PREVALENCE AND PLASMID CURING OF ANTIBIOTIC RESISTANT GRAM NEGATIVE BACTERIA ISOLATED FROM THE ENVIRONMENT

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ABSTRACT

This study was designed to determine the prevalence of antibiotic resistant Gram-negative bacteria in the environment and their ability to transfer resistance genes to other bacteria strains. A total of 138 isolates were identified from urban and rural sewage, water and soil samples collected in Nsukka, Nigeria using standard microbiological identification methods. Out of these 138 isolates, 70 were *Escherichia coli* while 68 were *Pseudomonas aeruginosa*. Twenty one (70 %), 12 (40 %), 5 (16.7 %) *Escherichia coli* isolates and 18 (60 %), 3 (10 %) and 15 (50 %) *Pseudomonas aeruginosa* were obtained from urban environmental samples of sewage, water and soil respectively while 16 (53.3 %), 9 (30 %), 7(23.3%) *Escherichia coli* and 12 (40 %), 1 (3.3 %), 19 (63.3 %) *Pseudomonas aeruginosa* isolates were isolated from rural environmental samples of sewage, water and soil respectively. The isolates from urban sewage recorded the highest antibiotic resistance frequency when Septrin was used with percentage resistance of 61.9 % and 77.8 % for *E. coli* and *P. aeruginosa* respectively. Isolates of *E. coli* from urban water showed the least resistance when Ciprofloxacin and Gentamycin were used and highest resistance frequency when Septrin was used. *P. aeruginosa* recorded the highest antibiotic resistance frequency when Augumentin, Streptomycin, Septrin and Amoxicillin were used with each having percentage resistance of 33.3 %. *E. coli* isolates from urban soil samples were completely resistant to Ofloxacin and Ciprofloxacin while resistance to Augumentin and Amoxicillin was observed with percentage resistance frequency of 40 % while *P. aeruginosa* showed different degrees of resistance to all the antibiotics used. *E. coli* and *P. aeruginosa* isolates from rural sewage recorded the least resistance when ciprofloxacin was used with percentage resistance of 12.5 % and 16.7 %, respectively. Both isolates recorded the highest antibiotic resistance frequency when Amoxicillin was used. *E. coli* was susceptible to Ofloxacin and Ciprofloxacin while it was highly resistant to Augumentin with percentage resistance frequency of 33.3 %. *P. aeruginosa* in water from rural environment was susceptible to all the antibiotics used. The *E. coli* and *P. aeruginosa* isolates from soil samples obtained from rural environment showed low resistance to the antibiotics. Five isolates (Two *E. coli* and three *P. aeruginosa* isolates) that were resistant to at least two different classes of antibiotics were subjected to plasmid curing. Result showed that some of the bacterial isolates haboured transferable resistance plasmids which can promote the transfer of resistance genes to other bacteria strains. This study reveals that antibiotic resistant *E. coli* and *P. aeruginosa* is present in the environment and also have the ability to transfer their resistance genes to other bacteria strains. Therefore, adequate treatment of sewage and proper disposal of waste in the environment is vital so as to reduce the risk of infection and antibiotic resistance in the environment.

Keywords: Antibiotic resistance, Plasmid curing, Gram negative bacteria, Environmental samples, *E. coli*, *P. aeruginosa*.

Introduction

A high-density patients' population in frequent contact with health care staff and the attendant risk of cross-infection contribute to the spread of antibiotic-resistant microorganisms in the environment (Bataineh *et al*., 2007). Occurrence and prevalence of these resistant strains in the environment is common in developing countries. The Gram-negative bacterium *Pseudomonas*

aeruginosa is a ubiquitous aerobe that is present in water, soil and on plants (Banerjee and Stableforth, 2000). Naturally, this organism is endowed with weak pathogenic potentials. However, its profound ability to survive on inert materials, minimal nutritional requirement, tolerance to a wide variety of physical conditions and its relative resistance to several unrelated antimicrobial agents and antiseptics, contributes enormously to its ecological success and its role as an effective opportunistic pathogen (Gales *et al*., 2001). The organism is pathogenic when introduced into areas devoid of normal defenses (Jawetz *et al.,* 1999) and infections are both invasive and toxigenic (Todar, 2002).

The organism has been incriminated in cases of meningitis, septicaemia, pneumonia, ocular and burn infections, hot tubs and whirlpool-associated follicultis, osteomyelitis, cystic fibrosis-related lung infection, malignant external otitis and urinary tract infections with colonized patients being an important reservoir within the environment (Hernandez *et al*., 1997). Cross-transmission from patient to patient may occur via the hands of the health care staff or through contaminated materials and reagent; therefore encourages the presence of these organisms in the environment (DuBois *et al*., 2001). However, it is believed that *Pseudomonas aeruginosa* is generally environmentally acquired and that person-to-person spread occurs only rarely (Harbour *et al*., 2002). As such, contaminated respiratory care equipment, irrigating solutions, catheters, infusions, cosmetics, dilute antiseptics, cleaning liquids, and even soaps have been reported as vehicles of transmission within the environs (Joklik *et al*., 1992; Berrouane *et al.,* 2000; DuBois *et al.,* 2001).

