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Isolation and Identification of Various *Pseudomonas* Species from Distinct Clinical Specimens and the Study of Their Antibiogram

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Original Research Article

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Abstract: *Pseudomonas* is a group of bacteria, which is widely distributed in soil, water, skin flora and most man- made environments throughout the world. Due to multidrug resistance patterns of *Pseudomonas*, it is imperative to know the institutional prevalent susceptibility profiles. This study was conducted to isolate different species of *Pseudomonas* from various clinical samples, to determine the antibiotic susceptibility pattern and to carry out the epidemiological investigation of the isolates. The study was conducted in a tertiary care hospital, over a period of 2 years. After identification of genus *Pseudomonas*, the speciation was done by biochemical tests and by VITEK 2. Antibiotic susceptibility was determined by disc diffusion method. Extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs) production were detected by the combined disc diffusion test. An epidemiological study of *Pseudomonas*was carried out. Of 5096 infected samples, 505 (9.9%) were non-fermenting Gram-negative bacilli, among which 1 were found to be *Acinetobacter*. The highest numbers of isolates were *Acinetobacter baumannii*, followed by *Acinetobacter lwoffii, Acinetobacter radioresistance, Acinetobacter calcoaceticus, Acinetobacter Haemolyticus,* and *Acinetobacter ursingii*. Highest incidences of susceptibility were to imipenem (60%), chloramphenicol, and gentamicin. ESBL and MBL productions were detected in 23% and 17%, respectively. A high level of antibiotic resistance was observed in this study and maximum isolation rate was in SSI. Most of the patients had high-risk factors such as prolonged hospitalization, indwelling catheters, and orthopedics implants *in situ* or other catheterization and diabetes.

Keywords: *Pseudomonas, Burkholderia cepacia complex, Sphingomonas paucimobilis, Strenotrophomonas maltophilia*, Multi drug resistance, Extended-spectrum β-lactamases, Metallo-β-lactamases.

INTRODUCTION

Pseudomonas is gram negative non sporing rods, which are straight or slightly curved [1].They are obligate aerobes, use oxygen as terminal electron acceptor [2]. They are widely distributed in nature as saprophytes, found in soil, water, sewage or as commensals on human skin or in the human gut and some of them found in hospital environment [1, 3, 4]. These bacteria remain stable under extreme conditions of temperature, humidity, and pH and in the presence of commonly used detergents, such as highly concentrated alcohol preparations and other antiseptics which normally inhibit the growth of other bacteria [5]. This stability offers *Pseudomonas* a growth advantage over other organisms in hospital environments. Hence, in the recent era they have emerged as an important nosocomial pathogen due to its ability for survival in the hospital environment on a wide range of dry and moist surfaces [4,6]. Their nutritional veracity is responsible for their ubiquity and is able to recycling of organic matter. They are resistant to physical and

chemical compounds like chlorhexidine and quaternary ammonium compounds [6].

Many distinct types of pigments are produced by *Pseudomonas*, like water soluble pigments are carotenoids (yellow orange), viocin (violet or purple) and phenazines (red, maroon) that impart distinctive colour in the colonies. Fluorescent *Pseudomonas* are characterised by production of water soluble pigment, which diffuse freely in the media and fluoresce brightly under U.V ray. Pyocyanin, a blue phenazine derivative which is water –soluble and diffusible pigments. Lemonnierin is an intracellular, insoluble blue pigment [1,2].

Earlier it was believed to be non pathogenic but recently they are more frequently isolated as primary pathogen. Usually they cause hospital acquired infection (HAI). These organisms are most commonly isolated from patients of serious underlying diseases, such as patients with prolonged antibiotic therapy, endotracheal intubation, catheterization, in burn patients and in extremes of age like in neonates, children and in geriatrics patients[4].

The isolation rate of *Pseudomonas has* been increasing recently in tertiary care hospital. Moreover, they pose a great threat to mankind as they are resistance to common antibioticsThese organisms are inherently resistance to many antibiotics by developing various efflux mechanisms and other methods. *Pseudomonas* species resistance to Ampicillin,Amoxicillin, Amoxicillin-Clavulanate, narrow spectrum and expanded-spectrum Cephalosporin, Cefotaxime and Ceftriaxone and several efflux pump system[1,7,8]. However, due to unpredictable multidrug resistance patterns of clinical strains of *Pseudomonas*, it is imperative to know the institutional prevalent susceptibility profiles. Hence, this study was conducted to isolate the *Pseudomonas* species from various clinical samples by a simplified phenotypic identification protocol and to determine the antibiotic susceptibility pattern of these isolates.

MATERIALS AND METHODS

This was a prospective study. The study was conducted in the Microbiology Department of Dr. D.Y. Patil Medical College, Hospital and Research Centre, over a period of 2 years (i.e. July 2012 to September 2014). A total 15,169 clinical samples of pus, blood, body fluid (pleural fluid, peritoneal fluid, synovial fluid etc.), urine, sputum, cerebral spinal fluid (CSF) & throat swab were carried out. The blood samples from the suspected patients of sepsis were collected in the adult and paediatric bottles of BACT/ALERT 3D system. The samples were taken from the suspected patients, admitted to different wards and various intensive care units (ICU) of this hospital. A detailed history was taken. The study was approved by the Ethical Committee of our institute (Dr. D.Y Patil Medical College and Research Centre). The statistical analysis was performed with the help of Microsoft EXCEL for WINDOWS 2007.

Samples were processed for culture by standard conventional methods. Genus *Pseudomonas* was identified by Gram staining (gram negative bacilli), cell and colony morphology,pigment production (Fig 1,2,3) positive catalase test, positive citrate test, triple sugar iron (alkaline slant/ no change butt), positive oxidase test and strongly motile by of motility test . Speciation of *Pseudomonas*was performed on the basis of Hugh and Leifson oxidative-fermentative test (O-F) for glucose, sucrose, lactose, mannitol; gelatin liquefaction, beta haemolysis on blood agar media, nitrate reduction test, urease hydrolysis test (Christensen), Decarboxylation of Arginine, Lysin and Ornithine and growth at 35 ^{0}C and at 42^0 C for 18-24 hours on two tubes of trypticase soy agar (TSA). The final identification and confirmation was done by the Vitek 2 system [2].

