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RESEARCH ARTICLE

A STUDY OF HEAVY METAL AND ANTIBIOTIC RESISTANCE IN HOSPITAL AQUATIC ENVIRONMENT

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ABSTRACT

Now a day, hospital aquatic environment is becoming under pressure due to antibiotics and heavy metal interference which highly affect the microbial diversity. These antibiotics and heavy metal have significantly increased the multi drug resistant microbes present in the aquatic environment which act as reservoirs including, hospital sewage, rivers and ocean water. Antibiotic resistance is commonly encoded on the same mobile genetic elements that carry heavy metal resistance genes. Those genes are certainly selected in presence of heavy metals along with them, antibiotic resistant gene are co-selected. The biological evolution is the marvellous example of bacterial resistance. The eutrophication facilitates of hospital environment is main cause of the growth and survival of microbial pathogens. In India there is no instruction for the use of antibiotics. Therefore, the aim of this review article is to increase the inspection of hospital aquatic environment polluted with various heavy metals and antibiotics and enlargement of hindrance strategies including reduction of excessive use of antibiotics in hospitals, wastewater treatment and suitable disposal of hospital wastes for protection of both environment as well as public health risk.

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INTRODUCTION

The discovery of antibiotics in the 20th Century marked a turning point in the treatment of infections. The ability to treat the bacterial infections of the pre-antibiotic era encouraged advances in medical fields and enlarges the scope of medical aid. Antibiotic resistance, a world problem, is particularly pressing in developing countries wherever the infectious disease burden is high and cost constrains the replacement of older antibiotics with newer, more costly ones (Sneha Verma and Anurag Rawat, 2014). The increased use of antibiotics has resulted in significant increase the numbers of antibiotic resistant bacteria present in marine environments. The incident of bacterial drug resistance was first documented in 1951. Management of common and toxic bacterial infections has been vitally compromised by the manifestation and quick spread of antibiotic-resistant bacteria. The microorganism infection burden in Asian nation is best among the all over world (World Health Organization, 2011); accordingly, antibiotics can play a very important role in limiting morbidity and mortality within the country (Mathew, 2009; Levine and Cherian, 2007). Drug Resistance refers to a condition in which the drugs that usually demolish the bacteria no longer do so." Antibiotic resistance has been detected in various marine environments which emerged as reservoirs including, hospital sewage, rivers ocean water and drinking water (Ash *et al*., 2002; Reinthaler *et al*., 2003; Schwartz *et al*., 2003). Increased introduction of antibiotics into the environment via medical therapy, municipal and hospital sewage (Brunea *et al*., 2004; Qadri *et al*., 2005; Hamelin *et al*., 2006), agriculture and animal husbandry has resulted in selective pressures on bacterial populations as well as the random use of antibiotics have extend drug-resistant microbes to all parts of the world. The eutrophication facilitates of hospital environment is main cause of the growth and endurance of microbial pathogens (Olapade *et al*., 2006). *P. aeruginosa* is a highly adaptable opportunistic pathogen. The ability of this bacterium to survive antimicrobial treatment represents a major challenge regarding treatment of infectious diseases (Labaer *et al*., 2004). In *P. aeruginosa* resistance is attributed to chromosomal mutations or acquisition of antibiotic resistance by genetic exchange mediated by plasmids, transposons or bacteriophages (Poole, 2011). Therefore, the aim of this review article is to increase the inspection of hospital aquatic environment polluted with various heavy metals and antibiotics and enlargement of hindrance strategies including reduction of excessive use of antibiotics in hospitals, wastewater treatment and suitable

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disposal of hospital wastes for protection of both environment as well as public health risk.

