ORIGINAL RESEARCH PAPER

Microbiology

Volume - 7 | Issue - 3 | March - 2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

Isolation and Characterization of Multi Drug Resistant Bacterial Pathogens in Treated Waste Water from Hospital Sewage Treatment Plant

KEYWORDS

Sewage Treatment Plant, Drug Resistance, *E. coli, Enterococci*

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ABSTRACT **Introduction**: Multiple Drug Resistant bacteria are often found in treated waste water from the hospital which leads to transfer of resistance to the bacteria in natural environment. **Aims:** The present study was aimed to know the spectrum of bacteria in Sewage Treatment Plants and resistance pattern of *E. coli* and *Enterococci* as indicator organisms. **Methodology:** A total of 120 water samples were processed by standard microbiological procedures. **Results:** The spectrum of common bacteria isolated from the STPs were *E. coli* (18.9%), *Klebsiella* (16.3%), *Pseudomonas*(15.7%), *Citrobacter* (14.8%), *Enterococci* (12.3%) *Micrococci* (11.6%)and *Staphylococci* (11.6%). All the isolates (100%) were resistant to Ampicillin. More than 75% isolates of *E.coli* and more than 70% *Enterococci* were resistant to more than four antibiotics. **Conclusion:** The present study highlights the importance of strict governance on functioning of STP and random microbiological testing to protect the environment.

Introduction

Antibiotics are used to treat and prevent diseases of humans as well as animals. However the modern medicine is facing a major problem due to development of increasing resistance to commonly used antibiotics. This leads to increase in morbidity and mortality. The increasing number of multiple antibiotic resistant pathogens has become a serious problem to the mankind.

Multiple Drug Resistant (MDR) bacterial strains are often found in untreated waste water from the hospital. Various studies have shown that hospital waste water is a highly selective environment and it leads to transfer of resistant bacteria in natural environment.

The bacterial drug resistance is of two types

1) Mutational drug resistance2) Transferrable drug resistance

Mutational drug resistance is mediated by mutations in the bacterial genome. It is highly dependent on natural selection and most of the time spontaneous. Transferrable drug resistance on the other hand is mediated by extrachromosomal genetic elements like plasmids. This type of resistance is transferred to other bacteria in the environment where there is more contact of bacteria. This type of resistance is the antibiotic resistance established and propagated in human and animal digestive systems.

The growth of resistant microbes in the environment like sewage treatment plant is due to high concentration of bacteria and sub lethal doses of antibiotics present in it. Hence these sewage treatment plants provide favorable condition for establishment and propagation of drug resistant bacteria. Many authors have reported that initially there is high concentration of antibiotics present in the sewage water which includes β lactam, Fluoroquinolones, Sulfonamides, Trimethoprims and Macrolides.[1,2] These antibiotics can be present as active or partially active forms in the sewage water in the STP with reduced concentration of antibiotics to some extent. High concentration of antibiotics and drug resistant bacteria have been reported in the STPs.[3,4] The reason could be the simultaneous excretion of both the components ie bacteria and antibiotics into the sewage and ultimately into the environment.

Sewage treatment process affects the relative rate of antibiotic resistance. Some scientists have observed that the sewage treatment leads to decrease in antibiotic resistance.[5] However there are also reports showing minimal or little difference in the level of resistance after sewage treatment.

Biological sewage treatment process reduces viable concentration of fecal bacteria. Hospital waste water contains high level of antibiotic resistant bacteria compared to other sewage sources. It has been reported that upto 50% coliforms are isolated from hospital waste water contain resistance plasmids in contrast to 2—5% coliforms isolated from other sewage plants.[6]

Various studies have shown that hospital waste water is a highly selective environment and it leads to transfer of resistant bacteria in natural environment. It has been shown that the presence of bacteriophages in the ecosystems act as vectors for horizontal transfer of antibiotic resistant genes and transfer this resistance to the environmental organisms inhabiting terrestrial and aquatic environment.[7]

E. coli are currently used by Environmental protection Agency (EPA-USA) as an indicator organism for fecal contamination in the water source. *E. coli* had been recognized as a contributor to dissemination of antibiotic resistance genes in the natural environment as well.[8,9] The major source of fecal contamination in the water source include humans[10], agricultural animals [11]and pets[12].

