International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

A Comparative Analysis of Multi-Drug Resistance Patterns in *Pseudomonas Aeruginosa* Isolated From Environmental Sources in Auchi, Edo State, Nigeria

Dania U. J[1]., Ojokoh A. O[2].

[1] Department of Science Laboratory Technology, Auchi Polytechnic, PMB 13, Auchi, Edo State, Nigeria

[2] Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria

Abstract: ***This study investigates the rate of sensitivity of multi-drug resistant P. aeruginosa from various environmental sources. A total of 72 Pseudomonas aeruginosa was obtained from the environmental sources which are waste water, soil and air. Isolated pure cultures of bacteria were subjected to various morphology and biochemical tests. The antibiotic susceptibility test was performed using disc diffusion method. Twelve (12) clinical pertinent antibiotics (Augmentin (Aug), Cloxacillin (Cxc), Gentamicin (Gen), Amikacin (Amk), Ceftazidime (Caz), Cefuroxime (Crx), Cetriazone (Ctr), Ciprofloxacin (Cpr), Ofloxacin (Ofl), Erythromycin (Ery), Imipenem (Imp) and Meropenem (Mer)) were tested against P. aeruginosa. Variation occurred in multidrug resistance patterns among various strains of Pseudomonas aeruginosa isolated. Among the antibiotics, the most effective were meropenem (carbepenems) and amikacin (aminoglycosides) with their resistant rate as 26.39% and 43.06%, respectively while the least effective were cloxacillin (penicillin) and cefuroxim (cephalosporin) both with resistant rate of 100% among the 72 P. aeruginosa strains. Six isolates were resistant to all the twelve antibiotics used. This study has shown that there is wide spread antimicrobial resistance patterns of some environmental strains of Pseudomonas aeruginosa from Auchi in Edo state, Nigeria.***

Keywords: *Pseudomonas aeruginosa*, Multi-drug, Susceptibility, Environmental sources, Auchi, Edo, Nigeria

1. Introduction

Pseudomonas aeruginosa is a gram-negative, rod-shaped, asporogenous, and monoflagellated bacterium that has an incredible nutritional versatility. It is a rod about 1-5 µm long and 0.5-1.0 µm wide. *P. aeruginosa* is an obligate respirer, using aerobic respiration (with oxygen) as its optimal metabolism, although can also respire anaerobically on nitrate or other alternative electron acceptors. *P. aeruginosa* can catabolize a wide range of organic molecules, including organic compounds such as benzoate. This, then, makes *P. aeruginosa* a very ubiquitous microorganism, for it has been found in environments such as soil, water, humans, animals, plants, sewage, and hospitals (Lederberg, 2000). In all oligotropic aquatic ecosystems, which contain high-dissolved oxygen content but low plant nutrients, *P.aeruginosa* is the predominant inhabitant and this clearly makes it the most abundant organism on earth (Costerton and Anwar, 1994).

Pseudomonas aeruginosa is an opportunistic pathogen commonly found in the environment mainly in soil and water, but is also regularly found on plants and sometimes on animals, including humans. It is a Gram-negative, rod-shaped bacterium that is motile by means of a single polar flagellum and known to be highly antibiotic resistant and able to grow in a variety of generally inhospitable environments, often through its ability to form resilient biofilms. The bacteria often produce the blue-green pigment pyocyanin, a redox-active phenazine, which is known to kill mammalian and bacterial cells through the generation of reactive oxygen intermediates. *Pseudomonas* infections often have a characteristic sweet odor and have become a substantial cause of infection in patients with immunodeficiencies. It is one of the main agents of hospital-acquired infections such as pneumonia, urinary tract infections (UTIs), and bacteremia (Drenkard *and* Ausubel, 2002*)*.

P. aeruginosa is an opportunistic pathogen that rarely causes disease in healthy individuals. Most infections are able to take hold by the loss of the integrity of a physical barrier to infection (eg, skin, mucous membrane) or the presence of immune deficiency. This bacterium has also minimal nutritional requirements and can tolerate a wide variety of physical conditions like temperatures up to 41 degrees Celsius.