Antibiotic Resistance in Bacteria

Microbial populations increase resistance to antimicrobials through several mechanisms. Of greater concern are cases of acquired resistance, where primarily greater populations of bacteria become resistant to antibiotics and proliferate and spread under the selective pressure of use of that antibiotic. The rate at which an individual gene mutates to express an antimicrobial resistance phenotype is a complex phenomenon in which environment, cell physiology, bacterial genetics, and population dynamics all play roles (Martinez and Baquero, 2000). Bacteria may obtain frequent genes for a metabolic pathway which eventually produce altered bacterial cell walls that no longer contain the binding site of the antibiotics, or bacteria may acquire mutations that limit access of antibiotic to the intracellular target site via down regulation of porin genes. Antibiotics at much higher concentrations that usually found in natural ecosystems can be found in hospital wastewater and soils (e.g. soils treated with manure and farm soils). However, these high concentrations are usually concentrated to areas of human population, whereas perfect environments usually have low concentrations of antibiotics. Risk assessments strength thus take into consideration mainly those areas with high antibiotic load and containing human-associated microorganisms (Baquero *et al*., 2008) for analyzing the effect of antibiotic pollution on natural aquatic environment. A different situation occurs for antibiotic resistance genes. It has been stated that acquisition of an antibiotic resistance phenotype produces a metabolic burden (Morosini *et al*., 2000; Andersson, 2006), and it was predicted that in the absence of selective pressure, resistance would disappear. Unfortunately, this condition is not always true. Achievement of antibiotic resistance may produce specific changes in the bacterial metabolism that can be even valuable for bacterial growth in some environment (Sanchez *et al*., 2002b; Alonso *et al*., 2004; Linares *et al*., 2006; Luo *et al*., 2005). Antibiotic resistance genes are found worldwide, as can be predicted due to their origin in environmental bacteria (Alonso *et al*., 2001). However, the wide propagation of genes frequently present in human pathogens in places without a high antibiotic load (Pallecchi *et al*., 2008) indicates that, once those elements are present in gene-transfer platforms, the probability for their maintenance in natural ecosystems can be high. For this reason, antibiotic resistance genes are being considered as pollutants themselves. Since antibiotic resistance genes are naturally situated in the chromosomes of environmental bacteria (D'Acosta *et al*., 2006; Martinez, 2008; Wright, 2007).

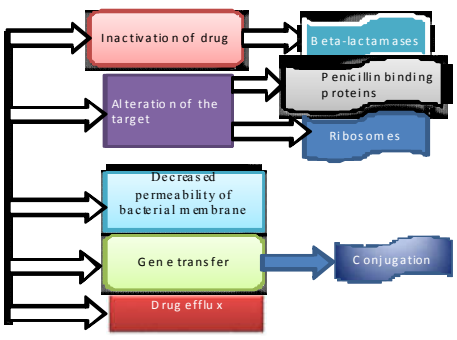

Figure 1. Schematic representation of the antibiotic resistance

Although widely in use, the effectiveness of antibiotics has decreased over time (World Health Organization 2012). This is a result of the distribution of antibiotic resistance bacteria; organisms capable of continuously increasing in toxic concentrations of these drugs. Millions of individuals have their health compromised by infections caused by resistant microorganisms, making resistance a global concern (Levy and Marshall, 2004). The problem is so big that resistance now found in all known classes of antibiotics (Costa *et al*., 2006). For some infections there is almost no drug available for their treatment (Tenover, 2006; Mcgowan, 2006). In addition, some bacterial diseases previously deemed treatable (e.g. gonorrhea and typhoid fever) or considered under control (e.g. tuberculosis) are once again a hazard due to the appearance of resistant strains (Pruden *et al*., 2006). Microorganisms resistant to at least three classes of antibiotics are considered multidrug-resistant. It is approximate that 400,000 cases of infections caused by multi-resistant bacteria occurred in Europe only during 2007, which can be linked to 25,000 deaths (European Centre for Disease Prevention and Control (ECDC) 2012). World Health Organization estimates that multidrug-resistant tuberculosis causes at least 150,000 deaths every year (World Health Organization, 2000). Multidrug-resistant pathogenic micro-organism is associated with increased morbidity and mortality, since they are much less susceptible to antibiotic treatment, our main weapon against microbial infections. Diseases caused by these microbes are more expensive to treat, because they usually require time taken treatments, more clinical trials and a larger number of drugs. This is of special significance in developing countries where little budget is directed for the acquisition of more efficient and expensive pharmaceuticals (World Health Organization, 2000; Levy and Marshall, 2004).