Identification of pigment production by King's A and King's B medium [9]

King's A medium [9]: Pyocyanin, a blue phenazine derivative characteristic of *P.aeruginosa*was diffusible and its production was enhanced by growth in 'King A (Fig 4)[9]'.

King's B medium [9]: Fluorescent *Pseudomonas* were characterised by production of water soluble pigment, which diffused freely in the media and fluoresce brightly under U.V ray. The organisms produced this pigments were *P.aeruginosa*, *P. putida, P. fluorescens, P.chlororaphis* etc. and was manifested in low iron containing media[6]. "King B "medium was the universally use medium for the production of fluorescent pigment [9].

Antibiotic susceptibility testing was determined by Kirby - Bauer disc diffusion method [2, 10]: Muller-Hinton agar media was used. Commercially available Himedia discs were used. The strength of the discs used and their zone size interpretation were carried out by National Committee for Clinical Laboratory Studies (NCCLS) guideline. The first line antibiotics, which were tested, Piperacillin (10mcg/disc), Carbenicillin, Cefotaxim (30mcg/disc), Ceftriaxone (30mcg/disc), Ceftazidime (30mcg/disc), Ciprofloxacin(5 mcq/disc) Gentamicin (10mcg/disc) Amikacin (30mcg/disc) and Imipenem (10mcg/disc). The second line antibiotics, which were tested, Tobramycin (10mcg/disc), Ofloxacin (5mcg/disc), Amoxicillin/Clavulanic acid (20/10mcg/disc), Piperacillin/Tazobactam (100/10mcg/disc), Tigecycline (15mcg/disc), Colistin (10mcg/disc) and Ertepenem (10mcg/disc).

Detection of multidrug drug resistance (MDR) strain

The isolates which were resistance to three or more than three groups of drugs were considered as MDR strain [11]. The groups of drugs we were tested are: - Penicillin (Piperacillin, Carbenicillin), Cephalosporin (Cefotaxim, Ceftriaxone, Ceftazidime), Aminoglycosides (Gentamicin, Amikacin, Tobramycin), Carbapenem (Imipenem, Ertepenem), Fluroquinolones (Ciprofloxacin, Ofloxacin), Glycylcyclines (Tigecycline) and Colistin.

Detection of Extended spectrum β-lactamases production [10, 12]

The Combine disk diffusion test (CDDT) was used to determine the prevalence of extended spectrum β-lactamases (ESBL) production. Muller-Hinton agar media was used. One Ceftazidime (CAZ) (30μg) disc was placed on a lawn culture of test isolates and at the distance of 15 mm on both side of CAZ disc, a combination disc of Ceftazidime/ Tazobactam (30/10 μg) and Ceftazidime / Clavulanic acid (30/10 μg) were placed. A≥ 5 mm increased in a zone diameter for either antimicrobial agent tested in combination with Clavulanic acid or Tazobactam versus the zone diameter of the agent when tested alone = ESBL producer (Fig 5)[10,12].

Detection of metallo β-lactamases production

Muller-Hinton agar media was used. One Imipenem (10μg) disc was placed on a lawn culture of isolates and at the distance of 15 mm a combination disc of 10μg of Imipenem and 100μl of EDTA disc was placed. Then it wasincubated at 35^0C for 18 - 24 hours. An increase in zone size ≥ 7 mm around the Imipenem - EDTA disc as compared to Imipenem disc alone was recorded as positive(Fig- 6)[10,12].

An epidemiological study of *Pseudomonas was* carried out by means of IN-USE test (Fig -7). With a sterile pipette, transferred 1 ml of the used disinfectant into 9 ml of nutrient broth in a sterile universal container. Placed 0.02ml drops of this mixture onto ten different areas of two well dried nutrient agar plates. Incubate one plate at 37^0C for 3 days and another one at room temperature for 7 days. Read the test as showing failure of disinfection if there was growth in more than five drops in either place [1,2]. To tract the source, 25 samples were isolated from inanimate objects and from disinfectants of different wards and ICUs [1].

RESULTS

In this study, out of 15169 clinical samples, total number of culture positive isolates were 5096(33.59 %) among which 1921(37.69%) were gram positive cocci (GPC) and 3175 (62.3%) were gram negative bacilli (GNB). Out of 3175 GNB, 505 (15.9%) were non-fermenting gram negative bacilli (NFGNB). Out of the total 505 isolates, 307(60.79 %) were different species of Pseudomonas. They were predominantly isolated fromPus , accounting for 40.1%(123 samples), followed by 26.38% of different type of body fluids , next to it was 15.31% of sputum, 11.72% of blood samples and 6.51% of urine samples . Maximum *Pseudomonas* species isolated were from male Surgical ward (23%) followed by Medicine Intensive Care Unit (MICU) (10.8%) next to it was Medicine ward (9.6%), male pulmonary word (8.4%). There was a higher incidence of infection among males (69.8%). In our study the patients were divided into eleven age groups, based subgroup as in table 5. The mean age of the patients was 44.44 years and the median age was 41.75 years. The majority of the patients belong to 51 to 60 years, accounting for 16.4%, followed by the age group of 41 to 50 years comprises 16%, and next to this is the age group of 31 to 40 years accounting for 15%.

The highest number of isolates were *Pseudomonas aeruginosa*, comprises 61.56% followed by *P. fluroscence* 13.68%, *P. putida* 9.44%, *P. stutzeri* 6.84%, then *Strenotrophomonas maltophilia* (previous designation: *Pseudomonas maltophilia*) 5.54%, *Burkhelderia cepacia complex* (BCC) (previous designation: *Pseudomonas cepacia*) and *Sphingomonas paucimobilis* (previous designation :*Pseudomonas paucimobilis)* each comprises of 0.98%, *P. alcaligens* 0.65% and one isolate of *Burkhelderia pseudomallei* (previous designation*: Pseudomona spseudomallei*).

