

Antimicrobial activity of Antibiotics and Antiseptics (Dettol and Betadine) against Clinical Isolates of *Pseudomonas aeruginosa*

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ABSTRACT- Hundred samples viz. urine, blood, wound, pus and sputum collected from different patients were found to harbour *Pseudomonas aeruginosa* (*P. aeruginosa*) (27%) with a maximum isolation from wound samples (33.33%) and minimum from blood samples (11.11%). The degree of resistance of *P. aeruginosa* isolates to different antibiotics like Ceftazidime (30 µg), Amikacin (30 µg), Imipenem (10 µg), Ciprofloxacin (30 µg), Tetracycline (30 µg), Gentamicin (10 µg), Norfloxacin (10 µg), Penicillin (30 µg), Chloramphenicol (30 µg), and Ofloxacin (5 µg) varied from 56% to 100%. Antiseptics i.e. Betadine and Dettol were found to be more effective against the MDR strain of *P. aeruginosa* at the dilutions of 10⁻¹ and 10⁻². Duration of the disease and hospitalization duration, evaluated as risk factors for *P. aeruginosa* colonization were found to be statistically significant while age and gender were found to be statistically non-significant. The incidence of multidrug resistance of *P. aeruginosa* is increasing fast due to the frequent use of antibiotics and antiseptics, which are used extensively in hospitals and healthcare centers, therefore it is a need to develop alternative antimicrobial agents for the treatment of infectious diseases.

Key-words- Antibiotic, Antiseptic, Betadine and Dettol, Disinfectants, *P. aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa is one of the leading causes of nosocomial infections, reported worldwide. The gram negative, rod shaped bacterium (0.5-0.8 µm and 1.5-3 µm in size) is ubiquitous with normal nutritional requirement and has emerged as the epitome of opportunistic pathogen of humans. There is hardly any tissue that it cannot infect, if the tissue defenses are compromised in some manner^[1]. *P. aeruginosa* is commonly encountered in health-associated infections. Multiple surveillance programs have reported the organism as one of the leading causes of nosocomial infection.^[2-4]

According to Center for Disease Control (CDC), incidence of *P. aeruginosa* infections in U.S. hospitals averages about 0.4% (4 per 1000 discharges) and the bacterium is the fourth most commonly isolated nosocomial pathogen accounting for 10.1% of all hospital acquired infections^[1].

Within the hospital, *P. aeruginosa* finds abundant reservoirs, such as disinfectants, respiratory equipment, food, sinks, taps, toilets, showers and mops. It is constantly reintroduced into the hospital environment on fruit-plants, vegetables as well as by visitors and patients transferred from other facilities. Spread occurs from patients, on the hands of hospital personnel, by direct patient get in touch with contaminated reservoirs, and by the ingestion of contaminated foods and water^[1].

Urinary Tract Infections (UTI) is a main hazard to human health. It is caused due to the various physiological changes of the urinary tract by the action of microbes^[5]. Urinary Tract infections has also been a most important type of hospital acquired infection (HAI)^[5]. Hospital acquired infections are of various types: UTI, Surgical Site Infection (SSI) Blood Stream Infection (BSI), and Respiratory Tract Infection (RTI), and the most common are Respiratory Tract (20-22%), and Urinary Tract (39%) infection^[5].

It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint-infections, gastrointestinal infections and a variety of systemic infections. Particularly in the patients with severe burns, cancer and AIDS, who are immune-suppressed, *P. aeruginosa* infection is a serious problem among them. The case fatality rate in these patients is nearly 50%^[1]. Most *Pseudomonas* infections

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are both invasive and toxinogenic. Particular bacterial determinants of virulence mediate the infection and are ultimately responsible for the distinguishing syndromes accompanying the diseases.

The ability of *P. aeruginosa* to invade tissues depends upon production of extracellular enzymes and toxins that breaks down physical barriers and damage host cells and are responsible for resistance to phagocytosis and the host immune-defenses. Two extracellular proteases have been linked with virulence that exerts their activity at the invasive stage-elastase and alkaline protease [1]. *Pseudomonas* pigment, Pyocyanin is also probably a determinant of virulence for the pathogen. *P. aeruginosa* produces two extra cellular protein toxins, exoenzyme S and exoenzyme A, that aid in the invasion of *P. aeruginosa*.

P. aeruginosa is notorious for its resistance to antibiotics and a particularly dangerous and dreaded pathogen. *P. aeruginosa* is naturally resistant to numerous antibiotics due to permeability barrier afforded by its Gram-negative outer membrane. Its tendency to colonize surface in a biofilm form makes the cells impervious to therapeutic concentration of antibiotics. Since its natural environment is the soil, living in association with bacilli, actinomycetes and molds, it has developed resistance to a variety of their naturally occurring antibiotics [1].

The heightened level of drug resistance is a result of the de novo emergence of resistance in a specific organism after exposure to antimicrobial as well as patient-to-patient spread of resistant organisms [6]. Accumulation of resistance after exposure to various antibiotics and cross resistance between agents may result in multi-drug resistant (MDR) *P. aeruginosa*.