Evolution and distribution of antibiotic resistance

The role of pollution in the increase and extend of resistant bacteria is not fully understood. However, a growing body of authentication suggests that pollution promotes proliferation of antibiotic resistance in bacteria within aquatic environments (Figure 2). In wastewater discharges from hospital and household sources affect the diversity of resistant bacteria (Czekalski *et al*., 2012; Thevenon *et al*., 2012). Those impacts also shape the genetic pool of water bodies by increasing abundance of antibiotic resistance genes within these environments (Tacão *et al*., 2012). As previously stated, in clinical circumstance, resistant microbes are the most successful and subsequently increase their numbers. Thus, hospital wastewaters have been shown to be rich in resistance genes (Schwartz *et al*., 2003) and resistant bacteria (Santoro *et al*., 2012). Multicellular organisms replace members of their related microbiota with other organisms and with their environment. Due to that, animals can increase antibiotic resistance bacteria among other animals, humans and throughout the environment (Allen *et al*., 2010). The increasing spread of antibiotic resistance among environmental bacteria has led some authors to consider antibiotic resistant bacteria and antibiotic resistance genes as emerging pollutants (Kümmerer, 2009). These entities have a exclusive property when compared to other contaminants: their capability to amplify and spread, survive in the environment. Since human populations are dependent on aquatic environments, the proliferation of antibiotic resistance bacteria and antibiotic resistance genes within these sites represents a severe risk to

Table 1. Antibiotic classes: action and resistance mechanisms

Class	Antimicrobial agent	Mechanism of action	Resistance mechanism
Aminoglycosides	Gentamycin, Kanamycin, Streptomycin	Inhibition of protein synthesis	Efflux, enzymatic inactivation, mutated target
Amphenicols	Chloramphenicol	Inhibition of protein synthesis	Efflux
Macrolides	Clarithromycin, Erythromycin	Inhibition of protein Synthesis	Efflux, mutated target
Tetracycline's	Tetracycline, Doxycycline	Inhibition of protein synthesis	Efflux
Beta-Lactam*	Penicillin, Aztreonam, Cefotaxime	Inhibition of cell-wall	Enzymatic inactivation, mutated target
Glycopeptides	Vancomycin, Bleomycin	Cell wall Inhibition of cell-wall synthesis	Cell wall modification, efflux
Quinolones	Nalidixic Acid, Ciprofloxacin	Inhibition of nucleic acids synthesis	Efflux, mutated target
Sulphonamides	Sulfamethoxazole	Inhibition of folate synthesis pathway	Alternative enzymes, mutated target
Lipopeptides	Daptomycin	Cell membrane depolarization	Cell membrane modification, mutations
Amino-acid derivates	Polymyxin B	Cell membrane permeabilization	Cell membrane modification