Multidrug resistant (MDR) *E.coli* is resistant to at least one antimicrobial agent among three or more antibiotic categories. Antibiotic resistance surveillance data show that *E. coli* has higher resistance for older generation human and veterinary antibiotics including Ampicillin, Streptomycin and Tetracycline and increasing resistance to even newer antibiotics such as Fluoroquinolones and Cephalosporins.[13]Antibiotic resistant bacteria through the environment again get colonized in the people especially in the hospitals causing various infections like urinary tract infections or sepsis.

Hence the present study was carried out with following aim and objectives

1. To know the spectrum of various bacteria in STPs
2. To isolate multi drug resistant *E. coli* and *Enterococci*

Material and Methods

Water sample was collected in 50 ml sterile flask from four corners storage tank of sewage treatment plant after the treatment of sewage water over a period of one month. They were processed within two hours of collection.

The samples were inoculated on MacConkey's agar, XLD medium and Blood agar. Representative bacteria *E. coli* and Enterococci were selected for the study. The bacterial colonies obtained after 18-24 hrs of incubation were inoculated in sterile peptone water and the density of overnight suspension was adjusted to 0.5 McFarland Standard. This suspension was used for identification and antibiotic sensitivity testing. Antibiotic susceptibility testing was carried out by

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Kirby Baur's Disc diffusion method.[14]

Results

A total of 120 water samples were collected and processed for the presence of drug resistant bacterial pathogens. It was observed that all the specimens grew minimum one pathogen. A total of 112 *E. coli* and 73 Enterococci were isolated. The spectrum of common bacteria isolated from the STP was as follows *E. coli* (18.9%), *Klebsiella* (16.3%), *Pseudomonas*(15.7%), *Citrobacter* (14.8%), *Enterococci* (12.3%) *Micrococci* (11.6%) and *Staphylococci* (11.6%). Antibiotic susceptibility pattern of *E. coli* and *Enterococci* was observed and analyzed.

Out of 112 *E.coli* isolates, the number of strains showing resistance to various antibiotics has been shown in the following Table 1.

Table 1: Percentage of *E. coli* showing resistance to Various Antibiotics

Antibiotics	AMP	AMC	CXM	CTX	TOB	OF	CEP	CIP	CPZ	TR
Percentage Resistance	100	85.7	77.7	75.9	57.1	56.3	50.9	42.9	28.6	42
Percentage Sensitivity	0	14.3	22.3	24.1	42.9	43.7	49.1	57.1	71.4	58

(AMP- Ampicillin, AMC- Amoxycillin+ Clavulanic Acid, CXM- Cefuroxime, CTX- Cefotaxime, TOB- Tobramicin, OF- Ofloxacin, CEP- Cephalothin, CIP- Ciprofloxacin, CPZ- Cefaparazone, TR- Trimethoprim)

It was observed that all the isolates (100%) were resistant to Ampicillin. More than 75% isolates of *E.coli* recovered from STP showed resistance to AMC, CXM, CTX. More than 50% isolates were resistant to TOB, OF, CEP and more than 25% isolates were resistant to CIP,CPZ and TR. More than 50% *E. coli* were resistant to more than 1 antibiotic.

Table 2 shows percentage of Enterococcal isolates showing resistant to various antibiotics.

Table 2: Percentage of *Enterococci* showing resistance to Various Antibiotics

Antibiotic	AMP	AMC	CX	P	AZM	COT	CXM	CIP	CZ	CLR	TE	GEN	CTX	LE	VA	LZ
Percentage Resistance	100	72.6	71.2	74	71.2	72.7	71.2	61.6	63	60.3	52	50.7	53.4	50.7	22	11
Percentage Sensitivity	0	27.4	28.8	26	28.8	27.3	28.8	38.4	37	39.7	48	49.3	46.6	49.3	78	89

(AMP- Ampicillin, AMC- Amoxycillin+ Clavulanic Acid, CX- Cloxacillin, P- Penicillin, AZM- Azithromycin, COT- Co-trimoxazole, CXM- Cefuroxime, CIP- Ciprofloxacin, CZ- Cefazolin, CLR- Claritromycin, TE- Tetracycline, GEN- Gentamicin, CTX- Cefotaxime, LE- Levofloxacin, VA- Vancomycin, LZ- Linezolid)

Amongst *Enterococci*,more than 70% isolates were resistant to AMC, CX, P, AZM, COT and CXM. More than 60% strains were resistant to CIP, CZ and CLR.More than 50% strains were resistant to TE, GEN, CTX, LE. More than 40% strains showed resistance to RIF, MRP. Only 22% *Enterococci* were resistant to Vancomycin and 11% *Enterococci* were resistant to Linezolid.

