ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2011; 2(2):724-733

ORIGINAL RESEARCH ARTICLE

"Incidence of Antibiotic-Resistant *Pseudomonas aeruginosa* Isolated from Drinking Water"

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Received 12 Jan 2011; Revised 21 Feb 2011; Accepted 04 Mar 2011

ABSTRACT

Pseudomonas aeruginosa has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several different epidemiological studies track its occurrence as a nosocomial pathogen and indicate that antibiotic resistance. *Pseudomonas aeruginosa* is a highly relevant opportunistic pathogen. One of the most worrisome characteristics of *P. aeruginosa* is its low antibiotic susceptibility. In present study drinking water samples were analyzed for its potability and presence of *P. aeruginosa*. Out of that 22 samples were found contaminated with *P. aeruginosa*. All 22 isolates showed maximum resistance to Levofloxin (50%) followed by Ciprofloxin (55%), and Gentamycin (51%) and Nitroflurantoin (51%), and Erythromycin (50%), and Co- trimaxazole (50%), and Oflaxacine (50%). The antibiotic like Tetracycline (46%), and Norfloxacin (46%), and Cephalexin (46%), and Metronidazole (46%), and Doxypal- Dr (46%), were moderately effective against the isolates and some antibiotic like Ampicillin (41%), Penicillin (41%) and Amixycellin (41%) were leess effective or minimum resistances against the isolates. The overall study concluded that the variation occurred in multiple antibiotic resistance patterns among various strains of *Pseudomonas* strains isolated from drinking water, indicated the emergence of antibiotic resistance, due to the indiscriminate use of antibiotics

Key Words: Opportunistic pathogen, drinking water quality and antibiotic resistance.

INTRODUCTION

Pseudomonas aeruginosa is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several different epidemiological studies indicate that antibiotic resistance is increasing in clinical isolates. All species and strains of *Pseudomonas* are Gram-negative rods, and have historically been classified as strict aerobes. Exceptions to this classification have recently been discovered in *Pseudomonas* biofilms (Cooper *et al* 2003).

Pseudomonas has the ability to metabolize a variety of diverse nutrients. Combined with the ability to form biofilms, they are thus able to survive in a variety of unexpected places. *P. aeruginosa* flourishes in hospital environments, and is a particular problem in this environment since it is the second most common infection in hospitalized patients. (Cornelis, 2008).

Selection of resistance during antipseudomonal therapy among initially susceptible isolates occurs frequently with this pathogen, resulting in the emergence of resistance to multiple drugs (Tacconelli *et. al*. 2008). Although multi drug-resistant *P. aeruginosa* (MDRPA) infections have been described in patients with cystic fibrosis or immunocompromised conditions and in isolated outbreaks in intensive care units, recent reports in critically ill patients in non outbreak settings have raised concerns because of the scarcity of novel agents to effectively treat MDRPA infections (Goossens *et al* 2005).

Pseudomonas aeruginosa has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several different epidemiological studies track its occurrence as a nosocomial pathogen and indicate that antibiotic resistance. *Pseudomonas*

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aeruginosa is a highly relevant opportunistic pathogen. One of the most worrisome characteristics of *P. aeruginosa* is its low antibiotic susceptibility. This low susceptibility is attributable to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes. Being Gram-negative bacteria, most *Pseudomonas spp.* are naturally resistant to penicillin and the majority of related beta-lactam antibiotics, but a number are sensitive to piperacillin, imipenem, ticarcillin, tobramycin, or ciprofloxacin (Muto *et al* 2003).

Their resistance to most antibiotics is attributed to efflux pumps which pump out some antibiotics before the antibiotics are able to act. The primary cause of antibiotic resistance is antibiotic use both within medicine and veterinary medicine (Krumperman, *et al*, 1983). The greater the duration of exposure the greater the risk of the development of resistance irrespective of the severity of the need for antibiotics. As resistance becomes more common there becomes a greater need for alternative treatments (Balcht *et al* 1994).Biofilms are more resistant to disinfection than planktonic bacteria and biofilms in drinking water distribution systems can act as a reservoir of pathogenic microorganisms causing outbreaks of infectious diseases. The challenge to avoid unwanted effects of bio-film growth in water distribution networks calls for new technologies for efficient microbial control (Paul *et al* 1997).

Antibiotic resistance has been called one of the world's most pressing public health problems. And organizations such as the Centers for Disease Control and Prevention have undertaken efforts to educate physicians and the public about antibiotic resistance (Wright *et al* 2009). Confirming a bacterial infection, selecting the appropriate antibiotic for an infection and educating patients about the importance of taking therapy exactly as prescribed are considered areas for improvement needed. In such scenario the study targeted to evaluate the prevalence and multidrug resistance of *Pseudomonas aeruginosa* (Poole, 2004).

MATERIALS AND METHODS

In present study a total of 44 drinking water samples were collected in sterile container pipe lines of various collection site of Neemuch City.

