www.ajlifesciences.com / ISSN: 2663-1040 *AJ Life Sci. 2021, 4 (1): 40-50*

Research Article

Abasyn Journal of Life Sciences

Open Access

DOI: 10.34091/AJLS.4.1.5

Antimicrobial Susceptibility of *Pseudomonas aeruginosa* Isolated from Hospital Environment

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Abstract

Pseudomonas aeruginosa is a leading cause of disease and death particularly in cystic fibrosis patients and also considered resistance to chemotherapeutic agents. Therefore, it is very difficult to remove the *Pseudomonas aeruginosa* from the hospital environment by using simple techniques. In the contemporary study, biofilm mediated mechanism of various antimicrobial responses were analyzed. For this purpose, different *Pseudomonas aeruginosa* clinical isolates were collected from Pakistan medical institute Islamabad (PIMS) hospital and were investigated for pellicle formation. *Pseudomonas aeruginosa* isolates were studied for different groups of antibiotics including imipenem, meropenem, ceftazidime, amikacin, tobramycin, gentamicin, piperacillin, cefoperazone, and cefotaxime. The goal was to check antimicrobial susceptibility of *pseudomonas aeruginosa* which shows resistant to tobramycin, imipenem, meropenem, amikacin, gentamicin, cefotaxime, piperacillin, ceftazidime, cefoperazone. Additionally, in this study, *Pseudomonas aeruginosa* strains were also investigated for pellicle formation. In conclusion, this research work wills highlights the useful mechanism of antibiotics resistance to *Pseudomonas aeruginosa* infections in clinical practice.

Key words: Antibiotics, Pseudomonas aeruginosa, antibiotics, Biofilm, Pelicle.

Article Info:
Received:
April 09, 2021
Received Revised:
July 26, 2021
Accepted:
August 8, 2021
Available online:
September 11, 2021

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1. INTRODUCTION

Pseudomonas aeruginosa causes nosocomial infections because of its ubiquitous nature, ability to survive in moist environments, and resistance to many antibiotics and antiseptics. A serious problem is the emergence of multidrug-resistance (MDR) Pseudomons aeruginosa strains resistance to β-lactams, aminoglycosides, and quinolones[1-3]. *Pseudomonas aeruginosa* is one of the most important opportunistic human pathogens[4]. It has emerged as a dominant pulmonary pathogen with biofilm-firming capability, resulting in progressive and intractable chronic pulmonary infections, especially in patients with cystic fibrosis. There is also an increasing awareness of the important role of *Pseudomonas aeruginosa* biofilms in the contamination of medical biomaterials such as catheters and prostheses[5].

Pseudomonas aeruginosa belong from Gram-negative bacteria, are the most important and known nosocomial pathogens predominant with alarming ratio[6]. The Gram-negative bacterium *Pseudomonas aeruginosa* is one of the novel and highly impact causing key human pathogen and prevalent aerobe inhabits vegetation, humid places and in wet soils[7, 8]. Because of its harmful opportunistic behaviour, awareness of extreme effects of *Pseudomonas aeruginosa* are observed in immuno depressed hospitalized patients like patients with thermal injury, where the defence system get suppressed with damaging of the skin host cells, orthopaedic related infections, respiratory diseases, immunosuppressed and catheterized patients are extremely exposed to the *Pseudomonas aeruginosa* infections in hospitals. In-addition, it may be the cause of the chronic debilitating pulmonary infection, which is the major factor in leading death of patients suffered with cystic fibrosis[9, 10]. Health associated infections system (HAIs) Infections caused by *Pseudomonas aeruginosa* are considered as a challenge because these organisms have potential to acquire resistance against number of drug classes and cause rigorous problematic diseases[11]. This adaptation in enhanced resistance ability resulted from improved genetic mutations in response of unproper and excessive use of extended-spectrum antibiotics[12], such as aminoglycosides, chloramphenicol, quinolones, tetracycline, β-lactams, quinolones, and analogues of sulphonamides, chloramphenicol and tetracycline. This situation presents a paradox condition for clinical infections control. Biofilm production is also one of the reasons for antibiotic resistance in Pseudomonas aeruginosa. Comparatively, bacteria colonized in biofilm forms have more tolerance and are resistant to several antibiotics than their free-living forms[13]. In the current research study, *Pseudomonas aeruginosa* strains from surgical wards of hospital were collected to examine the antimicrobial susceptibility and biofilm forming ability to mitigate possible infections.