Highest number of *Pseudomonas* species were isolated from surgical site infection (SSI), comprises of 35.18%, followed by 22.12% isolates were yield from the patients who were suffering from respiratory tract infection and 16.29% isolated were obtained from the patients who have developed septicaemia (Table 2).In this study we have analyzed the risk factors for colonization and infection with *Pseudomonas*. Major surgeries, trauma, SSI, prolonged hospitalization, mechanical ventilation, indwelling foreign devices (especially orthopedic implants), diabetes mellitus (DM), debilitating disease like tuberculosis (TB) and previous antimicrobial therapy all have identified as risk factors which are predisposing to acquisition this infection .In this study 28.71% isolates were obtained from the patients who were suffering from Diabetes Mellitus (DM).Around 18.6% isolates were obtained from the patients, who were on mechanical ventilators. Next to it was 15.31% of isolates from those patients who have admitted in this hospital for a long tenure. However, 12.92% isolates were yield from the patients who had indwelling catheters or orthopedics implants in situ, where as 9.57% of Pseudomonas species from the immunocompromised patients who were on chemotherapy (Table 3).

The isolates of *Pseudomonas aeruginosa* revealed good susceptibility toErtepenam (90.8%) followed by Imipenem (86.77%), Tobramycin (66.66%) next to it, was Amikacin (64.02%) (Fig 9). Likewise P. *fluroscence* also revealed good susceptibility to Ertepenam (95%) followed by Imipenem (88%),

Gentamicin and Piperacillin each comprises 71.4% (Fig-10). *P. putida* revealed 91.2% sensitivity to Ertepenam followed by 89.6% sensitivity to Imipenem, 68.9% sensitivity to Amikacin and Tobramycin & Tigecycline both showed 63.4% sensitivity (Fig-11). However, in case of *P. stutzeri* around 94 % showed sensitivity Ertepenam followed by Imipenem (95%), Gentamicin (90%) and Piperacillin (90%) (Fig 12). Around 86(28.01%) isolates of Pseudomonas species were MDR strains, among which 48 isolates (55.81%) were ESBL producer and 20 isolates (23.25 %) were MBL producer (Table 14).

28 samples from inanimate objects and from disinfectant were collected from different wards and ICUs. Out of these 28 samples 11(39.28%) were culture positive among all the culture positive isolates 72.72%% were different species of pseudomonas, predominantly *P.aeruginosa* (50%) (Table 15).

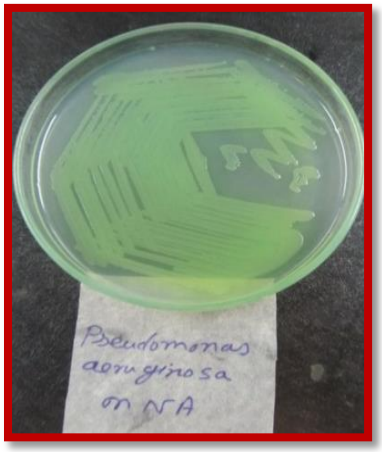


Fig-1: Growth of *Pseudomonas aeruginosa* on nutrient agar media

Fig-2: Growth of *Pseudomonas fluorescence* on nutrient agar media

Fig-3: Growth of *Pseudomonas aeruginosa* on nutrient agar media

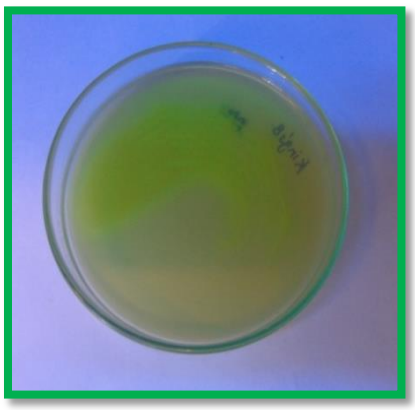


Fig-4: Kings B mediumunder U-V ray

Fig-5: ESBL producer

Fig-6: MBL producer

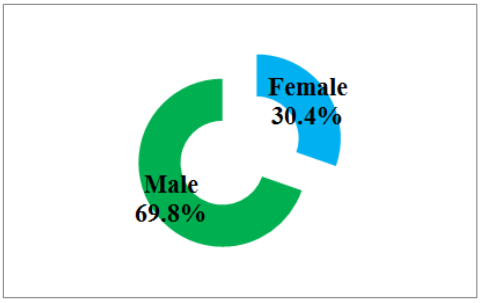


Fig-7: Gender distribution of the patients (n=307)

Test of proportion showed that the proportion of males (69.8%) were significantly higher than female

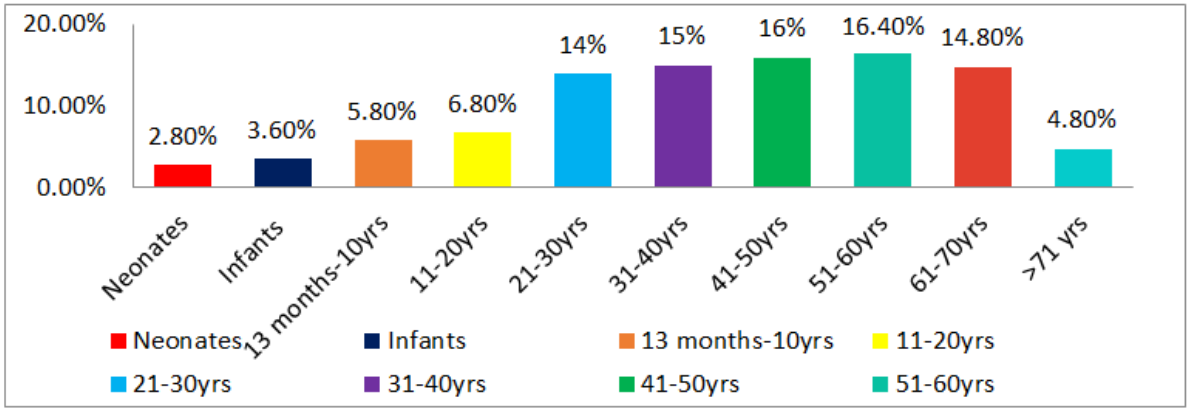


Fig-8: Age and Gender distribution of the patients (n=307)

The majority of the patient belong to 51 to 60 years.The lowest age group in this study is the neonates (till the 28th day of the life) and the highest age group is 81 to 90 years.