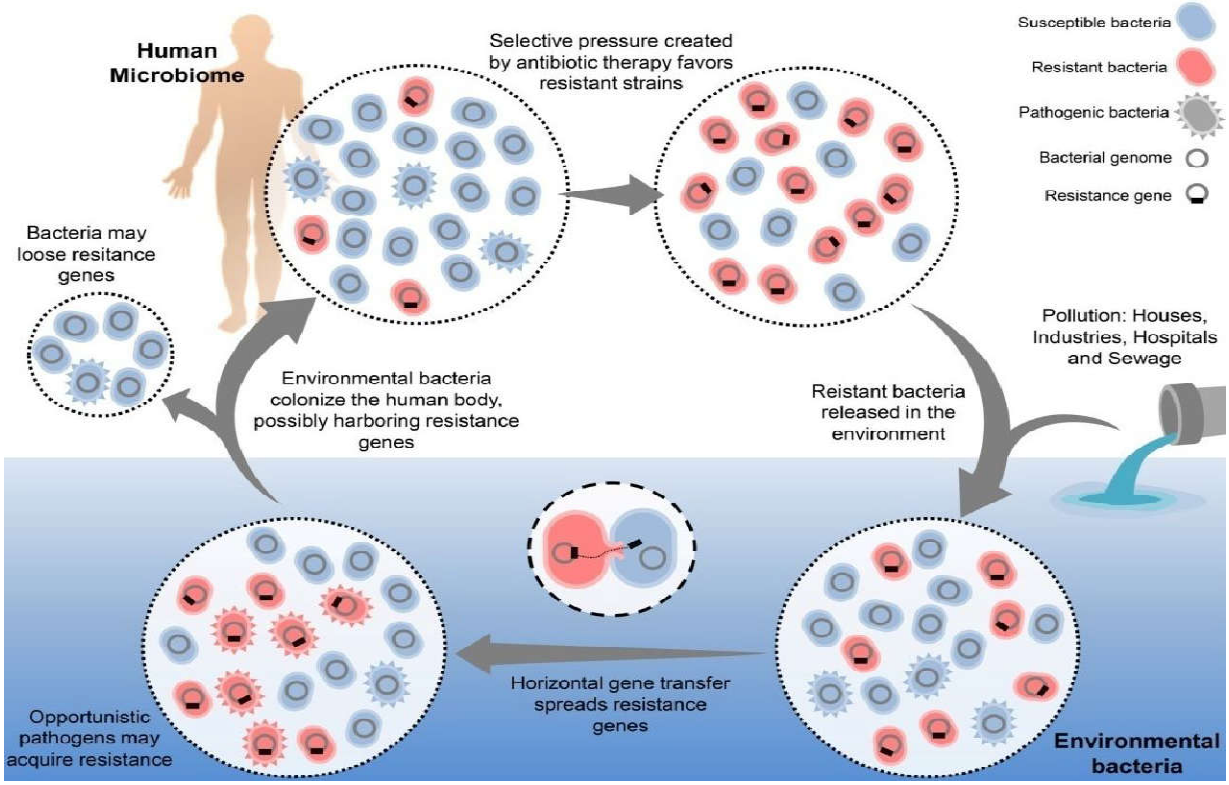

Figure 2. Schematic representation of the interactions between pollution, resistant bacteria and aquatic environments. (Hernandez *et al*., 1998)

public health (Jalal *et al*., 2012). However, antibiotic resistance is not just a medical issue but also an ecological matter. To know the process by which resistance propagates, it is necessary to consider not only hospital settings, but also the ecology and evolution of resistant organisms (Martínez, 2012). In earlier studies the appearance of resistance among the Gram negative bacterial population diverse significantly in different drug and water sampling sites. Gram negative bacteria showed lower drug resistant viable count ($1.23x10^4$-$7.0x10^2$) in site-II (receiving treated wastewater), as compared to more polluted site-I ($1.28x10^4$-$5.0x10^2$), III ($2.22x10^4$-$5.0x10^2$) and site-IV ($1.63\ x10^4$-$2.0\ x10^2$) respectively (Alam and Imran, 2015). Bacteria have very short life cycles compared to eukaryotes. This trait allows for the rapid emergence of new adaptations. In short periods of time, originally susceptible bacteria may become resistant through mutations or by acquiring resistance genes. When antibiotics are present, resistance traits tend to fast multiply among microbial populations (Zhang *et al*., 2011). These characteristics of bacterial evolution supply to a rapid development of multi-resistant pathogenic strains. Accordingly, once a new antibiotic is put into use, it does not take long awaiting it is challenged by novel resistant organisms.

Distribution of resistance is intensified as human populations grow and improve the use of antibiotics. The more these drugs are consumed, more intense is the selective pressure they inflict on bacterial communities. As a result, resistance mutations are evolutionary successful and therefore tend to increasingly improve their profusion among bacterial populations. This phenomenon takes place wherever bacterial communities are subjected to antibiotics as a related selective pressure, such as in hospitals. Water bodies are sites of genetic replace where environmental bacteria assist with microbes originated from humans and other animals, exchanging genes through horizontal gene transfer. Opportunistic infections often have large and adaptable genomes, prone to sharing genetic material. This trait helps these organisms to settle a more different set of environments. As consequence, aquatic ecosystems may become a hazard to human health when they are affected by pollution carrying resistant bacteria (Baquero *et al*., 2008). Gene transfer can be performed between pathogenic and environmental bacteria and even between very phylogenetically isolated organisms such as species of Gram negative and Gram-positive bacteria.