2. MATERIALS AND METHODS

2.1. Sampling of bacterial isolate

Different strains of *Pseudomonas aeruginosa* isolates were obtained from PIMS hospital Islamabad Pakistan were examined for different antibiotics susceptibility and biofilm formation. The sampling was done using sterile culture swabs from different inanimate sources such as wall, bed, floor, washbasin, table, water, doorknob, toilet, kidney tray, and from surgical wards.

2.2. Identification of bacterial isolates

Identification of all *Pseudomonas aeruginosa* was done using in the nutrient broth medium. The required nutrient broth was poured into all the culture swabs and kept the culture swabs in an incubator for incubation at the temperature of 37°C for 24 hours. After incubation, culture swabs were inoculated on Pseudomonas Cetrimide agar plates and positive isolates were noted producing colony forming units. Positive isolates were preserved in glycerol at -20°C for further analysis.

2.3. Preparation of cell suspension and antimicrobial Susceptibility Test

The cell suspension was prepared in saline water (0.85% NaCl) and was used for performing antimicrobial susceptibility tests. Cell density was adjusted by comparing it with McFarland (0.5) turbidity standard solution. A brief antibiotic resistance pattern of isolated bacterial pathogens were studied. For antibiotic susceptibility analysis of nine antibiotics, the method of Kirby Bauer Disc Diffusion was followed. An isolated colony from a culture, grown overnight on Pseudomonas Cetrimide agar plate, was added to sterile saline solution. The opaque appearance of the suspension was checked and balanced against turbidity

standard. Sterile culture swab was dipped into the suspension and the swab was sequentially streaked in three directions over the surface of the plate.

2.4. Pellicle formation assay

T-broth was used in pellicle formation assay contains bactotyptone and NaCl. Hydrolysis of bactotryptone polypeptide provides amino acids which are essential for bacterial growth while NaCl is used for osmotic balance. The T-broth nutrient was taken into test tubes of 6 mL volume. Cultures were inoculated and then test tubes kept in incubators at the temperature of 20-27°C till the formation of visible pellicles. After pellicle formation, their assay was done by visual inspection of the standing culture air-liquid interface. Pellicle formation was the result of multiplication of cells and the spreading of an extracellular matrix in a highly organized manner.

2.5. Congo red Assay

For the colony morphology analysis of bacterial cultures, Congo red assay was performed. In this method, agar (1 %) plates with tryptone layering without salt were prepared and supplemented with Coomassie brilliant blue (20 µg/ mL) and Congo red dye (40 µg /mL). With help of a toothpick bacteria were streaked on the entire surface of each plate. The One-Way ANOVA statistical approach was used to analyze the data in Statistics 8.1 LSD test. ($P<0.05$) was applied for significant differences among treatments. The random sampling method used to collect samples from hospital means.

3. RESULTS

3.1. Isolation of microbes

In the present study, *Pseudomonas aruginosa* strains were determined for its ability to form biofilms and their antimicrobial susceptibility for different groups of antibiotics. Total 52 isolates from the hospital environment were collected from 6 surgical wards with the help of sterile culture swabs. Sampling was done from PIMS surgical wards, from inanimate sources such as water, wash basin, wall, bed, floor, table, kidney tray, toilet, using sterile culture swabs, about 6 isolates were obtained from water, 11 from wash basin, 4 from kidney tray, 6 from the table, 7 from bed, 13 from toilet, 2 from floor, 1 from wall and 2 from door knob **(Table 1, Fig. 1).**

Table 1. Different antibiotics used for disc diffusion test

Ward No	Sample source								
	Water	Bed	Floor	Table	Toilet	Washbasin	Doorknob	Wall	Total
Surgical ward 1	2	0	0	0	3	4	0	1	10
Surgical ward 2	1	2	0	2	1	3	0	0	10
Surgical ward 3	0	1	1	3	3	1	0	0	10
Surgical ward 4	1	3	0	1	3	1	0	0	10
Surgical ward 5	1	1	1	0	1	1	1	0	6
Surgical ward 6	1	0	0	0	3	1	1	0	6
Total	6	7	2	6	14	11	2	1	52

