

ROLE OF UNTREATED LIQUID HOSPITAL WASTE TO THE DEVELOPMENT OF ANTIBIOTIC RESISTANT BACTERIA

M. J. ISLAM¹, M. S. UDDIN², M.A.HAKIM³, K. K. DAS⁴ AND M. N. HASAN⁵

¹Lecturer, Department of Pharmacy, University of Development Alternative, Dhanmondi, Dhaka, ²Assistant professor, ³Professor, Department of Microbiology, University of Chittagong, ^{4&5}Lecturer, Department of Biotechnology and Genetic Engineering, University of Development Alternative, Dhanmondi, Dhaka, Bangladesh

Accepted for publication: May 29, 2008

ABSTRACT

Islam M. J., Uddin M. S., Hakim M.A., Das K. K. and Hasan M. N. 2008. *Role of Untreated Liquid Hospital Waste to the Development of Antibiotic Resistant Bacteria.* *j. innov.dev.strategy 2(2): 17-21*

The study was carried out in the Department of Microbiology, University of Chittagong, Bangladesh during September to December 2007. To investigate the contribution of Liquid hospital waste to the development of antibiotics resistance in our environmental microflora, a total of four types of samples were collected. Samples includes relationship between the use of antibiotic and the development of resistance, 40 strains of *Escherichia coli* were isolated from the liquid hospital waste of Chittagong Medical College Hospital. *E. coli* strains were analyzed by agar disc diffusion to determine their susceptibility patterns to 7 antimicrobial agents. Plasmid DNA was isolated from ten isolates taken randomly by agarose-gel electrophoresis. Results of antibiotic susceptibility test showed that all of the isolates are multi-drug resistant (≥ 4) and were resistant to Tetracycline (100%), Ciprofloxacin (100%), Penicillin (100%), Erythromycin (100%), Gentamycin (50%) and Chloramphenicol (90%) but all of them were sensitive to Imipenem. Turbidometric analysis of ciprofloxacin resistance pattern showed all of the isolates were highly resistant to ciprofloxacin even at concentration of 3000 μ g/ml. Agarose-gel electrophoresis of plasmid DNA from 10 isolates showed that all of them contain a high-molecular weight plasmid DNA. Our study highlight that the resistance development was directly related to use of antibiotics. the results suggest that the multi-drug resistant *E.coli* & plasmid containing multidrug resistant genes are present in the hospital waste may act as a possible source of transfer of these highly resistant pathogens and their genes to human that could be threat for the treatment of disease by commercially available antibiotics.

Keywords: Hospital waste, multidrug resistance, *Escherichia coli*, plasmid

INTRODUCTION

Untreated liquid hospital waste containing unmetabolized antibiotics in low concentration contributes largely to the development of antibiotic resistance in our natural microflora/environmental microflora. Peoples of developing countries often bear antibiotic-resistant fecal commensal organisms (Calva, 1996; Lamikanra, 1989). Visitors to developing countries passively acquire antibiotic-resistant gut *Escherichia coli*, even if they are not taking prophylactic antibiotics, which suggests that they encounter a reservoir of antibiotic-resistant strains during travel (Murray, 1990). Apparently healthy people in developing countries carry potentially pathogenic, antibiotic-resistant organisms asymptotically (Woolfson, 1997). Several factors, such as improper sewage disposal and urban migration with crowding, encourage the exchange of antibiotic-resistant organisms between people and the exchange of resistance genes among bacteria, thereby increasing the prevalence of resistant strains. In Nigeria, resistant *E. coli* isolates from persons in an urban metropolis (Lagos) were significantly more likely to be resistant to ampicillin and streptomycin ($p < 0.05$), and possibly more resistant to sulphathiazole and tetracycline ($p < 0.10$), than isolates from residents of nearby smaller towns and villages (Lamikanra, 1997). Moreover, strains isolated from Lagos were more likely to show resistance to 4 to 6 of 7 antibiotics tested; whereas strains from rural areas were in most cases resistant to only 0 to 3 antibiotics (Lamikanra, 1997). Improper disposal of hospital waste accentuates such spread. Untreated hospital waste in Uganda was often dumped into public sewers or thrown into rubbish heaps ravaged by scavengers (Okello, 1997). Information from routine susceptibility testing of bacterial isolates and surveillance of antibiotic resistance, which provides information on resistance trends, including emerging antibiotic resistance, is essential for clinical practice and for rational policies against antibiotic resistance. Bacterial infections are often treated after they become life-threatening, which encourages empirical selection of broad-spectrum antibiotics (Okello, 1997; Shann, 1995). The antibiotic susceptibility pattern of bacterial isolates in much of the developing world is unknown, and little guides empirical prescribing. Susceptibility testing cannot be done readily because equipment, personnel, and consumables are scarce and expensive (Yang, 1993; Ringertz, 1993).

