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SCREENING FOR MULTIDRUG RESISTANCE BACTERIA PSEUDOMONAS AERUGINOSA IN HOSPITALIZED PATIENT IN HOSUR, KRISHNAGIRI (DT)

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ABSTRACT

Pseudomonas aeruginosa is a classic opportunistic pathogen because of its innate resistance to many antibiotics and disinfectants. It is also the most common Gram negative bacterium found in nosocomial infections causing various spectra of infections especially in neutropenic, immunocompromised, burns / tissue injury and cystic fibrosis patients all over the world. Recent advances in medicine such as the advent of more elaborate surgery and intensive care, the use of immunosuppressive drugs, the availability of invasive procedures and the increase in number of immunocompromised patients means there is a rise in patients with impaired immune defences liable to nosocomial infections. The increasing incidence of infections caused by multidrug resistant organisms have caused attention to be focused on measures for fighting resistance, foremost of which is susceptibility surveillance .This study therefore determined the prevalence, antibiotic susceptibility and resistant patterns of *Pseudomonas aeruginosa* strains from clinical specimens.

KEY WORDS

Antibiotic resistance, *Pseudomonas aeruginosa,* Nosocomial infection, MARS (Multiple antibiotic resistances).

INTRODUCTION

Pseudomonas aeruginosa is known for its ability to resist killing by a variety of antibiotics. The minimal nutritional requirements of *Pseudomonas*, as evidenced by its ability to grow in distilled water and its tolerance to a wide variety of physical conditions, contribute to its ecologic success and ultimately to its role as an effective opportunistic pathogen. *Pseudomonas aeruginosa* is primarily a nosocomial pathogen. In the annual Surveillance of nosocomial infections by the Centers for Disease Control (CDC) and Prevention from 1990 to 1996, it is the second most common etiology of nosocomial pneumoniae; 3rd for urinary tract infections and 4th for surgical site infections[5]**.** Likewise in a hospital-wide surveillance of nosocomial infections conducted by the Infection Control Committee of

the Philippine General Hospital in 1989. *Pseudomonas aeruginosa* was the most common organism isolated from all sites of infection (37%) [6].

Resistance to antimicrobial agents is an increasing clinical problem and is a recognized public health threat. *Pseudomonas aeruginosa* has a particular propensity for the development of resistance. It is naturally resistant to many antibiotics because of its relatively impermeable outer membrane and it can also easily acquire resistance, creating challenging therapeutic scenarios. All known mechanisms of β-lactam resistance can be found in this specie namely: β-lactamase production, altered outer-membrane permeability, active efflux and altered penicillin-binding proteins [12]. Thus, infections due to this organism are difficult to treat because of the possible coexistence of several mechanisms of resistance in the same strain; its capacity to produce a variety of virulence factors and the relatively limited choice of effective anti-pseudomonal antibiotics. Furthermore, emergence of resistance during therapy with these agents has been recognized as a cause of treatment failure. The reason that antibiotic resistance leads to adverse outcomes is due to the increased likelihood that the patient will receive ineffective or suboptimal antibiotic therapy. The development of resistance to all available antibiotics in some organisms then precludes the effectiveness of any antibiotic regimen. Organisms that are resistant to all known effective antimicrobials pose a serious threat to hospitalized patients. Thus two Gram-positive organisms have been described as being resistant to all antibiotics: vancomycin resistant enterococci and vancomycin resistant, S.aureus. Similarly Gram-negative bacteria may also become resistant to all available antibiotics. This is most likely to occur in an organism, such as *Pseudomonas aeruginosa* in which resistance to different classes of antibiotics has already been described [9].

Indiscriminate use of antibiotics leads to the development of resistance of initially sensitive strains of organisms, and possible destruction of the normal microbial flora. Local studies that quantitatively examine the health and cost impact of resistant organisms in our setting are lacking. Information on the emergence of resistance with different antibiotics can be of practical use in guiding empiric therapeutic choices. This prospective observational study aims to compare morbidity, mortality and costs of health care associated with infections due to antimicrobial resistant and susceptible strains of clinically significant *P. aeruginosa*. Baseline data derived from this study can be used as bases for instituting preventive measures and formulating recommendations on rational antibiotic use [3]. *Pseudomonas aeruginosa* has been increasingly recognized for its ability to cause significant hospital-associated outbreaks of infection, particularly since the emergence of multidrugresistant strains[15]. Outbreaks of multidrug-resistant *P. aeruginosa* colonization or infection have been reported on urology wards, a burn unit, hematology/oncology units, and adult and neonatal critical care units [7].Various medical devices and environmental reservoirs have been implicated in these outbreaks, including antiseptic solutions and lotions; endoscopy equipment; ventilator apparatus; and mouth swab [14]. These sources can easily be eliminated once identified. A greater challenge exists if the source of an outbreak involves permanent components of the hospital physical plant, such as plumbing fixtures[2].

The present investigation was carried out to isolate and identify the *Pseudomonas aeruginosa* from clinical samples obtained from Government hospital, Hosur. All the *Pseudomonas aeruginosa* isolates were identified by standard technique. Out of 60 clinical samples, a total of 32 *Pseudomonas aeruginosa* isolates were isolated and identified their sensitivity / resistant pattern against 16 antibiotics were recorded and described.

MATERIALS AND METHODS

(i)Sample collection: For this present investigation different clinical samples include sputum, urine and wound sample were collected

at Government hospital Hosur. Urine and sputum samples were collected from patient aseptically with the help of sterile wide mouthed screw capped plastic containers. Sterile cotton swabs were used for the collection of wound sample. All the swab samples were directly inoculated onto *Pseudomonas* selective agar (Certimide Agar, Himedia India Ltd.,). The urine sample was processed by pour plate method using cetrimide agar. The isolated strains were maintained on nutrient agar slants and stored at 4º C.