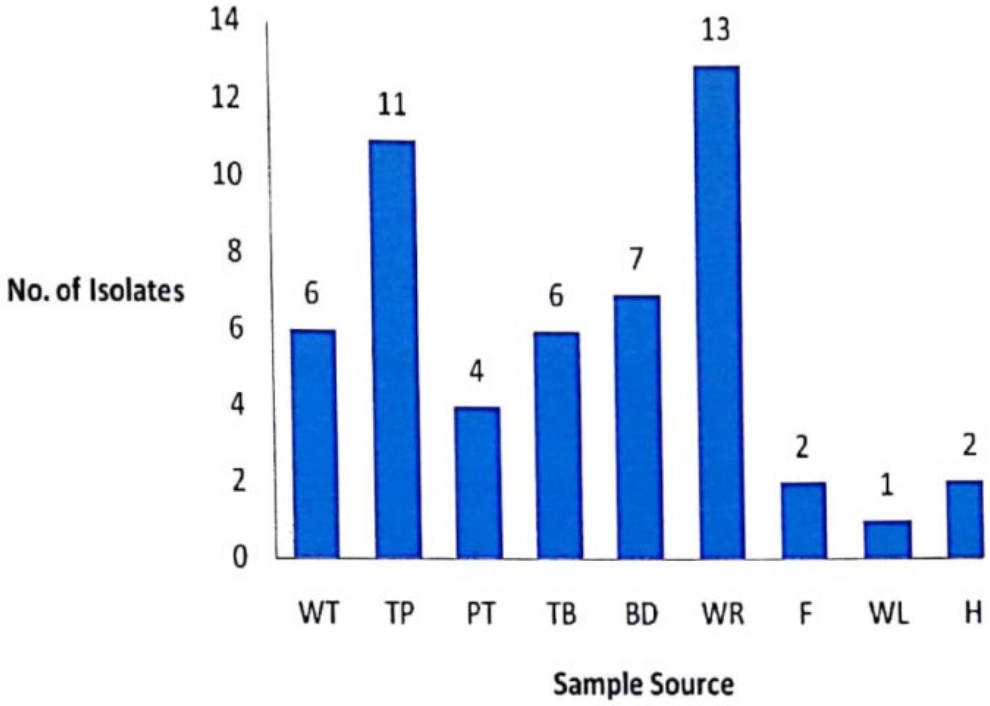

Fig. 1. Frequency of *Pseudomonas aeruginosa* in different sample sources

Out of total 52, 10 isolates were collected from surgical ward 1, 10 from surgical ward 2, 10 from surgical ward 3, 10 from surgical ward 4, 6 from surgical ward 5, and 6 from surgical ward 6**.** Isolates were cultured on Pseudomonas Cetrimide agar and identified by growth characteristics on medium *aeruginosa* colonies as blue-green, yellow-green, red-brown, or white-green.

3.2. Antibiotics Susceptibility Test

Pseudomonas aeruginosa isolates were tested in triplicates against a panel of antibiotics such as imipenem 10 µg; meropenem 10 µg, ceftazidime 10 µg, amikacin 10 µg; tobramycin 10 µg; gentamicin 10 µg; piperacillin 100 µg; cefoperazone 30 µg and cefepime 30 µg. Among 52 isolates, seven were resistant to tobramycin, 4 to imipenem, 3 to meropenem, 5 to amikacin, 11 to gentamicin, 22 to cefotaxime, 15 to piperacillin, 17 to ceftazidime, and 15 to cefoperazone **(Fig. 2, Table 2)**. Among all 16 were found to be multidrug resistance as shown in **Fig. 3**.

Table 2. Antibiotic susceptibility profile of isolates

S. No	Antibiotic Class	Resistant	Intermediate	Sensitive
1	Tobramycin	07	00	45
2	Imipenem	04	00	48
3	Meropenem	03	00	49
4	Amikacin	05	01	46
5	Gentamicin	11	01	40
6	Cefotaxime	22	13	17
7	Piperacilin	15	00	37
8	Ceftazidime	17	01	36
9	Cefoperazone	15	11	26