Heavy Metal Pollution in hospital effluents

Heavy metal pollution is also a severe environmental problem due to the increase in human and industrial activities (ASM COLLOQUIUM REPORT Antimicrobial Resistance 1999). Heavy metals are natural constituents of minerals found in the earth's crust, which sometimes can neither be degraded nor destroyed. A heavy metal is defined as any metallic chemical element that has a relatively high density and at low concentration; it is either toxic, or poisonous to all forms of life including bacteria (Lenntech, 2005). Hospital wastewater reveals the presence of molecules chlorinated in higher concentrations and in a punctual way the presence of heavy metals such as silver and mercury (Gartiser *et al*., 1996). Heavy metals naturally present in the environment and constitute a potential hazard for soils, waters, plants and sediments. Numerous studies have indicated that agro ecosystems receive inputs of Heavy metals from the increased use of agrochemicals, the application of metal containing wastes such as pig manure, sewage sludge, coal and wood ashes to soils, and from atmospheric deposition (Mhatre and Pankhurst, 1997). Although some of these metals are essential plant micronutrients and are necessary or are beneficial for plants and microbial growth and enhancement (Zn, Cu, Fe, Mn, Ni, Mo, Co), high contents and/or long-term presence of Heavy metals, in water and soils, are generally considered a matter of concern to society as they may adversely affect the quality of soil and water, and microbial diversity/population (Keller *et al*., 2002). Heavy metals are an important category of pollutants and as such have major detrimental impacts on both human health and the health of terrestrial and aquatic environment (Duruibe *et al*., 2007). A Much research on heavy metal pollutants (Cd, Cu, Cr, Hg, Pb, Ni, and Zn) has focused on their direct negative effects on organisms (Stepanauskas *et al*., 2006).

Heavy Metal Resistance

Strains of Gram negative bacteria are commonly resistant to heavy-metal ions and the resistances can often be transferred by conjugation. Plasmid-mediated resistance to Hg, Co, and Ni (Clausen, 2000) arsenite, and copper (Klonowska *et al*., 2008). In some instances resistance to these metals is mediated by the same plasmid that determines resistance to antibiotics (De *et al*., 2003). There are also reports of single plasmids in E.coli which bear genes for combinations of properties such as colicin production. Heavy metals such as lead, mercury, cadmium and nickel are metabolically poisonous at low concentrations. They reduce the activities of certain enzymes involved in the metabolic process (Midigan *et al*., 2003). At higher concentrations these metals form unspecific complex compounds in the micro-organism, which leads to lethal effects bacterial accumulation of and resistance to toxic heavy metals is a prevalent phenomenon. The mechanisms of heavy metal resistance have been reported to increase the antibiotic resistance capability of microorganisms. Among the microorganisms, bacteria, yeast and protozoa are generally the first category to be showing to heavy metals present in the environment. Microorganisms have acquired a range of mechanisms for amendment to the presence of toxic heavy metals. Among the various adaptation mechanisms, metal sorption, mineralization, uptake and increase, extracellular precipitation and enzymatic oxidation or reduction to a less toxic form, and efflux of heavy metals from the cell has been reported (Mergeay *et al*., 2003; Sharma *et al*., 2000; Nies, 2003).