Table-1: Distribution of different species of *Pseudomonas* in different clinical samples (n=307)

Name of the organism	Pus	Body fluid	Blood	Sputum	Urine	Total (n=307)	%
Pseudomonas aeruginosa	80	55	9	30	15	189	61.56%
Pseudomonas fluroscence	20	10	3	6	3	42	13.68%
Pseudomonas putida	15	8	2	2	2	29	9.44%
Pseudomonas stutzeri	7	6	2	6	0	21	6.84%
Pseudomonas alcaligens	0	0	0	2	0	2	0.65%
Burkholderia cepacia complex	0	0	3	0	0	3	0.98%
Burkholderia pseudomallei	0	0	1	0	0	1	0.32%
Strenotrophomonas maltophilia	1	2	14	0	0	17	5.54%
Sphingomonas paucimobilis	0	0	2	1	0	3	0.98%
Total	123 (40.1%)	81 (26.38%)	36 (11.72)	47 (15.31%)	20 (6.51%)	307	

Table-2: Diagnosis wise distribution of the *Pseudomonas* (n=307)

Name of the organism	SSI	Surface non healing ulcers	Burn	CSOM	Sepsis	RIT	CA	UTI	GIT	Total
P.aeruginosa	75	10	3	8	21	40	10	8	14	189
P.fluroscence	15	4	1	1	9	6	3	1	2	42
P. putida	11	2	1	0	2	7	2	2	2	29
P. stutzeri	5	1	0	2	2	8	1	2	0	21
P. alcaligens	0	0	0	0	0	2	0	0	0	2
BCC	0	0	0	0	3	0	0	0	0	3
B.pseudomallei	0	0	0	0	1	0	0	0	0	1
S. maltophilia	2	0	0	0	10	4	1	0	0	17
S. paucimobilis	0	0	0	0	2	1	0	0	0	3
Total	108	17	5	11	50	68	17	13	18	307
%	35.2%	5.5%	1.6%	3.6%	16.3%	22.1%	5.5%	4.2%	5.9%	

Table-3: Risk factors wise distribution of the organisms (n=209)

Name of the organism	DM	Chemo therapy due to malignancy	Prolonged hospitalization	Indwelling Intra vascular catheters	Ortho pedic implant	Venti lation	TB	Total (%)
P.aeruginosa	33	12	19	11	11	25	13	124
P.fluroscence	17	2	2	2	0	4	3	30
P.putida	4	3	1	4	2	1	0	15
P.stutzeri	4	2	2	1	0	4	1	14
P.alcaligens	0	0	1	0	0	1	0	2
BCC	1	0	2	0	0	0	0	3
B.pseudomallei	0	0	0	1	0	0	0	1
S.maltophilia	1	1	3	8	0	3	1	17
S.paucimobilis	0	0	2	0	0	1	0	3
Total	60	20	32	27	13	39	18	209
%	28.71%	9.6%	15.31%	12.92%	16.22%	18.66%	8.61%	

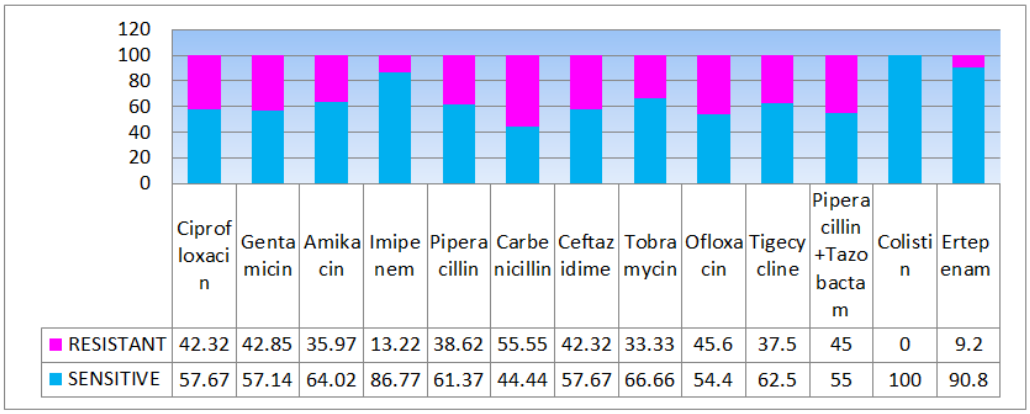


Fig-9: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* (n=189)

*Pseudomonas aeruginosa*shows a good sensitivity to Ertepenam (90.8%), Imipenem (86.77%), Tobramycin (66.66%) and Amikacin (64.02%).

P. fluroscence showed good sensitivity to Ertepenam (95%), Imipenem (88%), followed by Gentamicin (71.4%) and Piperacillin (71.4%) (Fig-10).

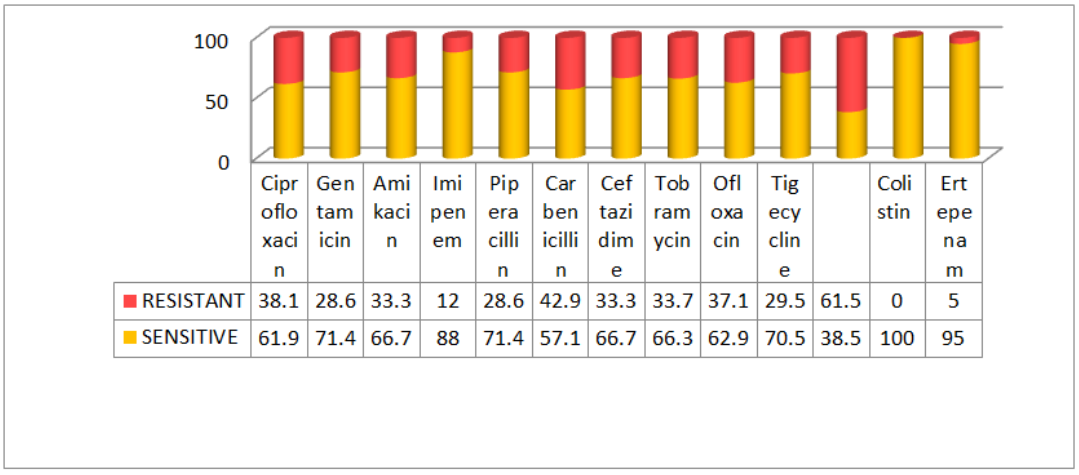
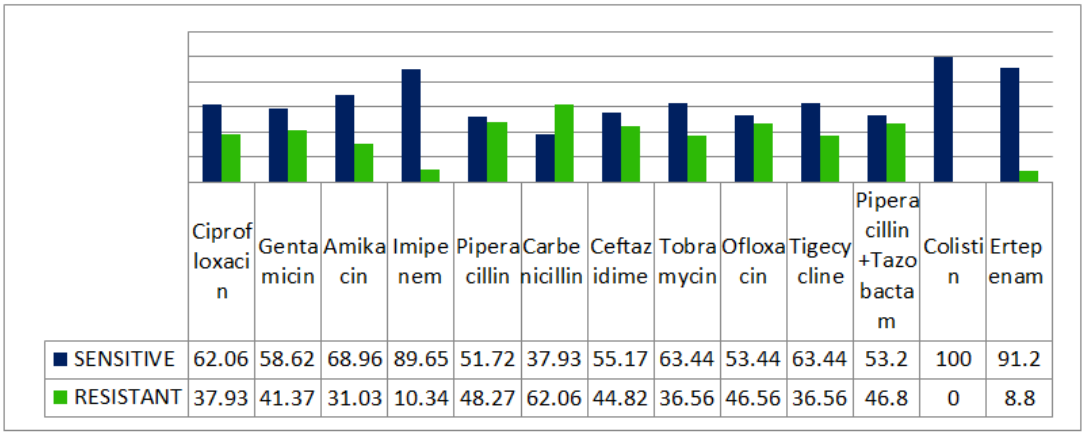