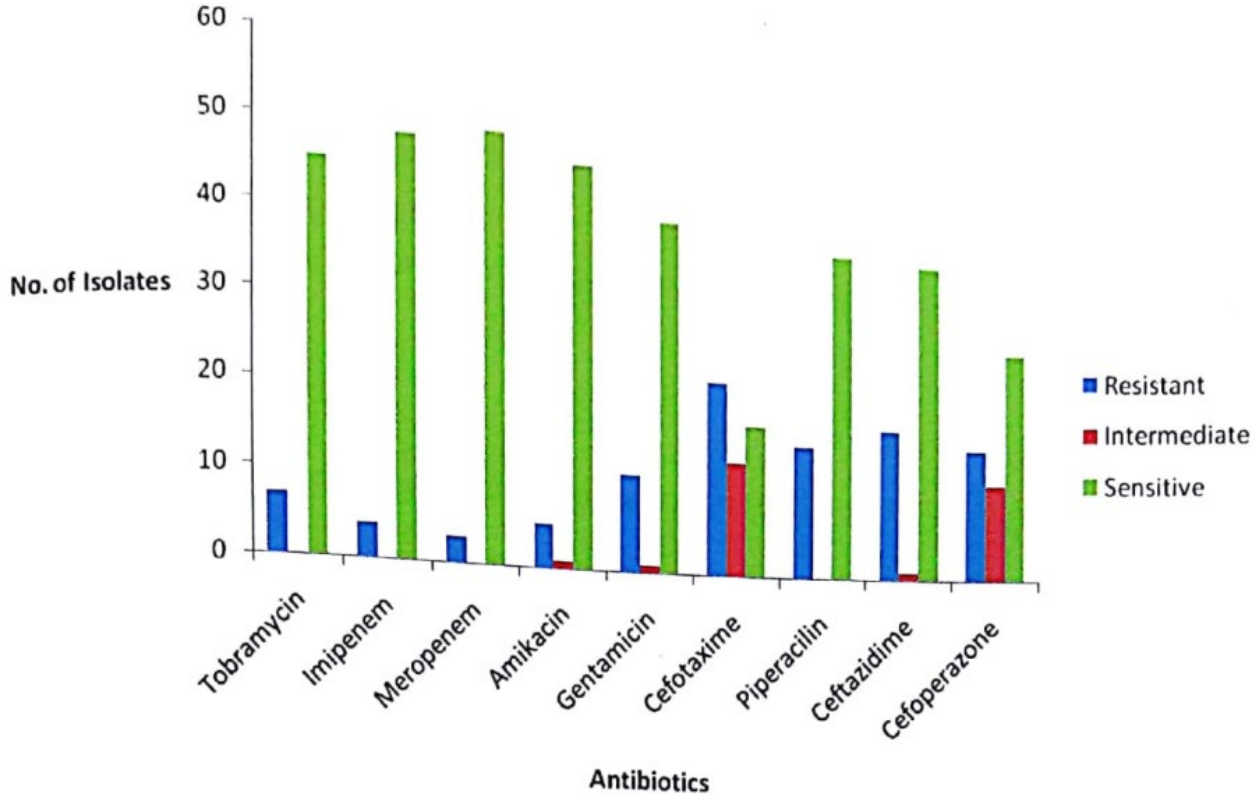

Fig. 2. Susceptibility of isolates against different antibiotics.

