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Prevalence and antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolated from non clinical samples of hospital environment

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Abstract: *Pseudomonas aeruginosa* is recognized as the second leading cause of gram- negative nosocomial infection and reduced antibiotic susceptibility among the strains from hospital environment is major treatment challenge. In view of this the present study was aimed to check the prevalence and antibiotic susceptibility pattern of *Pseudomonas aeruginosa* among the hospital environment. A total of 50 non clinical samples were collected from hospital environment. It was found that prevalence of *P. aeruginosa* was 46%. Highest occurence of bacteria was found from floor tiles (100%) and basins (80%) of hospital settings. High resistance was exhibited among the isolates. Many isolates showed multiple drug resrstance (MDR). The highest resistance was shown towards Ampicillin and Amoxyclav (100%) followed by Erythromycin (82.60%), Chloramphenicol (69.56%) and Tetracycline (65.21%), Gentamicin (60.86%) and Ciprofoxacin (56.52%).

Key Words: *Pseudomonas aeruginosa, non clinical, antibiotic susceptibility.*

1. INTRODUCTION:

Nosocomial infection or hospital-acquired infections (HAIs) are one of the most important problems in the worldwide. These infections are more dangerous than other infections. because they are caused by bacteria have a high resistance to antibiotics. These infections area important cause of increased morbidity, mortality and healthcare costs worldwide (Maheswaran *et al.,* 2007).According to a study cited by the WHO (World Health Organization), over 1.4 million people worldwide suffer from HAI at any given time (*WHO,* 2002). The risk of nosocomial infection depends on a number of factors. These include the ability of pathogens to remain viable on a surface. The rate at which contaminated surfaces are touched by patients and healthcare workers, the context in which the patient is exposed, and the levels of contamination that result in transmission to patients (Boyce, 2007).

Pseudomonas are diverse group of established and emerging pathogen and are major agents of nosocomial and community-acquired infections, widely distributed in the hospital environment where they are particularly difficult to eradicate (Ravichandra *et al.*, 2012). Most members of the genus (especially *Pseudomonas aeruginosa*) are opportunistic pathogens often associated with infections of the urinary tract, respiratory system, soft tissue, bone and joint, gastrointestinal infections, dermatitis. Bacteremia and a variety of systemic infections, particularly in patients with severe borns, cancer and AIDS (Pirmay *et al.*, 2005).

The overall prevalence of antibiotic-resistant *P. aeruginosa* is increasing, with up to 10% of global Isolates found to be multi-drug resistance. It is recognized as the second leading cause of gram- negative nosocomial infection and a major treatment challenge for *Pseudomonas aeruginosa(*Nasreen *et al.*,2015*).*Multidrug-resistant (MDR) strains of *P. aeruginosa* are often isolated among patients suffering from nosocomial infections, particularly those in the intensive care unit (ICU), *(Tassios ,et al.*,1997*).*Thus, infections caused by *P. aeruginosa* are par-ticularly problematic because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs (Gales *et al.*,2001).

Due to its nominal nutritional requirement *P.aeruginosa* has the ability to survive in soil, plant surfaces, waste water, moist environment, surface water, even on inert materials (Remington*;* Schimpff .,1981). *P. aeruginosa* is mostly acquired From environment and spread person-to-person rarely (Anthony . *et al* .,2002). *P. aeruginosa* is highly

ubiquitous in water system and Capable to acquire antibiotic resistancedue to its low outer membrane permeability and extensive efflux pump system (Kato ,*et al.*,2001; Lister *et al.*,2009). Thus this study has been undertaken to check the prevalence and antimicrobial susceptibility of *Pseudomonas aeruginos*a isolated from various non clinical samples in the hospital setting.