P. aeruginosa was first described as distinct bacterial specie at the end of the nineteenth century, after the development of sterile culture media by Pasteur. In 1882, the first scientific study on *P. aeruginosa*, entitled "On the blue and green coloration of bandages," was published by a pharmacist named Carle Gessard. This study showed *P. aeruginosa's* characteristic pigmentation: *P. aeruginosa* produced water-soluble pigments, which, on exposure to ultraviolet light, fluorescene blue-green light. This was later attributed to pyocyanine, a derivative of phenazine, and it also reflected the organism's old names: *Bacillus pyocyaneus*, *Bakterium aeruginosa*, *Pseudomonas polycolor*, and *Pseudomonas pyocyaneus* (Botzenhardt and Doring, 1993). *P. aeruginosa* has many strains, including *Pseudomonas aeruginosa* strain PA01, *Pseudomonas aeruginosa* strain PA7, *Pseudomonas aeruginosa* strain UCBPP-PA14, and *Pseudomonas aeruginosa* strain 2192. Most of these were isolated based on their distinctive grapelike odor of aminoacetophenone, pyocyanin production, and the colonies' structure on agar media (Gilardi, 1985).

P.aeruginosa is an opportunistic human pathogen. It is "opportunistic" because it seldom infects healthy individuals. Instead, it often colonizes immunocompromised patients, like those with cystic fibrosis, cancer, or AIDS (Botzenhardt and Doring, 1993). It is such a potent pathogen that firstly, it attacks up two third of the critically-ill hospitalized patients, and this usually portends more invasive diseases. Secondly, *P.aeruginosa* is a leading Gram-negative opportunistic pathogen at most medical centers, carrying a 40-60% mortality rate. Thirdly, it complicates 90% of cystic fibrosis deaths; and lastly, it is always listed as one of the top three most frequent Gram-negative pathogens and is linked to the worst visual diseases (Fick, 1993). Furthermore, *P.aeruginosa* is a very important soil bacterium that is capable of breaking down polycyclic aromatic hydrocarbons and making rhamnolipids, quinolones, hydrogen cyanide, phenazines, and lectins. It also exhibits intrinsic resistance to a lot of different types of chemotherapeutic agents and antibiotics, making it a very hard pathogen to eliminate (Lederberg, 2000).

2. Methodology

Collection of samples

Samples of environmental materials (soil, waste water and air) from which *P. aeruginosa* was isolated were collected from Mechanic village, Auchi Edo State at different locations, of which 20 samples each of soil and water were collected while 20 plates of prepared nutrient agar were taken to the site to collect air samples, by exposing the plates in the mechanic pit and allowing air to filtrate the agar.

Bacteriological Analysis

The collected microbial samples were transported to the laboratory following Cheesbrough, (2000) method. Samples were plated primarily onto nutrient agar and Mac conkey agar and the plates were incubated at 37^0C for 24–48 hrs. The Suspicious isolates were presumptively identified by using colony morphology, pigment formation, mucoidy, haemolysis on blood agar, positive oxidase test, grape-like odour, growth at 42^0C on nutrient agar, positive motility test, and Gram reaction (Cheesbrough, 2000).

Further, the *P. aeruginosa* isolates were confirmed by biochemical tests which include; oxidase, catalase, coagulase, indole, urease, methyl red, voges prokuer, citrate, triple sugar iron and sugar fermentation test.

Antibiotic Susceptibility Testing

The agar disc diffusion method of Bauer (Bauer *et al*, 1966) modified based on National Committee for Clinical Laboratory Standards (NCCL, 2000) CLSI was followed to perform the susceptibility test for the *P. aeruginosa* isolates. A small inoculum of each bacterial isolate was emulsified in 3ml sterile normal saline in bijou bottles and the turbidity compared with barium chloride standard (0.5 McFarland). A sterile cotton swab was dipped into the standardized solution of *P.aeruginosa* cultures and used to evenly inoculate Mueller-Hinton plates and the plates were allowed to dry. Thereafter, the clinically pertinent 12 antibiotic discs (Abtek Biologicals Ltd Liverpool, L9 7AR UK) with the following drug contents Augmentin (Aug), Cloxacillin (Cxc), Gentamicin (Gen), Amikacin (Amk), Ceftazidime (Caz), Cefuroxime (Crx), Cetriazone (Ctr), Ciprofloxacin (Cpr), Ofloxacin (Ofl), Erythromycin (Ery), Imipenem (Imp) and Meropenem (Mer) were placed on the plate. After 24 hrs, clinical interpretation [resistant (R), and sensitive (S)] of the size of the zone was evaluated based on the MIC susceptibility value as determined by the diameter from the zone of inhibition and compared with ATCC 27853 strain of *P. aeruginosa*.