(ii)Identification of Pseudomonas aeruginosa isolates: The different clinical specimen received from the government hospital Salem were cultured on blood agar and MacConkey agar plates and incubated at a temperature of 37˚C for 24 hours and on Mueller Hinton agar plates to assess pigment production Plate-1. The culture plates were processed using standard microbiological procedures, Characterization and identification of *P. aeruginosa* was carried out using a combination of colonial morphology, Gram stain characteristics, motility tests, pigmentation, oxidation-fermentation tests, catalase and oxidizer activity tests and pyocyanin production [4].

(iii)Kirby- Bauer Disc Diffusion method: Antibiotic susceptibility was determined on Mueller Hinton agar using the disc diffusion method according to the modified Kirby-Bauer technique (Vandepitte *et a*l, 1999). All the isolated *P. aeruginosa* strains were tested for their sensitivity to the following Antibiotics:Tetracycline(30mcg),Rifampicin(5mcg) ,streptomycin(10mcg),Carbenicillin(100mcg), Ciprofloxacin(5mcg), Chloramphenicol (30mcg) ,Cotrimaxazole (25mcg), Tobramycin(10mcg) ,Imipenem(10mcg),Norfloxacin(10mcg),sparfloxac in(5mcg),Amoxyclave(30mcg), Piperacillin(100mcg), Lomefloxacin(10mcg), Ceftizoxime(30mcg), and Gentamicin(10mcg). Isolates were considered multidrug resistant if they showed resistance to 3 or more of the tested antibiotics. The multiple antibiotic resistance MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested [10].

RESULTS

The present investigation was carried out to isolate and identify the *Pseudomonas aeruginosa* from clinical samples. All the *Pseudomonas aeruginosa* isolates were identified by standard technique Table -1. Out of 60 clinical samples, a total of 32 *Pseudomonas aeruginosa* isolates were isolated and identified their sensitivity / resistant pattern against 16 antibiotics were recorded and described in Table 3 - 6, and Graph 1- 4.

Table -1

Colony Morphology and Biochemical characteristics of isolated Pseudomonas aeruginosa

S.No	Tests	Results
1	Gram staining	Gram Negative, Single rods
2	Motility	Motile
3	Colony Morphology Nutrient Agar	Bluish green colours colonies
	MacConkey agar	Non lactose fermenting colonies
	Blood Agar	Hemolytic colonies
	Cetrimide agar	Bluish green colour colonies
4	Oxidase	Positive
5	Catalase	Positive

6	Growth at temperature 5ºC	Negative
	15ºC	Positive
	37ºC	Positive
	42ºC	Positive
7	Growth at pH a)5.7	Positive
	b)6.8	Positive
	c)8.0	Positive
8	Growth on Nacl (25%)	Positive
9	Oxidative on O-F medium	Positive
10	Simmon's citrate medium	Positive
11	Urease	Negative
12	Indole	Negative
13	Methyl Red	Negative
14	Vogues Prosker	Negative
15	Nitrate reduction	Positive
16	Gelatin hydrolysis	Positive
17	Malonate	Negative
18	ONPG	Negative
19	Glucose	Positive
20	Sucrose	Negative
21	Lactose	Negative
22	Maltose	Negative
23	Mannitol	Positive
24	Cellobiose	Negative
25	Xylose	Negative
26	Inositol	Negative
27	Trehalose	Negative
28	Raffinose	Negative
29	Arabinose	Positive
30	Adonitol	Negative
31	Salicin	Negative
32	Sorbitol Tests	Negative Results
33	Arginine dihydrolase	Negative
34	Tween 20 hydrolysis	Positive
35	Tween 80 hydrolysis	Positive
36	Starch hydrolysis	Negative

The report of characterization is represented in Table- 1. On the basis of these characterizations, the isolated bacteria species have been identified as *Pseudomonas aeruginosa.*

Table - 2
Distribution of Pseudomonas aeruginosa in Clinical Samples

S.No	Name of the Sample	No of specimen collection	Positive for *Pseudomonas aeruginosa*
1	Wound	26	22(84.6%)
2	Urine	20	8(40%)
3	Sputum	14	2(14.2%)

The wound sample was the predominant. Out of 26 wound samples, 22 (84.6%) were positive for *Pseudomonas aeruginosa*. Next predominant were urine samples, out of 20 samples 8 (40%) were positive and very low number of *Pseudomonas aeruginosa* were isolated form sputum sample, 2 (14.2%) out of 14 Table - 2 & Graph - 5.