Mechanism of Metal Resistance

At higher concentrations, however, heavy-metal ions form unspecific complex compounds in the cell, which leads to toxic effects. Some heavy-metal cat ions, e.g. Hg2+, Cd2+ and Ag+, form strong toxic complexes, which makes them too dangerous for any physiological Function Most cells solve metal toxicity problem by using two types of uptake system for heavy-metal ions: one is fast, unspecific and, since it is used by a variety of substrates, constitutively expressed. These fast systems are frequently driven only by the chemo osmotic gradient across the cytoplasmic membrane of bacteria. The second type of uptake system has high substrate specificity, is slower and often uses ATP hydrolysis as the energy source, sometimes in addition to the chemiosmotic gradient, and these expensive uptake systems are only produced by the cell in times of need, starvation or a special metabolic situation; they are inducible (Nies, 2003). Ni2+, Co2+, Zn2+, and Mn2+ are accumulated by the fast and unspecific CorA magnesium uptake system in Gram-negative bacteria. When a cell faces a high concentration of any heavy metal that is accumulated by such an unspecific system, the specific heavy-metal ion is move into the cytoplasm in spite of its high concentration, because these unspecific transporters are constitutively expressed. Thus, the gate cannot be closed. This ``open gate'' is the first reason why heavy-metal ions are toxic (Nies, 2003). There is significant evidence that micro-organisms can rapidly adapt to the toxic metals by altering their chemistry and mobility. The mechanisms of resistance include metal reduction or transformation to more unstable or less toxic forms. Some bacteria including *E.coli, Pseudomonas*, and *Clostridium* enzymatically reduce Hg2+ to Hg0 which is highly explosive and diffuses away from the bacterial cell. Others have specific metal efflux systems, which are the most frequently found mechanism of plasmid mediated metal resistance (Silver and Phung, 2005). Other microbes tolerate metals through binding by extracellullar polysaccharides (precipitation and exclusion) mediated by construction of low molecular weight binding proteins such as phytochelatins (Angle and Chaney, 1989). Chelation and complexion of metal species with the media components and organism induced pH changes can also contribute to metal tolerance. Chromate tolerance is achieved through methylation, reduction and precipitation at the cell surface, blocking cellular uptake by altering the uptake pathway and removal from cytoplasm by efflux pumps. In many cases these responses appear to be plasmid mediated (Silver and Phung, 2005). Cadmium binds to sulfhydryl groups on essential proteins thus interfering with important cellular functions. It can also cause single-stranded breakage of DNA in E.*coli*. Two cadmium plasmid mediated resistance efflux systems, that is, *cad* in *Staphylococcus aureus* and *czc* in *Acinatobacter eutrophus* are well characterized and documented (Smith *et al*., 2002). The *czc* system 28 also confers resistance to zinc and cobalt. Another system that confers resistance to zinc and cobalt that is chromosomally encoded is known to exist (Bruins *et al*., 2000). Cadmium resistance genes located on transposons have been reported in *Listeria monocytogenes*. Resistance to mercury is based on its redox potential and its low vapour pressure. Resistant bacteria are capable to reduce Hg2+ to metallic mercury (Hgo), which does not remain in the cell but leaves the cell by passive

diffusion. Bacterial tolerance to heavy metals has been reported in both Gram negative and Gram positive bacteria.

Co-Relation between Heavy Metal and Antibiotic Resistance

Hospital wastewater has a high amount of both inorganic and organic matter, as well as high densities of living organisms, including pathogenic, and environmental bacteria. It has been suggested that genes encoding resistance to heavy metals can be located together with antibiotic resistance genes on either the same or different genetic structure (eg. plasmid), within the same bacterial strain (Guardabassi and Dalsgaard, 2002). McArthur and Tuckfield, (2000) had recommended that antibiotic and metal resistance among bacteria are linked very closely together and that expression of antimicrobials resistance may be dependent on exposure to metals. This is supported by the result that unspecific mechanisms conferring and antibiotics exist in some bacterial species and that indirect evidence that bacteria isolated from heavy-metal polluted marine sediment are considerably more resistant to antibiotics than their counterparts isolated from unpolluted sites. Similarly drug and metal resistance among bacteria have been shown higher proportional along industrial contamination gradients (De Jaysankar *et al*., 2007) and use of metal based antimicrobial agents. Nakahara *et al.* (2001) have suggested that the combined expression of metal tolerance and antibiotic resistance may not be an accidental phenomenon but rather is caused by selection resulting from metals present in an environment. Antibiotic and heavy metals resistance another connection between heavy metals and pathogen incidence is concern that metal pollutants may act as co-selection agents for antibiotic resistance in bacteria. Co-selection occurs when selection for one trait simultaneously selects for a second trait: in this case, selection for metals resistance also selects for antibiotics resistance (Baker-Austin *et al*., 2006). In fact, one explanation for the evolution of antibiotic resistance genes, some of which have had broad evolutionary histories prior to broad human uses of antibiotics, is their ability to function in heavy metal resistance (Aminov *et al*., 2001): essentially preadapting them to present human uses of antibiotics.