P. aeruginosa has previously shown high levels of resistance against most antibiotics like, Ceftazidime (13.3%), Ofloxacin (11.6%), Cefotaxime (15%), Ceftriaxone (8.4%) and Ciprofloxacin (8.3%), rekindling interest caused by MDR-*P. aeruginosa*, which are also resistant to high concentration of salts and dyes weak antiseptics and many commonly used antibiotics [4,7]. It is tolerant to a broad diversity of physical conditions, including temperature. In the last decade there have been increasing reports of resistance to Carbapenems the life saving antimicrobial *P. aeruginosa* infection that may develop due to impermeability [8]. The epidemiology of MDR-*P. aeruginosa* is complex. MDR-*P. aeruginosa* infections are associated with severe adverse clinical outcomes [9].

P. aeruginosa is a major cause of nosocomial infection and an opportunistic pathogen. Despite advances in sanitation facilities and the introduction of wide variety of antimicrobial agents with antipseudomonal activities, life-threatening infections caused *P. aeruginosa* continue to be a matter of concern in hospital. The incidence of multidrug resistance of *P. aeruginosa* is also increasing fast due to the frequent use of antibiotic as well as antiseptics and disinfectants used extensively in hospitals and healthcare setting for a variety of topical and hard surface applications. The widespread use of antiseptics and disinfectant products has prompted some speculation

on the development of microbial resistance, in particular cross-resistance to antibiotics. Utmost incidences of bacterial infection were determined in poor economic status patients followed by those of high and middle economic status respectively, due to the deficiency of education about the infection and unhygienic surroundings [10].

MATERIALS AND METHODS

Place of work- The present study entitled “Clinical distribution of *Pseudomonas aeruginosa* and evaluation of its sensitivity against common antimicrobial agents” was conducted in the Department of Microbiology and Microbial Technology, AIDU, Allahabad in the year of 2009.

Collection of samples- Total of 100 samples i.e. 20 each of urine, blood (Sterile sample bottles) wound, pus and sputum (Stuart transport medium) were collected. The name, age, sex, economic status was recorded in a predesigned Performa. Samples were collected from different hospitals of Allahabad, India.

Transport of samples- Urine and Blood samples were transported in sterile sample bottles. Wound, Pus and Sputum samples were obtained using sterile swabs which were transported in Stuart transport medium.

Processing of samples- Samples were processed for the isolation and identification of the organism preferably within 2 hrs after the collection from the hospitals. Samples were stored at 4°C till further processing.

Isolation of *P. aeruginosa*- All the samples were first inoculated under aseptic conditions into selective medium i.e. Cetrimide agar (Fig. 1) to maximize the isolation of *Pseudomonas aeruginosa* and avoid overgrowth of other organisms. Each sample was sub cultured into Nutrient Agar medium (Fig. 2) and incubated aerobically at 37°C for 24 to 48 hrs.

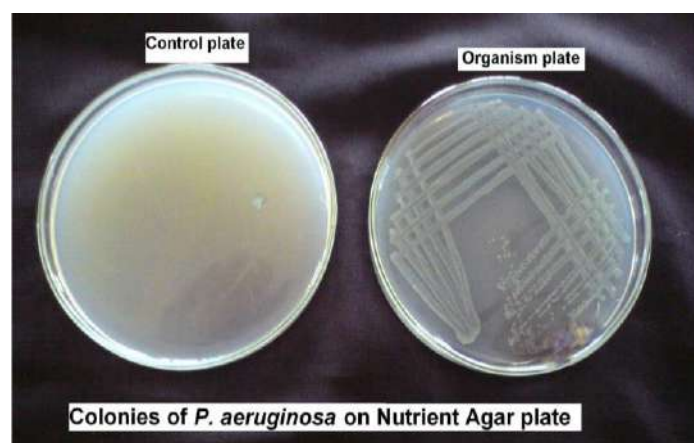


Fig. 1: Colonies of *Pseudomonas aeruginosa* on Nutrient agar plate

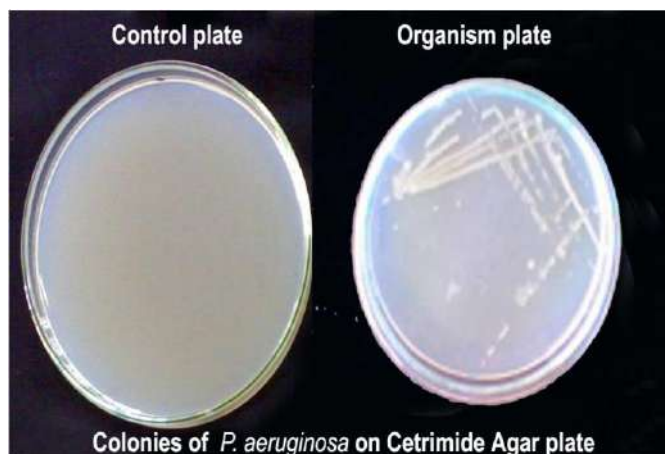


Fig. 2: Colonies of *Pseudomonas aeruginosa* on Cetrimide agar plate

Identification of *P. aeruginosa*

The isolates were identified on the basis of cultural, morphological, and biochemical characteristics as per Bergey's Manual of Systemic Bacteriology^[11].

Cultural characteristics- Plates were observed for production of large, opaque, irregular colonies with the distinctive, small, rough, strongly cohesive colony.