2. MATERIAL AND METHODS:

- **Collection of samples**

A total of 50 non-clinical samples were collected from Government Medical Hospital, Akola during the September to December 2023. The samples were collected using sterilized cotton swabs from various sites and hospital wards into saline suspension (0.85%) and transported to laboratory,The collection of samples along with the data is shown in table 1

- **Table 1:- Collections of samples**

SN	Samples	Codes	Sites of samples collection
1	Bed	B1	Department of radiology
2	Bed	B2	Ward no. 1
3	Bed	B3	Ward no. 1
4	Bed	B4	Orthopedic preparative room no.6
5	Bed	B5	Ward no . 12
6	Wall	W1	Physiotherapy department room no. 112
7	Wall	W2	Ward no. 22
8	Wall	W3	Ward no. 3
9	Wall	W4	Ward no. 1
10	Wall	W5	Ward no. 13
11	Basin	Ba1	Physiotherapy department room no. 112
12	Basin	Ba2	Ward no. 23
13	Basin	Ba3	Ward no. 3
14	Basin	Ba4	Ward no.1
15	Basin	Ba5	(TCU) Ward no. 26
16	Electrical board	E1	Ward no. 10
17	Electrical board	E2	Ward no. 13
18	Electrical board	E3	(TCU) Ward no. 26
19	Electrical board	E4	Ward no. 19
20	Electrical board	E5	ENT room no. 124
21	Floor	F1	Ward no. 10
22	Floor	F2	Ward no. 12
23	Floor	F3	ENT Anesthesia room
24	Floor	F4	Ward no 19
25	Floor	F5	Ward no. Ward no. 23 unit II
26	Door	D1	Ward no. 8 (ICU)
27	Door	D2	Ward no. 7
28	Door	D3	Ward no. 6
29	Door	D4	Ward no. 5
30	Door	D5	Ward no. 2
31	Window	Wd1	ICCU recovery room
32	Window	Wd2	Ward no. 7
33	Window	Wd3	Ward no. 6
34	Window	Wd4	Ward no. 5
35	Window	Wd5	Ward no.9
36	Chair	C1	Ward no. 23 Unit II
37	Chair	C2	Ward no. 1

38	Chair	C3	Ward no. 12
39	Chair	C4	Ward no. 10
40	Chair	C5	Ward no. 21
41	Table	T1	Ward no. 23 NICU
42	Table	T2	Ward no. 1,room no. 1
43	Table	T3	Ward no. 12
44	Table	T4	Ward no. 11
45	Table	T5	Ward no. 21
46	Trey	Ty1	Ward no. 19
47	Trey	Ty2	Ward no. 24
48	Trey	Ty3	Ward no. 21
49	Trey	Ty4	Ward no. 11
50	Trey	Ty5	Ward no. 23

- **Isolation and identification of *Pseudomonas aeruginosa* from Samples**

The sample collected from non clinical sources were inoculated on Sterilized cetrimide agar and plates were incubated at 37^{O}C for 24 hours. After incubation the isolated colonies were further streaked on nutrient agar slants and preserved at 4^{O}C for further study. The pure cultures were then identified by standard conventional methods which includes morphological cultural and biochemical characteristics

- **Determination of antibiotic susceptibility/resistance pattern of isolates.**

Antimicrobial susceptibility was performed on Mueller Hinton agar by standard disc diffusion method recommended by clinical and standards institute. This was done by dipping a sterile swab into broth culture of test bacteria, carefully swabbing the entire surface of Muller Hinton agar plates the antibiotics used against *P.aeruginosa* were as follows:

Gentamicin (GEN^{10}) , Ciprofoxacin (CIP^{5}), chloramphenicol (C^{30}), Erythromycin (E^{15}), Ampicillin AMP^{10}, Amoxyclav AMC^{30}, and Tetracycline (TE^{30}). Then, the antibiotic discs were placed on the surface of the inoculated plates and gently pressed. The plates were incubated at 37^{O}C for 18-24 h. The diameter of inhibition zone was measured in millimeters and isolates were scored as sensitive or resistant by comparing with values recommended on standard charts.