3. Result

A total of 72 *Pseudomonas aeruginosa* isolates from environmental sources (waste water, soil and air) were obtained from different spots in mechanic village in Auchi area of Edo State as shown in table 1.

Table 1: Sources of Isolates

Sources	*Number of samples collected*	*Number of positive*
Waste water	50	15(30%)
Soil	50	20(40%)
Air	50	37(74%)
Total	150	72(48%)

The antibiotic sensitivity and resistance patterns of various source isolates are shown in Table 2 - 4. Twelve (12) most commonly used drugs for *Pseudomonas* infection were used for antibiotic susceptibility assay which are; Augmentin (Aug), Cloxacillin (Cxc), Gentamicin (Gen), Amikacin (Amk), Ceftazidime (Caz), Cefuroxime (Crx), Cetriazone (Ctr), Ciprofloxacin (Cpr), Ofloxacin (Ofl), Erythromycin (Ery), Imipenem (Imp) and Meropenem (Mer).

Table 2, shows sensitivity and resistance patterns of 15 *Pseudomonas aeruginosa* isolates from waste water. Out of 15 isolates, 2(13.33%) were sensitive to Augumentin, 4(26.67%) to Gentamycin, 10(66.67%) to Amikacin, 8(53.33%) to Ciprofloxacin, 6(40%) to Ofloxacin, 2(13.13%) to Erythromycin, 9(60%) to Imipenem and 12(80%) to Meropenem. While all the 15 isolates were resistants to Cloxacillin, Ceftazidine, Ceftriazone and Cefuroxime.

Table 2: Antibiotic resistance patterns of the 15 *Pseudomonas aeruginosa* isolates from the waste Water

Class of Antibiotic	Type of Antibiotic	Number and Percentage of Resistant	Number and Percentage of Susceptible
Penicillin	Augmentin (30ug)	13(86.67%)	2(13.33%)
	Cloxacillin (5ug)	15(100.0%)	0 (0%)
Aminoglycoside	Gentamycin (10ug)	11(73.33%)	4(26.67%)
	Amikacin (30ug)	5(33.33%)	10(66.67%)
Cephalosporin	Ceftazidime (30ug)	15(100.0%)	0(0.00%)
	Cefuroxime (30ug)	15(100.0%)	0(0.00%)
	Ceftriaxone (30ug)	15(100.0%)	0(0.00%)

Quinolones	Ciprofloxacin (5ug) Ofloxacin (5ug)	7(46.67%) 9(60.00%)	8(53.33%) 6(40.00%)
Macrolides	Erythromycin (5ug)	13(86.67%)	2(13.33%)
Carbepenems (ß -Lactamase inhibitors)	Imipenem (10ug) Meropenem(10ug)	6(40.00%) 3(20.00%)	9(60.00%) 12(80.00%)

The sensitivity and resistance patterns of 20 *Pseudomonas aeruginosa* isolates from soil sample are shown in Table 3. The result shows that 5(25%) of the isolate were susceptible to Augumentin, 7(35%) to Gentamycin, 10(50%) to Amikacin, 1(5%) to Ceftazidine, 1(5%) to Ceftriazone, 10(50%) to Ciprofloxacin, 8(40%) to Ofloxacin, 1(5%) to Erythromycin, 14(70%) to Imipenem and 16(80%) to Meropenem. Cloxacillin and Cefuroxime were not effective against the 20 isolates.

Table 3: Antibiotic resistance patterns of the 20 *Pseudomonas aeruginosa* isolates from the Soil

Class of Antibiotic	Type of Antibiotic	Number and Percentage of Resistant	Number and Percentage of Susceptible
Penicillin	Augmentin (30ug) Cloxacillin (5ug)	15(75.00%) 20(100%)	5(25.00%) 0 (0.00%)
Aminoglycoside	Gentamycin (10ug) Amikacin (30ug)	13(65.00%) 10(50.00%)	7(35.00%) 10(50.00%)
Cephalosporin	Ceftazidime (30ug) Cefuroxime (30ug) Ceftriaxone (30ug)	19(95.00%) 20(100.00%) 19(95.00%)	1(5.00%) 0(0.00%) 1(5.00%)
Quinolones	Ciprofloxacin (5ug) Ofloxacin (5ug)	10(50.00%) 12(60.00%)	10(50.00%) 8(40.00%)
Macrolides	Erythromycin (5ug)	19(95.00%)	1(5.00%)
Carbepenems (ß -Lactamase inhibitors)	Imipenem (10ug) Meropenem(10ug)	6(30.00%) 4(20.00%)	14(70.00%) 16(80.00%)