Morphological characteristics- A suspected colony was picked from the plate and smear preparation was made on clean glass slide and Gram staining was performed and observed under 100 X objectives.

Biochemical characteristics- Various biochemical tests were performed for the identification of *P. aeruginosa*. Following biochemical were been undertaken-

Catalase Test- Catalase test was performed by adding 3% hydrogen peroxide (H_2O_2) solution to trypticase soy agar slant culture. Release of free oxygen gas (O_2) bubbles indicated positive catalase test. Alternatively the test was performed as slide test in which the inoculum from a plate culture was picked and placed on a clean glass slide. One drop of hydrogen peroxide was added and appearance of bubbles indicated positive catalase test. The appearance of bubble occurred due to breakdown of hydrogen peroxide to water and oxygen by catalase enzyme present in some microorganisms which help them in their survival.

Oxidase Test- The oxidase test (also known as the cytochrome oxidase test) was used to look for oxidase enzymes produce by certain bacteria. Oxidase catalyses electron transport between substrate acting as electron donors in the bacterium and N,N,N',N' -tetramethyl-p-phenylenediamine (TMPD)- a redox dye present as hydrochloride. The dye was reduced to deep-violet blue color in the presence of oxidase enzymes. The test was performed by wetting strips of filter paper with a fresh 1% TMPD dye solution. The colony was picked & tested with a loop and rubbed on to moistened strip. Change in

color to deep-violet was observed which show the positive test for oxidase.

Nitrate Reduction Test- Nitrate reduction medium was inoculated with the organism and incubated aerobically at $37^\circ C$ for 24 to 48 hrs. After incubation, α -naphthylamine and sulfanilic acid were added. These two compounds react with nitrite and turn red in color. In few tubes nitrate was further reduced to ammonia or nitrogen gas. To distinguish between these two reactions, zinc dust was added. Zinc reduces nitrate to nitrite. The tubes were turn red because α -naphthylamine and sulfanilic acid are already present in the tube. The test organisms were able to reduce nitrate. Bright red color after the addition of α -naphthylamine and sulfanilic acid and no color change upon the addition of zinc was recorded as positive nitrate reduction test.

Carbohydrate Fermentation- In this test, a test tube with an inverted Durham's tube containing a basal medium and the particular sugar (1% conc.) along with a suitable indicator (Bromocresol purple) was used. The medium was inoculated with the help of a loop containing the test organism and then incubated aerobically at $37^\circ C$ for 24 to 48 hrs. Change in color of the medium from purple to yellow indicates acid production and the fermentation of the particular sugar. The presence of air-bubble in the Durham's tube indicates gas production. The organism was tested for glucose, lactose, sucrose, maltose, and mannitol fermentation. *P. aeruginosa* showed acid production with glucose and mannitol sugars.

Indole hydrolysis- In this test, tryptone broth was used containing large amount of tryptophan. In presence of tryptophanase enzyme tryptophan was hydrolyze into an indole and pyruvic acid. The isolated organism was inoculated in tryptone broth. All the inoculated and uninoculated (control) tubes were incubated at $37 \pm 0.2^\circ C$ for 48 hrs. After incubation, 5 drops of Kovac's reagent was added, a red layer at the top of broth indicates positive test, where as no change in color indicated negative test.

Methyl red test- The Methyl Red test involved adding the pH indicator methyl red to MR-VP broth. If the organism uses the mixed acid fermentation pathway and produces stable acidic end products, the acids will overcome the buffers in the medium and produce an acidic environment in the medium. The isolated microorganisms were inoculated in Methyl Red-Voges Proskauer broth. All the inoculated and uninoculated (control) tubes were incubated at $37 \pm 0.2^\circ C$ for 48 hrs. After incubation, 5-6 drops of methyl red reagent was added. Red color of medium indicates positive test, while no color change in the medium indicated negative result.

Voges- Proskauer test- This test was performed to determine the capability of microorganism to produce non-acidic end products such as ethanol and acetoin (acetyl methyl carbinol) from the organic acid. The

isolated microorganisms were inoculated in Methyl Red-Voges Proskauer broth. All the inoculated and uninoculated (control) tubes were incubated at $37\pm0.2^{\circ}\text{C}$ for 48 hrs. After incubation, 12 drops of freshly prepared VP-reagent I (naphthol solution), 2-3 drops of VP-reagent II (40% KOH) was added in all the inoculated and uninoculated tubes. Development of crimson to pink (red) color indicated positive test where as no change in color indicated negative test.

Citrate utilization- Citrate test was performed to determine the ability of microorganisms to utilize citrate as carbon source. The utilization of citrate depends on the presence of an enzyme citrase that breakdown citrate to oxaloacetic acid and acetic acid. The isolated organism were inoculated in Simmon's Citrate Agar slant and incubated at $37\pm0.2^{\circ}\text{C}$ for 48 hrs. After incubation, tubes were examined for change in coloration of slant from green to blue indicating positive test for citrate utilization. If color did not change, it indicated negative test for citrate utilization.

Motility test- This test was done to check the motility of the bacterium. Tube containing motility agar was stab inoculated. Positive test is indicated by the growth around the stab line that has radiated outwards in all directions while no growth around the stab line indicates negative test.

