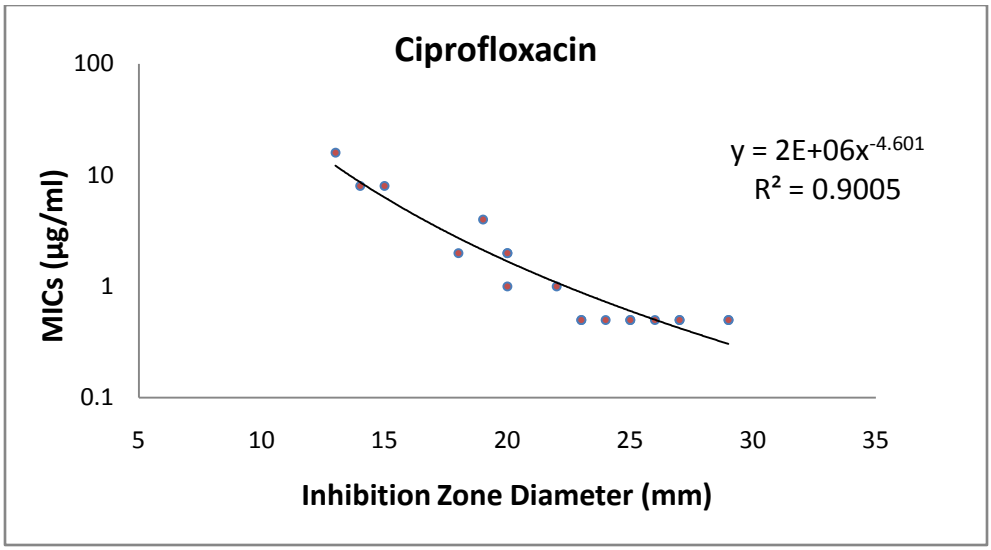


Figure2. Relationships between inhibition zone diameter (mm) in Log scale and MICs (µg/ml) for gentamicin (top), and ciprofloxacin (bottom).

The equations described the relationship between the MICs and inhibition zone diameters are:

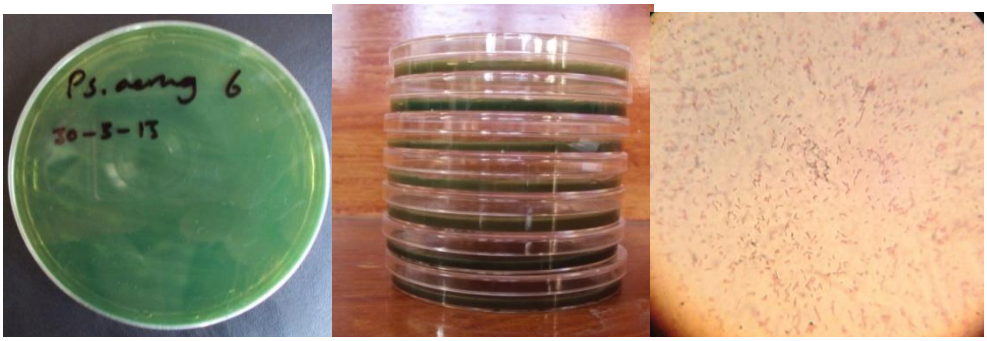
$$\text{MICs} = 1\text{x}10^{8} \cdot \text{InZon}^{-6.286} \text{ For gentamicin}$$

$$\text{MICs} = 2\text{x}10^{6} \cdot \text{InZon}^{-4.601} \text{ For ciprofloxacin}$$

Where In Zone is the inhibition zone diameter in millimetre.

Resistance through β-lactamase production

All the isolates were subjected to β-lactamase detection. The disappearance of the blue colour of starch and iodine was indicative of β-lactamase production. And all the isolates were β-lactamase producers (100%).



A **B** **C**

D **E** **F**

Figure3. Example sensitivity test plates. **A & B** are greenish blue pigmented isolates; **C** is Gram stain of *P. aeruginosa* showing gram-negative bacilli (1000 x); **D & E** are pure cultures of two isolates before pigment production; and **F** is a pure culture of non-pigmented isolate.