3. RESULTS AND DISCUSSION:

Pseudomonas aeruginosa is a major cause of nosocomial infection. Despite advances in sanitation facilities and the introduction of a wide variety of antimicrobial agents with antipseudomonal activities, life threatening infections caused by *Pseudomonas aeruginosa* continue to be hospital infections. A critical factor in the survival of *Pseudomonas aeruginosa* in an unfavorable environment is its ability to transform from a mobile "swarmer" cell to a glycocalyx enclosed microcolony which serves to protect the organisms against the active phagocytes, surfactants, enzymes and high levels of specific antibodies Nowadays, the prevalence of *Pseudomonas aeruginosa* and the new resistant strains continue in both community-acquired pathogens and hospital originated infections. Maniatis *et al*., (1997)

In the present study a total of 50 non-clinical samples were collected from Government medical Hospital. Total 23 samples were found to be positive for the presence of *P. aeruginosa*. Amongst the non clinical samples 46% of prevalence of *P. aeruginosa* was observed from the hospital environment (fig.1) while 54% of samples were found to be negative for the presence of *P. aeruginosa.* Altayar *et al.*, (2016) reported only 6% of *P. aeruginosa* from hospital environment, Savas e*t al.*, (2005) reported 16.4% of *P. aeruginosa* isolated from intensive care unit of hospital. In a study carried out in turkey, Inan *et al.(*2000*)* isolated 68%of *Pseudomonas aeruginosa* strains and 60-83% of the antibiotics resistant strains from ICU patients. In the same study, resistance was detected against ceftazidime (34%), Imipenem (26%) , and amikacin (26%) .

In the study occurrence of *P. aeruginosa* from different samples were checked. (Table 1). It was found that all floor samples were found to be positive for *P. aeruginosa*. It was found that 80% of basin samples were positive for *P. aeruginosa.* It was followed by bed and tray (60%), chair and table (40%).Wall, electric board, door, window showed 20% of occurrence of *P. aeruginosa.* Similarly altayyar *et al*., (2016) also reported 50% of isolates obtained from sinks, 16.7% from ground , 8.3% from walls, and chair.

P. aeruginosa often has been isolated from sinks and other moist sites in hospitals. The Possible transmission of *P. aeruginosa* from sinks to patients has been reported. Recent reports showed extensive bacteriological screening of the inanimate Hospital environment has identified *Pseudomonas spp*. In the majority of moist sites of hospital. The important and environmental sources of *Pseudomonas spp*. are Humid places. The role of contamination of the Environment with *Pseudomonas* in hospital has been (Panagea *et al*., (2005); Pal *et al*.,(2010).

The antibiotic susceptibility pattern of all the isolates was checked by kirby-Bauer method (Fig 3). It was found that *P. aeruginosa* isolates showed multiple drug resistance as resistance was shown to more than two antibiotics among the isolates. Further high resistance was exhibited among the isolates. The highest resistance was shown towards ampicillin and Amoxyclav (100%). It was followed by Erythromycin (82.60%), Chloramphenicol (69.56%) and Tetracycline (65.21%), Gentamicin (60.86%) and Ciprofoxacin (56.52%). The present results are in accordance with the studies of Masood and Zahra (2014) ; Altayyar et *al*., (2016), Savas *et al*,(2005). The present results are in accordance with the studies of Masood and Zahra (2005); Altayyar *et al*., (2016), Savas *et al*.,(2005).

Fig. 1: Prevalence of *P. aeruginosa* isolated from non-clinical samples.