All the drinking water samples were analyzed for its potability and presence of *P. aeruginosa*. Out of that 22 samples were found contaminated with *P. aeruginosa*. All the samples were collected from drinking water pipe line. *P. aeruginosa* was isolated by using selective culture medium (Hi-media) and identified by biochemical tests.

All the isolates were subjected to perform antibiotic sensitivity test by Kirby and Buyer (1966) method. Study data were subjected for analysis. In present study 15 antibiotics were used against 22 isolates (**Table 1**). Circular zone of inhibition created by each test compound and that of standard compound was measured by Hi-Antibiotic Zone Scale (Hi-Media, Mumbai). The zone measurement scale can read inhibition zones ranging from 10 mm to 40 mm. Cellulosic discs of diameter 6 mm were used to be impregnated with the test compounds.

Table 1:- Antibiotics Used in Study

SN	Antibiotics	Quantity	SN	Antibiotics	Quantity
1	Levofloxin	15 mcg	9	Metronidazole	05 mcg
2	Tetracycline	30 mcg	10	Penicillin G	10 mcg
3	Ciprofloxin	05 mcg	11	Doxypal-dr	13 mcg
4	Ampicillin	10 mcg	12	Erythromycin	15 mcg
5	Gentamycin	10 mcg	13	Amixycellin	07 mcg
6	Nitroflurantoin	300 mcg	14	Co-trimaxazole	25 mcg
7	Norfloxacin	10 mcg	15	Oflaxacine	01 mcg
8	Cephalexin	30 mcg			

RESULTS AND DISCUSSION

In this study total 44 drinking water samples of various sources (Pipeline and tap water), were analyzed for the presence of pseudomonas contamination in drinking water. A total number of 22 isolates identify as *P. aeruginosa*. The antibiotic susceptibility data was analyzed (**Table 1**). The data analysis indicated that all 22 isolates showed maximum resistance to Levofloxin (50%) followed by Ciprofloxin (55%), and Gentamycin (51%) and Nitroflurantoin (51%), and Erythromycin (50%), and Co- trimaxazole (50%), and Oflaxacine (50%). The antibiotic like Tetracycline (46%), and Norfloxacin (46%), and Cephalexin (46%), and Metronidazole (46%), and Doxypal-dr (46%), were moderately effective against the isolates and some antibiotic like Ampicillin (41%), Penicillin (41%) and Amixycellin (41%) were less effective or minimum resistances against the isolates. Several workers reported higher degree of sensitivity of

pseudomonas to Gentamycin (Nikadio, 2009). Similerily in this profile indicated that Pseudomonas is frequently resistant to many commonly used antibiotics.

Table 2:- Antibiotic Susceptibility data and Zone of Inhibition)

SN	Isolates	Antibiotic Susceptibility test														
		Zone of Inhibition (mm)														
	Psuedomonas Spp	Levofloxin	Tetracycline	Ciprofloxin	Ampicillin	Gentamycin	Nitroflurantoin	Norfloxacin	Cephalexin	MetrOnidazole	Penicillin G	Doxypal-dr	Erythromycin	Amixycellin	Co-trimaxazole	Oflaxacine
1	PS 1	0	12	0	20	0	0	30	0	0	28	0	30	33	0	20
2	PS 2	32	0	0	0	15	0	0	18	15	13	24	21	0	30	30
3	PS 3	32	0	0	32	0	20	22	18	20	0	20	0	30	0	0
4	PS 4	0	15	30	35	0	18	12	0	25	0	0	0	20	12	0
5	PS 5	0	0	20	0	10	0	15	0	0	25	30	0	0	10	35
6	PS 6	15	20	0	0	20	30	0	22	0	0	31	21	0	0	0
7	PS 7	0	0	0	21	0	0	19	0	0	35	25	30	21	0	0
8	PS 8	35	21	32	22	0	22	39	26	0	0	0	25	28	0	39
9	PS 9	30	0	25	0	20	0	0	21	20	14	0	0	0	21	12
10	PS 10	18	33	0	35	25	0	21	0	23	20	12	0	25	25	24
11	PS 11	0	21	31	0	0	31	0	0	0	0	30	12	30	0	10
12	PS 12	0	35	0	0	21	35	0	19	24	24	0	30	0	0	0
13	PS 13	25	0	0	0	0	21	18	0	27	21	25	0	21	12	0
14	PS 14	0	0	12	12	0	0	20	25	0	0	0	0	33	30	30
15	PS 15	21	18	0	16	18	14	0	30	18	25	18	20	0	35	0
16	PS 16	0	0	21	0	35	0	35	0	0	30	30	0	0	0	35
17	PS 17	0	21	14	0	24	0	0	24	25	0	0	0	35	0	0
18	PS 18	35	0	30	15	0	0	0	23	30	30	0	31	0	0	23
19	PS 19	0	20	0	16	30	23	12	0	0	0	0	20	33	12	0
20	PS 20	23	0	0	30	0	30	20	35	13	21	30	0	31	20	15
21	PS 21	21	30	20	23	0	0	0	19	0	35	35	30	0	0	0
22	PS 22	0	12	0	32	23	19	0	0	35	0	0	0	20	25	0

Results showed that maximum isolates were found to resistant to Ciprofloxine as compare to other antibiotics while other isolates were showed sensitivity towards Ampicillin, penicillin G and Amlyxicillin. The results of table 3 showed the overall spectrum of antibiotic resistance among isolates of *P. aeruginosa*. As indicated that maximum isolates were found to Sensitive to Ampicillin and penicillin G as compare to other antibiotics while other isolates were showed Resistance towards Ciprofloxin, and Ofloxacine (**Table 2**).