The sensitivity and resistance patterns of 37 *Pseudomonas aeruginosa* isolates from air sample are shown in Table 4. The result shows that 2(5.41%) of the isolate were susceptible to Augumentin, 16(43.24%) to Gentamycin, 23(62.16%) to Amikacin, 1(2.70%) to Ceftazidine, 1(2.70%) to Cefuroxime, 15(40.54%) to Ciprofloxacin, 13(35.14%) to Ofloxacin, 1(2.70%) to Erythromycin, 18(48.65%) to Imipenem and 25(67.58%) to Meropenem. Cloxacillin and Ceftriazone were not effective against the 37 isolates.

Table 4: Antibiotic resistance patterns of the 37 *Pseudomonas aeruginosa* isolates from the Air

Class of Antibiotic	Type of Antibiotic	Number and Percentage of Resistant	Number and Percentage of Susceptible
Penicillin	Augmentin (30ug) Cloxacillin (5ug)	35(94.59%) 37(100.0%)	2(5.41%) 0 (0.00%)
Aminoglycoside	Gentamycin (10ug) Amikacin (30ug)	21(56.76%) 14(37.84%)	16(43.24%) 23(62.16%)
Cephalosporin	Ceftazidime (30ug) Cefuroxime (30ug) Ceftriaxone (30ug)	36(97.30%) 37(100.0%) 36(97.30%)	1(2.70%) 0(0.00%) 1(2.70%)
Quinolones	Ciprofloxacin (5ug) Ofloxacin (5ug)	22(59.46%) 24(64.86%)	15(40.54%) 13(35.14%)
Macrolides	Erythromycin (5ug)	36(97.30%)	1(2.70%)
Carbepenems (ß -Lactamase inhibitors)	Imipenem (10ug) Meropenem(10ug)	19(51.35%) 12(32.43%)	18(48.65%) 25(67.57%)

Table 5, shows sensitivity and resistance patterns of the 72 *Pseudomonas aeruginosa* isolates from the mechanic village (environment). Out of 72 isolates, 9(12.5%) were sensitive to Augumentin, 27(37.5%) to Gentamycin, 43(59.72%) to Amikacin, 2(2.78%) to Ceftazidine, 2(2.78%) to Ceftriazone, 33(45.83%) to Ciprofloxacin, 27(37.5%) to Ofloxacin, 4(5.56%) to Erythromycin, 41(56.94%) to Imipenem and 53(73.61%) to Meropenem. While all the 15 isolates were resistants to Cloxacillin and Cefuroxime.

Table 5: Antibiotic resistance patterns of the 72 *Pseudomonas aeruginosa* isolates from environmental sources

Class of Antibiotic	Type of Antibiotic	Number and Percentage of Resistant	Number and Percentage of Susceptible
Penicillin	Augmentin (30ug) Cloxacillin (5ug)	63(87.5%) 72(100%)	9(12.5%) 0 (0%)
Aminoglycoside	Gentamycin (10ug) Amikacin (30ug)	45(62.5%) 29(40.28%)	27(37.5%) 43(59.72%)
Cephalosporin	Ceftazidime (30ug)	70(97.22%)	2(2.78%)

	Cefuroxime (30ug) Ceftriaxone (30ug)	72(100.00%) 70(97.22%)	0(0%) 2(2.78%)
Quinolones	Ciprofloxacin (5ug) Ofloxacin (5ug)	39(54.17%) 45(62.5%)	33(45.83%) 27(37.5%)
Macrolides	Erythromycin (5ug)	68(94.44%)	4(5.56%)
Carbepenems (ß -Lactamase inhibitors)	Imipenem (10ug) Meropenem(10ug)	31(43.06%) 19(26.39%)	41(56.94%) 53(73.61%)

4. Discussion

In this study, twelve antibiotics were tested against *P. aeruginosa* from different environmental samples. These were (penicillin) augumentin, cloxacillin, (aminoglycosides) gentamicin, amikacin, (cephalosporins) ceftazidime, cefurozime, ceftriazone, (quinolones) ciprofloxacin, ofloxacin, (macrolide) erythromycin, (carbepenemes) imipenems and meropenems. The reason choosing this antimicrobial was their wide use in the hospital as antipsedudomonal agents. Therefore, this kind of study could provide appropriate guidelines to the hospital regarding the prescription of these antimicrobials according to their sensitivity to *P. aeruginosa*.