Graph - 5
Distribution of Pseudomonas aeruginosa in Clinical Samples

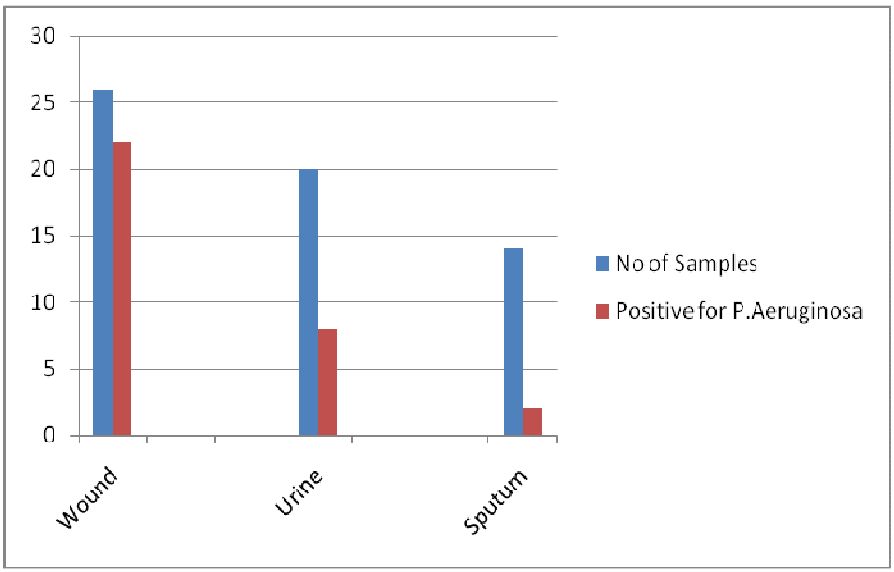
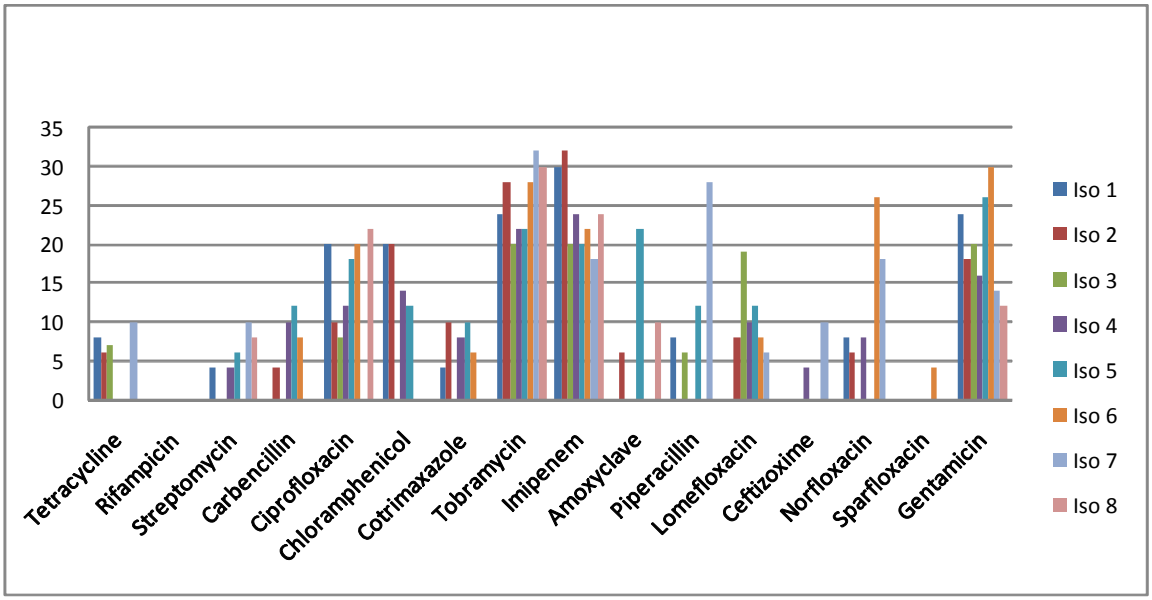


Table - 3
Antibiotic sensitivity and resistance pattern for 1-8 isolates

SNo	Name of the Antibiotic	Strength	Diameter of zone of inhibition							
			Isolate1	Isolate2	Isolate3	Isolate4	Isolate5	Isolate6	Isolate7	Isolate8
1	Tetracycline	30mcg	8 Resistant	6 Resistant	7 Resistant	0 Resistant	0 Resistant	0 Resistant	10 Resistant	0 Resistant
2	Rifampicin	5mcg	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant
3	Streptomycin	10 mcg	4 Resistant	0 Resistant	0 Resistant	4 Resistant	6 Resistant	0 Resistant	10 Resistant	8 Resistant
4	Carbenicillin	100mcg	0 Resistant	4 Resistant	0 Resistant	10 Resistant	12 Resistant	8 Resistant	0 Resistant	0 Resistant
5	Ciprofloxacin	5mcg	20 Resistant	10 Resistant	8 Resistant	12 Resistant	18 Resistant	20 Resistant	0 Resistant	22 Resistant
6	Chloramphenicol	30mcg	20 Resistant	20 Sensitive	0 Resistant	14 Resistant	12 Resistant	0 Resistant	0 Resistant	0 Resistant
7	Cotrimaxazole	25mcg	4 Resistant	10 Resistant	0 Resistant	8 Resistant	10 Resistant	6 Resistant	0 Resistant	0 Resistant
8	Tobramycin	10mcg	24 Sensitive	28 Sensitive	20 Sensitive	22 Sensitive	22 Sensitive	28 Sensitive	32 Sensitive	30 Sensitive
9	Imipenem	10mcg	30 Sensitive	32 Sensitive	20 Sensitive	24 Sensitive	20 Sensitive	22 Sensitive	18 Sensitive	22 Sensitive
10	Amoxyclave	30mcg	0 Resistant	6 Resistant	0 Resistant	0 Resistant	22 Sensitive	0 Resistant	0 Resistant	10 Resistant
11	Piperacillin	100mcg	8 Resistant	0 Resistant	6 Resistant	0 Resistant	12 Resistant	0 Resistant	28 Sensitive	0 Resistant
12	Lomefloxacin	10mcg	0 Resistant	8 Resistant	19 Resistant	10 Resistant	12 Resistant	8 Resistant	6 Resistant	0 Resistant
13	Ceftizoxime	30mcg	0 Resistant	0 Resistant	0 Resistant	4 Resistant	0 Resistant	0 Resistant	10 Resistant	0 Resistant
14	Norfloxacin	10mcg	8 Resistant	6 Resistant	0 Resistant	8 Resistant	0 Resistant	26 Sensitive	18 Resistant	0 Resistant
15	Sparfloxacin	5mcg	0 Resistant	0 Resista Nt	0 Resistant	0 Resistant	0 Resistant	4 Resistant	0 Resistant	0 Resistant
16	Gentamicin	10mcg	24 Sensitive	18 Sensitive	20 Sensitive	16 Sensitive	26 Sensitive	30 Sensitive	14 Resistant	12 Resistant

