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Review article

ASSESSMENT OF DISINFECTANT AND ANTIBIOTIC RESISTANT BACTERIA IN HOSPITAL WASTEWATER

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Abstract

Large quantities of antibiotics and disinfectants are used in hospitals for patient care and disinfection process, respectively. These products are partially metabolized and residual quantities reach hospital wastewater, exposing bacteria to wide range of biocides that could act as selective pressure for development of resistance. The aim of the study review paper is to assess disinfectant and antibiotic resistant bacteria. Hospital effluents tested contain antibiotic resistant bacteria which are released to receiving water bodies resulting in huge public health threat.

Key words: antibiotics, biocides, disinfectants, heterotrophic count, hospital influent, indicator, minimum inhibitory concentration, most probable number

Introduction

Wastewater is referred to any water, whose quality has been adversely being abused by anthropogenic influence. This includes liquid waste discharged from domestic home, agricultural commercial sectors, pharmaceutical and hospital. Hospitals are an essential asset of any society, and waste production is inevitable outcome of service delivery. In hospitals water consumed by various parts such as hospitalization, surgery rooms, laboratories, administrative units, laundry, health services, kitchen and in the process its physical, chemical and biological quality decreased and converted to wastewater (Mahvi et al, 2009). Health care waste consists of solid, liquid and gaseous waste contaminated with organic and inorganic substance including pathogenic microorganisms, radiological chemicals, partially metabolized antibiotics which are usually generated from laboratory analysis of tissues and body fluids as well as excreted from patients (Nuñez & Moretton, 2007).

A variety of substances such as pharmaceuticals, radionuclides, antiseptics, disinfectants and solvents are used in hospitals for treatment, medical diagnostics, disinfection and research. After application many non-metabolized drugs excreted from patients and residual chemicals enter into wastewater which finally interacts with microflora of Hospital sewage. These microfloras are composed by saprophytic bacteria from the atmosphere, soil, medical devices and water employed in the hospital practice; the pathogens are mainly released with the patient excreta. These bacteria that survive in Hospital wastewaters may be exposed to a wide range of biocides that could act as a selective pressure for the development of resistance. Due to heavy antibiotic use, hospital wastewater contains larger numbers of resistant organisms than domestic wastewater (Abdelraouf et al, 2006). When the antimicrobial agents attack disease-causing bacteria, they also affect non-pathogenic bacteria in their course, thus they exterminate these bacteria and make room for more resistant bacterial growth (Nuñez & Moretton, 2007). It is clear that microorganisms can adapt to a variety of environmental, physical and chemical conditions, and it is therefore not surprising that resistance to extensively used antibiotic, antiseptics and disinfectants has been reported. Many of these reports of resistance have often paralleled issues including inadequate cleaning, incorrect product use, or ineffective infection control practices, which cannot be underestimated.

Resistance can be either a natural property of an organism (intrinsic) or acquired by mutation or acquisition of plasmids or transposons. The nature of biofilm structure and the physiological attributes of biofilm organisms confer an inherent resistance to antimicrobial agents, whether these antimicrobial agents are antibiotics, disinfectants, or germicides (Rodney and Costerton,
2002). Intrinsic resistance is mostly demonstrated by gram-negative bacteria, bacterial spores and mycobacterium. Acquired, plasmid-mediated resistance commonly exists in both gram negative and positive bacteria and most widely associated with many antimicrobial agents which are conferred by R-factor (Olowe et al, 2004). In recent years, rotation of disinfectants and antibiotics in hospitals and elsewhere, e.g. in the pharmaceutical, agricultural and food industries, has been advocated to prevent the development of bacterial resistance (Russell, 2002). It has been speculated that low-level resistance may aid in the survival of microorganisms at residual levels of antibiotics, antiseptics and disinfectants; any possible clinical significance of this remains to be tested.