Urease test- Urea is a major organic waste product of protein digestion. This test was performed to determine the ability of microorganisms to produce enzyme urease. The urease is hydrolytic which attacks the carbon and nitrogen bond amide compounds (e.g. urea) with the liberation of ammonia. Urease test was performed by growing the organism on urea agar medium containing the pH indicator phenol red (pH 6.8). During incubation, microorganism possessing urease reduces ammonia that raises the pH of the medium. As the pH become higher, the phenol red was changed from a yellow color (pH 6.8) to a red or deep pink color, which indicated positive test where as no change in color indicated negative test.

Antibiotic susceptibility pattern of *P. aeruginosa*-

Antibiotic susceptibility pattern of *P. aeruginosa* isolates was studied using Disc Diffusion method described by Bauer *et al.* [12]. In this test the isolates obtained were swab inoculated on Mueller Hinton agar plate. Under sterile conditions, antibiotic discs were placed on the surface of the inoculated plate. The plates were incubated at 37°C , for 24 hrs, observed for the zone of inhibition and compared with CLSI (Clinical and Laboratory Standards Institute) scale [13]. For conducting the antibiotic susceptibility test the following antibiotics discs purchased from Hi-media (Mumbai) were used: Ceftazidime (Ca), Amikacin (Ak), Imipenem (I), Ciprofloxacin (Cf), Tetracycline (T), Gentamicin (G), Norfloxacin (Nx), Penicillin (P), Chloramphenicol (C), Ofloxacin (Of).

Effect of antiseptic on *P. aeruginosa*- Antiseptic test was done by using filter paper disk method. In this test the isolates obtained were swab inoculated on Nutrient agar plate. Sterile disk was prepared by dipping in alcohol with the help of the forceps, in front of the flame and was then dipped half way in to a beaker containing the chemical agents (Betadine and Dettol). Chemical agents were diluted up to 10^{-4} . Antiseptic discs were prepared by soaking them in dilutions from 10^{-1} to 10^{-4} . Under sterile condition impregnated antiseptic disc was placed on the surface of the inoculated plate. The plates were inoculated at 37°C for 24 hrs and observed for the zone of inhibition (mm) [14].

STATISTICAL ANALYSIS

The data obtained during the course of investigation was statistically analyzed by applying χ^2 -test at 5% probability level as well as Z-test and t-test was interpreted accordingly (Panse and Sukhatme [15]).

RESULTS AND DISCUSSION

In the present study, of the hundred clinical samples collected from different patients a high prevalence rate of bacterial pathogen (51%) was observed. Screening of the clinical samples for incidence of *P. aeruginosa* showed the percentage occurrence to correspond to 53% (Table 1 & Fig. 3). *P. aeruginosa* is reportedly the most frequently isolated non-fermentative gram negative bacterium that has emerged as a major nosocomial pathogen. With respect to the observations made in the present study most literature report a similar prevalence rate of *P. aeruginosa* ranging from 44.7% - 91.67% [16-18]. Certain reports, have however documented a lower incidence of the pathogen in the range 13%- 31.52% [19-21]. Incidence of *P. aeruginosa* was affected by geographical factors, duration of investigation, culture technique and geographical variations.

Table 1: Incidence of *P. aeruginosa* in clinical samples

Total Samples	Positive samples for bacterial isolates (%)	<i>P. aeruginosa</i> (%)
100	51(51)	27(53)

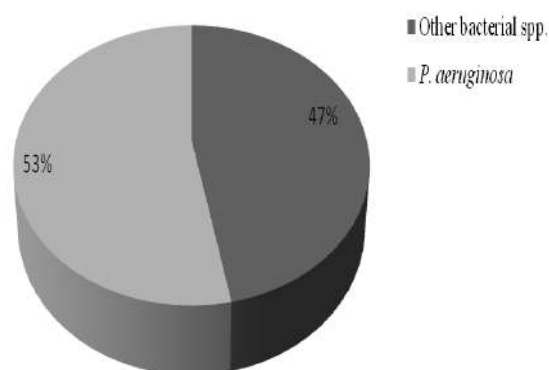


Fig. 3: Percentage incidence of *P. aeruginosa* in clinical samples

The different samples screened for *P. aeruginosa* included urine, blood, wound, pus and sputum (20 each). Distribution pattern showed highest isolation rate from wound (33.33%) followed by sputum (22.22%), pus (18.52%) and urine (14.81%). The least number of isolates were obtained from blood samples (11.11%). On

analyzing the data, the incidence of bacterial pathogen with respect to samples type were found to be statistically non-significant (Table 2 & Fig 4). Similar studies have reported maximum isolation of *P. aeruginosa* from wound and pus varying in the range 27.81% - 33.30% [9,22].