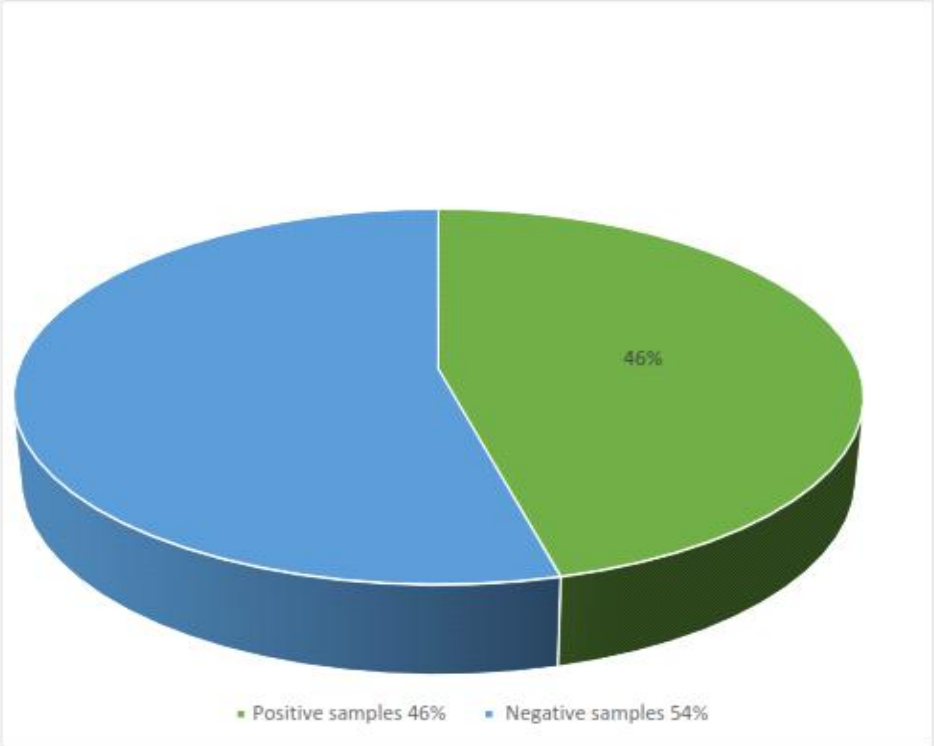


Table 1: Occurrence of *P. aeruginosa* isolated from non-clinical samples.

Sr.No	Sample	Occurrence of *P. aeruginosa*	
		Numbers	**Percentage (%)**
1	Bed	3	60%
2	Wall	1	20%
3	Basin	4	80%
4	Electrical Board	1	20%
5	floor	5	100%
6	Door	1	20%
7	Window	1	20%
8	Chair	2	40%
9	Table	2	40%
10	Trays	3	60%
	Total	**23**	**46%**

Fig. 2 : Occurrence of *P. aeruginosa* isolated from non-clinical samples.