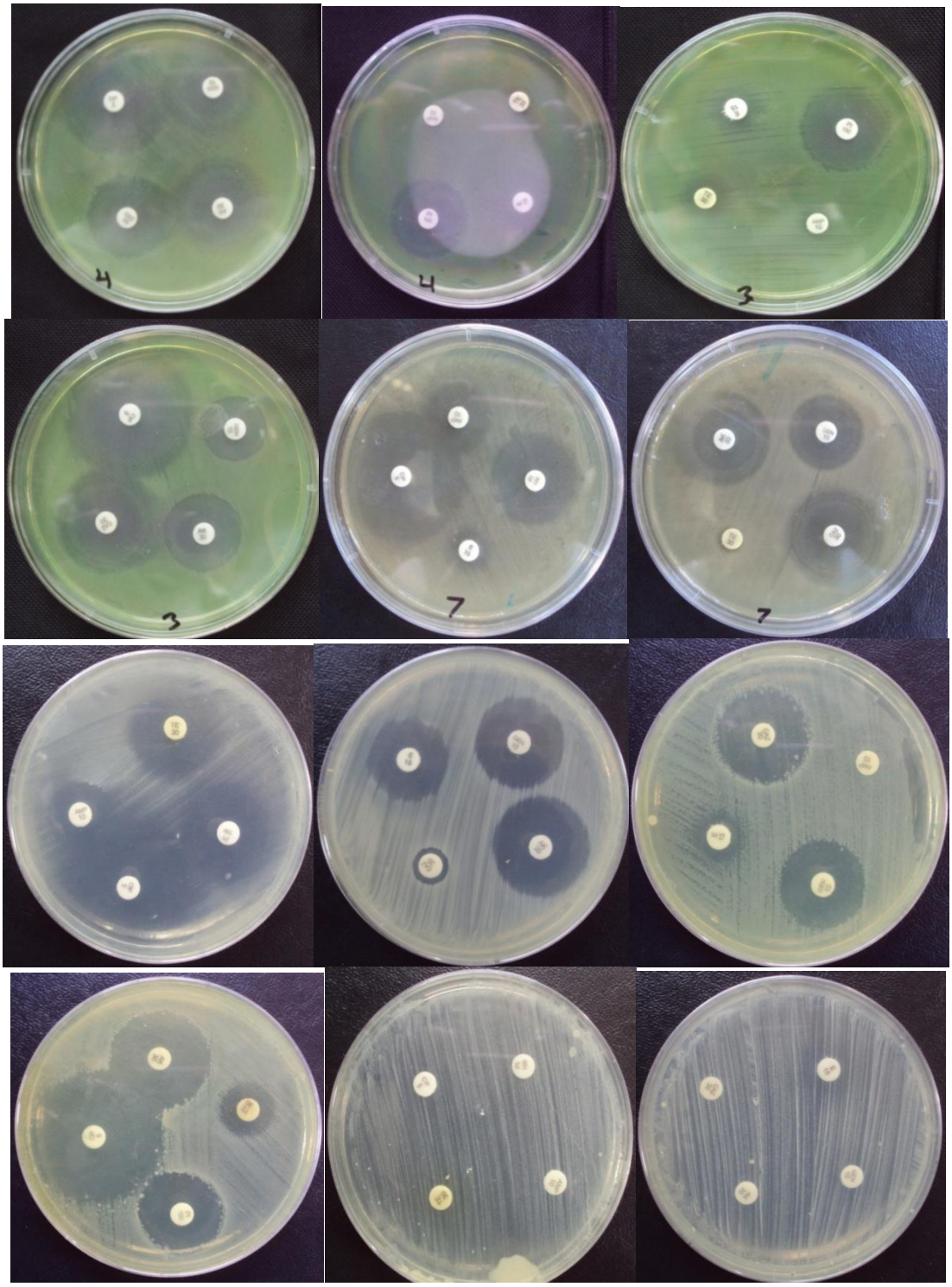


Figure4. Examples of disk-diffusion test plates showing different anti-*P. aeruginosa* activity around the antibiotic disks.

Figure5. Iodometric method results for β-lactamase detection (a) control, (b) positive result

Discussion

This is the first study in the Department of Microbiology and in the University of Dhamar that evaluated the activity of commonly prescribed antibiotic against *P. aeruginosa* and determined the MICs values for gentamicin and ciprofloxacin in 20 isolates from infected patients.

One isolate (isolate number 18) did not respond to any antibiotic under study, therefore it was excluded from the statistical calculation of zone diameter. Likewise, this isolate was also excluded from statistical calculation of MICs as it gave an extremely outlier value and will skew the statistical distribution to the right side. However, the reason for this outlier is not clear, most probably due to either extreme resistance or a technical error during the procedure of identification, storage. Thus, the overall statistical analyses were done for 19 isolates.