(Table 3: - Isolates Resistance to Antibiotics)

SN	Levofloxin	Tetracycline	Ciprofloxin	Ampicillin	Gentamycin	Nitroflurantoin	Norfloxacin	Cephalexin
1	PS 1	PS 2	PS 1	PS 2	PS 1	PS 1	PS 2	PS 1
2	PS 4	PS 3	PS 2	PS 5	PS 3	PS 2	PS 6	PS 4
3	PS 5	PS 5	PS 3	PS 6	PS 4	PS 5	PS 9	PS 5
4	PS 7	PS 7	PS 6	PS 9	PS 7	PS 7	PS 11	PS 7
5	PS 11	PS 9	PS 7	PS 11	PS 8	PS 9	PS 12	PS 10
6	PS 12	PS 13	PS 10	PS 12	PS 11	PS 10	PS 15	PS 11
7	PS 14	PS 14	PS 12	PS 13	PS 13	PS 14	PS 17	PS 13
8	PS 16	PS 16	PS 13	PS 16	PS 14	PS 16	PS 18	PS 16
9	PS 17	PS 18	PS 15	PS 17	PS 18	PS 17	PS 21	PS 19
10	PS 19	PS 20	PS 19		PS 20	PS 18	PS 22	PS 22
11	PS 22		PS 20		PS 21	PS 21		
12			PS 22					
Total	11	10	12	9	11	11	10	10

SN	Metr0nidazole	Penicillin G	Doxypal-dx	Erythromycin	Amixycellin	Co-trimaxazole	Oflaxacine	
1	PS 1	PS 3	PS 1	PS 3	PS 2	PS 1	PS 3	
2	PS 5	PS 4	PS 4	PS 4	PS 5	PS 3	PS 4	
3	PS 6	PS 6	PS 8	PS 5	PS 6	PS 6	PS 6	
4	PS 7	PS 8	PS 9	PS 9	PS 9	PS 7	PS 7	
5	PS 8	PS 11	PS 12	PS 10	PS 12	PS 8	PS 12	
6	PS 11	PS 14	PS 14	PS 13	PS 15	PS 11	PS 13	
7	PS 14	PS 17	PS 17	PS 14	PS 16	PS 12	PS 15	
8	PS 16	PS 19	PS 18	PS 16	PS 18	PS 16	PS 17	
9	PS 19	PS 22	PS 19	PS 17	PS 21	PS 17	PS 19	
10	PS 21		PS 22	PS 20		PS 18	PS 21	
11				PS 22		PS 21	PS 22	
Total	10	9	10	11	9	11	11	

The overall study concluded that, When 22 isolates analysed for antibiotic sensitivity, data analysis showed that maximum 12 isolates were resistant to Ciprofloxin as compare to other isolates. The overall resistance patterns are as follow, 11 isolate resistant to Levofloxin, 10 isolate R to Tetracycline, 9 isolates R to Gentamycin, 11 isolate R to Nitroflurantoin and followed by Doxypal-xr (10), Erythromycin 11, Co trimaxzole 11 and Oflaxacine 11 isolates showed resistance (**Fig 1**).

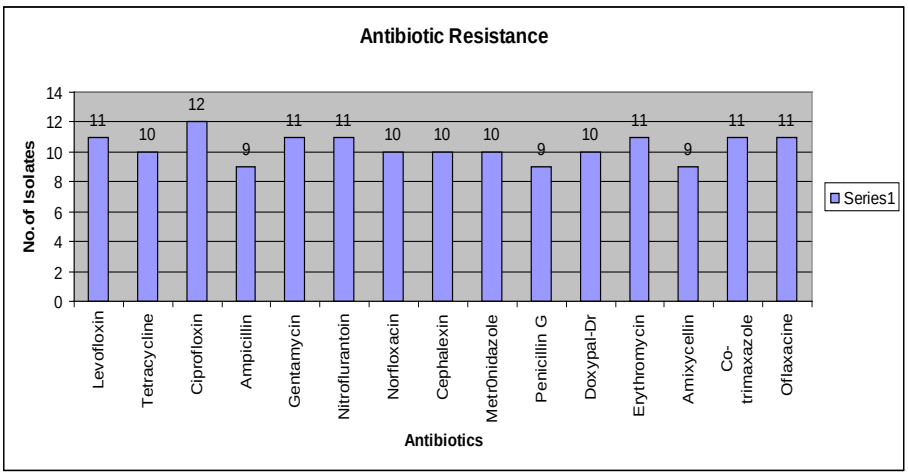

Fig 1: - Isolates Resistance to Antibiotics

Where as, 13 isolates were showed sensitivity towards, Ampicillin, Pennicillin G, 12 isolates showed sensitivity for Norfloxcin, Cephalexin, metronidazole and Amixycellin. Where as 10 isolates showed sensitivity towerds ciprofloxacin and Ofloxcine. Result indicated that less number

of isolate showed sensitivity for these 15 antibiotics (**Fig 2**). Silmilar results also found by Tambekar *et al* In 2007, they found that all the isolated strains of *Ps. Aeruginosa* were highly sensitive (100%) to Gatifloxacine, Ofloxacin, Gentamicin and Imipenem follwed by Tetracycline, Ciprofloxacin Levofloxacin and Amikacin.