Among the twelve antibiotics, maximum sensitivity was found with meropenem (73.61%) among carbepenems followed by amikacin (59.72%) among the aminoglycoside, while other drugs showed decrease in susceptibility pattern. Maximum sensitivity was demonstrated by these two classes of drugs in comparison to other antibiotics used in this study. One of the reasons for these drugs still remaining sensitive might be due to their restricted use in ICU (intensive care unit) and also limited use in critical care unit.

In earlier studies by Quinn (1998), it was reported that increased resistance rates of *Pseudomanas aeruginosa* have been detected against carbapenems, quinolons and third-generation cephalosporins across the globe. In this study, resistance rates against carbapenems such as meropenems and imipenem, were 26.39% and 43.06% and against aminoglycosides such as amikacin was 40.28%. In yet another study by Bouza *et al* (1999), it was reported that resistance to imipenem was 14% in Spain, 19.3% in Italy and 68% in Saudi Arabia. The National Nosocomial Infections Surveillance (NNIS) system reported the incidence of imipenem resistance as 18.5% among isolates of *Pseudomanas aeruginosa* from ICU patients (CDC, 1999).

The resistance of *P. aeruginosa* to the antibiotic in the quinolone group is variable in different centers. In a prospective study, resistance to ciprofloxacin was reported as 8-31% in ICU patients (Tassios *et al*., 1998). This study reveals that the resistance rate against ciprofloxacin was found as 54.17% while it was 32% in Indian (Sivaraj *et al*., 2012), 23% in Spain (Bouza *et al*.,1999), 31.9% in Italy (Bonfiglio *et al*., 1998), and 28.8% in Latin America. *P. aeruginosa* isolates had high resistant against ofloxacin 62.5% another quinolone used in the study. The difference in the resistance patterns to the various quinolones is similar to a study in Turkey where a wide range of resistance status against various quinolones was also recorded (Algun *et al*, 2004). The main mechanism of resistance to fluoroquinolones has been reported to be the decrease in binding of the target quinolones to enzymes as a result of changes in DNA gyrase and or topoisomerase enzymes. Mutations occur in gyr A and par C genes. This is usually against all quinolones. However, resistance due to mutations of gyr B, though less common may not be against all quinolones (Algun *et al*, 2004).

The resistant rate of ceftazidime (97.22%) was very high compared to Ciprofloxacin. According to earlier reports, resistance to ceftazidime was 15%-22% in the world (Jones, 2001), 36% in Indian (Sivaraj *et al*., 2012). Resistance to Ceftriaxone and cefuroxime was higher 97.22% and 100.00%, similar to ceftazidime. Resistance rates of anti-pseudomonal antibiotics were quite low in the United Kingdom: 5% for ceftazidime, 10% for ciprofloxacin, and 11% for imipenem (Spencer, 1996). In a study by Akingbade, *et al* (2012), *Pseudomonas aeruginosa* isolates showed 72.7% resistance to erythromycin which is in line 94.44% resistance to erythromycin in this study.

The air isolates were more resistant to the antibiotics which were more effective against the isolates when compared to the soil and waste water isolates; from the air isolates, resistant to meropenem, amikacin and imipenem were 32.43%, 37.84% and 51.35% respectively, in soil isolates, resistant to meropenem, amikacin and imipenem were 20.00%, 50.00% and 30.00% respectively, while in waste water isolates, resistant to meropenem, amikacin and imipenem were 20.00%, 33.33% and 40.00% respectively, All the isolates were resistant to five or more antibiotics; of this 6(8.33%) of isolates were resistance to all twelve antibiotics used.

5. Conclusion

Our results indicate that the resistance of *P. aeruginosa* is on the rise which may be due to the activities of industrial effluent in the environment. As a result, immunization may fail to recover by constant exposure of resistance microbes. Even though of medical improvement, the antimicrobial resistance still becomes an age - old problem. So, proper implementation of antibiotic policies and guideline must be there in every hospital to local susceptibility pattern. Currently, the treatment of *P. aeruginosa* infections is based on combination antibiotic therapy that traditionally includes ß-lactam agents and aminoglycosides, in addition to this; treatment with fluoroquinolones has offered new perspectives. The development of effective vaccine against *P. aeruginosa* is necessary in the modern world.