Escherichia coli are Gram–negative bacterium and the main aerobic commensal bacteria species (Alhaj, 2007; Von and Marre, 2005). The native habitat of *Escherichia coli* is the enteric tract of humans and other warm-blooded animals. Therefore, *Escherichia coli* are widely disseminated in the environment through the faeces of humans and other animals and its presence in water is generally considered to indicate faecal contamination; therefore called indicator of water contamination and the possible presence of enteric pathogens. *Escherichia coli* are able to acquire resistance easily within environment. Antibiotic resistant *Escherichia coli* may pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *Staphylococcus aureus*, through a process called horizontal gene transfer. *Escherichia coli* often carry multidrug resistant plasmids and under stress, readily transfer those plasmids to other species. Thus, *Escherichia coli* are important reservoirs of transferable antibiotic resistance in the environs (Salyers *et al*., 2004). It has been observed that antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Hassan, 1995).

Escherichia coli are an opportunistic pathogen in neonatal and immune-compromised patients (Annette, 1998). Bacteremia, wound infections, urinary tract infection, and gastrointestinal infections are the diseases associated with *Escherichia coli* and are often fatal in newborns (Raina *et al*., 1999). The organism is of clinical importance due to its cosmopolitan nature in the environment and the ability to initiate, establish and cause various kinds of infections (Okeke *et al*., 2000; Olowe *et al.,* 2003; Tobih *et al*., 2004). Infections with antibiotic resistant bacteria make the therapeutic options for infection treatment extremely difficult or virtually impossible in some instance (El-Astal, 2004). Therefore, the determination of antimicrobial susceptibility of environmental and clinical isolate is often crucial for optimum antimicrobial therapy of infected patients.

Increase in antibiotic resistance level is now a global problem. *Pseudomonas aeruginosa* is naturally resistant to many of the widely used antibiotics, so chemotherapy is often difficult. Resistance is

due to the transfer of a resistance plasmid (R-plasmid) which is a plasmid carrying genes encoding proteins that detoxify various antibiotics out of the cell. Low antibiotic susceptibility, which is a worrying characteristic, is attributable to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes e.g. mexAB-oprM,mexXY, etc (Poole, 2004), and low permeability of the bacterial cellular envelopes. Besides intrinsic resistance, *Pseudomonas aeruginosa* easily develops acquired resistance either by mutation in chromosomally-encoded genes, or by the horizontal gene transfer of antibiotic resistance determinants. Development of multidrug resistance by *Pseudomonas aeruginosa* isolates requires several different mutations and/or horizontal transfer of antibiotic resistance genes. Hyper-mutation favours the selection of mutation-driven antibiotic resistance in *Pseudomonas aeruginosa* strains producing chronic infections, whereas the clustering of several different antibiotic resistance genes in integrons favours the concerted acquisition of antibiotic resistance determinants. Some recent studies have shown that phenotypic resistance associated with biofilm formation or to the resistance determinants (Cornelis, 2008). This study is aimed on determining the resistance patterns of *Pseudomonas aeruginosa* and *Escherichia coli* isolates from environment to some commonly used antibiotics and plasmid curing.

Materials and methods

Sample size and site

A total of 180 environmental samples were randomly collected and examined, 60 samples each (from sewage, water and soil). Sewage effluent and water samples were aseptically collected with sterile containers while soil samples were collected with a sterile polythene bags from the top 0-15cm. These samples were collected from both urban and rural areas of Nsukka, Enugu State, Nigeria.

Isolation and characterization of bacteria isolates

These environmental samples were processed as follows:

Water samples: Ten-fold serial dilution method was used by adding one ml of each sample into 9ml of sterile distilled water in a test tube. Diluents were plated out on Pseudomonas base agar and MacConkey agar. The plates were incubated at 37^0C for 24 hrs. Discrete colonies were picked from the agar plates based on size and colour of colonies and were stored on agar slant for further identification.

Soil samples: One gram of each soil sample was mixed with 9ml of sterile distilled water and shaken for some minutes. The resulting suspension was allowed to settle and the supernant was serially diluted and plated. The plates were incubated at 37^0C for 24 hrs, and then repeatedly sub cultured on nutrient agar so as to obtain pure cultures of the isolates.

Sewage effluents: One ml of each sample was added into 9ml of sterile distilled water in a test tube. Diluents were plated out on Pseudomonas base agar and MacConkey agar. The plates were incubated at 37^0C for 24 hrs. Discrete colonies were picked from the agar plates based on size and

colour. These were further purified to obtain pure cultures. The pure isolates were stored on agar slants for further use. Pure cultures of *E. coli* and *P. aeruginosa* isolates were identified biochemically using conventional identification techniques including oxidase test, sugar fermentation, indole, citrate, catalase, methylred test, voges-proskauer test, Gram staining, and colonial morphology such as pigmentation (Cheesbrough, 2006).