The majority of antibiotics used is only partially metabolized after administration, and are released via patient excreta into the municipal sewage system. Antibiotics used in hospitals and private households and released into effluent and municipal sewage indicates a selection pressure on bacteria. (Kümmerer, 2003). Low concentrations of antibiotics in the environment may select for resistant bacteria (Jeannette, 2007). These resistant bacteria from environments may be transmitted to humans, in whom they cause disease that cannot be treated by conventional

antibiotics (Khachatourians, 1998). We aimed to investigate the use of antibiotics in the hospital sectors, their release in hospital waste and the development of antibiotics resistant bacteria. With the aim of investigation of possible played by untreated liquid hospital waste to the resistance development, we collect the liquid hospital waste from the Chittagong medical college hospital (CMCH), Chittagong, Bangladesh.

MATERIALS AND METHODS

Collection of samples: We collected four types of samples from the Chittagong Medical College Hospital (CMCH), Chittagong, Bangladesh. Samples are hospital waste, hospital waste discharge from the sewage drain, liquid waste from the municipal sewerage drain and liquid waste from the safety tank of Shaheed Abdur Rab hall (University of Chittagong) which has no connection with the CMCH. Samples were transported to the laboratory of Department of Microbiology, University of Chittagong and performed all of the analysis

Collection of treatment history: To envisage the correlation of waste disposal and resistance development, the treatment histories of antibiotics used in the CMCH was noted down during sample collection time. The treatment history is important for the prediction the correlation between resistance development and waste disposal. We have collected the samples during the time period September to December 2007 and collected the treatment records of antibiotics during this time.

Bacteriological enumeration: All the bacteriological enumerations were carried out by plate count agar (Foster *et al.* 1958, Staurt scientific, U.K). In this case total number of bacteria and total number of resistant bacteria were counted. In case of total resistant count ciprofloxacin 30 μ g/ml was mixed with the PCA agar, shacked well to mix homogenously and kept it in refrigerator at 4^oC until used.

Concentration dependent analysis of resistance pattern: Concentration dependent analysis of ciprofloxacin resistance pattern also carried out by counting the total number of bacteria in different concentration of ciprofloxacin and drawing a graph using the chart of total number of bacteria found in different concentrations of ciprofloxacin.

Isolation and Identification ciprofloxacin resistant Escherichia coli from hospital wastes: Single colonies were picked up by sterile tooth picks randomly from the PCA plates then patched on an Eosine Methylene Blue (EMB) agar plate containing 30 μ g/ml ciprofloxacin. A single patch represented a single colony. Suspected colonies showing green metallic sheen on EMB agar are then identified by conventional biochemical test.

Antimicrobial susceptibility test: The standardized disc diffusion method also known as Kirby Bauer method (Bauer, 1999) was used for the *in vitro* determination of the sensitivity to the antimicrobial agents. Antibiotics are chosen so that some of them are used during sample collection (e.g. tetracycline 30 μ g/ml and ciprofloxacin 5 μ g/ml), some of them are continuously used in hospital in addition to the running antibiotics, some of them were moderately or rarely used (e.g. penicillin 10 μ g/ml, Erythromycin 15 μ g/ml) in hospital, some of them were not using (e.g. Imipenem and Gentamycin). All discs were obtained from Oxoid (Unipath Ltd, Basings take, UK). Interpretation of the followed criteria recommended in the National Committee for clinical Laboratory Standards.