References

[1] Akingbade, O.A., Balogun, S.A., Ojo, D.A., Afolabi, R.O., Motayo, B.O., Okerentugba, P.O. and I.O. Okonko (2012): Plasmid profile analysis of multidrug resistant *Pseudomonas aeruginosa* isolated from wound infections in South West, Nigeria. *World Applied Sciences Journal*. 20 (6): 766-775

[2] Algun, U., Arisoy, A., Gunduz, T. and Ozbakkaloglu, B. (2004): The resistance of *P. aeruginosa* strains to fluoroquinolone group of antibiotics. *Indian J. Med. Microbiol*. 22: 112-114.

[3] Bauer, A.W., Kirby, M.M., Sharis, J.L. and Turck, M. (1966): Antibiotic susceptibility testing by a standard single disk method. *American J of Clin Pathol*. 45: 493–496.

[4] Bonfiglio, G., Carciotto, V, and Russo, G. (1998): Antibiotic resistance in *Pseudomonas aeruginosa*: An Italian survey. *Antimicrob Agents Chem*. 41: 307-310.

[5] Botzenhardt, K. and Doring, G. (1993): Ecology and epidemiology of *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa as an Opportunistic Pathogen*. 1-7.

[6] Bouza, E., Garcia-Gorrote, F., Cercenado, E., Marin, M. and Diaz, M.S. (1999): *Pseudomonas aeruginosa*: a survey of resistance in 136 hospitals in Spain. The Spanish *Pseudomonas aeruginosa* Study Group. *Antimicrob Agents Chem*. 43: 981-982.

[7] Centers for Disease Control and Prevention (1999): National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1990-May 1999. *Am J Infect Control*. 27: 520-32.

[8] Cheesbrough, M. (2000): *Pseudomonas* and related organisms; Biochemical test to identify bacteria; Antimicrobial susceptibility testing. In: District Laboratory Practice in Tropical Countries Part II. Low price edition 2000. Cambridge University 8 Press, Cambridge. 193–194; 63–70; 132–143.

[9] Costerton, W. and Anwar, H. (1994): *Pseudomonas aeruginosa*: The Microbe and Pathogen. *Pseudomonas aeruginosa* Infections and Treatment.1-17.

[10] Fick, R. (1993): *Pseudomonas aeruginosa*—the Microbial Hyena and Its Role in Disease: An Introduction. *Pseudomonas aeruginosa*: *The Opportunist*. 1-6.

[11] Drenkard, E. *and* Ausubel, F.M. *(*2002*):Pseudomonas* biofilm formation and antibiotic resistance are linked to phenotypic variation. *Nature*. 416*:* 740–743.

[12] Gilardi, G. (1985): Cultural and Biochemical Aspects for Identification of Glucose-Nonfermenting Gram-Negative Rods. Non-fermenting Gram-Negative Rods. 17-24.

[13] Lederberg, J. (2000): *Pseudomonas*. Encyclopedia of Microbiology. Second Edition. San Diego. 3:876-891.

[14] National Committee for Clinical Laboratory (2000): Standards Performance Standards for Antimicrobial Disk Susceptibility Testing. Document M100-S9. National Committee for Clinical Laboratory Standards, Wayne, PA.

[15] Quinn, J.P. (1998): Clinical problems posed by multiresistant nonfermenting gram-negative pathogens. *Clin Infect Dis*. 27: 117-4.

[16] Spencer, R. C. (1996): An 8-year microbe based survey of the epidemiology, frequency and antibiotic susceptibility of *Pseudomonas aeruginosa* hospital isolates in the United Kingdom. *J Antimicrob Chem*. 37: 295-301.

[17] Sivaraj, S., Murugesan, P., Muthuvelu, S., Purusothaman, S. and Silambarasan, A. (2012): Comparative study of *pseudomonas aeruginosa* isolate recovered from clinical and environmental samples against antibiotics. *International Journal of Pharmacy and Pharmaceutical Sciences*. 4(3): 103-107

[18] Tassios, P. T., Gennimata, V. and Maniatis. A. (1998): Emergence of multidrug resistance in ubiquitous and dominant *Pseudomonas aeruginosa* serogroup O:11. *J Clin Microbiol*. 36: 897-901.