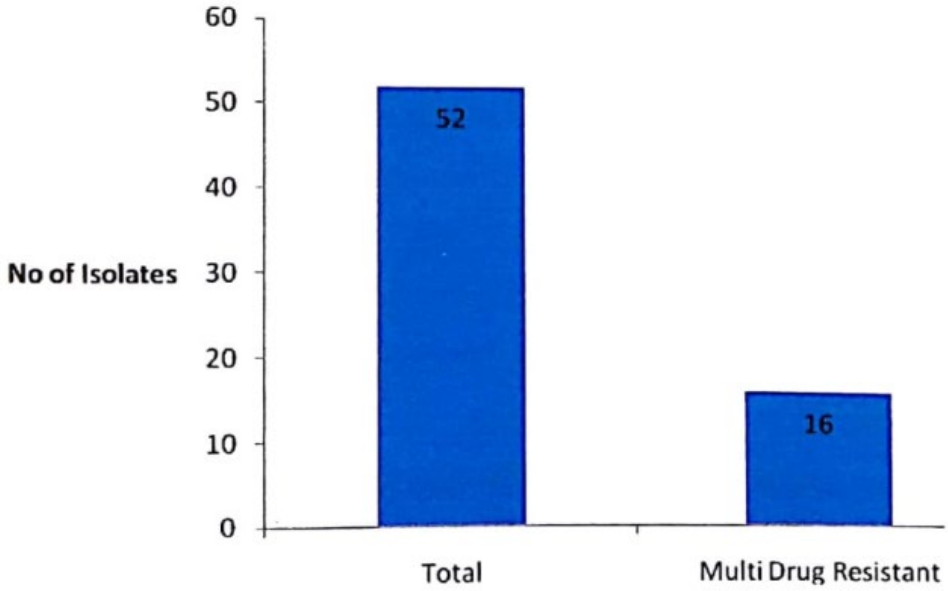

Fig. 3. Frequency of multidrug-resistant isolates

3.3. Pellicle formation assay

Pellicle formation assay was conducted for a total of 52 isolates. 33 of the total isolates were capable of forming a pellicle. The pellicle is formed at the air-liquid interface and about 19 isolates were imperfect in forming a pellicle as shown in **Table 3 and Fig. (4, 5)**.

Table 3. Pellicle formation assay

S. No.	Sample No	Pellicle Formation Assay		S. No	Sample No.	Pellicle Formation Assay	
		Positive	Negative			Positive	Negative
1	1WR1		-	27	3TB3	+	
2	1WR2		-	28	4PT1	+	
3	1WT1		-	29	4WT1	+	
4	1WL1	+		30	4BD1		-
5	1TP2		-	31	4BD2	+	
6	1WR3	+		32	4WR1	+	
7	1TP3	+		33	4TB1	+	
8	1TP4		-	34	4BD3		-
9	1TP3	+		35	4TP1	+	
10	2TB1	+		36	5H1	+	
11	2TP1		-	37	5WR1	+	
12	2TP2		-	38	5TP1		-
13	2TB2	+		39	5F1	+	
14	2PT1	+		40	5BD1	+	
15	2WT1		-	41	6H1		-
16	2WR1		-	42	6WR1	+	
17	2TP3		-	43	6WR1	+	
18	3TB1	+		44	6TP1	+	
19	3TB2	+		45	6WR2	+	
20	3WR1		-	46	6WT1	+	
21	3BD1	+		47	2BD1	+	
22	3WR2	+		48	1WT2	+	-
23	3PT1	+	-	49	2BD2	+	-
24	3WR3	+	-	50	4WR2	+	
25	3TP2			51	4WR3		
26	3F1			52	5WT1		

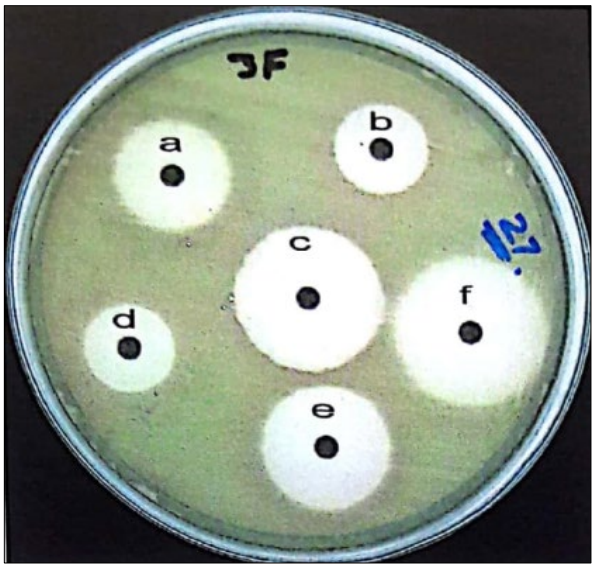

Fig. 4. Kirby-Bauer disc diffusion test for antibiotic susceptibility testing on Mueller-Hinton agar (a cefotaxime; b, cefoperazone; c, imipenem; d, tobramycin; e, amikacin; f, meropenem)

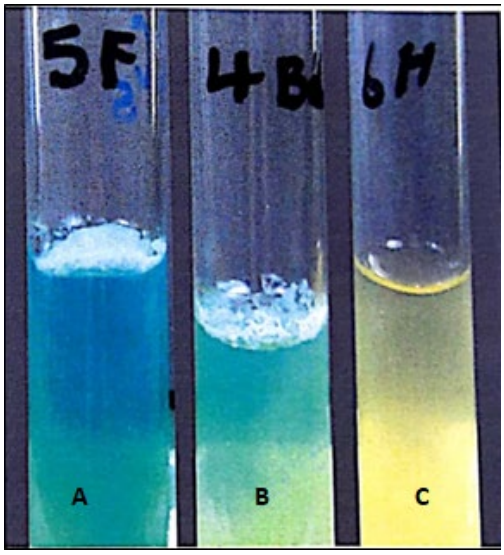

Fig. 5. A and B show pellicle and C shows no pellicle. C is deficient in pellicle formation

3.4. Congo red assay

Similarly, Congo red assay was done for total 52 isolates. It was observed that 33 out of 52 isolates formed red-colored rugose colonies. Red and rugose colony morphology is the characteristic of pellicle forming isolates **(Table 4 Fig. 6 (a, b)**.