Table 2: Distribution of *P. aeruginosa* in different sample sites

Total Isolates	Samples sites				
	Urine (%)	Blood (%)	Wound (%)	Pus (%)	Sputum (%)
	4 (14.81%)	3 (11.11%)	9 (33.33%)	5 (18.52%)	6 (22.22%)

$\chi^2 = 3.93$, NS= Non-significant, $\chi^2 (5\%) = 9.49$

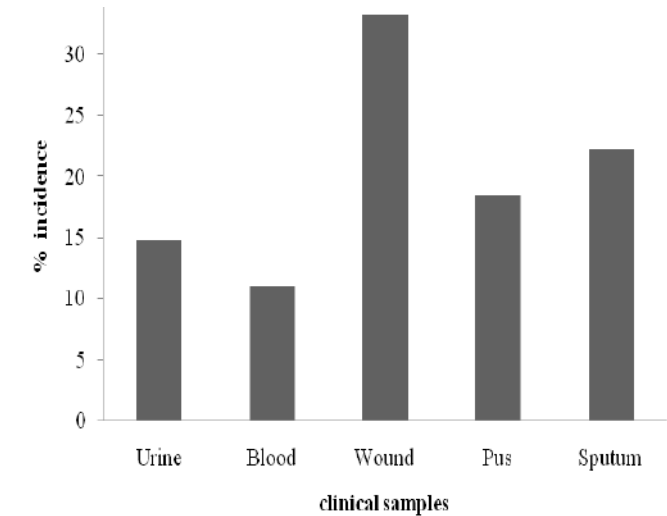


Fig. 4: Distribution of *P. aeruginosa* with respect to clinical samples

The clinical isolates of *P. aeruginosa* were subjected to antibiotic sensitivity test. Results revealed an increasing trend towards development of antibiotic resistance. The resistance rates corresponded to 100% for Penicillin, Chloramphenicol and Tetracycline, 92% for Ceftazidime, 85% for Amikacin, Ciprofloxacin [23], Gentamicin, 81% for Ofloxacin, 74% for Norfloxacin, and 56% for Imipenem. On analyzing the data, antibiotic sensitivity pattern (Table 3) against *P. aeruginosa* with respect to antibiotics were found to be statistically non-significant (Table 4, Fig. 5-6). Several studies have documented a similar pattern of resistance among *P. aeruginosa* isolates. Resistances have been recorded against Penicillin (80.4%), Amikacin (81%-92.68 %), Tetracycline and Gentamycin (71.6%), Norfloxacin (25.5%), Ciprofloxacin (75.8%-79%), Chloramphenicol (100%), Ceftazidime (80%) and Ofloxacin (81.6%). [17,20,24] Imipenem was the only drug in the present investigation towards, which *P. aeruginosa* had shown least resistivity. However, resistance rate up to 56% was observed. The resistance rate to imipenem has been previously shown to be increasing (24%-60%) [4,25]. The susceptibility pattern of *P. aeruginosa* has been reported

to be influenced by collateral damage from previous exposure to antibiotics. Further reduced permeability *i.e.* down regulation of porin channels in outer lipopolysaccharide membrane effectively reduces entry of carbapenems and is sufficient for acquisition of resistance.

Table 3: Antibiotic susceptibility pattern of *P. aeruginosa* isolates

S. No.	Antibiotic Disc	Conc. (µg)	<i>P. aeruginosa</i>
1	Ca	30	S
2	Ak	30	S
3	I	10	S
4	Cf	30	S
5	T	10	R
6	G	10	S
7	Nx	30	S
8	P	30	R
9	C	30	R
10	Of	5	S

S= Sensitive, R= Resistant
Ca = Ceftazidime, G = Gentamicin
Ak = Amikacin, Nx= Norfloxacin
I = Imipenem, P = Penicillin
Cf = Ciprofloxacin, C = Chloramphenicol
T = Tetracycline, Of= Ofloxacin

Table 4: Percentage resistance of Clinical isolates of *P. aeruginosa* against antibiotics

Total samples No.	Pa isolates	Percentage resistance against Antibiotics (%)									
		Ca	Ak	I	Cf	T	G	Nx	P	C	Of
100	27	92	85	56	85	100	85	74	100	100	81

$\chi^2= 5.40$, NS= Non-significant, χ^2 (5%) = 9.49, Pa= *Pseudomonas aeruginosa*

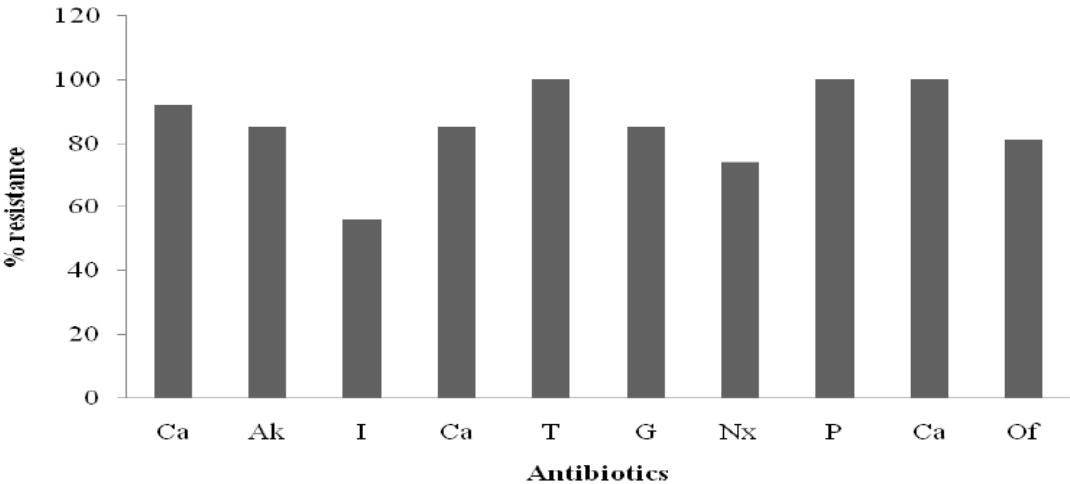


Fig. 5: Percentage resistance of clinical isolates of *P. aeruginosa* against antibiotics