Interactions between water pollution and antibiotic resistant bacteria

Untreated sewage from household, clinical, industrial and agricultural origins is constantly released into the bay's waters (Cardoso *et al*., 2012). Mixing of marine, wastewater and freshwater grants this environment had high microbial diversity (Vieira *et al*., 2008). Currently, we are exploring the links between wastewater discharges and the diversity of antibiotic resistance bacteria within the bay and nearby aquatic environments. For that purpose, we analyzed the variety of cultivable bacteria resistant to ampicillin in water bodies located in the state of Rio de Janeiro. Inoculants for mixed bacterial cultures were obtained from each of the six sampling sites. Culturing was performed on four antibiotic concentrations. Bacteria were grown in clinical resistance and super-resistance ''50 times higher'' (Dantas *et al*., 2008) concentrations of ampicillin. Super resistance cultures showing growth were inoculated on culture media supplemented with antibiotic concentrations 600 times higher than clinical levels. Bacteria were also cultivated on ampicillin-free media for control. All cultures were grown in Luria-Bertani liquid media for 24 hours at 37°C followed by DNA extraction for construction of 16S rRNA gene libraries.

Resistance transfer from the environment to the hospital

Patients admitted to hospital are credible to obtain bacteria which are multiplying in the hospital environment, such as *Pseudomonas aeruginosa* and these may well be antimicrobial resistant. It has been demonstrated that the general environment of the hospital, mainly sites such as sink drains, mop heads and other wet environments will act as sources not only for bacteria capable of directly causing nosocomial infection but which can also act as a gene pool for antibiotic resistance genes (Levy and Marshall, 2004). Outside of this apparent interaction of patients with the hospital environment, it is maybe food that is the major route of flow of resistance genes from the more general environment to man. The human gut carries a large number of commensal bacteria; usually of the order of ≥1010/g of faeces and this figure only apply to culturable bacteria. The bowel flora is constantly being challenged by new bacteria in the food and although the leading flora remains, colonisation of antibiotic resistant bacteria, mainly enterococci / *Enterobacteriaceae* and staphylococci occurs in those individuals not receiving antimicrobials. A study undertaken as long ago as 1979, showed that in individuals with no history of fresh utilization of antibiotics, 10% or more of the total aerobic Gram negative bacteria were resistant to one or more antimicrobials (Levy and Marshall, 2004). A wide study by (Buschmann *et al*., 2012) showed that the incidence of seven primary antibiotic resistance markers against the staphylococcal flora in antibiotic untreated subjects was tetracycline 87.5%, erythromycin 68.8%, fusidic acid 56.3%, trimethoprim 42.4%, chloramphenicol 25%, clindamycin 9.4% and gentamycin 4.7%. We now identify that against staphylococci there is sufficient opportunity and genetic mechanisms for the mobilisation of those genes into potentially infectious species (Buschmann *et al*., 2012).

Potential aspects

Presence of antibiotic and heavy metal resistance bacteria in the hospital aquatic environment may be an indication that the area is contaminated with antibiotics and chemical pollutant. Such an area may foster adaptation and selection leading to antibiotic resistant organisms. The growing quantity of antibiotics/antimicrobial solvents used will probably increase antibiotic resistivity in pathogenic bacteria of the hospital aquatic system. The issue of antibiotic and heavy metal resistance has received considerable attention due to the problem of the emergence and rapid expansion of antibiotic-resistant pathogenic bacteria. Many scientists now show that bacteria in polluted hospital aquatic environment become resistant to large number of antibiotics and heavy metals.

Conclusion

Whether antimicrobial are given as treatments to humans or animals, their misuse is at the spirit of the antibiotic-resistance problem. Antibiotic and metal resistances among bacteria are linked very closely together and that expression of antimicrobials resistance may be dependent on exposure to metals. Appearance of heavy metal and antibiotic resistance in pathogenic bacteria of the hospital effluents is directly linked with arbitrary and excess use of antibiotics, Redionucleocides and antimicrobial solvents for treatment of infectious disease.

Therefore, now the hospital aquatic environment acts not only as a reservoir of resistance genes, but also as a medium for the spread and evolution of resistance. Detection of multi-antimicrobial-agent and heavy metal resistant bacteria in hospital aquatic environment is alarming. Therefore, increased inspection of hospital effluents and development of hindrance strategies for protection of public health is necessary.

Competing interests

The authors declare that there are no competing interests.

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