Table 4. Congo red assay

S. No.	Sample No.	Colony Morphology	S. No.	Sample No.	Colony Morphology
1	**1WR1**	**Smooth**	**27**	**3TB3**	Red and rugose
2	**1WR2**	**Smooth**	**28**	**4PT1**	Red and rugose
3	**1WT1**	**Smooth**	**29**	**4WT1**	Red and rugose
4	**1WL1**	**Red and rugose**	**30**	**4BD1**	Smooth
5	**1TP2**	**Smooth**	**31**	**4BD2**	Red and rugose
6	**1WR3**	**Red and rugose**	**32**	**4WR1**	Red and rugose
7	**1TP3**	**Red and rugose**	**33**	**4TB1**	Red and rugose

8	**1TP4**	**Smooth**	**34**	**4BD3**	Smooth
9	**1TP3**	**Red and rugose**	**35**	**4TP1**	Red and rugose
10	**2TB1**	**Red and rugose**	**36**	**5H1**	Red and rugose
11	**2TP1**	**Smooth**	**37**	**5WR1**	Red and rugose
12	**2TP2**	**Smooth**	**38**	**5TP1**	Smooth
13	**2TB2**	**Red and rugose**	**39**	**5F1**	Red and rugose
14	**2PT1**	**Red and rugose**	**40**	**5BD1**	Red and rugose
15	**2WT1**	**Smooth**	**41**	**6H1**	Smooth
16	**2WR1**	**Smooth**	**42**	**6WR1**	Red and rugose
17	**2TP3**	**Smooth**	**43**	**6WR1**	Red and rugose
18	**3TB1**	**Red and rugose**	**44**	**6TP1**	Red and rugose
19	**3TB2**	**Red and rugose**	**45**	**6WR2**	Red and rugose
20	**3WR1**	**Smooth**	**46**	**6WT1**	Red and rugose
21	**3BD1**	**Red and rugose**	**47**	**2BD1**	Red and rugose
22	**3WR2**	**Red and rugose**	**48**	**1WT2**	Red and rugose
23	**3PT1**	**Smooth**	**49**	**2BD2**	Smooth
24	**3WR3**	**Red and rugose**	**50**	**4WR2**	Red and rugose
25	**3TP2**	**Smooth**	**51**	**4WR3**	Red and rugose
26	**3F1**	**Red and rugose**	**52**	**5WT1**	Smooth

Fig. 6. (a): Smooth colony morphology of no pellicle forming *Pseudomonas aeruginosa*

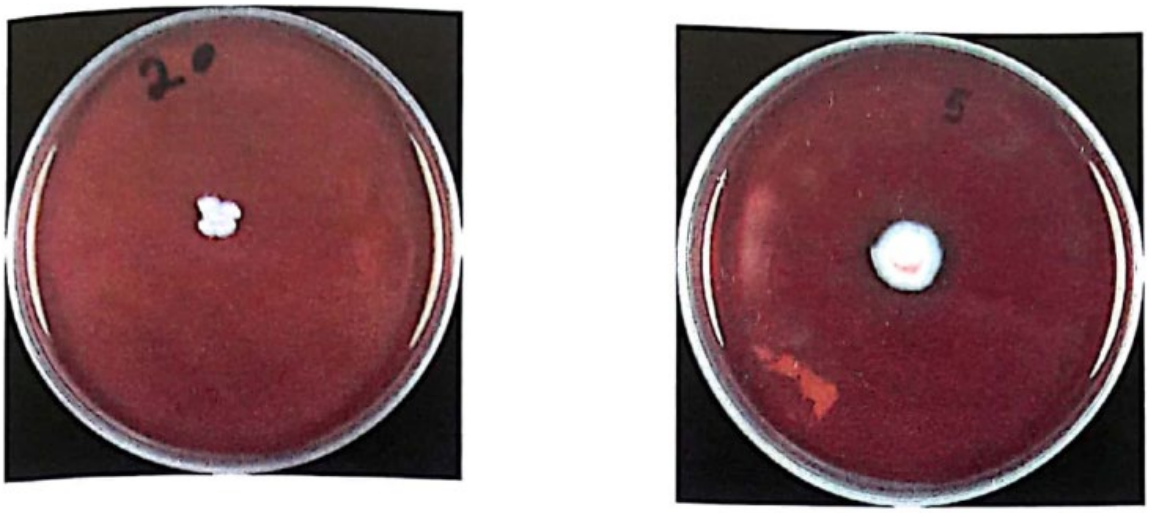

Fig. 6. (b): Red and rugose colony morphology of pellicle forming *Pseudomonas aerugi*