Fig-10: Antibiotic susceptibility pattern of *Pseudomonas fluroscence* (n=42)

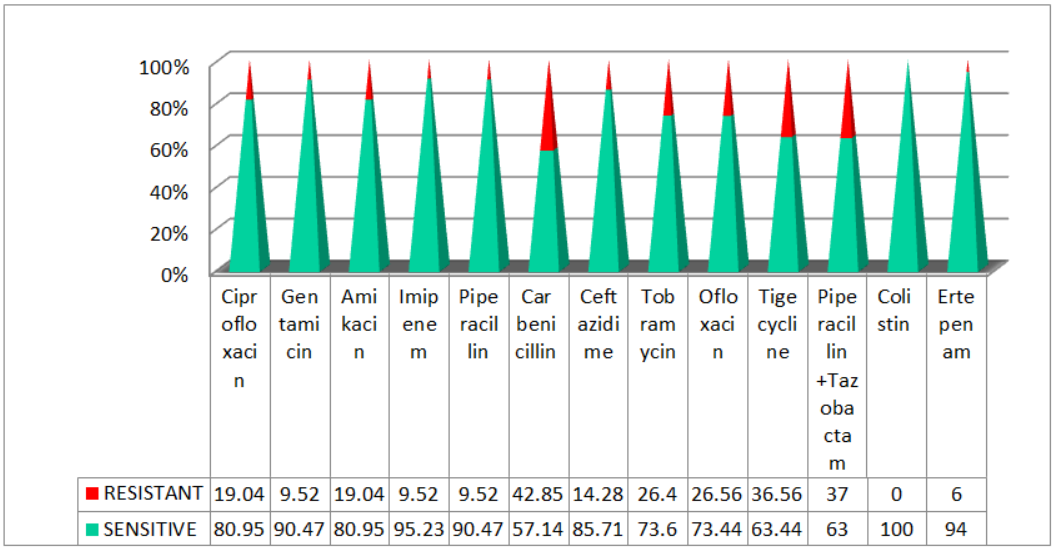


	Ciprofloxacin	Gentamicin	Amikacin	Imipenem	Piperacillin	Carbenicillin	Ceftazidime	Tobramycin	Ofloxacin	Tigecycline	Piperacillin +Tazobactam	Colistin	Ertepenam
SENSITIVE	62.06	58.62	68.96	89.65	51.72	37.93	55.17	63.44	53.44	63.44	53.2	100	91.2
RESISTANT	37.93	41.37	31.03	10.34	48.27	62.06	44.82	36.56	46.56	36.56	46.8	0	8.8

Fig-11: Antibiotic susceptibility pattern of *Pseudomonas putida*(n=29)

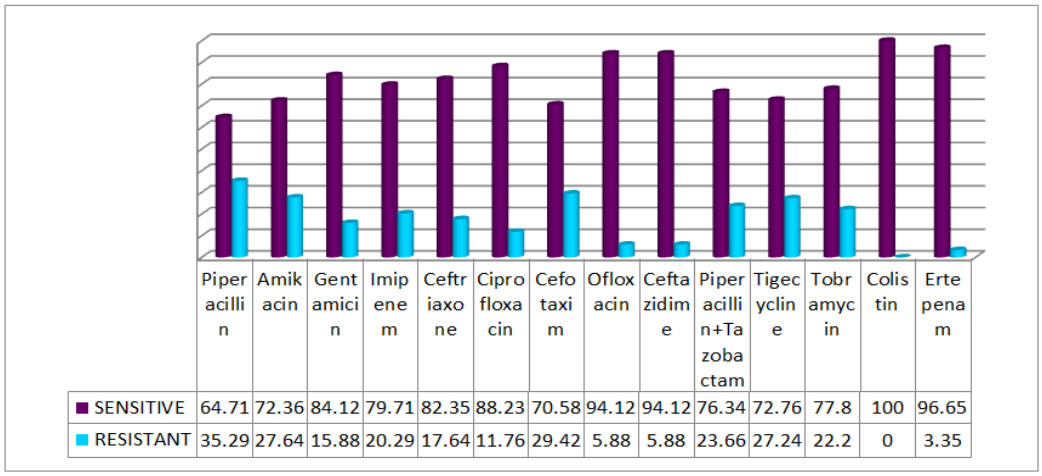
P.putida showed a good sensitivity to ertepenam (91.25%), imipenem (89.65%), followed by amikacin (68.96%) and tigecycline (63.44%) & tobramycin (63.44%).

P. stutzeri showed a good sensitivity to ertepenam (94%), imipenem (95.2%), gentamycin (90.5%) and piperacillin (90.5%) (Fig -12).