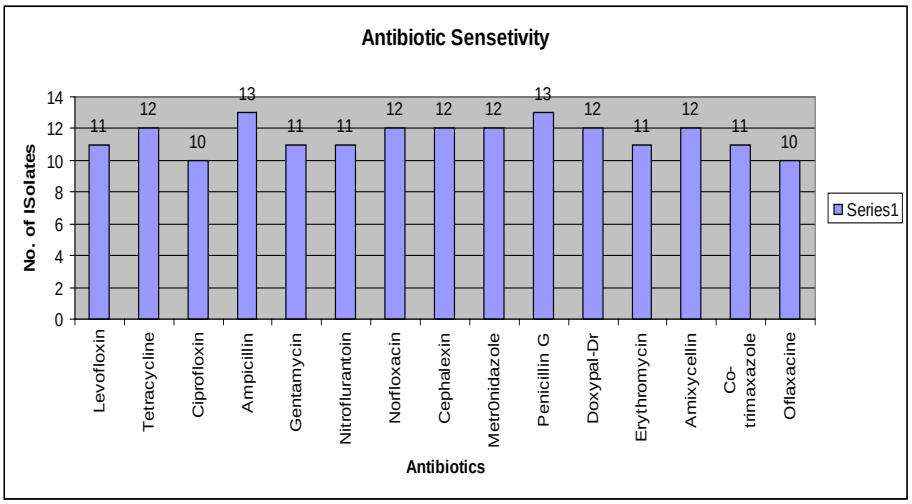

Fig 2: - Isolates Sensitive to Antibiotics

Similarly, investigators also found that, this approach has been largely unsuccessful with the penicillin family. However, among the cephalosporins and cephamycins a number of compounds that resist hydrolysis by b-lactamases have been developed. Plasmid mediated b-lactamases hydrolyse extended spec- trum cephalosporins and are inhibited by clavulanic acid (Nordmann *et al.* 1993), whereas chromosomally mediated cephalosporinases are usually not inhibited by clavulanic acid. Such enzymes exhibit clinical resistance in *Pseudomonas aeru- ginosa* (Barthelemy *et al.* 1988).

When data analyzed for individual isolates it is found that PS (I) and PS (II) showed 32 mm zone of inhibition for Levifloxin, 20 mm for Ciprofloxin, 30 mm for Norfloxacin, 27 for penicillin G, 33 mm for Co trimaxazole and 20 mm for Ofloxcin. The data showed that PS I showed resistance for 9 antibiotics and PS II showed resistance for 7 antibiotics. Study showed three antibiotics, Ciprofloxin, Nitroflurantion and amphycillin did not showed zone of inhibition, indicated the resistance toward these two isolates (**fig 3**).

PS 3 showed resistance towards Ciprofloxin, Nitroflurantion, as compare to Isolate PS 4. Besides this PS 4 showed 15 mm for Tetracycline, 30 mm for Ciprofloxin, 35 mm for Amphycillin, 25 mm for Metronidazole, 20 mm for Amixycellin and 12 mm for Co-trimaxazole. PS 4 showed highly resistance towards Ciprofloxin, Gentamycin, and Cephalexin and Penicillin G (**Fig 4**). PS 5 showed highly resistance to Ampicillin, Norfloxacin and Metronidazole. PS6 showed resistance for Ampicillin Nitroflurantion Norfloxin, amphycillin Oflaxacine which did not showed any zone of inhibition (**Fig 5**).

Data showed that the highest sensitivity showed in the case of Norfloxacin and Oflaxacine by PS 7(Fig 6). PS 8 showed o mm zone for Gentamycin, Metronidazole and co- trimaxazole indicated highly resistance of Isolate PS7. Data showed that the highest sensitivity showed in the case of Norfloxacin and Oflaxacine by PS 8 (**Fig 6**).

The data also indicated that PS 9 showed resistance for nitroflurantoin and Erythromycin. The fig 7 showed that Out of 15 antibiotic used while PS 9 show resistance against 4 antibiotics. Data showed that the highest sensitivity showed in the case of Ampicillin and Tetracycline by PS 10 (**Fig 7**).