Congo red was not bound to 19 isolates and had smooth colony morphology which indicating non-pellicle forming isolates. Congo red binds with the extracellular matrix and the colony morphology become red and wrinkled. Smooth colony morphology indicates that no extracellular matrix was present; therefore, we found a deficient in pellicle formation. **Figure-7 (a)** showed smooth colonies which were deficient in pellicle formation, while **Figure-7(b)** showed red and rugose colonies which were the characteristic of pellicle forming *Pseudomonas aeruginosa.*

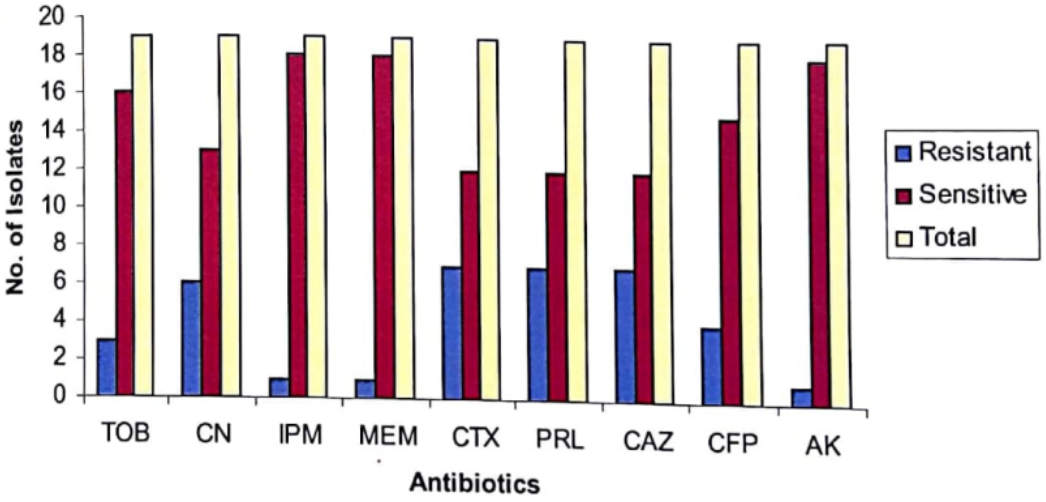

Fig. 7. (a) Antimicrobial susceptibility of pellicle former isolates

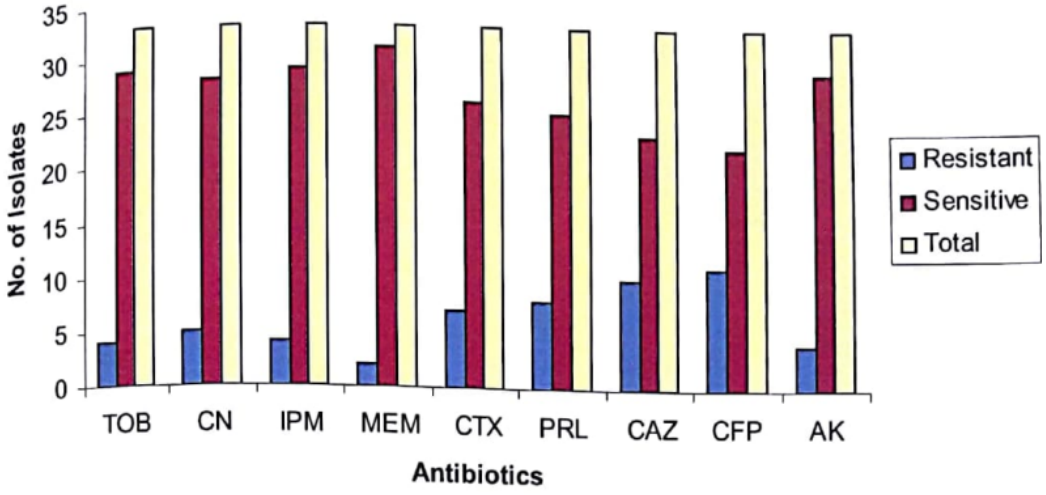

Fig. 7. (b) Antimicrobial susceptibility of non-pellicle former isolates

4. DISCUSSION

In the present study, *Pseudomonas aeruginosa* isolates were collected from different intimate sources such as water, wash basin, wall, bed, kidney tray, toilet, floor, and table and door knob from surgical wards. *Pseudomonas aeruginosa* can survive and replicate within the hospital environment, where they colonize skins and hospital distilled water system[14]. It was reported that the role of tap water as a source of endemic *Pseudomonas aeruginosa* infections in the intensive care unit (ICU). He also reported that water outlets hardboard distinct genotypes of *Pseudomonas aeruginosa* over prolonged periods[15]. *Pseudomonas aeruginosa* persist on inanimate sources for 6 hours to 16 months in a moist environment and on dry floor for 5 weeks[16]. Pathogens were present in potable water, cooling tower water, distilled water, nebulizers, contaminated respiratory therapy solution, room humidifiers, vaporizers, mist tents, sinks, hydrotherapy pools, whirlpools, lithotripsy therapy tanks, dialysis water, eyewash stations, endoscopes, and flower vases[17].

Pseudomonas aeruginosa isolates were tested against a panel of antibiotics. In which, 22 were resistant to cefotaxime, 17 to ceftazidime, and 15 to cefoperazone. These three antibiotics belong cephalosporin group.

Cephalosporin group was ineffective against these isolates. 15 were resistant to piperacillin which belongs to Penicillin group. In the present study, we have 13.4% resistant *Pseudomonas aeruginosa* isolates to imipenen and meropenem, 9.6% to Amikacin, 21% to Gentamicin, 13.4% to tobramycin, 42.3% to cefotaxime and 28.8% to Cefoperazone. Shashikala et al., (2006) reported 10.9% *Pseudomonas aeruginosa* isolates resistant to imipenem/merpenem. Although about 6.3% to tobramycin, 16% to cefotaxime, and 14% resistance was found against Cefoperazone[18-20]. We