Graph - 1

Shows the sensitivity / resistance pattern of Pseudomonas aeruginosa isolates 1-8 against 16 antibiotics



All the isolates are found to be resistant to Tetracycline, Rifamibicin, Streptomycin, Carbenicillin, Ciprofloxacin, Cotrimaxazole, Sparfloxacin, Ceftizoxime and Lomefloxacin. Norfloxacin, Piperacillin, Amoxyclave and Chloramphenical not active against all the isolate except isolate 6, 7, 5 and 2 respectively. Tobramcyin active against all the isolates Imiphenem except isolates 7, Gentamycin except the isolates 7 and 8 found to be sensitive to the remaining isolates Table 3 and Graph 1.

Table - 4

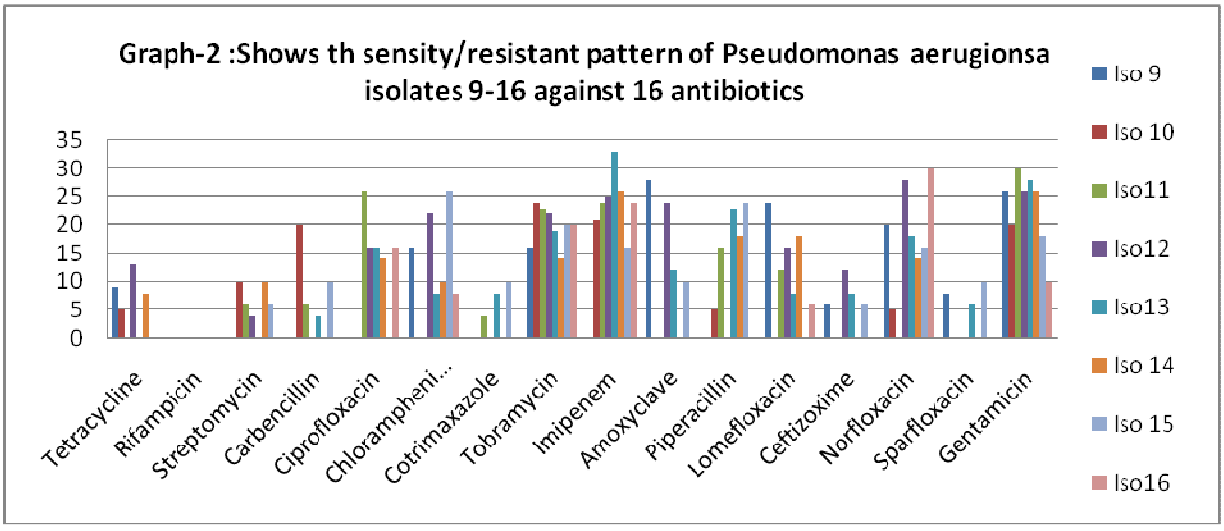
Antibiotic sensitivity and resistance pattern for 9-16 isolates

S.No	Name of the Antibiotic	Strength	Diameter of zone of inhibition							
			Isolate9	Isolate10	Isolate11	Isolate12	Isolate13	Isolate14	Isolate15	Isolate16
1	Tetracycline	30mcg	9 Resistant	5 Resistant	0 Resistant	13 Resistant	0 Resistant	8 Resistant	0 Resistant	0 Resistant
2	Rifampicin	5mcg	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant
3	Streptomycin	10 mcg	0 Resistant	10 Resistant	6 Resistant	4 Resistant	0 Resistant	10 Resistant	6 Resistant	0 Resistant
4	Carbenicillin	100mcg	0 Resistant	20 Resistant	6 Resistant	0 Resistant	4 Resistant	0 Resistant	10 Resistant	0 Resistant
5	Ciprofloxacin	5mcg	0 Resistant	0 Resistant	26 Sensitive	16 Resistant	16 Resistant	14 Resistant	0 Resistant	16 Resistant
6	Chloramphenicol	30mcg	16 Resistant	0 Resistant	0 Resistant	22 Sensitive	8 Resistant	10 Resistant	26 Sensitive	8 Sensitive

7	Cotrimaxazole	25mcg	0 Resistant	0 Resistant	4 Resistant	0 Resistant	8 Resistant	0 Resistant	10 Resistant	0 Resistant
8	Tobramycin	10mcg	16 Resistant	24 Resistant	23 Sensitive	22 Sensitive	19 Sensitive	14 Resistant	20 Sensitive	20 Sensitive
9	Imipenem	10mcg	0 Resistant	21 Sensitive	24 Sensitive	25 Sensitive	33 Sensitive	26 Sensitive	16 Resistant	24 Sensitive
10	Amoxyclave	30mcg	28 Sensitive	0 Resistant	0 Resistant	24 Sensitive	12 Resistant	0 Resistant	10 Resistant	0 Resistant
11	Piperacillin	100mcg	0 Resistant	5 Resistant	16 Resistant	0 Resistant	23 Resistant	18 Resistant	24 Sensitive	0 Resistant
12	Lomefloxacin	10mcg	24 Resistant	0 Resistant	12 Resistant	16 Resistant	8 Resistant	18 Resistant	0 Resistant	6 Resistant
13	Ceftizoxime	30mcg	6 Resistant	0 Resistant	0 Resistant	12 Resistant	8 Resistant	0 Resistant	6 Resistant	0 Resistant
14	Norfloxacin	10mcg	20 Resistant	5 Resistant	0 Resistant	28 Sensitive	18 Resistant	14 Resistant	16 Resistant	30 Resistant
15	Sparfloxacin	5mcg	8 Resistant	0 Resistant	0 Resistant	0 Resistant	6 Resistant	0 Resistant	10 Resistant	0 Resistant
16	Gentamicin	10mcg	26 Sensitive	20 Sensitive	30 Sensitive	26 Sensitive	28 Sensitive	26 Sensitive	18 Sensitive	10 Resistant