Isolation of Plasmid DNA: Plasmid extraction was carried out by alkaline lysis technique (Bonfiglio G. *et al.* 1995). The extracted plasmid was then isolated using a horizontal 1% agarose gel electrophoresis technique.. Plasmid DNA was separated by horizontal electrophoresis in 1% agarose slab gels in a Tris-Acetate EDTA (TAE) buffer at room temperature at 80 volt (50 mA) for 3 hrs.

RESULTS AND DISCUSSION

The result bacteriological enumeration showed that the total number of bacteria found in different samples is varies from sample to samples (Figure 1) but the total number of resistant bacteria was decreased with the increase of distance of sample collection site from waste disposal site (Figure 2).

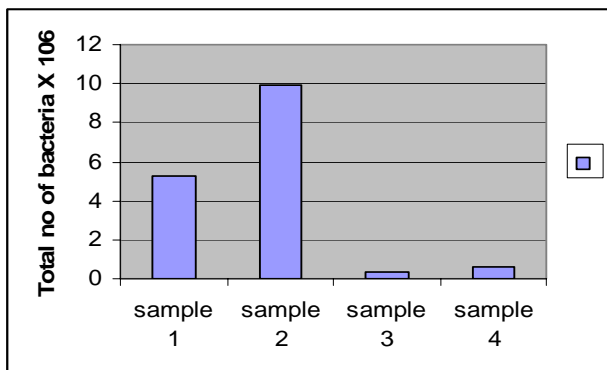


Figure 1. Results of total resistant bacterial count in all the samples collected from CMCH

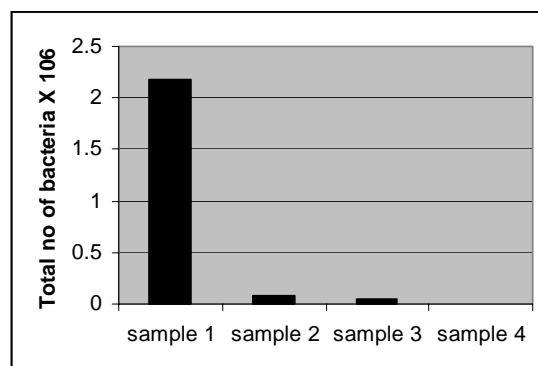
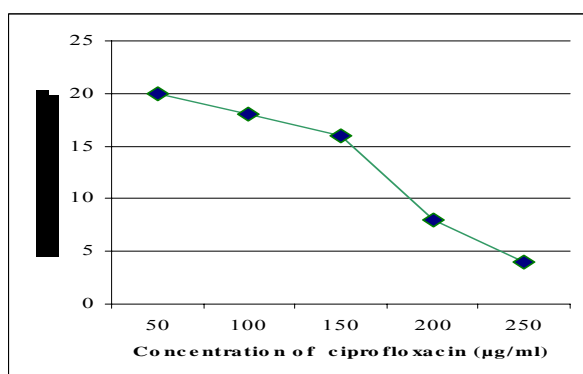
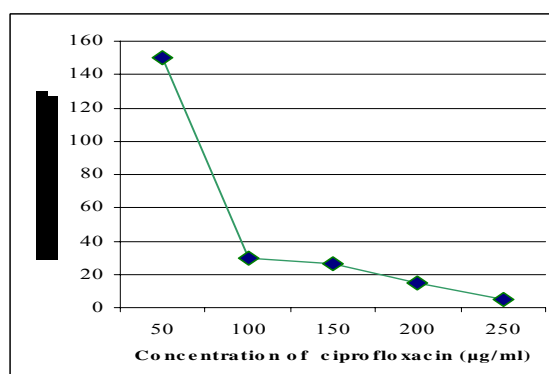