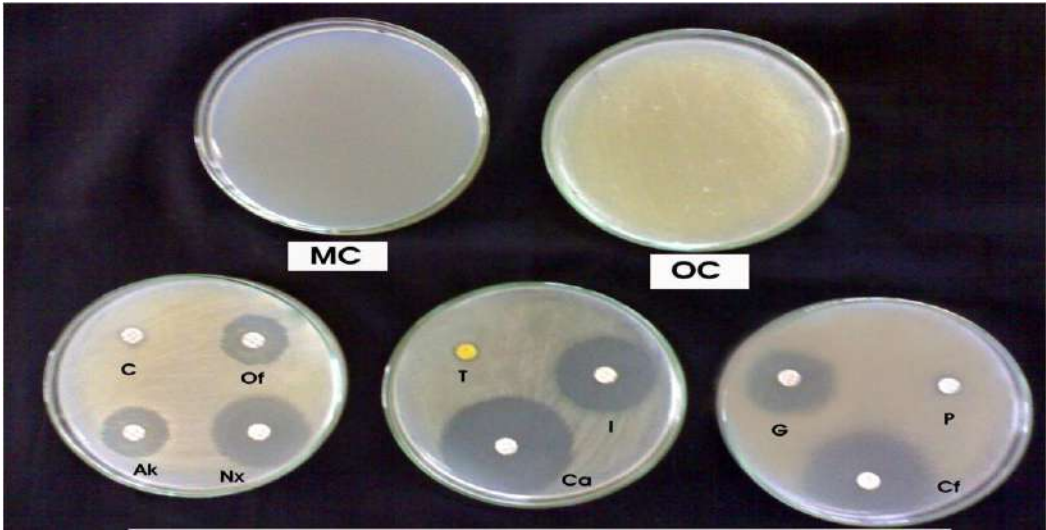


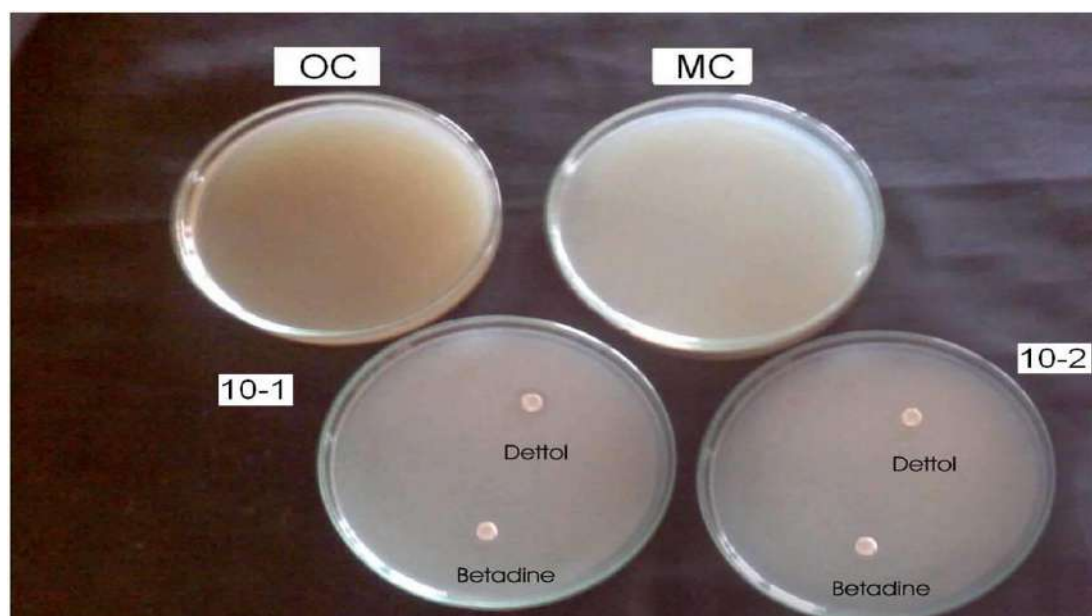
Fig. 6: Antibiotic susceptibility pattern of *P. aeruginosa*

Antiseptics are used extensively in hospitals and healthcare settings for a variety of topical applications. In particular they are an essential part of infection control practices and aid in the prevention of nosocomial infections. The activity of two commonly used antiseptics (Betadine & Dettol) was evaluated against *P. aeruginosa*. As per the results obtained both antiseptics were effective against *P. aeruginosa*. Dettol exhibited slightly greater inhibition (in terms of zone diameter) as compared to Betadine (Table 5 & Fig. 7). Both antiseptics were effective at dilutions up to 100 fold. However, no activity was observed at 1000 fold dilution. Thus Dettol and Betadine shown MIC at 100 fold dilution for *P. aeruginosa*.