Antibiotic susceptibility test

The antimicrobial susceptibility test was carried out using Kirby Bauer disk diffusion method. The antibiotics used include; Augmentin (30µg), Gentamycin (10µg), Pefloxacin (30µg), Ofl oxacin (10µg), Streptomycin (30µg), Chloramphenicol (30µg), Sparfloxacin (10µg), Ciprofloxacin (1 0µg), Amoxicillin (30µg), Septrin(30µg) (Oxoid, UK). Pure cultures of bacteria isolates standardized to 0.5 McFarland turbidity standards were aseptically streaked on Mueller Hinton (MH) Agar (Oxoid UK) using sterile cotton swab sticks. The antibiotic disks were aseptically placed on the MH agar plates; and these were incubated at 37^0C for 24 hrs. The inhibition zone diameters were measured to the nearest millimeter using meter rule and the results were interpreted according to the guidelines of the Clinical Laboratory Standard Institute, (CLSI, 2009).

Plasmid curing

Plasmid curing was conducted on multidrug resistant isolates. This was done to determine whether the gene coding for resistance is chromosomally-mediated or plasmid-mediated. Plasmid being an extra chromosomal DNA molecule is eliminated from host bacteria after exposure to sub-lethal concentrations of intercalating agents such as sodium deodecylsulphate. The experiment was done according to Tomoeda, (1968).

Use of sodium dodecyl sulphate (sds)

Two concentrations (1 % and 5 %) of SDS in nutrient broth were used in this experiment. Nutrient broth was prepared and supplemented with 1g of SDS in one batch of 99 ml and 5 g of SDS in the second batch of 95 ml to achieve a final concentration of 1 % and 5 % (w/v) SDS respectively. It was then sterilized by autoclaving at 121^oC for 15min.

Selected overnight cultures of isolates were standardized to 0.5 McFarland turbidity standards using sterile saline. From these, 0.1 ml of each culture was inoculated separately into 5 ml of SDS supplemented nutrient broth in test tubes and incubated at 37^oC for 24 h. After incubation, cultures were standardized and spread on Mueller Hinton agar and susceptibility testing carried out.

Statistical analysis: The data obtained was analyzed using one way and two way analysis of variance (ANOVA)

RESULTS

Isolation of organisms

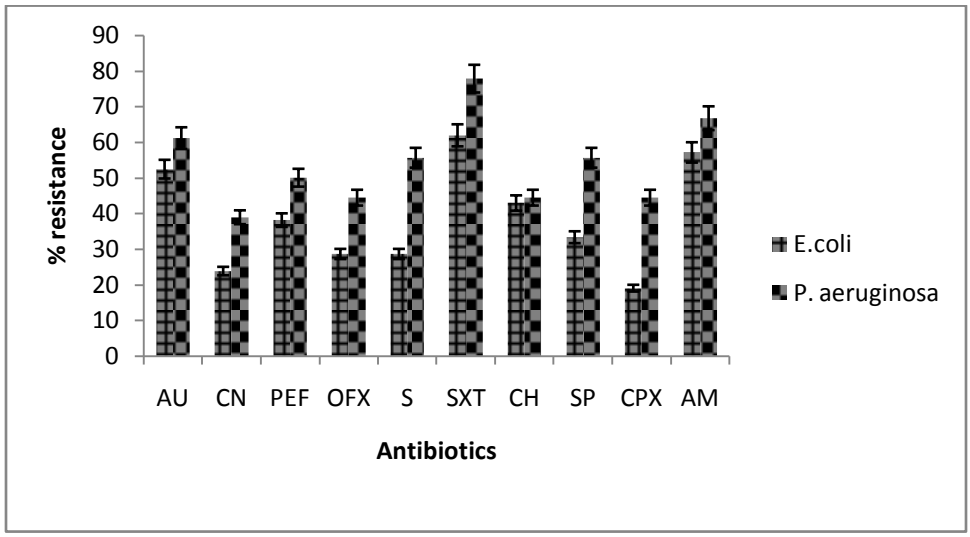
The organisms were isolated from sewage, water and soil using standard bacteriological procedure. The number of isolates from different sites is shown in Table 1.

Table1: Isolates from urban and rural environment.