Discussion: It has been reported that various classes of antibiotics including β Lactams, Sulfonamides, Trimethoprim, Macrolides, Fluoroquinolones and Tetracyclines are detected in sewage.[1,2]

Bacteria and antibiotics both are excreted by humans and discharged into the environment via sewage outfall. Many authors have reported correlation between presence of high concentration of antibiotic in sewage and increased level of antibiotic resistance observed in sewage bacteria.[3,4] In this study, the most frequently isolated bacteria were *E. coli, Klebsiella, Pseudomonas, Citrobacter* spp. and amongst the Gram positive *Enterococci* and *Staphylococci*. According to study by Felek *et al.*[15] The most frequently isolated bacteria is *Klebsiella* (29.2%) followed by *Pseudomonas*(21.5%), *E.coli* (12.3%), *Citrobacter* (7.7%), *S.aureus*(8.2%), *Shigella*(3.1%) and *Enterococcus*(7.7%). In another study from Brazil Chagas *et al.* reported that the most common multidrug resistant GNBs from hospital waste water were *Klebsiella pneumoniae, Enterobacter cloacae* and *E. coli*.[16] Ekhaise and Omavwoya showed that *Klebsiella, Pseudomonas* and *Serratia* were most frequently associated GNBs in hospital waste.[17]

In this study, *E. coli* and *Enterococci* were selected as indicator organisms from the Gram negative and Gram positive group of bacteria and their antibiotic sensitivity pattern was studied. A very high level of resistance was noticed in both the indicator organisms. It was observed that all the isolates (100%) were resistant to Ampicillin. According to the study by Felek *et al.* the overall resistance of GNBs to Ampicillin was found to be 97% followed by Cephalothin 49%, Co-timoxazole 38%, Tetracycline 37%, Nalidixic acid 36%, Cefotaxime 33% and Ciprofloxacin 12%.[15]

In our study 42.9 % *E.coli* were found to be resistant to CIP. A study from Bangla Desh reported 100% resistane to CIP.[18] Felek *et al.* have reported that 12% of *E.coli* resistant to CIP.[15]In this study, it was observed that 86.6% *E. coli* were resistant to more than 2 antibiotics and 56.2% *E.coli* isolates were resistant to more than 5 antibiotics. Katouli *et al.* have reported that 92% of E. coli strains were resistant to more than two antibiotics (MDR Strains) and about 7% strains found to be resistant to 12 antibiotics.[19] They have reported that the highest resistance observed against Aztreonam, Gentamicin, Augmentin, Ceftazidime, cefepime ranging from 89% to 79% with lowest resistance found to Ciprofloxacin, Norfloxacin, Nalidixic Acid, Nitrofurantoin and Chloramphenicol ranging between0-12%. Whereas Felek *et al.* in their study reported that 46.2 % of Gram negative bacteria were resistant to more than 5 antibiotics.[15]

In this study, more than 70% isolates of *Enterococci* were found resistant to more than 7 antibiotics and more than 50% isolates were resistant to 9 antibiotics. Our study shows that 63% of *Enterococci* were resistant to Ciprofloxacin whereas Katouli et al. reported 13% *Enterococci* resistant to Ciprofloxacin.[19]

Some studies have also shown that the relative rate of resistance is different in raw and treated sewage.[20,21] While some authors have reported elevation of drug resistance, others have also reported decline in drug resistance.[5,20,21] These contradictory reports indicate that the resistance is dependent on variety of factors like the specific bacteria and antibiotic, type and stage of treatment and environmental factors. However in this study the presence of antibiotic in the sewage was not checked, hence the correlation was not obtained.

Conclusion

In this study we carried out identification of multidrug resistant bacterial strains in the treated sewage. Hospitals act as concentrated source of drug resistant bacteria which gets released in the sewage. Various procedures in the sewage treatment plant like chlorination eliminate these pathogens. However as shown in this study certain strains of bacteria are able to survive the treatment process and may get released into the municipal waste water or surface water. Hence this study highlights the importance of strict governance on functioning of sewage treatment plant and random microbiological testing of the same in order to protect the environment.