Graph - 2

Shows the sensitivity / resistance pattern of Pseudomonas aeruginosa isolates 9-16 against 16 antibiotics

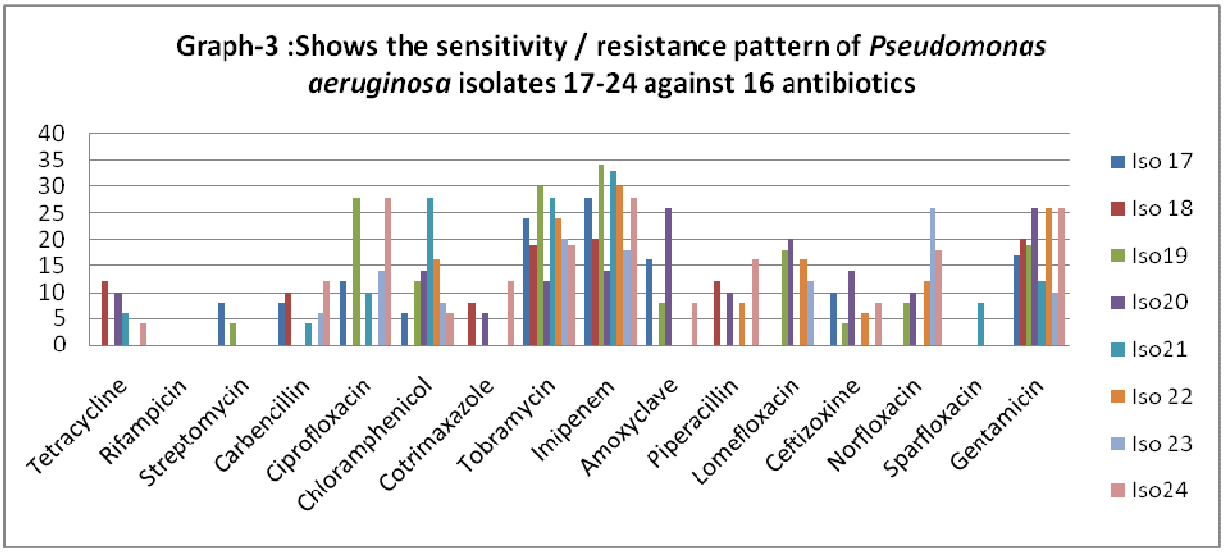


All the isolates 9-16 are found to be resistant to Tetracycline, Rifampicin, Streptomycin, Carbenicillin, Cotrimaxazole, Lomefloxacin, Ceftizoxyme and Sparfloxacin. Ciprofloxacin,

Piperacillin, Ceftizoxime and Norfloxacin are also not active against all the isolates except isolates 11, 15 and 12 respectively. All the isolates are found to be resistant to chloramphenical except 12 and 14, Isolates 11, 12, 13 and 15 are sensitive to tobramycin, Isolates 9 and 12 are sensitive to Amoxyclave and Isolate 16 only resistant to Gentamycin. Table - 4 and Graph - 2.

Table - 5.
Antibiotic sensitivity and resistance pattern for 17-24 isolates

S.No	Name of the Antibiotic	Strength	Diameter of zone of inhibition							
			Isolate17	Isolate18	Isolate19	Isolate20	Isolate21	Isolate22	Isolate23	Isolate24
1	Tetracycline	30mcg	0 Resistant	12 Resistant	0 Resistant	10 Resistant	6 Resistant	0 Resistant	0 Resistant	4 Resistant
2	Rifampicin	5mcg	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant
3	Streptomycin	10 mcg	8 Resistant	0 Resistant	4 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant
4	Carbenicillin	100mcg	8 Resistant	10 Resistant	0 Resistant	0 Resistant	4 Resistant	0 Resistant	6 Resistant	12 Resistant
5	Ciprofloxacin	5mcg	12 Resistant	0 Resistant	28 Sensitive	0 Resistant	10 Resistant	0 Resistant	14 Resistant	28 Sensitive
6	Chloramphenicol	30mcg	6 Resistant	0 Resistant	12 Resistant	14 Resistant	28 Sensitive	16 Resistant	8 Sensitive	6 Sensitive
7	Cotrimaxazole	25mcg	0 Resistant	8 Resistant	0 Resistant	6 Resistant	0 Resistant	0 Resistant	0 Resistant	12 Resistant
8	Tobramycin	10mcg	24 Sensitive	19 Resistant	30 Sensitive	12 Resistant	28 Sensitive	24 Sensitive	20 Sensitive	19 Sensitive
9	Imipenem	10mcg	28 Sensitive	20 Sensitive	34 Sensitive	14 Resistant	33 Sensitive	30 Sensitive	18 Resistant	28 Sensitive
10	Amoxyclave	30mcg	16 Resistant	0 Resistant	8 Resistant	26 Sensitive	0 Resistant	0 Resistant	0 Resistant	8 Resistant
11	Piperacillin	100mcg	0 Resistant	12 Resistant	0 Resistant	10 Resistant	0 Resistant	8 Resistant	0 Resistant	16 Resistant
12	Lomefloxacin	10mcg	0 Resistant	0 Resistant	18 Resistant	20 Resistant	0 Resistant	16 Resistant	12 Resistant	0 Resistant
13	Ceftizoxime	30mcg	10 Resistant	0 Resistant	4 Resistant	14 Resistant	0 Resistant	6 Resistant	0 Resistant	8 Resistant
14	Norfloxacin	10mcg	0 Resistant	0 Resistant	8 Resistant	10 Resistant	0 Resistant	12 Resistant	26 Sensitive	18 Resistant
15	Sparfloxacin	5mcg	0 Resistant	0 Resistant	0 Resistant	0 Resistant	8 Resistant	0 Resistant	0 Resistant	0 Resistant
16	Gentamicin	10mcg	17 Sensitive	20 Sensitive	19 Sensitive	26 Sensitive	12 Resistant	26 Sensitive	10 Resistant	26 Sensitive