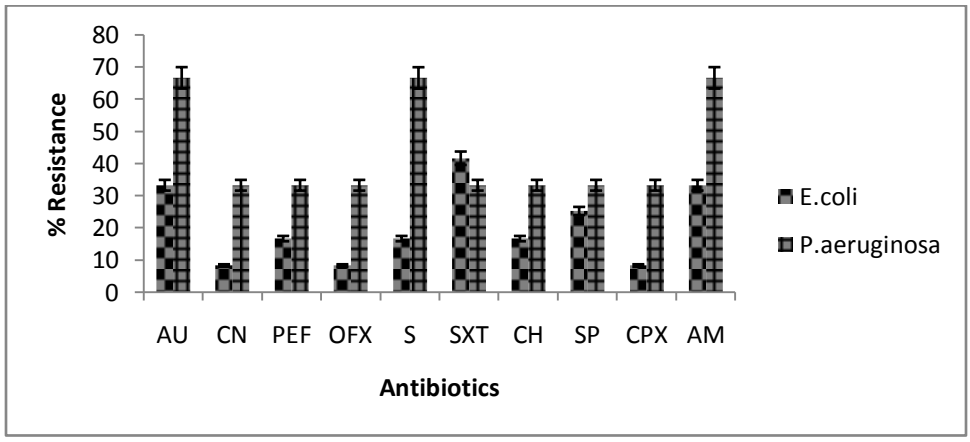
Site	Sample	No. of samples	*E. coli*	*P. aeruginosa*
Urban Environment	Sewage	30	21 (70 %)	18 (60 %)
	Water	30	12 (40 %)	3 (10 %)
	Soil	30	5 (16.7 %)	15 (50 %)
Rural Environment	Sewage	30	16 (53.3 %)	12 (40 %)
	Water	30	9 (30 %)	1 (3.3 %)
	Soil	30	7 (23.3 %)	19 (63.3 %)

Antibiotic resistance patterns of isolates from sewage, water and soil from urban environment.

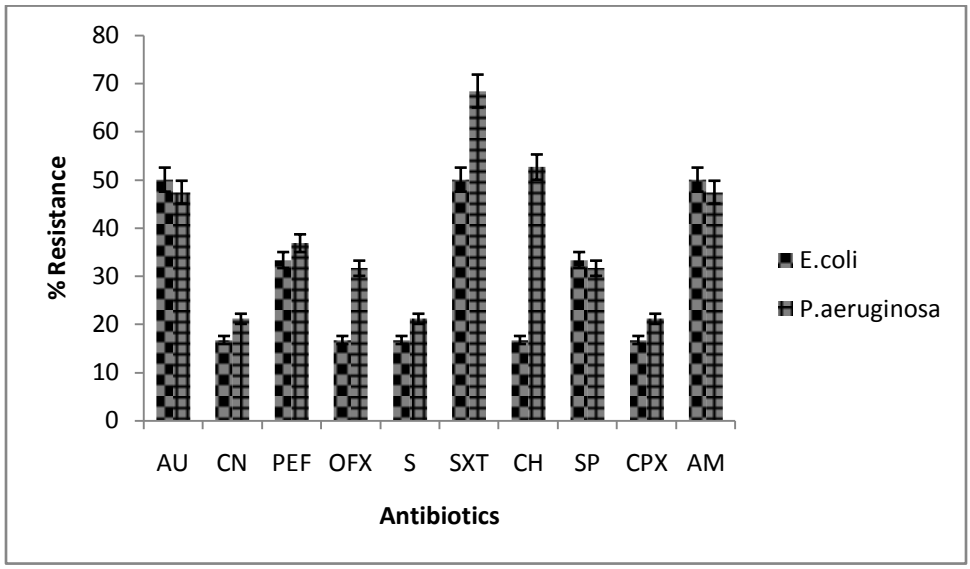
The results of the antibiotics resistance of the isolates to the antibiotics are presented in Fig 1 to 3. In Fig 1, the percentage antibiotics resistance of isolate from sewage is presented. The result showed that all the isolates recorded the highest resistance when Septrin was used with percentage resistance value of 61.9 % and 77.8 % for *E. coli* and *P. aeruginosa* respectively. The result showed that there is a statistical significant difference ($P \leq 0.5$) in the percentage resistance values between *E. coli* and *P. aeruginosa* when compared. *E. coli* recorded the least resistance when Ciprofloxacin was used while *P. aeruginosa* recorded the least resistance when Gentamycin was used. The result of the percentage resistance of the isolates from water to different antibiotics is presented in Fig 2; the result showed different resistance to antibiotics with *E. coli* having the least resistance value when Ciprofloxacin and Gentamycin were used while high resistance was observed when Septrin was used. *P. aeruginosa* recorded highest resistance value (33.3 %) when Augumentin, Streptomycin, Septrin and Amoxacillin were used. Fig 3 showed the result of the percentage resistance of the isolates from soil to different antibiotics. *E. coli* showed zero tolerance to Ofloxacin and Ciprofloxacin and highest resistance to Augumentin and Amoxacillin with percentage resistance value of 40 % while *P. aeruginosa* showed different degrees of resistance to all antibiotics. There is a significant difference ($P \leq 0.05$) in the percentage resistance to antibiotics by the isolates.



AU-augumentin, CN-gentamycin, PEF-perfloxacin, OFX-ofloxacin, S-streptomycin, SXT-septrin, CH-chloramphenicol, SP-sparfloxacin, CPX-ciprofloxacin, AM-amoxicilin.
Fig.1: Percentage antibiotics resistance of sewage isolates from urban environment.