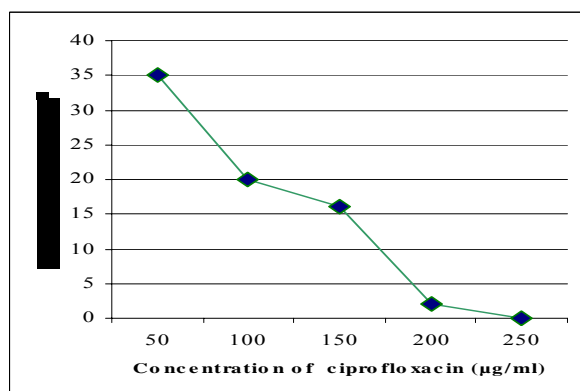
Figure 2. Results of total resistant bacterial count in all the samples collected from CMCH



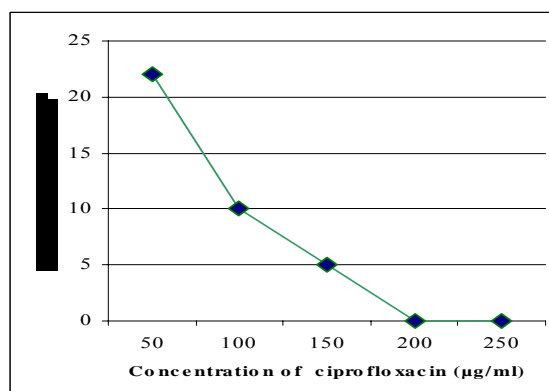
Sample 1



Sample 2



Sample 3



Sample 4

Figure 3. The number of ciprofloxacin resistant bacterial count depends on the sampling sites. Samples collected from the smaller distance of antibiotic use which one getting more time to expose to unmetabolized antibiotics showed the greater resistance pattern.

Analysis of treatment history collected from the CMCH showed that they used mainly Ciprofloxacin (50%), Penicillin (19.2%), Tetracycline (20%) for the treatment purposes. Results of antibiotic susceptibility test showed that all of the isolates are multi-drug resistant (≥ 4) and were resistant to Tetracycline (100%), Ciprofloxacin (100%), Penicillin (100%), Erythromycin (100%), Gentamycin (50%) and Chloramphenicol (90%) but all of them were sensitive to Imipenem. Turbidometric analysis of ciprofloxacin resistance pattern showed all of the isolates are highly resistant to ciprofloxacin even at concentration of 3000µg/ml. Agarose-gel electrophoresis of plasmid DNA from 10 isolates showed that 6 of them contain a high-molecular weight plasmid DNA.