PS 11 showed 21 mm zone for Tetracycline, 31 mm zone for Ciprofloxin. PS 11 showed highest resistance against Ampicillin (0 mm zone of inhibition) simultaneously also found in Norfloxacin and co- trimaxazole. Data showed that the highest sensitivity showed in the case of Norfloxacin and Oflaxacine by PS 11 (**Fig 8**). As per the result of Fig 8, PS 12 showed 35 mm zone for Tetracycline, 21 mm zone for Ciprofloxacin, 35 mm zone of inhibition for Nitroflurantoin, 19 mm for Cephalexin and Nitroflurantoin, 39 mm for Norfloxacin, 26 mm, 25 mm Erythromycin, and 24 mm zone of inhibition observed by PS 12 against Metronidazole and Penicillin G.

As per the result Data showed that the highest sensitivity showed in the case of Levofloxin, Metronidazole and Amixycellin by PS 13, whereas PS 14 showed o mm zone for Gentamycin, and Gentamycin indicated highly resistance of Isolate PS14 (**Fig 9**). For Amixycellin, Tetracycline and Levofloxin PS 15 showed 0 mm zone of inhibition, which indicated the resistance for these antibiotics. PS 16 showed o mm zone for Gentamycin, Norfloxine Metronidazole and co- trimaxazole indicated highly resistance of Isolate PS16 (**Fig 10**). PS 17 showed o mm zone for Gentamycin, Norfloxine Metronidazole and co- trimaxazole indicated highly resistance of Isolate PS17. As per the result of Fig 11, PS 18 showed 35 mm zone for Levofloxin, 30 mm zone for Ciprofloxacin, Norfloxacin, 26 mm Cephalexin, 25 mm Erythromycin, and 22 and 23 Amixycellin and Oflaxacine (**Fig 11**). PS 19 showed 35 mm zone for Levofloxin whereas, PS 20 showed o mm zone for Gentamycin, Norfloxine Metronidazole and co- trimaxazole indicated highly resistance of Isolate PS20 (**Fig 12**).

PS 21 showed 21 mm zone for Levofloxin, 30 mm zone for Tetracycline, 20 for Ciprofloxin 19 mm zone of inhibition for cephalexin, 35 mm for Penicillin G and Doxypal-xr, 30 mm for Erythromycin. Data showed that the highest sensitivity showed in the case of Norfloxacin and Oflaxacine by PS 21 (**Fig 13**). PS 21 showed o mm zone for Gentamycin, Metronidazole and co-trimaxazole indicated highly resistance. PS 22 showed 15 mm zone for Tetracycline, 33 mm zone for Ampicillin, 25 for Gentamycin 35 mm zone of inhibition for Metronidazole, 35 mm for Penicillin G and Doxypal-xr, 30 mm for Erythromycin. Data showed that the highest sensitivity showed in the case of Metronidazole and Ampicillin by PS 22 (Fig 13). PS 22 showed o mm zone for Norfloxin, and Oflaxacine indicated highly resistance.