have performed antimicrobial susceptibility before pellicle formation assay of these isolates. No significant difference in the antibiotics resistance pattern of pellicle formers and non-pellicle formers was observed before pellicle formation which indicates that greater resistance to different antibiotics demonstrated by *Pseudomonas aeruginosa* biofilms may majorly be because of impermeability of microbial biofilms to different antibiotics and their might be no extraordinary inherent resistance mechanism in biofilms-formers. In this study, we found that 63.5% of *Pseudomonas aeruginosa* isolates were biofilm forming as evident by pellicle formation through pellicle formation assay while no pellicle was produced in 36.5% of isolates[21]. Observed a striking difference in different strains of Haemophilus influenza concerning ability to form biofilms. The pel genes were needed for the production of a pellicle in the *Pseudomonas aeruginosa* PA14 strain when grown as standing liquid culture for 72h at room temperature. They further reported that the carbohydrate content of the pellicle's material was a glucose rich substance[22].

The biofilm production capability of *Pseudomonas aeruginosa* isolates was detected through Congo red assay. It was observed that 63.5% isolates formed red coloured rugose colonies white 36.5% produced smooth white colonies. Red and rugose colony morphology is the characteristic of pellicle formation[23]. Reported that when grown on agar plates containing Congo red, *Pseudomonas aeruginosa* PA14 colonies were dark red whereas the pel mutants were pale pink. They reported that the pellicle forming colonies also had a wrinkled or 'rugose' morphology, whereas the non-pellicle forming colonies were smooth. Showed that colonies that strain red under these conditions do so because of Cingo red absorption[24].

4. CONCLUSIONS

In this research work, *Pseudomonas aeruginosa* strains were isolated from inanimate sources of surgical wards of hospital. These strains were investigated for antibiotic resistance in the laboratory experiments and significance levels of resistance were observed against cephalosporin. Further study confirmed that most of the *Pseudomonas aeruginosa* strains were susceptible to Carbapenemes, aminoglycoside, and Penicillin. Present study also concludes that out of 52 strains, 16 isolates were recognized as multidrug resistant towards various groups of antibiotics and 33 isolates were capable forming pellicle.

ACKNOWLEDGMENT

None declare

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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