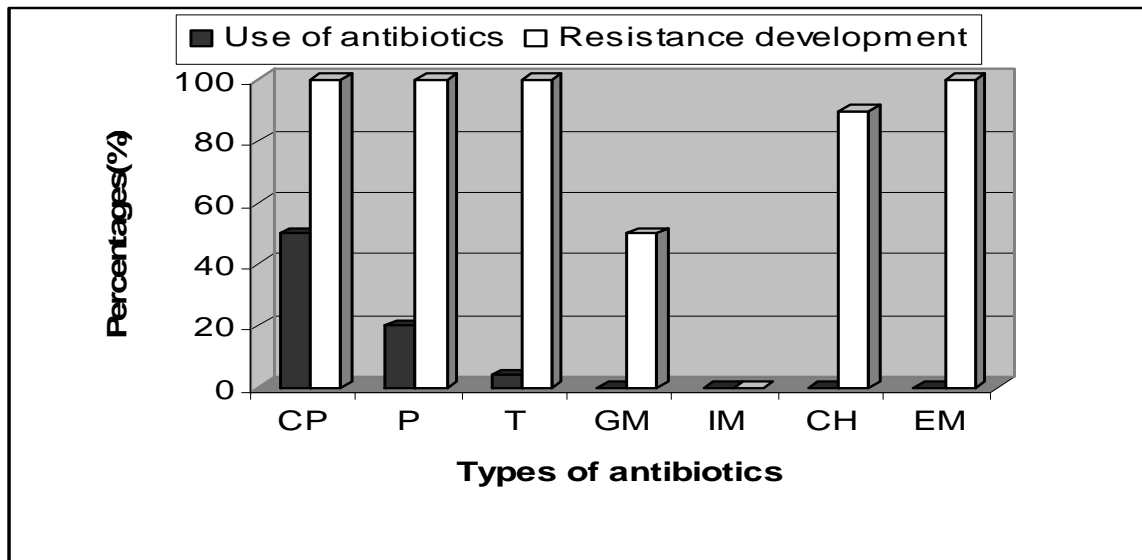


Figure 4. Correlation of antibiotics use and resistance development. Ciprofloxacin (CP), Penicillin (P), Tetracycline (T), Gentamycin(GM), Imipenem (IM), Chloramphenicol (CH), Erythromycin (EM).

We investigated the resistance pattern of 10 isolates of *Escherichia* seven antimicrobials of which 3 were commonly used by the practitioner during sample collecting periods clearly demonstrated the high resistance rate to all of these three tested antibiotics. This result suggests that the extent of resistance to an antibiotic is associated with the extent of antibiotic use. *E. coli* isolated from the hospital waste was highly resistant to Ciprofloxacin even at 3000µg/ml which could be the result of unmetabolized antibiotics released from the hospital in low concentration (Thomas *et al.* 2007; Guardabassi, 1998; Jeannette, 2007) and repeated use of antibiotics by the practitioners against which resistance already developed. High resistance rate was also noted against chloramphenicol and penicillin could be associated with the misuse of them. All of the isolates are sensitive to Imipenem which suggests that a need for regular monitoring of antimicrobial susceptibility rates in different human population that would be helpful to identify relevant factors that contribute to the spread of resistance pathogens and would support the prudent use of antibiotics (Erb *et al.*, 2007). All of the isolates found multiple antibiotic resistance (>=4).

We analyzed randomly 10 isolates for the presence of Plasmid DNA and all of them found to contain high molecular weight plasmid with high resistant rate. Our study untreated liquid hospital waste contributes to the development of antibiotic resistance in our natural microflora and multiple antibiotics resistant *E.coli* isolates & plasmid containing multidrug resistant genes are present in the hospital waste may act as a possible source of transfer of these highly resistant pathogens and their genes to human that could be threat for the treatment of disease by commercially available antibiotics.