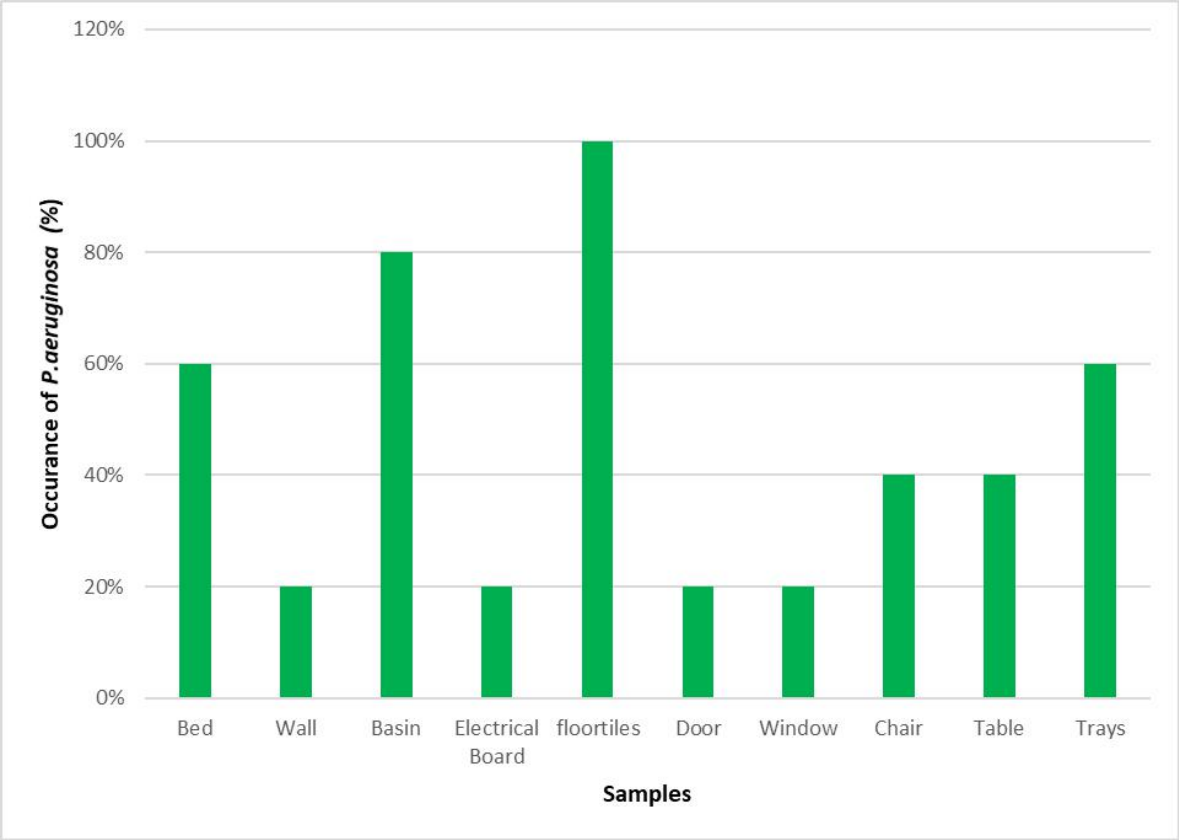
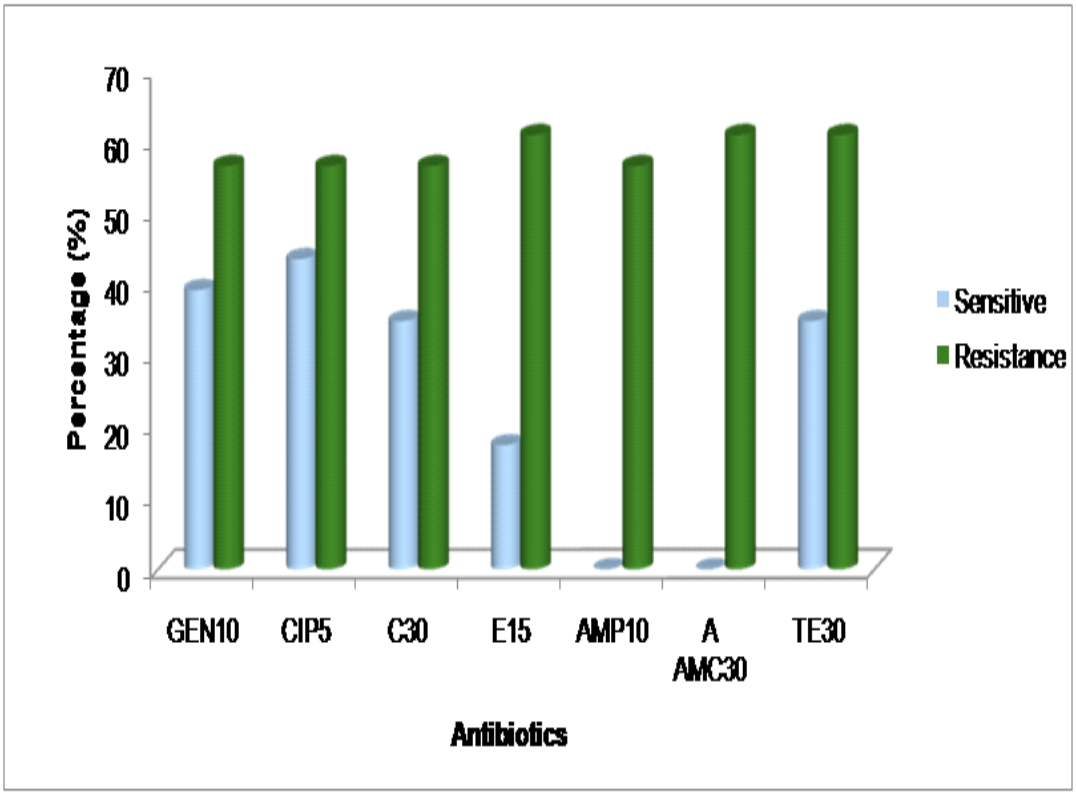