The Isolates 17, 19 and 22 are sensitive to Tobramycin, Imipenem and Gentamycin, Isolate 18 sensitive to Imipenem and Gentamycin, Isolate 20 sensitive to amoxyclave and Imipenem and Gentamycin, Isolate 21 sensitive to Chloramphenical, Tobramycin and Imipenem, Isolate 23 sensitive to Imiphenem and Norfloxacin, Isolate 24 sensitive to Ciprofloxcacin and Isolate 26 sensitive to Gentamycin only Table - 5, Graph - 3.

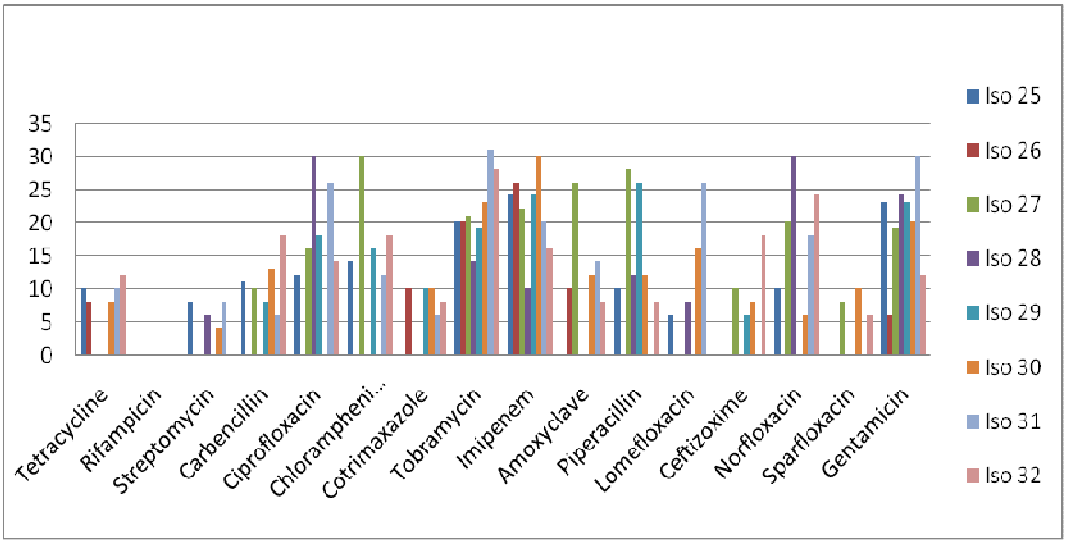
Table - 6.
Antibiotic sensitivity and resistance pattern for 25-32 isolates

S.No	Name of the Antibiotic	Strength	Diameter of zone of inhibition							
			Isolate25	Isolate26	Isolate27	Isolate28	Isolate29	Isolate30	Isolate31	Isolate32
1	Tetracycline	30mcg	10 Resistant	8 Resistant	0 Resistant	0 Resistant	0 Resistant	8 Resistant	10 Resistant	12 Resistant
2	Rifampicin	5mcg	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant	0 Resistant
3	Streptomycin	10 mcg	8 Resistant	0 Resistant	0 Resistant	6 Resistant	0 Resistant	4 Resistant	8 Resistant	0 Resistant
4	Carbenicillin	100mcg	11 Resistant	0 Resistant	10 Resistant	0 Resistant	8 Resistant	13 Resistant	6 Resistant	18 Resistant
5	Ciprofloxacin	5mcg	12 Resistant	0 Resistant	16 Resistant	30 Resistant	18 Resistant	0 Resistant	26 Sensitive	14 Resistant
6	Chloramphenicol	30mcg	14 Resistant	0 Resistant	30 Sensitive	0 Sensitive	16 Resistant	0 Resistant	12 Resistant	18 Sensitive
7	Cotrimaxazole	25mcg	0 Resistant	10 Resistant	0 Resistant	0 Resistant	10 Resistant	10 Resistant	6 Resistant	8 Resistant
8	Tobramycin	10mcg	20 Sensitive	20 Sensitive	21 Sensitive	14 Resistant	19 Sensitive	23 Sensitive	31 Sensitive	28 Sensitive

9	Imipenem	10mcg	24 Sensitive	26 Sensitive	22 Sensitive	10 Resistant	24 Sensitive	30 Sensitive	20 Sensitive	16 Sensitive
10	Amoxyclave	30mcg	0 Resistant	10 Resistant	26 Sensitive	0 Resistant	0 Resistant	12 Resistant	14 Resistant	8 Resistant
11	Piperacillin	100mcg	10 Resistant	0 Resistant	28 Sensitive	12 Resistant	26 Sensitive	12 Resistant	0 Resistant	8 Resistant
12	Lomefloxacin	10mcg	6 Resistant	0 Resistant	0 Resistant	8 Resistant	0 Resistant	16 Resistant	26 Sensitive	0 Resistant
13	Ceftizoxime	30mcg	0 Resistant	0 Resistant	10 Resistant	0 Resistant	6 Resistant	8 Resistant	0 Resistant	18 Sensitive
14	Norfloxacin	10mcg	10 Resistant	0 Resistant	20 Resistant	30 Sensitive	0 Resistant	6 Resistant	18 Resistant	24 Sensitive
15	Sparfloxacin	5mcg	0 Resistant	0 Resistant	8 Resistant	0 Resistant	0 Resistant	10 Resistant	0 Resistant	6 Resistant
16	Gentamicin	10mcg	23 Sensitive	6 Sensitive	19 Sensitive	24 Sensitive	23 Sensitive	20 Sensitive	30 Sensitive	12 Resistant

Graph - 4.