With growing concerns about the development of biocide resistance and cross-resistance with antibiotics, it is clear that clinical isolates should be under continual surveillance and possible mechanisms should be investigated (Gerald and Russell, 1999). The public health impact of release of resistant bacteria to receiving environment can be explained by many ways. First, if the resistant bacteria are carrying transmissible gene, they transfer resistant genes through conjugation or transduction so that infection caused by these bacteria are usually difficult to treat and also decrease antibiotic pool for treatment of bacterial infection. Second, this organism may act as vector or reservoir of resistant genes. Third, there will be increased nosocomial infection. Fourth, if infection occurs, it will increase cost of treatment and hospitalization (Nuñez & Moretton, 2007).

In developed countries domestic wastewater and Hospital effluents are discharged, usually, in the urban sewer system where they mix with other effluents and finally reach the sewage treatment plant. The last step of this process is the release of purified wastewaters to a river, a lake, or seawaters. Some of these water bodies may serve as sources of drinking water at somewhere in the community. As result, dissemination of antimicrobial resistance bacteria in the environment will be minimized. This problem in developed country less severe compared to developing country, mainly due to proper antibiotic usage, effective infection control program and better management of hospital wastewater. The situation in developing country, like Ethiopia is overwhelming as the above factors are less practiced. In addition to this most hospitals in Ethiopia neither have hospital wastewater treatment plant nor discharge waste to urban sewer system, which worsen the problem.

Literature review

Large quantities of disinfectants and antibiotics are used in hospitals for disinfection process and patient treatment respectively. Most of the antibiotic taken by the patients is partially metabolized and excreted through feces and urine. After use, residual quantities of these products reach the wastewater, exposing the bacteria that survive in hospital wastewaters to a wide range of biocides that could act as a selective pressure for the development of resistance (Nuñez and Moretton, 2007). Increasing attention has been directed recently to the resistance of bacteria to antibiotics and disinfectants. The resistant bacteria isolated were diverse in nature. For example, study conducted in Buenos Aires City hospital, Brazil, the bacterial population resistant to disinfectants was mainly composed by *Enterobacteriaceae*, *Staphylococcus* spp, and *Bacillus* spp, which are highly associated to nosocomial infections (Nuñez and Moretton, 2007).

Co-resistance to antimicrobial agents among organisms are reported in different studies and an indication of the risks posed by the untreated effluents to public health as well as increasing evidence about the role of hospital wastewaters as environmental reservoir of multi-drug resistant bacteria. Study conducted in Nigeria showed that organisms belonging to seven genera of public health importance such as *Pseudomonas, Streptococcus*, *Serratia*, Staphylococcus, *Klebsiella*, *Proteus* and *Bacillus* showed varying degrees of resistance to the test antimicrobial agents ranging from 0% to 77.8%. Furthermore the report explained, among 25 organisms isolated from hospital A were recognized 16 phenotypic patterns of co-resistance to the test disinfectants and antibiotics; while from hospitals B and C were recognized 13 and 9 patterns, respectively, from among 18 and 14 isolates (Adelow et al, 1997). However in study conducted to compare disinfectant and antibiotic activities, the result showed all strains tested were susceptible to sodium hypochlorite, glutaraldehyde and to the association quaternary ammonium formaldehyde - ethyl alcohol disinfectants. Further susceptibility of strains to phenol and to one quaternary ammonium compound was variable. Among twenty-one antibiotic-multiresistant strains (methicillin-resistant *Staphylococci*, *Enterococcus* spp, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*, Serratia *marcescens* and *Escherichia coli*) eleven (52%) and eight (38%) strains were resistant to the quaternary ammonium and phenol compounds, respectively. Among six isolates that demonstrated susceptibility to antibiotics (*Staphylococci*, *Enterococcus* spp, *P. mirabilis*, *E. cloacae* and *E. coli*) two strains (33%) showed resistance to these disinfectants. This result demonstrated that lack of correlation between antibiotic-susceptibility and susceptibility to disinfectants in hospital strains (Aparecida et al, 2000). However, one study demonstrated cross-resistance between triclosan and antibiotics in Pseudomonas aeruginosa showed that exposure of a clinically significant bacterium to the antiseptic triclosan efficiently can select for multi-drug resistant (MDR) derivatives, including high-level resistance to an ant pseudomonas drug (Rungtip et al, 2001).