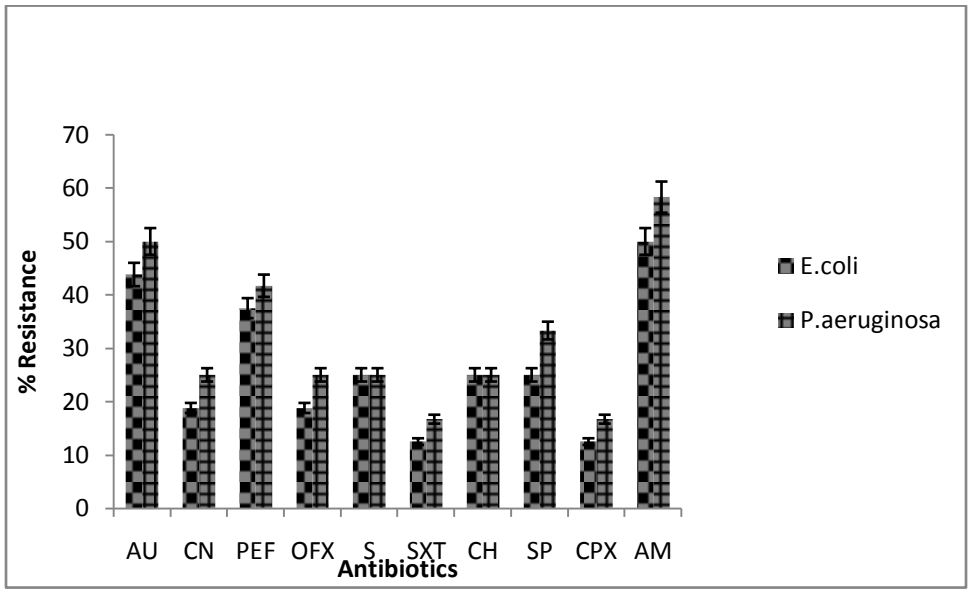
AU-augumentin, CN-gentamycin, PEF-perfloxacin, OFX-ofloxacin, S-streptomycin, SXT-septrin, CH-chloramphenicol, SP-sparfloxacin, CPX-ciprofloxacin, AM-amoxicilin.
Fig.2: Percentage antibiotics resistance of water isolates from urban environment.



Au-augumentin, CN-gentamycin, PEF-perfloxacin, OFX-ofloxacin, S-streptomycin, SXT-septrin, CH-chloramphenicol, SP-sparfloxacin, CPX-ciprofloxacin, AM-amoxicilin.
Fig.3: Percentage antibiotics resistance of soil isolates from urban environment.

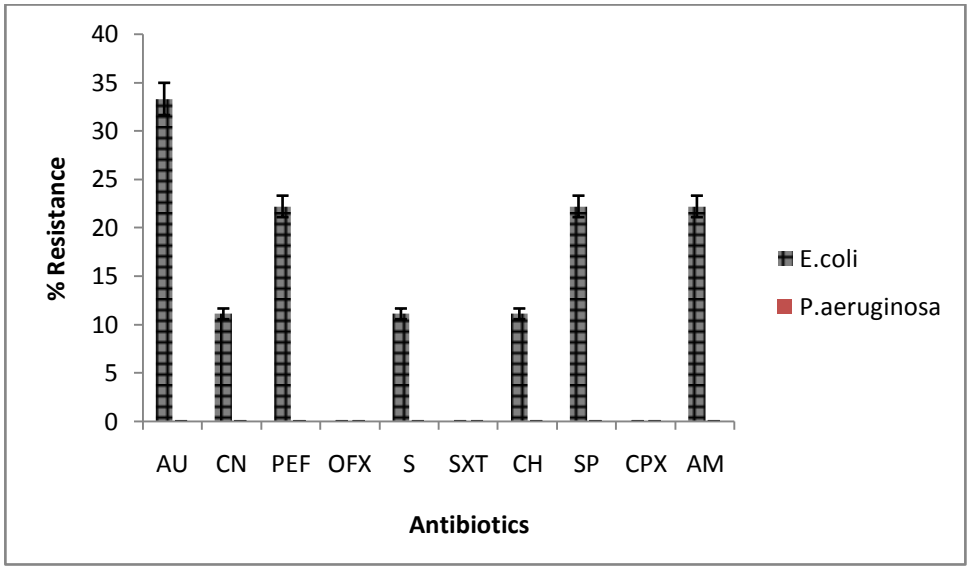
Resistance patterns of isolates from sewage, water and soil from rural environment to different antibiotics