There was no activity against the pathogen on subsequent (Betadine & Dettol). Similar to the present study other reports have documented 100 fold dilutions of antiseptics as Betadine, Lysol, Dettol, and Savlon to have an inhibitory effect towards *P. aeruginosa* [14,26]. Over dilution i.e. dilution of 1000 fold and above has been also previously shown to have very negligible or no inhibitory effect against the pathogen [27,28]. The effectiveness of antiseptics in controlling infection is often compromised by the fact that these agents get contaminated during the preparation process. Further, resistance due to incorrect product use and ineffective infection control practices cannot be underestimated.

Table 5: Antimicrobial activity of antiseptics (Betadine & Dettol) *P. aeruginosa*

S. No.	Antiseptics	Mean value of zone of inhibition (mm)			
		10^{-1}	10^{-2}	10^{-3}	10^{-4}
1	Dettol	7.6	5.7	0	0
2	Betadine	5.6	4.3	0	0

**Fig. 7:** Antimicrobial activity of antiseptics (Betadine and Dettol) on *P. aeruginosa*

The study population was divided into three categories based on their age: 0-30 yrs, 31-60 yrs and 61-90 yrs. Results revealed higher occurrence of *Pseudomonas aeruginosa* (50%) among patients of the age group 31-60 yrs followed by age 0-30 yrs (28.33%) and least in the age 60-90 (21.66%) (Table 6 & Fig 8). The data however lost significance, when analyzed statistically. Similar data have been cited in other literature where greater isolation

was obtained in patients of the age groups 0-29 yrs (15%) and 21- 40 yrs (43.2%)^[19,21,22].

In contrast most studies document the old age group (<50 yrs) to be predisposed to *P. aeruginosa* infection^[29-31]. Since the majority of patients were in the intermediate age, hence the greater isolation rates. Infection is influenced by general health of a patients and risk increases with the exposure to the pathogens.

Table 6: Age as a risk factor causing infection due to *P. aeruginosa* in among patients

S. No	Age group (year)	Total patients	Occurrence of <i>P. aeruginosa</i>	
			Total no. of +ve cases	Total no. of -ve cases
1	0-30	29	17(28.33%)	12(30%)
2	31-60	51	30(50%)	21(51.22%)
3	61-90	20	13(21.67%)	7(17.50%)

t- test= 0.84, NS= Non-significant, t_{table} = 12.6

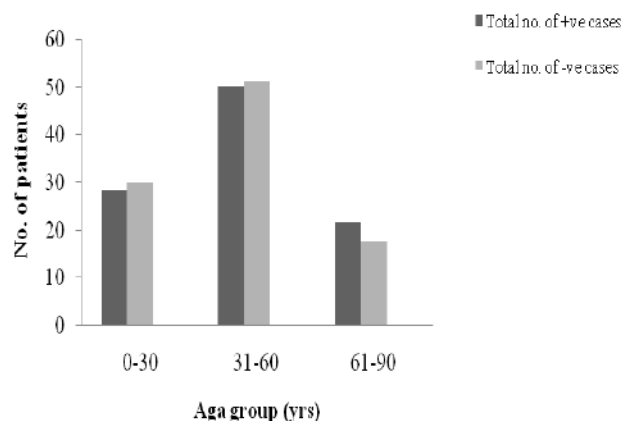


Fig. 8: Age as a risk factor causing infection due to *P. aeruginosa* among patients

Occurrence of *P. aeruginosa* on the basis of gender is shown in Table 7 & Fig 9. The results showed higher occurrence in males (59.26%) and females patients (40.74%). The difference was however found to be non-significant. The present findings were supported by other studies where male patients showed higher incidence [21,22,31]. Maintenance of hygienic standards contributes to acquisition of pathogen by the individual.

Table 7: Gender as a risk factor causing infection due to *P. aeruginosa* in among patients

S. No	Gender	Total patients	Occurrence of <i>P. Aeruginosa</i>	
			Total no. of +ve cases	Total no. of -ve cases
1	Male	63	16(59.26%)	47(64.38%)
2	Female	37	11(40.74%)	26(35.62%)

Z-test = 0.29, NS= Non-significant, χ^2 (5%) = 1.96

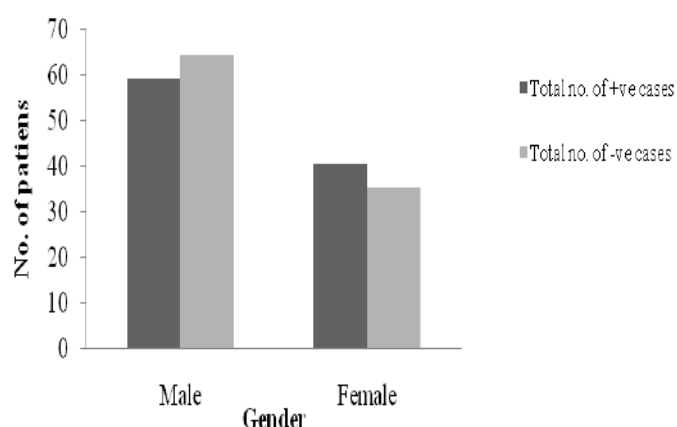


Fig. 9: Gender as a risk factor causing infection due to *P. aeruginosa* among patients

Duration of disease in patient was another factor analyzed for incidence of *P. aeruginosa* infection. Two categories were defined on the basis of disease duration: 1-10 days and 11-20 days.