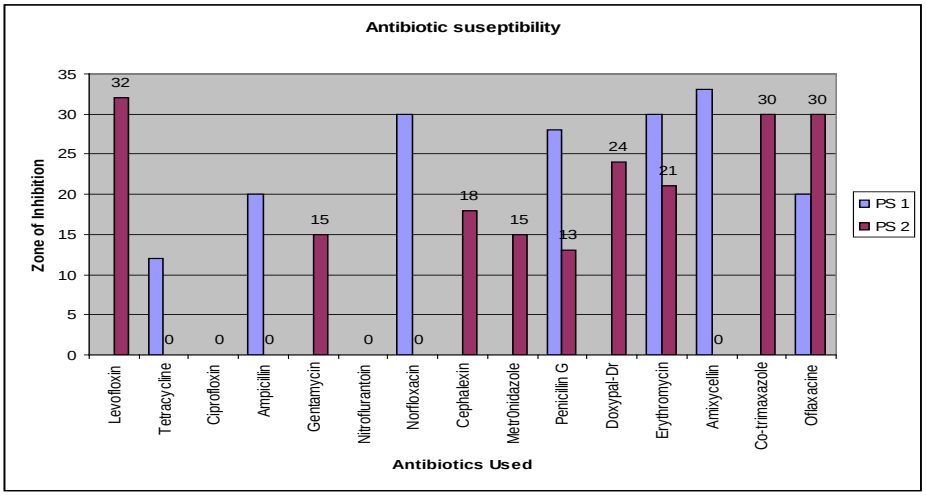

Fig 3:- Zone of Inhibition by PS I and PS II

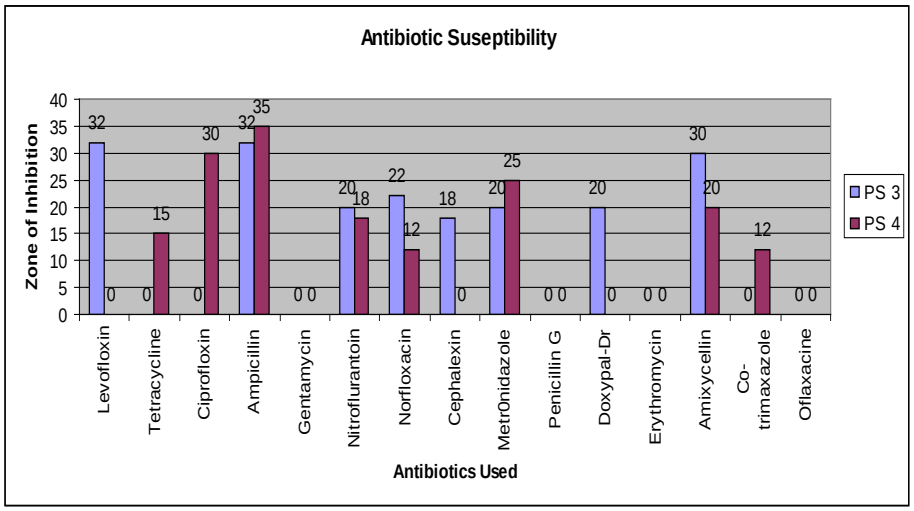

Fig 4:- Zone of Inhibition by PS 3 and PS 4

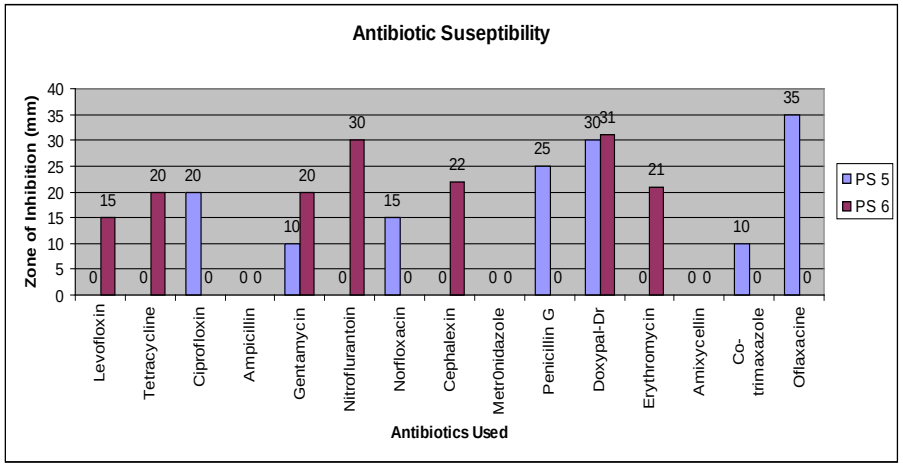

Fig 5:- Zone of Inhibition showed by PS 5 and PS 6

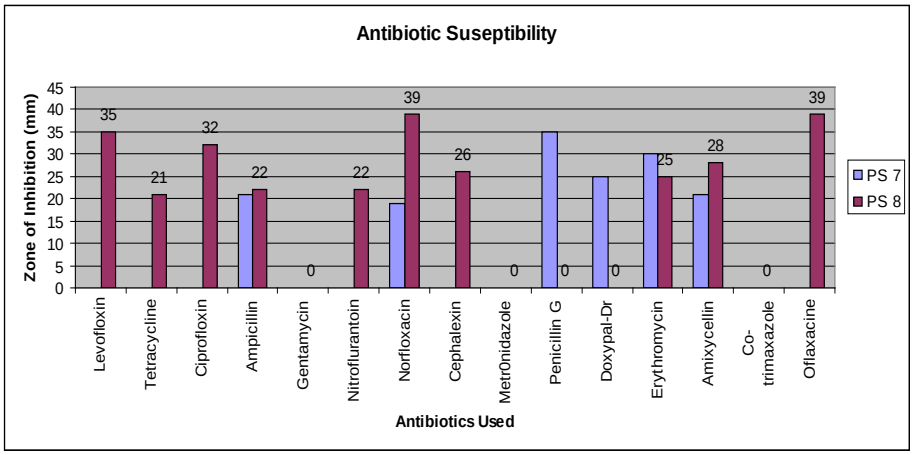

Fig 6:- Zone of Inhibition by PS 7 and Ps 8

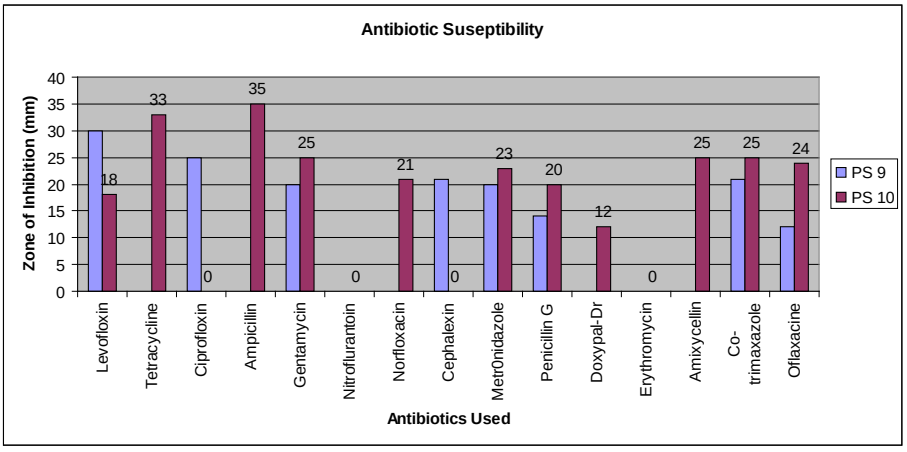

Fig 7:- Zone of Inhibition by PS 9 and Ps 10

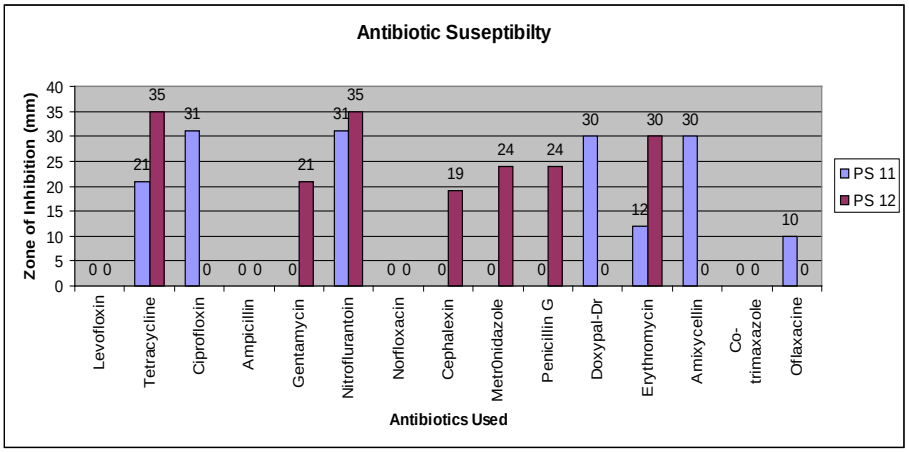

Fig8:- Zone of Inhibition by PS 11 and Ps 12

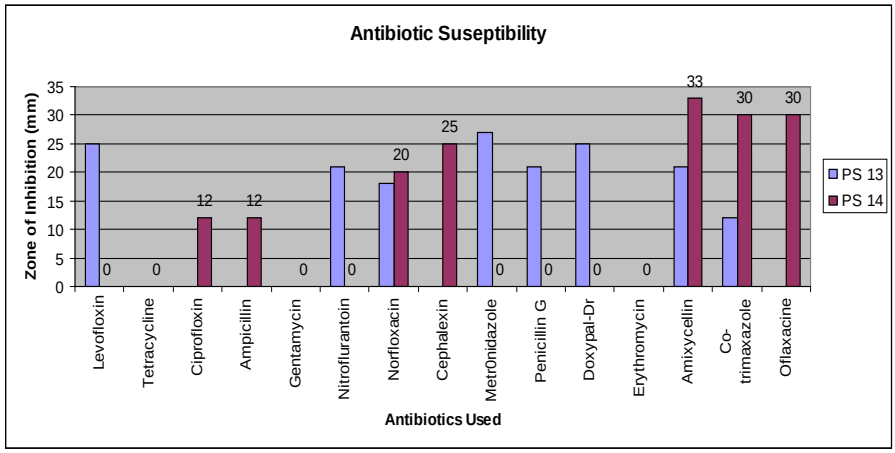

Fig 9:- Zone of Inhibition by PS 13 and Ps 14

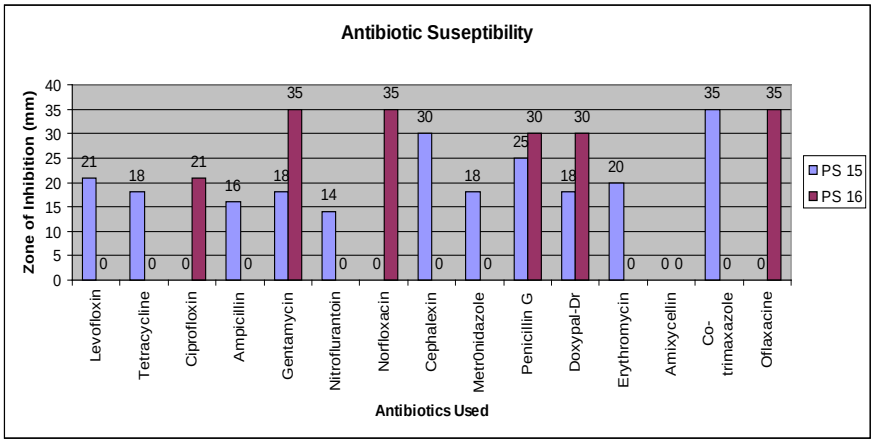

Fig 10:- Zone of Inhibition by PS 15 and Ps 16