The resistance of isolates from sewage, water and soil from rural environment to different antibiotics were also evaluated. The result is presented in fig 4 to 6. Fig 4 showed the percentage resistance of sewage isolate from rural environment to different antibiotic*. E. coli* and *P. aeruginosa* recorded the least resistance when ciprofloxacin was used with percentage resistance values of 12.5 % and 16.7% respectively. Both isolates recorded the highest resistance when Amoxicillin was used. The result of percentage resistance of waters isolates to different antibiotic is presented in Fig 5; *E. coli* showed zero resistance to Ofloxacin and Ciprofloxacin and highest resistance to Augumentin with percentage resistance value of 33.3 %. *P. aeruginosa* in water from rural environment showed zero resistance to all the antibiotics used. The percentage resistance of soil from rural environment is presented in fig 6. The isolates of soil from rural environment also showed low resistance to the antibiotics. The highest resistance recorded by *E.coli* was 42.8 % which occurred when Amoxicilin and Augumentin were used whereas the highest recorded resistance by *Pseudomonas aeruginosa* was 47.4 % which occurred when Augumentin and amoxicillin were used.



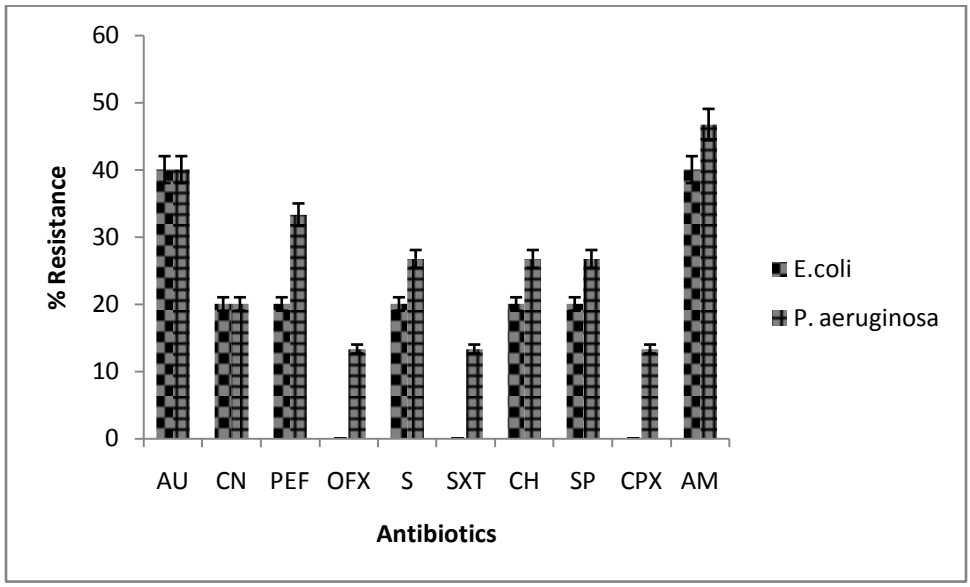
AU-augumentin, CN-gentamycin, PEF-perfloxacin, OFX-ofloxacin, S-streptomycin, SXT-septrin, CH-chloramphenicol, SP-sparfloxacin, CPX-ciprofloxacin, AM-amoxicilin.

Fig.4: Percentage antibiotics resistance of sewage isolates from rural environment.



AU-augumentin, CN-gentamycin, PEF-perfloxacin, OFX-ofloxacin, S-streptomycin, SXT-septrin, CH-chloramphenicol, SP-sparfloxacin, CPX-ciprofloxacin, AM-amoxicilin.

Fig.5: Percentage antibiotics resistance of water isolates from rural environment.



AU-augumentin, CN-gentamycin, PEF-perfloxacin, OFX-ofloxacin, S-streptomycin, SXT-septrin, CH-chloramphenicol, SP-sparfloxacin, CPX-ciprofloxacin, AM-amoxacilin.
Fig. 6: Percentage antibiotics resistance of soil isolates from rural environment.

Table 2: Effect of SDS mediated plasmid curing on resistant bacteria isolates from urban samples

Isolates	Resistant antibiotics	Effect at 1 % concentration	Effect at 5 % concentration
E. coli from sewage	Resistant to AU, SXT, AM	Resistant to SXT	No resistance
E. coli from soil	Resistant to AU, SXT, AM	No resistance	No resistance
P. aeruginosa from sewage	Resistant to AU, SXT, AM	Resistant to SXT	No resistance
P. aeruginosa from water	Resistant to AU, S, AM	No resistance	No resistance
P. aeruginosa from soil	Resistant to AU, SXT, CH, AM	Resistant to SXT	No resistance

DISCUSSION

A total of 180 environmental samples were randomly collected and examined; 60 samples each from sewage, water and soil. These samples were examined for the presence of multidrug resistance *E. coil* and *Pseudomonas aeruginosa* and the effect of SDS mediated plasmid curing on antibiotics resistance patterns of the isolates were evaluated using 1 % and 5 % concentration.