References:

1. Cha JM, Yang S., Carlson AH, Trace determination of β Lactam antibiotics in surface water and urban waste water using liquid chromatography combined with electroscopy tandem mass spectrometry. Journal of Chromatography,2016;1115(1-2):

Volume - 7 | Issue - 3 | March - 2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

46-57.
2. Karthikeyan KG, Meyr MT. Occurrence f antibiotics in waste water treatment facilities in Wisconsin, USA. Science of the Total Environment, 2006; 361 (1-3): 196-207.
3. Iwane T, Urase T, Yamamoto K. Possible impact of treated waste water discharge on incidence of antibiotic resistant bacteria in river water. Water Science and Technology.2001;43 (2): 91-99.
4. Kim S, Aga DS, Potential ecological and human health impacts of antibiotics and antibiotic resistant bacteria from waste water treatment plant. Journal of Toxicology and Environmental health, Part B,10 (8): 559-573.
5. Garcia S, Wade B, Baur C, Craig C, Nakaoka K, Lorowitz W. The effect of waste water treatment on antibiotic resistance in E. coli and Enterococcus spp. Water Environment Research. 2007;79(12): 2387-2395.
6. Caplin JL, Hanlon GW, Taylor HD. Presence of Vancomycin and Ampicillin Resistant Enterococcus faecium of epidemic clonal complex-17in wastewaters from the south coast of England. Environmental Microbiology. 2008; 10(4): 885-892.
7. Colomer-Lluch M, Imamovic L, Jofre J, Muniesa M: Bacteriophages carrying antibiotic resistance genes in fecal waste from cattle, pigs, and poultry. Antimicrob Agents Chemother. 2011; 55: 4908-4911.
8. Henriques IS, Fonseca F, Alves A, Saavedra M J, Correia A. Occurrence and diversity of integrons and β-lactamase genes among ampicillin-resistant isolates from estuarine waters. Res. Microbiol. 2006 ; 157 : 938 – 947.
9. Zhao J, Zhu Y, Li Y, Mu X, You L, Xu C, et al. Coexistence of SFO-1 and NDM-1 - lactamase genes and fosfomycin resistance gene fosA3 in an Escherichia coli clinical isolate. FEMS Microbiol. 2015; Lett. 362: 1–7.
10. Bernhard AE, and Field, KG. A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides–Prevotella genes encoding 16S rRNA. Appl. Environ. Microbiol.2000; 66 : 4571– 4574.
11. Shanks OC, Atikovic E, Blackwood AD, Lu J, Noble R T, Santo Domingo J, et al. Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. Appl. Environ. Microbiol. 2008 ;74: 745–752.
12. Ervin JS, Van De Werfhorst LC, Murray J L, Holden PA. Microbial source tracking in a coastal California watershed reveals canines as controllable sources of fecal contamination. Environ. Sci. Technol. 2014; 48: 9043–9052.
13. Tadesse DA, Zhao S, Tong E, Ayers S, Singh A, Bartholomew M J, et al. Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950–2002. Emerg. Infect. Dis. , 2012; 18:741–749.
14. AW, Kirby Bauer WMM, Sherries JC,Jurek M, Antibiotic susceptibility testingby a standardized singlemethod. American Journal of ClinicalPathology.1966;45: 493-496.
15. Feleke M, Mengistu E,Yeshambel B,Walelegn W. Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia BMC Research Notes2014, 2013; 7:215.
16. Chagas TP, Seki LM, Cury JC, Oliveira JA, Dávila AM, Silva DM, Asensi MD: Multiresistance, beta-lactamase-encoding genes and bacterial diversity in hospital wastewater in Rio de Janeiro Brazil. J Appl MIcrobiol. 2011; 111 (3):572-581.
17. Ekhaise FO, Omavwoya BP: Influence of hospital wastewater discharged from university of Benin teaching hospital (UBTH), Benin City on its receiving environment. American-Eurasian J Agric Environ Sci. 2008; 4 (4): 484-488.
18. Islam MJ, Uddin MS, Hakim MA, Das KK, Hasan MN: Role of untreated liquid hospital waste to the development of antibiotic resistant bacteria. J Innov Dev Strategy. 2008; 2 (2): 17-21.
19. Katouli M, Thompson, J.M. , Gündoğdu, A. Stratton, H.M. Antibiotic Resistant Bacteria in Hospital Wastewaters and Sewage Treatment PlantsScience Forum and Stakeholder Engagement: Building Linkages, Collaboration and Science Quality Page 225-229.
20. Espigares E, Bueno A, Espigares M, Galvez R. Isolation of Salmonella serotypes in wastewater and effluent: Effect of treatment and potential risk. International Journal of Hygiene and Environmental Health. 2006; 209(1):103-107.
21. Martins da Costa P, Vaz-Pires P, Bernardo F. Antimicrobial resistance in Enterococcus spp. isolated in inflow, effluent and sludge from municipal sewage water treatment plants. Water Research,2006; 40(8):1735-1740.