Table 2: Morphological, Cultural and Biochemical characteristics isolates

Characteristics	Isolates
Morphological characteristics	
Shap	Rod Shape Bacteria
Motility	Single polar flagella
Gram-character	Gram negative bacteria
Size	0.5 to 0.8 by 1.5 to 3 micro meter
Cultural characteristics	
Size	1 mm
Shape	Circular
Margin	Entire
Elevation	Flat
Opacity	Opaque
Consistency	Soft
Colour	Green pigment in the medium
Biochemical character IMViC test	
Indole test	Negative
Methyl red test	Negative
Voges-Proskauer test	Negative
Citrate utilization test	positive
Enzyme Test	
Nitrate	Negative
H2S gas production	positive
Amylase	Positive
Catalase	Positive

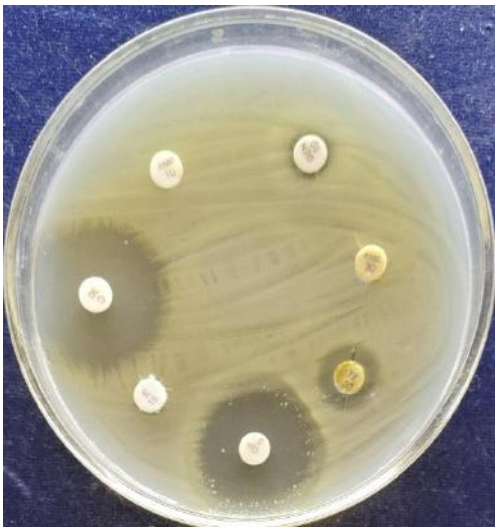
Urease	Negative
Gelatinase	Positive
Sugar fermentation test	
Glucose	Positive
Lactose	positive
Mannitol	Negative
Sucrose	Positive

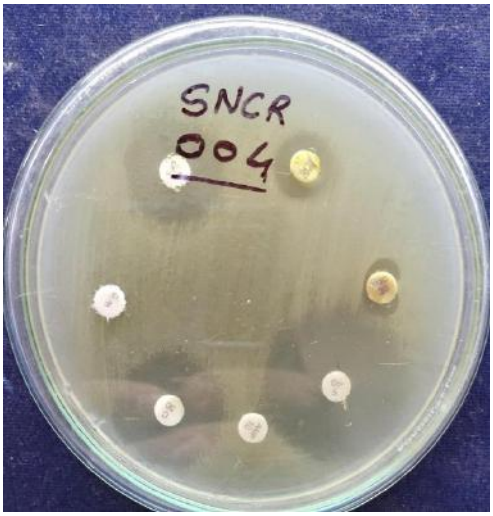
Fig. 3 :- Overall antibiotic sensitivity/ resistance pattern of *P. aeruginosa*



Collection of non-clinical samples from hospital.

Antibiotics susceptibility patterns of *P. aeruginosa*





4. CONCLUSION:

In the present study high prevalence of *P. aeruginosa* was found as 46% of non-clinical samples were found to be contaminated by the bacteria. MDR was also found to be present amongst the isolates. This study will help to improve the knowledge of antibiotic resistance pattern among physicians. Every effort should be made to prevent selection and spread of resistant organism. Frequent hand washing to prevent spread of organism should be encouraged. Better surgical and medical care should be provided to patients during hospital stay.

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