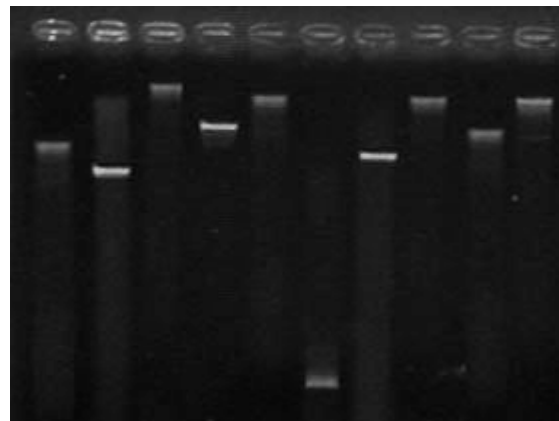


Figure 5. Plasmid DNA bands found in the ciprofloxacin-resistant isolates

REFERENCES

- ARI Program for the control of acute respiratory infections, Geneva. 1994. World Health Organization.
- Bauer A.W., Kirby W.M.M., Sherris J.C. and Turk M. 1999. Antibiotic susceptibility testing by a standard single disk method. *American Journal of Clinical Pathology*. 45: 493-496
- Bonfiglio G. and Livermore D.M. 1994. β -lactamase types amongst *Staphylococcus aureus* isolates in relation to susceptibility to β -lactam inhibitor combinations. *Antimicrob Chemother*. 33:465-481
- Calva J.J., Sifuentes-Osornio J. and Ceron C. 1996. Antimicrobial resistance in fecal flora: longitudinal community-based surveillance of children from urban Mexico. *Antimicrob Agents Chemother*. 40:1699-1702
- Erb A., Stürmer T., Marre R. and Brenner H. 2007. Prevalence of antibiotic resistance in *Escherichia coli*: overview of geographical, temporal, and methodological variations. *European Journal of Clinical Microbiology & Infectious Diseases*. 36 (2):83-90
- Foster, E. M., Nelson, F. E., Speck, M. L., Doetsch, R. N. and Olson, J. C. 1958. *Dairy Microbiology*
- Guardabassi L., Petersen A., Olsen J.E. and Dalsgaard A. 1998. Antibiotic resistance in *Acinetobacter* spp. isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant. *Appl Environ Microbiol*. 64(9):3499-3502
- Jeannette Muñoz-Aguayo, Kevin S. Lang, Timothy M. LaPara, Gerardo González and Randall S. Singer. 2007. Evaluating the Effects of Chlorotetracycline on the Proliferation of Antibiotic-Resistant Bacteria in a Simulated River Water Ecosystem. *Applied and Environmental Microbiology*. 73 (7): 5421-5425
- Khachatourians. G.G. 1998. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *CMAJ*. 159(9):1129-1136
- Kümmerer K, Henninger A. 2003. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clin Microbiol Infect*. 9(12):1203-1214
- Lamikanra A., Fayinka S.T. and Olusanya O.O. 1989. Transfer of low level trimethoprim resistance in faecal isolates obtained from apparently healthy Nigerian students. *FEMS Microbiol Lett*. 50:275-278
- Lamikanra A. and Okeke I.N. 1997. A study of the effect of the urban/rural divide on the incidence of antibiotic resistance in *E. coli*. *Biomedical Letters*. 55:91-97
- Murray B.E, Mathewson J.J., DuPont H.L., Ericsson C.D. and Reves R.R. 1990. Emergence of resistant fecal *Escherichia coli* in travelers not taking prophylactic antimicrobial agents. *Antimicrob Agents Chemother*. 34:515-518
- National committees for Clinical Laboratory Standard (NCCLS). 1993. Performance Standards for Antimicrobial Disk Susceptibility Test Approved Standard M2-A5. 5th edn. NCCLS. Villanova, Pa.
- Okello D., Konde-Lule J., Lubanga R. and Arube-Wani J. 1997. Waste disposal in private medical clinics in Kampala, Uganda. *J Clin Epidemiol* 50. 1:45S
- Ringertz S., Muhe L., Krantz I., Hathaway A., Shamebo D. and Freij L. 1993. Prevalence of potential respiratory disease bacteria in children in Ethiopia. Antimicrobial susceptibility of the pathogens and use of antibiotics among the children. *Acta Paediatr*. 82:843-848
- Shann F. 1995. The management of pneumonia in children in developing countries. *Clin Infect Dis*. 21 Suppl 3:S218-225
- Thomas S., Holger V., Slike K., Wolfgang K., Katja S., Bernd J. and Ursula O. 2007. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms, *FEMS Microbiology Ecology*. 43 (3): 325-335
- Woolfson A., Huebner R., Wasas A., Chola S., Godfrey-Faussett P. and Klugman K. 1997. Nasopharyngeal carriage of community-acquired, antibiotic-resistant *Streptococcus pneumoniae* in a Zambian paediatric population. *Bull World Health Organ*. 75:453-462
- Yang YH, Fu SG, Peng H, Shen AD, Yue SJ, Go YF, et al. 1993. Abuse of antibiotics in China and its potential interference in determining the etiology of pediatric bacterial diseases. *Pediatr Infect Dis J*. 12:986-8.