Evaluation of environmental samples showed that *E. coli* is more frequents in sewage and water when compared to soil, whereas *P. aeruginosa* is more frequent in soil; this occurred in both urban and rural environment. The percentage resistance of isolates from urban environment was also evaluated and the isolates from the urban sewage showed varying degree of resistance to the antibiotics. *E. coli* and *Pseudomonas aeruginosa* recorded higher resistance when Septrin was used with percentage resistance values of 61.9 % and 77.8 % respectively and least resistance when Ciprofloxacin and Gentamycin were used. *Pseudomonas aeruginosa* also showed high resistance to Augumentin and Amoxicillin with percentage resistance of 61.1 % and 66.7 %, respectively. This is in line with the work of Guardabassi and Dalsgaard, 2002. There is a significant difference ($p \leq 0.05$) in the percentage resistance displayed by the two isolates. The *E. coli* isolated from water obtained in the urban area showed very low resistance to antibiotics.

The highest resistance recorded was 41% when Septrin was used. *P. aeruginosa* on the other hand also showed low resistance to the antibiotics. It recorded the highest resistance when Augumentin and Amoxacilin were used with percentage resistance values of 33.3 % each. *E. coli* showed zero tolerance to Ofloxacin and Ciprofloxacin and highest resistance to Augumentin and Amoxacillin with percentage resistance of 40 % while *P. aeruginosa* showed different degrees of resistance to all antibiotics. This agrees with the work of Iheau *et al.,* 2002. There is no significant difference between the isolates from soil and that from water ($p \geq 0.05$) in the percentage resistance values to antibiotics by the isolates.

The isolates of water from rural environment showed little or no resistance to the antibiotics, *E. coli* showed no resistance to Pefloxacin, Ofloxacin and Septrin and low resistance to other antibiotics whereas *P. aeruginosa* showed no resistance to the entire antibiotic. This result agrees with the work of Tambekar *et al*. (2008) which reported that *P. aeruginosa* was highly sensitive to Ofloxacin and Gentamycin. It also reaffirmed the work of Schluter *et al.,* 2007. The different level of resistance to this antibiotic expressed by these organisms as observed in our study might be attributed to antibiotic bacterial emergence because of improper and extensive use of this antibiotic, antibiotic discharge in various amounts in the environment, indiscriminate use of antibiotic in medical, veterinary and agricultural practices leads to multiple antibiotic resistances in bacterial pathogens. This is in line with the work of Diab *et al*. (2002). This result also affirms the work of Chikere *et al*. (2008) who reported the sensitivity of Gram negative isolates to Pefloxin, Gentamycin and Ciprofloxacin to be 100 %. The isolates of soil from rural environment also showed low resistance to the antibiotics. There is a significant difference between the isolates from soil and that from water. The highest resistance record by *E. coli* was 42.8 % which occurred when Amoxacilin and Augumentin were used, whereas the highest recorded resistance by *Pseudomonas aeruginosa* was 47.4 % which occurred when Augumentin and Amoxacillin were used. This is in line with the findings of Shahid and Malik (2005) who reported that environmental isolates of *Pseudomonas aeruginosa* were less resistant to commonly used antibiotics. Five isolates of *E.coli* (2) and

P.aeruginosa (3) that were resistant to at least two different classes of antibiotics were subjected to plasmid curing. Result showed that when the isolates were treated with 1% SDS, most of them become susceptible to almost all the antibiotic except Septrin (SXT); when it was increased to 5 % SDS, the number that was susceptible increased to 100 %. This result showed that some of the strain had resistance plasmid which can promote the transfer of resistance gene to other strains. This study has revealed the prevalence of antibiotic resistant *E. coli* and *P. aeruginosa* in environmental samples and the possible transfer of antibiotic resistance to other bacteria strains. We therefore recommend adequate treatment of sewage and proper disposal of waste in the environment as this will reduce the risk of infection and antibiotic resistance among bacteria population in the environment.

REFERNCES

Alhaj,N., N.S., Mariana, A.R.Raha,R and Ishak,Z. (2007). Prevalence of antibiotic Resistance among *Escherichiacoli* from Different sources in Malaysia. *International Poultry Science* **6**: 293 – 297.

Annette,P (1998). Review of epidemiological studies on health effects from exposure to recreational water. Oxford University Press.

Banerjee, D. and Stableforth,D (2000).The treatment of respiratory pseudomonas infection in cystic fibrosis: what drug and which way? Drugs, **60**: 1053 –1064.

Bataineh, H.A., Khalid,M. andAlrashed, K.M.(2007). Resistant gram –negative bacilli and antibiotic consumption in Zarqa, *Jordan. Pak. Journal of Science*.**23**: 59 - 63.

Berrouane, F.Y., McNutt, L. andBuschellman, B.J. (2000). Outbreak of severe *P. aeruginosa* infection caused by contaminated drains in a whirlpool bathtubs. *Clinical Infectious Diseases* **31:** 1331 – 1337.

Cheesbrough, M. (2006). District laboratory Practices in Tropical countries. Cambridge University Press. P. 434.

Chikere et al (2008). Multidrug resistant bacteria isolated from different Drinking Water sources.

Cornelis, P. (2010). Iron uptake and metabolism in pseudomonads. *Journal of Applied and Biotechnology*. **86:** 1637- 1645.