Shows the sensitivity / resistance pattern of Pseudomonas aeruginosa isolates 25-32 against 16 antibiotics



The Isolates 25,26 and 30 are sensitive to Tobramycin, Imiphenem and Gentamycin, Isolate 27 sensitive to Chloramphenical, Tobramycin, Imiphenem, Amoxyclave, Piperacillin and Gentamycin, Isolate 28 sensitive to Chloramphenical, Norfloxacin and Gentamycin, Isolate 29 sensitive to Tobramycin, Imiphenem, Piperacillin and Gentamycin, Isolate 31 sensitive to

Ciprofloxacin, Tobramycin, Imiphenem, Lomefloxacin and Gentamycin and Isolate 32 sensitive to Chloramphenical, Tobramycin, Imiphenem, Cefrofloxacin and Norfloxacin Table - 6 & Graph- 4.

It was observed that only Gentamycin, Imiphenem and Tobramycin are activity against *pseudomonas aeruginosa* and remaining were not active. To assess the statistical significance of mean of antibacterial activity of *pseudomonas aeruginosa* the independent t-test was carried out and it was found to be significantly invariable at 0.0000 (P<0.000) Table- 7.

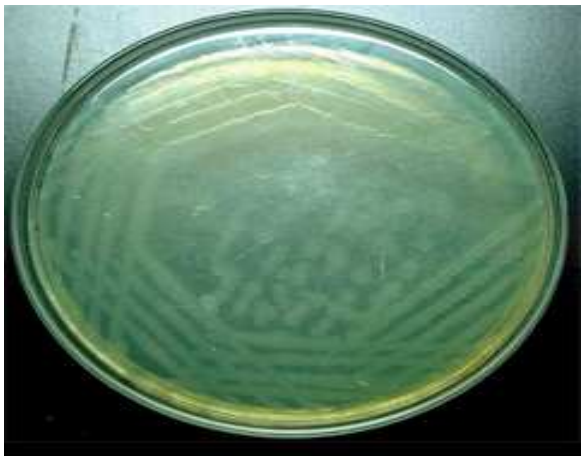
Table - 7

Mean and Standard Error of Antibacterial Activity of Pseudomonas aeruginosa

S.No	Name of the Antibiotics	Disc Diffusion method M ± x SE of (x)
1	Tetracycline	4.5625 ± 0.8339
2	Rifampicin	0.0000 ± 0.0000
3	Streptomycin	3.3125 ± 0.6575
4	Carbenicillin	5.6250 ± 1.0227
5	Ciprofloxacin	12.1875 ± 1.7537
6	Chloramphenicol	10.7500 ± 1.6233
7	Cotrimaxazole	4.0625 ± 0.7854
8	Tobramycin	22.0625 ± 5.8361
9	Imipenem	23.875 ± 1.0374
10	Amoxyclave	6.7850 ± 1.4998
11	Piperacillin	8.8125 ± 1.6586
12	Lomefloxacin	8.4063 ± 1.4423
13	Ceftizoxime	4.0625 ± 0.9002
14	Norfloxacin	11.8438 ± 1.7972
15	Sparfloxacin	1.8750 ± 0.6087
16	Gentamicin	20.5313 ± 1.1518

Statistical inference: significant at p < 0.000

Plate : 1 ***Pseuomonas aeruginosa*** Cetrimide agar showing fluorescent colonies



DISCUSSION

Out of 60, 32 isolates of pseudomonas aeruginosa were recovered from various clinical specimen include urine and sputum samples. Among the 3 samples, wound sample was predominant for *pseudomonas aeruginosa* isolates.Most of the isolates were found to be resistant to antimicrobial agents, Tetracyclin, Rifampicin, Streptomycin, Carbenicillin, Ciprofloxacin, Chloramphenical, Cotrimixazole, Amoxyclav, Piperacillin, Lomefloxacin, Ceftizoxime, Norfloxacin, and Sparfloxacin by disc diffusion method.

[1] Total of 6 *pseudomonas aeruginosa* strain from hospital environment in their result, out of 60 strains, 55 were found to be multiple drug resistant (resistant to three or more than three antibiotics with a MAR index of 7= 0.25). Among penicillin's, the highest level of resistance was against Ticarcillin (100%). Tetracyclines were not effective with 86% resistance. 93.4, 88.5 and 83.6% isolates were sensitive to Polymyxin, Ciprofloxacin and Norfloxacin respectively. Overall polymyxin B and Gentamycin were the most active agents were identified; twenty-seven antibiotypes were amongst the 48 isolates recovered in the hospital environment. Twenty-two were resistant to four antibiotics (MAR index= 0.33) and 11 to 5 antibiotics (MAR index= 0.047).National Committee of Clinical Laboratory 1997 also reported that three unusual isolates of *pseudomonas aeruginosa* recovered from various clinical specimens from two patients were found to be resistant to 12 antimicrobial agents (Cefoperazone, Ceftazidime, Aztreonam, Piperacillin, Ticarcillin – Clavulanic Acid, Imiphenem, Minocycline, Gentamycin, Tobramycin, Amikacin, Ofloxacin and Ciprofloxacin) by the route disc diffusion method.