The occurrence of *P. aeruginosa* was higher in duration of disease for 11-20 days (81.82%) as compared to 1-10 days (18.18%). The data was found to be significant when analyzed statistically (Table 8, Fig 10). Duration of disease over 5 days has been identified previously as a major risk factor predisposing a patient to acquire infection due to *P. aeruginosa* [31]. Duration of disease disposes a patient to acquire nosocomial infection due to immune compromised state of the individual.

Table 8: Duration of disease as a risk factor causing infection due to *P. aeruginosa* among patients

S. No	Duration of disease (days)	Total patients	Occurrence of <i>P. Aeruginosa</i>	
			Total no. of +ve cases	Total no. of -ve cases
1	1-10	42	8(18.18%)	34(60.71%)
2	11-20	58	36(81.82%)	22(39.29%)

$\chi^2 = 18.3$, S= Significant, χ^2 (5%) = 3.84

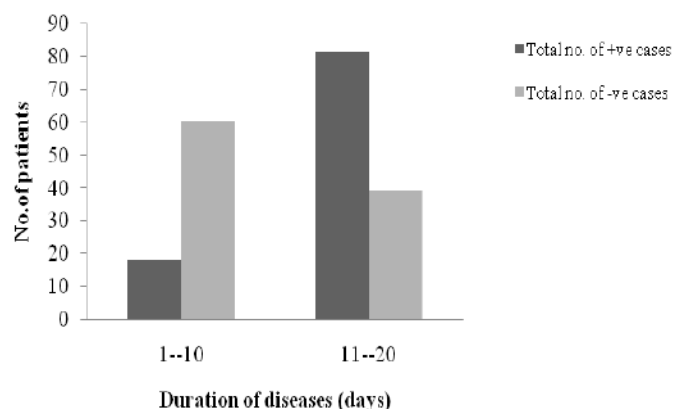


Fig. 10: Duration of disease as a risk factor causing infection due to *Pseudomonas aeruginosa* among patients

On the basis of duration of hospital stay 3 groups were categorized: 1-10 days, 11-20 days and 21-30 days. Highest incidence was observed in 21-30 days duration (53.33%) followed by 11-20 days (33.33%) and least in 1-10 days (13.33%). On analyzing the data, the incidence of *P. aeruginosa* infection on the basis of hospitalization duration were found to be statistically significant (Table 9 & Fig 11). Similar results were reported by Oguntibeju and Rau [19] and Talon *et al.* [32], who identified duration of hospital stay as a major risk factor. Length of hospitalization affects colonization due to exposure of the individual to various pathogens harbored in the environment and by constant handling by health care workers.

Table 9: Hospitalization duration as a risk factor causing infection due to *P. aeruginosa* in among patients

S. No	Hospitalization duration (Days)	Total patients	Occurrence of <i>P. aeruginosa</i>	
			Total no. of +ve cases	Total no. of -ve cases
1	1-10	17	2(13.33%)	15(60%)
2	11-20	14	5(33.33%)	9(36%)
3	21-30	9	8(53.33%)	1(4%)

$\chi^2= 16.89$, S= Significant, χ^2 (5%) =5.99

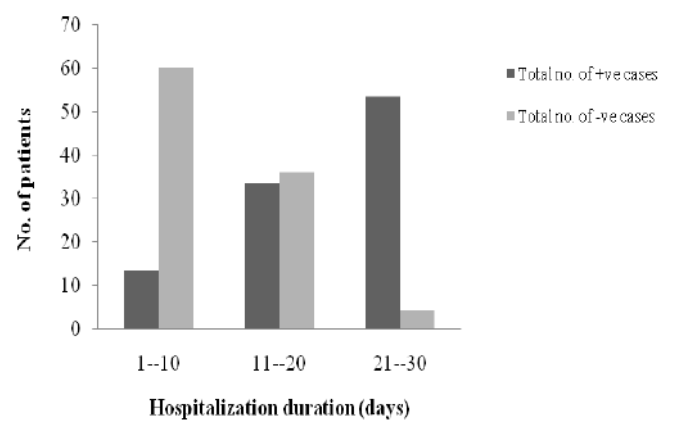


Fig. 11: Incidence of *P. aeruginosa* infection on the basis of Hospitalization duration

CONCLUSIONS

P. aeruginosa is a major cause of nosocomial infection. Despite advances in sanitation facilities and the introduction of wide variety of antimicrobial agents with anti-pseudomonal activities of *P. aeruginosa* continue to be cause of life-threatening infections. With growing concerns about the development of biocidal resistance and cross-resistance with antibiotics, clinical isolates should be under continual surveillance and other possible mechanisms of resistance should be investigated. Also, antiseptic and disinfectant products can vary significantly in their activity despite containing similar levels of biocides, which underlies the need for close inspection of efficacy claims. It will also make for more efficient use of antipseudomonal agents clinically with the potential for the design of newer, more effective compounds and products.

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