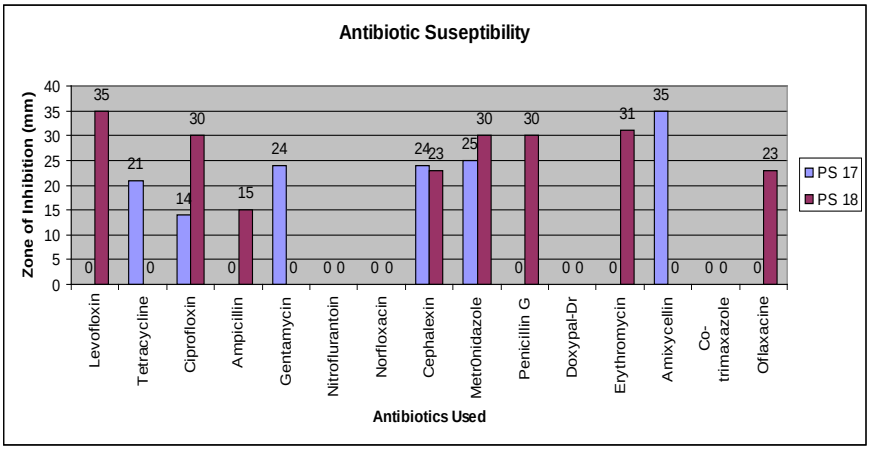

Fig 11:- Zone of Inhibition by PS 17 and Ps 18

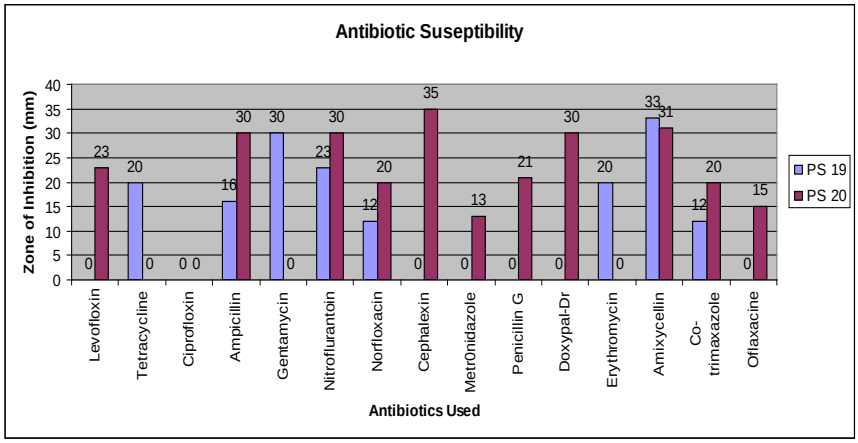

Fig 12:- Zone of Inhibition by PS 19 and Ps 20

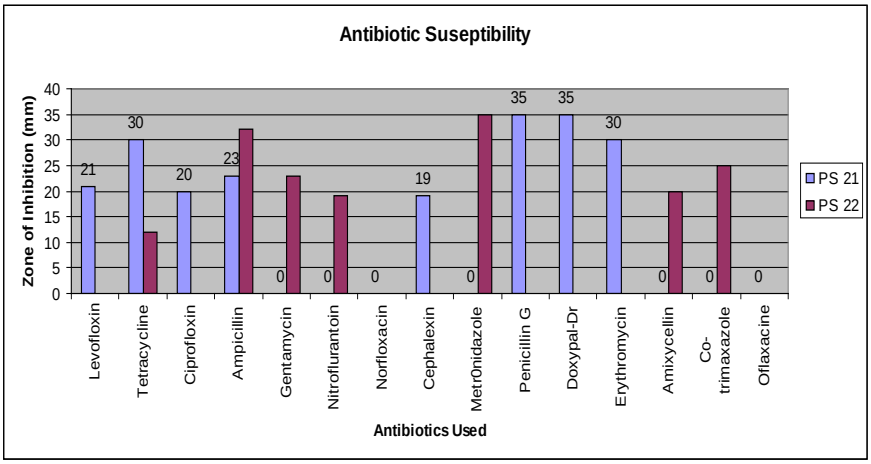

Fig 13:- Zone of Inhibition by PS 21 and Ps 22

CONCLUSION

The overall study concluded that the variation occurred in multiple antibiotic resistance patterns among various strains of *P. aeruginosa* isolated from drinking water, indicated the emergence of antibiotic resistance, due to the indiscriminate use of antibiotics,The overall resistance patterns are as follow, 11 isolate resistant to Levofloxin, 10 isolate resistance to Tetracycline, 9 isolates resistance to Gentamycin, 11 isolate resistance to Nitroflurantoin and followed by Doxypalxr (10), Erythromycin 11, Co trimaxzole 11 and Oflaxacine 11 isolates showed resistance. Comparatively high antibacterial sensitivity observed due to rare or occasional of the drug and could be attributed to the fact these drug were seldom used. The high level resistance to these antibiotics might be attributed to antibiotic and antibiotic resistance bacterial emergence in drinking water sources because of improper and higher use of these antibiotics.

ACKNOWLEDGMENT:

We are thankful to Dep't. of Life Sciences of MIST, supporting staff and editor of journal. We also thanks full to BRNSS management who provide the facilities.

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