Healthcare liquid waste composed of residual quantity of disinfectant, antibiotics, other variety of chemicals, saprophytic microorganisms, commensal and pathogenic bacteria. Due to diverse interaction of these organisms (particularly pathogenic group) with themselves and chemical environment, there will be evolving of resistant bacteria that will difficult for antibiotic treatment. Study carried out in Nepal found out that healthcare liquid wastes were loaded with multiple drug resistance bacteria and seemed to pose a huge public health threat in the transfer of such resistance to the bacterial pathogens causing community acquired infections, thereby limiting our antibiotic pool (Sharma et al, 2010). Also study conducted in Sweden demonstrated that high prevalence of Vancomycin Resistant Enterococci (VRE) in Swedish sewage possibly due to antimicrobial drugs or chemicals released into the sewage system may sustain VRE in the system (Aina et al, 2002).

Selection and dissemination of resistant bacteria in nature should be avoided in order to ensure effective treatment against infectious disease in humans and maintain an ecological balance that favors the predominance of a susceptible bacterial flora in nature. Studies indicated difference in existence of antibiotic resistant bacteria in different sewages. For instance study conducted in Al Shifa hospital, Gaza compared contribution of hospital wastewater to the spread of antibiotic resistance with non-health institution. The most frequently identified bacterium was Pseudomonas sp. (33.1%) followed by E. coli (30.5%), Enterococcus sp. (21.4%), Klebsiella sp. (10.4%) and Proteus sp. (4.5%). There was high incidence of antibiotic resistance among both gram-negative and gram-positive isolates and those isolated from wastewater samples from Al-9 Shifa hospital and laboratory building of Islamic University of Gaza contain higher number of antibiotic resistant bacteria than bacteria that isolated from other sites (Abdelraouf, 2006).

Antimicrobial resistance may spread in aquatic environment (drinking and recreational water) and its role is not only as reservoir of clinical resistance genes, but also as a medium for spread and evolution of resistance genes and their vectors (Hilary-Kay, 1993). Therefore the potential for indigenous aquatic organisms to provide the source of new resistance genes and their associated genetic vectors and to function as hosts for the continued evolution of clinically important resistance genes deserves more intense and detailed investigation (Hilary-Kay, 1993).

Study conducted in Brazil detected extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in effluents and sludge of a hospital sewage treatment plant and indicated the hospital wastewater treatment plant did not show a satisfactory efficacy in removing pathogenic micro-organisms, allowing for the dissemination of multiresistant bacteria into the environment (Pardo et al, 2007). However study conducted in U.S wastewater research division, municipal environmental research laboratory, U.S. Environmental Protection Agency observed the effect of UV light disinfection on antibiotic resistant coliforms in wastewater effluents and indicated UV irradiation effectively disinfected the wastewater effluent, the percentage of the total surviving coliform population resistant to Tetracycline or Chloramphenicol was significantly higher than the percentage of the total coliform population resistant to those antibiotics before UV irradiation and the finding was attributed to the mechanism of R-factor mediated resistance to tetracycline (Mark, 1982). The influence of hospital wastewater containing resistant bacteria discharged to the receiving environment should not be underestimated. Public health impact is one of the most serious and requires urgent response from all stakeholders. One study conducted in Belgium to compare the antimicrobial tolerance of Oxytetracycline-resistant heterotrophic bacteria isolated from hospital sewage and freshwater fishfarm water generally showed, Oxytetracycline-resistant hospital heterotrophs displayed a higher frequency (84%) of Ampicillin (Amp) tolerance compared to the Oxytetracycline-resistant heterotrophs from the freshwater fishfarm site (22%) (Huys et al, 2001). Similar study conducted in Austria to evaluate antibiotic resistance of E. coli in sewage and sludge, the highest resistance rates were found in E. coli strains of a sewage treatment plant which treats not only municipal sewage but also sewage from a Hospital (Reinthaler et al, 2003). Other study conducted in Nigeria demonstrated that hospital wastewater was observed to play a significant role in the influence on the qualities of the bacteriological and physiochemical parameters on the receiving environment due to increased amount of organic matter and essential nutrients in hospital wastewater (Ekhaise and Omavwoya, 2008). Most of the time hospital effluents with other sewage released to water bodies (lakes, rivers or streams) or underground which will be used as medium for transfer of resistant bacteria and their genes in aquatic system. As these water bodies are one of the major sources of water, directly or indirectly, for human and animal consumption, its pollution may contribute to the maintenance and even the spread of bacterial antibiotic resistance (Mesdaghinia et al, 2009).