Except Imiphenem, Gentamycin and Tobramycin all antibiotics were resistant to most of the isolates. Some unusual resistance of these antibiotics also observed this may due to presence of conjucative plasmid. The resistant of Imiphenem, Cefetazidime, Piperacillin, Amoxyclav, Carbenicillin may due to the presence of chromosomal lactamase.

[8] The higher level of resistance seen in isolate ABD (MIC, 623 ug / ml) was associated with a PI 6.4 β lactamase encoded by a plasmid, pMLH 52, with a molecular mass of ca. 100 MDa, Production of this enzyme transferred to pseudomonas aeruginosa PU21 in plate mating but not to *Escherichia coli*. PU21 transconjugants that acquired the pI 6.4 enzymes expressed resistance to Ceftazidime, other Penicillin's, and Cephalosporins and to β lactamase inhibitor combinations, although not to Imiphenem. Resistance to Chloramphenicol, Sulfonamides, and various Aminoglycosides was contransferred with β lactamase. A pI 8.3 β lactamase also

produced by stain ABD, did not transfer and was presumed to be the chromosomal class C enzyme typical of *pseudomonas aeruginosa*. When cloned into *Escherichia coli*, the pI 6.4 enzyme gave Ceftazidime resistance, albeit at a lower level than in *pseudomonas aeruginosa.*The resistance of Chloramphenicol, Tetracycline, may due to efflux system. [13] Reported that the natural function of the multidrug efflux systems of *pseudomonas aeruginosa* and indeed, all bacteria is the subject of some debate. In some instance a case can be made for antimicrobials / Xenobiotics being the intended substrates and thus protection from these agents in the primary role for the efflux systems.

These observations suggest that resistance against few antibiotics of *pseudomonas aeruginosa* is plasmid mediated. The review of the literature showed that multidrug resistance of *Pseudomonas aeruginosa* could be due to combination of several factors.

REFERENCE

1. Arun Jyothi Mathius, Philip S and Fernandas J, Invitro activity of Antibacterial agent in *pseudomona*s infection Clinical Microbiology. Rev.50: 162-190, (1996).
2. Bukholm G, Tannaes T, Kjelsberg ABB and Smith-Erichsen N, An outbreak of multidrug-resistant *Pseudomonas aeruginosa* associated with increased risk of patient death in an intensive care unit. *Infect Control Hosp Epidemiol* . 23: 441-446, (2002).
3. Carmeli Y, Troillet N, Eliopoulos GM and Samore MH, Emergence of antibiotic-resistant *Pseudomonas aeruginosa*: comparison of risks associated with different antipseudomonal agents. Antimicrob Agents Chemother . 43(6):1379-1382, (1999).
4. Cheesbrough M, Medical Laboratory Manual for Tropical Countries Vol.II Microbiology. Butterworth Heinemann Ltd. Linacre House, Jordan Hill Oxford OX2 8DP. 264-265, (1993).
5. Cordero CP, Ngelangel C, Beltran A, *et al*, Derivation of a mortality prediction model at the intensive care unit of the Philippine General Hospital. Phil J Internal Medicine .32:275-279, (1994).
6. Fonbuena EG, Fadreguilan E, Timbreza F, Alejandria M and Lansang M, Comparison of the predictive accuracy of two scoring systems in medical patients with sepsis at the Philippine General Hospital. Phil J Microbiol Infect Disease. 31(3):95-100, (2002).
7. Gillespie TA, Johnson PRE, Notman AW, Coia JE and Hanson MF, Eradication of resistant *Pseudomonas aeruginosa* strain after a cluster of infections in a hematology/oncology unit. *Clin Microbiol Infect.* 6: 125-130, (2000).
8. Hall LMC, Ewarg V and Davis Y, *pseudomonas aeruginosa* Resistance through chromosome. Journal of Pharmacology. (l) 125:129-135, (1999).
9. Howard D, McGowan J, Packard R, Scott RD andSolomon SL, Measuring the economic costs of antimicrobial resistance in hospital settings: summary of the Centers for Disease Control and Prevention-Emory Workshop. Clin Infect Disseases; 33(9):1573-1578, (2001).
10. Krumpernam PH, Multiple antibiotic resistance indexing *Escherichia coli* to identify risk sources of faecal contamination of foods. Appl. Environ. Microbiol. 46: 165-170, (1983).
11. Olayinka AT, Olayinka BO and Onile BA, Antibiotic susceptibility and plasmid pattern of *Pseudomonas aeruginosa* from the surgical unit of a university teaching hospital in north central Nigeria International Journal of Medicine and Medical Sciences,Vol 1.(3) : 079-083, (2009).
12. Pechere JC and Kohler T, Patterns and modes of β-lactam resistance in *Pseudomonas aeruginosa.* Clin Microbiol Infect. 5 (1) : S15-S18, (1999).

13. Ramakrishnan SV, Sridhar PV and Senthil CS, *Escherichia coli* diarrhoea. Baillieres clinical Gasteroenterology . 243-261, (1998).
14. Silva CV, Magalhaes VD, Pereira CR, Kawagoe JY, Ikura C and Ganc AJ, Pseudo-outbreak of *Pseudomonas aeruginosa* and *Serratia marcescens* related to bronchoscopes. *Infect Control Hosp Epidemiol.* 24:195-197, (2003).
15. Susy Hota MD, Zahir Hirji MHSc, Karen Stockton MHSc, Camille Lemieux MD LLB, Helen Dedier MLT, Gideon Wolfaardt and Michael A. Gardam, Outbreak of Multidrug-Resistant *Pseudomonas aeruginosa* Colonization and Infection Secondary to Imperfect Intensive Care Unit Room Design. Infection control and hospital epidemiology. (30): 26-33. (2009).