The spectrum rank order of the antimicrobial against *P. aeruginosa* in terms of percentage of susceptibility was: piperacillin (73.7%) > ciprofloxacin (57.9%) > amikacin (47.4) > ceftazidime (36.8%) = gentamicin (36.8%) > tetracycline (0%) = erythromycin (0%) = ampicillin (0%). In spite of the difficulties in comparing results that show regional differences, the results obtained from this study are in agreement with those obtained for, ciprofloxacin in previous study (Tripathi et al., 2011). However, the studied isolates in this show less susceptibility for ceftazidime, but higher than that reported for gentamicin (Tripathi et al., 2011). Another explanation is the small size in this study 19 isolates compared with the previous study (102 isolates). In a Malaysian study (Pathmanathan et al., 2009) which reported that the antipseudomonal penicillin; piperacillin, is the most potent amongst the tested panel of selected antibiotics with a resistance percentage of 7%, but this difference, with the findings in this thesis (0%), is attributed to many factors including geographical, technical, and chronological since the breakpoint values of piperacillin was re-evaluated and adjusted in 2013 by CLSI and some breakpoint values of piperacillin inhibition zone, in previous publications of CLSI, which were considered resistant have been assigned to a new category ; the intermediate, in 2013 publications.

In another recent study (Fatima et al., 2012) with larger sample size (n=120 isolates), the susceptibility and resistant rate obtained for amikacin were 65% and 35%, which is slightly higher than those obtained in this work 47% and 21%, respectively. On the other hand another study (Gad et al., 2007) with a sample size of 107 isolates, the susceptibility found was ampicillin (0%), tetracycline (0%), amikacin (51%), ciprofloxacin (23%). These results are in a very good agreement with those obtained in this thesis. Lodise et al., 2007 reported that the *P. aeruginosa* susceptibility to amikacin is greater than the susceptibility to ciprofloxacin. This trend is in agreement with the results obtained in this study. Both Haleem et al., (2011) and (Jombo et al., 2008) reported an absence of susceptibility of *P. aeruginosa* toward ampicillin, erythromycin and tetracycline. This is in line with the results obtained in this thesis.

For determining the MICs for the isolates, only two antibiotics, namely, ciprofloxacin and gentamicin were used because it is an expensive technique and time-consuming. As the materials required are not available in the laboratory, a decision taken to investigate only two drugs at a specific concentration. A separate study can be design to test MICs of all antibiotics. The MICs for ciprofloxacin 5µg (2.6±4.0µg/ml) was lower than that obtained for gentamicin 10µg (16.7±23.1µg/ml) see table 7. This result is in line with previously reported values for gentamicin (12-19µg/ml) in Gad et al., (2007) and for ciprofloxacin (range: 0.12- 64µg/ml) reported by Ibrahim-Elmagboul

(1997). A high variability in both cases was obtained. This is most probably due to the different *P. aeruginosa* strains in different isolates. Very high negative correlations between the MICs and the inhibition zone diameter for both ciprofloxacin and gentamicin, indicating the lower MICs values for the wide zones. In other words, those isolates with high susceptibility to the effect of the antibacterial agent can be treated successfully with a combination of this drug and aminoglycosides, since monotherapy with either antipseudomonal penicillins or cephalosporins can result in selection of stably induced mutant that produce a massive amounts of chromosomally mediated β-lactamase (Yu et al., 1999).

Those isolates with low diameter (high MICs values), a combination therapy with two or more antimicrobial agents is required. It is well-documented that P. aeruginosa isolates produce beta-lactamase that is involved in the mechanism of resistance of these bacteria to antibiotics (Vedel 2005; Zhao et al., 2010). The recovery of this phenomenon during this experimental work in the lab. Indicates the good level of handling the samples during the course of the study.

Conclusion

Not all antibacterial agents on the market possess activity against *P. aeruginosa*. Among the antibiotics tested piperacillin was the most active drug against *P. aeruginosa*, followed by ciprofloxacin, ceftazidime, gentamicin, and amikacin. *P. aeruginosa* was fully resistant to ampicillin, erythromycin, and tetracycline. All the isolate showed β-lactamase production. The findings obtained in this study are in very good agreement with previously reported studies from different countries (see discussion).

The resistance shown by these isolated are really problematic issue, at least in Yemen. During infectious diseases treatment, especially when caused by pathogens that are often drug resistant like *P. aeruginosa*, sensitivity tests must be used to select effective antimicrobial drugs. Combination of two or more antimicrobial drugs with intermediate sensitivity that works via different mechanism of action is an alternative way for rational treatment. This approach needs further well-designed study and experimental analysis using larger sample size with different antimicrobial concentration and good laboratory conditions.

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