	Ciprofloxacin	Gentamicin	Amikacin	Imipenem	Piperacillin	Carbenicillin	Ceftazidime	Tobramycin	Ofloxacin	Tigecycline	Piperacillin +Tazobactam	Colistin	Ertepenam
RESISTANT	19.04	9.52	19.04	9.52	9.52	42.85	14.28	26.4	26.56	36.56	37	0	6
SENSITIVE	80.95	90.47	80.95	95.23	90.47	57.14	85.71	73.6	73.44	63.44	63	100	94

Fig-12: Antibiotic susceptibility pattern of *Pseudomonas stutzeri* (n=21)



	Piperacillin	Amikacin	Gentamicin	Imipenem	Ceftriaxone	Ciprofloxacin	Cefotaxim	Ofloxacin	Ceftazidime	Piperacillin+Tazobactam	Tigecycline	Tobramycin	Colistin	Ertepenam
SENSITIVE	64.71	72.36	84.12	79.71	82.35	88.23	70.58	94.12	94.12	76.34	72.76	77.8	100	96.65
RESISTANT	35.29	27.64	15.88	20.29	17.64	11.76	29.42	5.88	5.88	23.66	27.24	22.2	0	3.35

Fig-13: Antibiotic susceptibility pattern of *Strenotrophomonas maltophilia* (n=17)

S. maltophilia showed a good sensitivity to Ertepenam (96.65%), Ofloxacin (94.12%), Ceftazidime (94.12%) and Ciprofloxacin (88.23%)

Table-14: distribution of Multidrug resistance strains amongst different species of *Pseudomnas*

Name of the organism	Total number of isolates	Total number of MDR	ESBL	MBL
P.aeruginosa	189	61	35	14
P. fluroscence	42	10	7	1
P.putida	29	10	5	3
P.stutzeri	21	1	0	0
P.alcaligens	2	1	1	0
BCC	3	1	0	0
B.pseudomallei	1	0	0	0
S. maltophilia	17	2	0	2
S. paucimobilis	3	0	0	0
Total	307	86(28.01%)	48(55.81%)	20(23.25%)

Table-15: Epidemiological study

Sr no	Inaimate objects or disinfectants	ward	Growth
1	Wash basin	MSW	*P.aeruginosa*
2	Wash basin	FSW	MRSA
3	Wash basin	MMW	*A.boumannii*
4	Wash basin	FMW	No Growth
5	Air condition machine	NICU	No Growth
6	Warmer	NICU	No Growth
7	Humidifier	PICU	No Growth
8	Wash basin	PICU	*P.stutzeri*
9	Humidifier	SICU	No Growth
10	Wash basin	SICU	*P.aeruginosa*
11	Disinfectant	MSW	*P.aeruginosa*
12	Disinfectant	FSW	MRSA
13	Disinfectant	MMW	*P.fluorescence*
14	Disinfectant	MOW	*P.stutzeri*
15	Disinfectant	FOW	*P.fluorescence*
16.	Disinfectant	OBGY	*P.aeruginosa*
17.	Disinfectant	M. Opthal Ward	No Growth
18.	Disinfectant	F. Opthal Ward	No Growth
19.	Disinfectant	M. ENT	No Growth
20	Disinfectant	F. ENT	No Growth
21	Disinfectant	PICU	No Growth
22	Disinfectant	NICU	No Growth
23	Disinfectant	SICU	No Growth
24	Disinfectant	MICU	No Growth
25	Disinfectant	OT (NEURO OT)	No Growth
26	Disinfectant	OT (OPTHAL OT)	No Growth
27	Disinfectant	OT (ORTHO OT)	No Growth
28	Disinfectant	OT (SURGERY OT)	No Growth

DISCUSSION

Pseudomonas is ubiquitous in nature as saprophytes. Earlier it is believed to be non pathogenic.But recently they have emerged as primary opportunistic pathogens in hospitalized patients as well as immunocompromised patients and responsible for causing variant infections [12]. They are very hard to desiccate, difficult to eradicate and has numerous intrinsic and acquired mechanisms of drug resistance, thus they possess a great threat to the clinician as well as to microbiologists. They can stay alive within disinfectants and can create problem in health care facilities spreading by cross contamination. They are posing a great threat to human race as they are resistant to routinely used antibiotics. The abuse and the unjudicial practice of antibiotics are responsible for the burgeoning resistance of commonly used antibiotics towards *Pseudomonas.* More over the multidrug resistance among these organisms makes the treatment of this infection difficult and expensive [12]. Antibiotic susceptibility pattern of *Pseudomonas* may vary geographically. Due to multidrug resistance patterns of

Pseudomonas, it is imperative to know the institutional prevalent susceptibility profiles.

A total of 15,169 clinical samples of pus, wound swab, different body fluid, blood, sputum and urine were carried out. Out of these total sample processed, 5096 (33.59 %) were culture positive. A total of 505(15.9%) NFGNB were obtained from the culture positive samples. Among these 505 NFGNB, the leading number of isolates were different species of Pseudomonas (189 isolates), followed by 170 isolates of different species of Acinetobacter.

However, among all the culture positive clinical samples, processed. *Pseudomonas aeruginosa*is the most common isolate, accounting for 189 (61.56%), *Pseudomonas fluroscence* accounting for 42 (13.68%). Next to it was *Pseudomonas putida* 29 (9.44%), *Pseudomonas stutzeri* 21 (6.84%), *Strenotrophomonas maltophilia* 17 (5.54%), next to it were *Burkholderia cepacia complex* (BCC) and *Sphingomonas paucimobilis* accounting for 3 isolates (0.98%) and Pseudomonas *alcaligens* 2 (0.65%) respectively. We have yielded only one *Burkholderia Pseudomallei multivorans*. Similar result were obtained by Patel P. H. et al in 2013, yield 76.97% *Pseudomonas aeruginosa,*which was the commonest one, followed by *Pseudomonas* species 0.54%, *Strenotrophomonas maltophilia* 0.2%,and *Pseudomonas putida* 0.8%[13]. Another similar study done by Memish Z.A. *et al*. in 2012 yield 72.9% *Pseudomonas aeruginosa*, which was the commonest one, followed by Stenotrophomonas *maltophilia* 1.8%[14].