Some microorganisms inherently developed resistance to disinfectant and even grow in disinfectant solutions. The problem becomes worst, if these organisms are present in hospital environment and easily transmitted to patients from medical devices and pose significant health problem. For instance study showed that resistant cells of Pseudomonas aeruginosa and
waterborne Pseudomonas sp.(strain Z-R) were able to multiply in nitrogen-free minimal salts
solution containing various concentrations of commercially prepared, ammonium acetate buffered benzalkonium chloride (CBC), a potent antimicrobial agent (Frank et al, 1969).

Moreover the efficacy of disinfectant should be monitored regularly to insure proper activity against major medically important organisms. One study conducted to show the efficacy o variety of disinfectant against Listeria spp. in the presence and absence of organic matter revealed that the presence of whole serum and milk (2% fat) further reduced the disinfectant capacities of most of the formulations studied and findings emphasize the need for caution in selecting an appropriate disinfectant for use on contaminated surfaces, particularly in the presence of organic material (Best et al, 1960). For instance study conducted to reveal factors influencing the occurrence of high number of iodine-resistant bacteria in iodinated swimming pool, iodine appeared to be more effective than chlorine against both the standard fecal indicators, coliform bacteria and enterococci, and against staphylococci derived from the mouth, nose, and skin (Martin et al, 1966) and explained that high iodine concentrations were easily maintained, and that bathing load, sunlight, and presence of organic matter had less of a depleting effect on the iodine concentrations than on the chlorine concentrations (Martin et al, 1966).

Serious problem of release of antibiotic or disinfectant resistant bacteria to the environment is potential of transferring of the resistant gene to community bacteria. Infection caused by resistant bacteria will challenge the antibiotic therapy and increase the cost of treatment and hospitalization. Study conducted in Philadelphia to examine transfer of plasmids pBR322 andpBR325 in wastewater from laboratory strains of Escherichia coli to bacteria indigenous to the waste disposal system showed that bacterial strains isolated from raw wastewater or a plasmid free E. coli laboratory strain served as recipients. Transfer of the pBR plasmid into the recipient strain occurred during a 25-h co-incubation in either L broth or sterilized wastewater (Michael etal, 1985). After the co-incubation, recipients exhibited both plasmid-encoded phenotypic characteristics and an altered plasmid profile, as shown by agarose gel electrophoresis of purified plasmid DNA (Michael et al, 1985). However study conducted in France to trace whether antibiotic-resistant Pseudomonas aeruginosa isolated from hospitalized patients recovered in the hospital effluents, the result showed genotyping of both clinical and wastewater isolates was determined by using pulsed-field gel electrophoresis (PFGE). There was no common PFGE pattern in antibiotic-resistant *P. aeruginosa* from humans and wastewater therefore antibiotic resistance profile of wastewater isolates was different from that of clinical isolates (Tumeo et al, 2008).

Continued updating of the susceptibility pattern of recent clinical isolates, isolates from hospital wastewater and follow-up in changes of antibiotic patterns are periodically necessary in every country. This is particularly important to prevent population of bacteria resistant to antibiotics and select antibiotic of choice for particular condition. Study conducted in France to compare vitro activity of Cefoxitin with Metronidazole and Clindamycin against 322 strains of anaerobic bacteria collected from several hospitals during 1982 and tested by an agar dilution method showed that Metronidazole and Cefoxitin inhibited at least 89% of strains tested, whereas Clindamycin was less active (Dubreuil et al, 1984).

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