DuBois, V., Arpin, C. and Melon, M.(2001). Nosocomial outbreak due to a multi-resistance strain of *Pseudomonas aeruginosa* P.12: efficacy of cefepime –amikacin therapy and analysis of β-lactam resistance. *Journal of clinical Microbiology***39:**2072 – 2078.

El-Astal, Z.(2004) Bacterial pathogens and their antimicrobial susceptibility in Gaza strip, *Palestine. Pak Journal of Medical Sciences* .**20**: 365 –370.

Gales, A.C., Jones,R.N., Turnidge, J., Rennie, R and Ramphal, R. (2001). Characterization of *Pseudomonas aeruginosa* isolates: occurrence rate, antimicrobial susceptibility pattern and molecular typing in the Global SENTRY antimicrobial surveillance program 1997 – 1999. *Clinical Infectious Disease* **32:** S146 – 155.s

Guardabassi, L. and Dalsgaard, A (2002). Occurrence and fate of antibiotic resistant bacteria in sewage. Paper presented to Danish Environmental Protection Agency. **722**: 1-59

Harbour, C., Anthony, M. and Rose, B.(2002).Genetic analysis of *Pseudomonas aeruginosa* isolates from the sputa of Australian adult cystic fibrosis patients. *Journal of Clinical Microbiology***40:** 2772 – 2778.

Hernandez, J., Ferrus, M.A., Hernandez, M and Owen, R.J. (1997). Arbitrary primed PCR fingerprint and serotyping of clinical *Pseudomonas aeroginosa*strains. *FEMS Immunology and Medical Microbiology* **17**: 37 – 47.

Iheanu, O., Femi, A.S., Timothy, A and Ayoteju, A. (2002) Plasmid encoded transferable antibiotics resistance in gram negative bacteria isolated from drinking water in Somalia city. *Pakistan Journal of Biological Sciences*. **5**:774-779.

Jawetz, E., Melnick, J.L., Adelberg, E.A., Brooks, G.F., Butel,S.J.andOrnston, L.N. (1999). Pseudomonas, Acinetobacter and uncommon gram –negative bacteria. In: review of Medical Microbiology, 19thedn. Appleton Langue Publishing Prentice Hall, Los Algeluss, California, USA. Pp. 224 – 229.

Joklik, K.W., Willet, P.H., Amos, B.D and Catherine,M.W.(1992). Pseudmonas. In: Zinser Microbiology, 20thedn. Durham, Nolwalk, USA. Pp. 576 – 583.

National Committee for clinical Laboratory Standards, NCCLS (2004). Performance standards for antimicrobial susceptibility testing. National Committee for Clinical Laboratory Standards, Wayne, PA.

Okeke, I. N., Fayinka, S. T. and Lamikanra, A. (2000). Antibiotics resistance in Escherichia coli from Nigerian students, 1986- 1998 . Emerging infectious Disease.6:393-396

Olowe, O.A., Olayemi, A.B., Eniola, K.I.T and Adeyeba, A.O.(2003). Aetiological agents of diarrhea in children under 5 years of age in Oshogbo. *African Journal of Clinical and Experimental Microbiology* **4**: 62 – 66.

Poole, K.(2004). Efflux –mediated multi-resistance in gram –negative bacteria. *Clinical Microbiol. Infections* 10:12-26.

Raina, P.S., Pollari, F.L., Teare, G.F., GOSS, M.J., Barry, D.A., Wilson, J.B.(1999). The relationship between *E. coli* indicator bacteria in well-water and gastrointestinal illness in rural families. *Canada journal of public Health*.**90**: 172 – 5.

Salyers, A.A.,Gupta,A and Wang,Y.(2004). Human intestinal bacteria as reservoirs for antibiotic resistance genes, *Trends Microbiology*, **12:** 412 – 416.

Shahid, M. and Malik, A.(2005).Resistance due to aminoglycoside modifying enzymes in *Pseudomonas aeruginosa* isolates from burns patients. *Indian journal of Medical research*,**122:324-329.**

Schluter, A., Szczepanowki, R., Puhler, A.(2007). Top EM. Genomics of hicp-I antibiotic resistance plasmids isolated from waste water treatment plants provide evidence for a widely accessible drug resistance gene pool. FEMS Microbiology Reviews .**31**: 449-477.

Tambekar et al (2008). Assessment of coliform contamination in drinking water. wedc.lboro.ac.uk.

Tobih, J.E., Taiwo, S.S., Olowe, O.A., Olaosun,O.A.andAdejumo, S.O. (2006). Microbiological profiles of discharging ears in Osogbo, Nigeria.

Todar, K.(2002).Todar's online textbook of bacteriology. Pp. 1- 8.

Tomoeda, (1968). Effective elimination of drug resistance and sex factor in *Escherichia coli* by sodium dodecyl sulfate. *Journal of Bacteriology*. **95**: 1078-1089.