*Pseudomonas aeruginosa*is a pathogen associated with a wide range of nosocomial infection. This organism can cause disease in hospitalized patients, predominantly surgical site infection (SSI), pneumonia, septicemia, urinary tract infection, soft tissue infections, non-healing ulcers and chronic suppurative otitis media (CSOM)[15]. In this present study majority of *P. aeruginosa* (42.32%) were isolated from pus and wound discharge and from different types of body fluid (55 isolates) like pleural fluid, peritoneal fluid, knee aspiration etc. Similar result was obtained by Rit K. He has isolated 101 (50.24 %) *P. aeruginosa* from pus [8]. Another previous study byand Yoshodhara *et al*. isolated majority of the *Pseudomonas aeruginosa* from the pus [16]. In this present study 39.68of *Pseudomonas aeruginosa*were encountered as surgical site infection (SSI), followed by 21.16% isolates yield from respiratory tract infection.Moreover, around 11.11% isolates of *P.aeruginosa* were isolated from the blood cultures of the patients diagnosed with septicemia. The National Nosocomial Infection Surveillance system from 1986-2003 reported that *Pseudomonas aeruginosa*is the second most common cause of pneumonia (18.1%), third most common cause of urimary tract infection (16.3%) and eight most frequently isolated pathogen from blood stream (3.4%)[16].

Similarly *P. aeruginosa, P. fluroscence, P. putida* and *P. stutzeri*, are commonly isolated from pus and wound discharge. In the current study we have isolated different strains of *Pseudomonas stutzeri*, mainly from respiratory tract infection (38.1%) and most of the patients were on mechanical ventilators.Only 2(0.65%) isolates of *Pseudomonas alcaligens* were yield in this study from sputum among which one patient was admitted in ICU with the diagnosis of pneumonia with septic shock and he was on mechanical ventilator and another was admitted in pulmonary ward with the diagnosis of lower pneumonia with consolidation for a long tenure.

Pigment production of *Pseudomonas* is an important characteristics of identifying different species of *Pseudomonas.*Many distinct types of pigments are produced *Pseudomonas*, like water soluble pigments are carotenoids (yellow orange), viocin (violet or purple) and phenazines (red, maroon) that impart distinctive colour in the colonies.Water –soluble and diffusible pigments are fluorescein (pyoverdin), pyocyanin, pyorubin, melanin and miscellaneous other pigmented by- product [2]. Fluorescent *Pseudomonas* are charecterised by production of water soluble pigment, which diffuse freely in the media and fluoresce brightly under U.V ray.The organisms produce this pigments are *P. aeruginosa, P. putida, P. fluorescens, P. chlororaphis, P. syringae, P. Cichorii* and *P. Flavescens* and is manifested in low iron containing media [6] "King B "medium is the universally use medium for the production of fluorescent pigment [9]. Pyocyanin, a blue phenazine derivative characteristic of *P.aeruginosa*is diffusible and its production is enhanced by growth in" King a [9]". Other phenazine pigments are characteristic of *P. chlororaphis* (chlororaphin, green: frequently crystallizes in medium) and of *P. chlororaphis* (phenazine-α-carboxylate, orange, soluble) [6]. Some strains of *Pseudomonas* produce phenazine-α-carboxylate and other produce a variety of phenazine pigments of chlororaphin family, aside from phenazine-α-carboxylate [17, 18]. Lemonnierin is an intracellular, insoluble blue pigment characterised by *P. fluorescens* biotype IV [6]. In this study we have yielded also different types pf pigments (Fig 1,2,3). The production of pigments was enhanced by use of King A and King B media (Fig B).

Burkholderia cepacia complex (BCC) found in many niches of both natural and clinical environments .BCC is emerging as an important cause of morbidity and mortality in hospitalized patients because of high intrinsic antibiotic resistance, such as aminoglycosides, chloramphenicol and polymyxins. An upsure of septicaemia due to BCC is documented in various studies [19]. We have isolated 2 isolates of BCC and one isolate of *Burkholderia pseudomallei* from the

patients, were diagnosed with sepsis and admitted in the ICU. However other studies, like Gautam V *et al*. in 2006-2007 isolated pretty much high number of BCC (39 isolates)from various specimens, including blood cultures[20]. But Rit *et al*. in 2013 have isolated 14(6.96%) *B. cepacia*, mostly from pus [21].

Stenotrophomonas maltophilia is water borne organisms and recently emerged as an important opportunistic pathogen in debilitated host. They are enraging as a known cause of infection in the nosocomial settings. The isolates of this emerging pathogen from respiratory tract is quite difficult to interpret as primary pathogen. However if this isolate yields from a site which is supposed to be sterile, such as from blood, operated site any fluid from body cavity, drain tip or CVP tip, then this isolate represents as true or primary pathogen. Similarly in this current study we have isolated 17 *Stenotrophomonas maltophilia*, in which 14(82.35%) were from blood of sepsis patients, where central venous catheter in situ. We have isolated 2 (11.76%) isolates, one from drain tip of a cholecystectomy patient and another obtained from intraoperated sample of an appendix. One isolate was from pleural fluid of a patient of pleural effusion. Muder *et al.* report same kind of study where he was reported a series of 91 patients with *Stenotrophomonas maltophilia* bacteraemia, among them 56% did not reveal any clinically apparent portal of entry but 84 % of these individuals had central venous catheter in place.

In this current study we have isolated 3 *Sphingomonas paucimobilis*, 2 from blood samples and one from sputum samples. These isolates were obtained from the blood cultures of two young patients who were admitted in ICU and female medical ward for a long tenure with the diagnosis of septicaemia. However the isolate from the sputum yield from a 25 years male patient admitted in with the diagnosis of pneumonia with lower lobe consolidation and he was on mechanical ventilator. A study done by Malini *et al*. revealed a high percentage of (5.2%) this isolates [22].

There are some predisposing factors which accelerate the occurrence of the infection due to this organisms, such as growing number of operative intervention, increased use of broadspectrum antibiotics, prolonged hospital stay or being bed ridden in ICU, on mechanical ventilators, malignant disorder ,neutropenia due to chemotherapy and diabetics melitis[15]. In this study the leading predisposing factors are DM (28.71%), followed by on mechanical ventilator (18.66%) and prolonged hospitalization (15.31%).

These NFGNB are posing a great threat to human race as they are resistant to routinely used antibiotics. The abuse and the unjudicial practice of antibiotics are responsible for the burgeoning resistance of commonly used antibiotics towards NFGNB.The resistance to antimicrobials is increasing in recent years and almost resistance to all commonly used antibiotics.

In this study, all these bacterial isolates were highly resistant to major antimicrobial agents. A significant proportion of *P. aeruginosa* (32.28%) was MDR and also resistant to Imipenem. Such high resistance rates of major bacterial isolates from Hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP), which remained the important causes of morbidity and mortality. We have isolated 28.01 % MDR strains, among which 55.81 % were ESBL–producer and 23.255 were MBL producer (Table 14). This increasing antimicrobial resistance has aroused the concern of the failure of antibiotic treatment. A remarkable thing we have observed that Colistin showed 100 % sensitivity to all the isolates (n=307) as well as Ertepenam revealed more than 90 % sensitivity to all these isolates. Which is very alarming, as these isolates were revealed only good sensitivity to such higher groups of drugs.

Pseudomonas aeruginosa shows a good sensitivity to ertapenem (90.8%), imipenem (86.77%), tobramycin (66.66%) and amikacin (64.02%). This is almost similar to the study by Patel P.H. *et al*. who reported 94% sensitivity to this drug [13]. A study by Rit K *et al.* reported that *P.aeruginosa* were highly susceptible to colistin (100%), imipenem (91.8%) and amikacin (69.3%) [21]. similarly In case of Pseudomonas *fluroscence*, ertapenam (95%) and imipenem (88%) show the highest sensitivity. Similarly, a study by Rit K *et al*. reported 100 % sensitivity to imipenem [21]. In this current study gentamicin and piperacillin each of them show 71.4% sensitivity. Unlikely, Rit K *et al*. revealed a low sensitivity rate to gentamicin (33.33%) [21].

Likewise, ertepenam (91.2%), imipenem (89.65%) and amikacin (68.96%) shows the highest sensitivity for *Pseudomonas putida*, almost similar to this study, a study by Patel P.H. *et al*. revealed 100% susceptibility to Imipenem[13]. The other isolates of *P. putida* show a moderate susceptibility pattern towards Tigecycline and Tobramycin (63.44%of each).While discussing about of *Pseudomonas stutzeri*, similarly it revealed 100 % sensitivity to colistin followed by ertepenam (94%), imipenem (95.23%) and gentamicin (99.5%). Similarly Patel P.H. *et al*. revealed exactly same sensitivity rate of imipenem (100%)[13]. Where as Rit K *et al*. reported only 75 % sensitivity to imipenem and reported a low susceptibility rate to gentamicin (50%), ceftazidime (50%) and amikacin (25%)[21].

S. maltophilia revealed good sensitivity to ertapenam (96.65%), ofloxacin (94.12%), ceftazidime (94.12%), ciprofloxacin (88.23%) and ceftriaxone (82.35%). A study by Rit K *et al.* Reported a good

susceptibility to Ceftazidime (66.7%) [21]. In this study fluoroquinolones revealed a good sensitivity. Similarly done by Nayyar C *et al*. revealed that 70.5% strains were susceptible to fluoroquinolones, unlikely another Abdel-Aziz N *et al*. reported 16.67% susceptibility to fluoroquinolones [23,24]. In the current study, Gentamicin and Amikacin, accounting for 84.12% and 72.36%, where as a study by Juyal D *et al*. Reported only 16.67% susceptibility to gentamicin and almost resistant to amikacin (33.33%)[15]. We have observed 79.71% sensitivity rate to imipenem, where as a study done by Juyal D *et al*. reported almost resistant to imipenem [15].

As rates of infection have increased, so the incidence of infection with MDR isolates of Pseudomonas species is also increasing. Providing effective treatment for infections caused by MDR Pseudomonas is a challenge. MDR strains typically require therapy with Colistin, an older and relatively toxic polymyxin antimicrobial and aminoglycosides or with the newer antimicrobial agent like ertapenem or tigecycline[25].

However to trace the source of *Pseudomonas*, we carried out an epidemiological study in different words and ICU (NICU, PICU, SICU). The samples were collected from wash basin, disinfectants or air condition machine and IN-USE test were carried out. We observed a very remarkable thing that disinfectants of all major surgical wards revealed the presence of different species of *Pseudomonas* (Table 15). This was an alarming findings as in this study majority of the isolates were obtained from the surgical wards. Pseudomonas is hydrophilic and can readily recover from moist environments, such as drains vegetables, wash basins, sinks and even antibiotic solutions as well as disinfectants.

However, none of these environmental reservoirs pose a great threat to most individuals unless ingested and induce gastrointestinal colonization due to antibiotic therapy altered the normal bacterial flora [11].

CONCLUSION

A large number of different species of *Pseudomonas*are isolated as primary pathogen from different clinical specimens of the patients, admitted in different wards and ICUs. Most of the patients had high risk factors, like prolonged hospitalization, immunocompromised due to chemotherapy, indwelling catheters and orthopedics implants in situ or other catheterization (urinary or intravenous), diabetics and burns.

Pseudomonas aeruginosa is the commonest pathogen obtained from SSI. They are encountered as a major hospital acquired pathogen. They are transmitted to human body by sources like intravascular catheter, drain tubes & surgical interventions. These NFGNB were mainly from wounds, degloveing injuries, SSI, bed sores, fracture sites, implants, and cellulitis and space infection. These organisms have possibly come from inanimate objects like ventilator, humidifier, and wash basin and from diluted disinfections.

Most effective antibiotics are imipenem, amikacin, gentamicin, ciprofloxacin, cefotaxim and cotrimaxazole. All the strains were sensitive to Colistin. A quite high number of isolated Acinetobacter species are MDR strains and most of them areresistant to commonly used antibiotics. This is an alarming indication that theseNFGNB need to be taken more seriously as primary pathogen and should not be discarded as mere contaminant or non pathogen. Hence, proper isolation and identification of these organisms can enlighten their prevalence rate and role of pathogenicity among hospitalized patients.

However, the antibiotic susceptibility can change from hospital to hospital set up and there may be a gross geographical variation. Hence treating these pathogens should be based on the laboratory data after identifying the proper causativeagents and antibiotic susceptibility result. Minimized the use and abuse of antimicrobial agents, proper surveillance of antibiotic panel, strict infection control measures and even simple yet proper hand washing and by using disinfections of inanimate objects, can prevent the emergence of Pseudomonas and can reduce the rate of MDR strains. It is imperative that every hospital should monitor a proper antibiogram profile for these isolated from time to time to serve as a basic empirical therapy to prevent the development of Multi drug resistance (MDR) cases.

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