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Review

Pseudomonads: A versatile bacterial group exhibiting dual resistance to metals and antibiotics

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Pseudomonads is a versatile and cosmopolitan bacterial group that can occur in metal contaminated as well as clinical environment and many of them possess the ability to proliferate resistance to bacteria from their own or other group. Antibiotic resistance in clinical bacteria is a growing concern for mankind. Various reports have shown dual resistance of pseudomonads to metal and antibiotic involving different inheritable or non-inheritable mechanisms like co-resistance, cross-resistance, co-regulation, biofilm production, persistence and swarming. Increasing rate of metal pollutant in environment contributed by various anthropogenic activities may constantly provide a selective pressure in proliferation of antibiotic resistance among bacteria.

Key words: Pseudomonads, dual-resistance, metals, antibiotics, inheritable, non-inheritable.

INTRODUCTION

Metal pollutants pose a severe threat to ecological system due to their negative impact on most life forms. Though some heavy metals are essential trace elements, most can be, at high concentrations, toxic to all Forms of life, including microbes, by forming complex compounds within the cell. Although, higher organisms readily succumb to the toxic influence of metals, microbes are known to possess a wide array of genetic composition that allows them to circumvent metallic stress (Appanna et al., 1996; Valdman et al., 2001). Since heavy metals are increasingly found in microbial habitats due to natural and environmental processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals (Adarsh et al., 2007). This increasing heavy metal tolerance has another implication in the environment as it may contribute to the maintenance of antibiotic resistance genes by increasing the selective pressure of the environment. The occurrence of multiple metal and antibiotic resistance property in microbial community poses a potential threat towards human and environmental health. There is concern that metal contamination functions as a selective agent in the proliferation of antibiotic resistance (Baker-Austin et al., 2006). Many have speculated and have even shown that a correlation exists between metal tolerance and antibiotic resistance in bacteria (Spain, 2003; Nostrand et al., 2007; Timoney et al., 1978; Oyetibo et al., 2009; Calomiris et al., 1984). Documented associations between the types and levels of metal contamination and specific patterns of antibiotic resistance suggest that several mechanisms underline this co-tolerance process. These co-tolerance mechanisms include co-resistance (different resistance determinants present on the same genetic element) and cross- resistance (the same genetic determinant responsible for resistance to antibiotics and metals). Indirect but shared regulatory responses to metal and antibiotic exposure such as biofilm induction also represent potential co-tolerance mechanisms used by prokaryotes (Baker-Austin et al., 2006).

Microorganisms change/reduce the toxicity of metallic contaminants through permeability barriers, intra- and extra-cellular sequestration, efflux pumps, enzymatic detoxification, reduction, pH changes and are capable of accumulating toxic metal ions by two well defined processes *viz*: (i) biosorption: an energy- independent binding of metal ions to cell walls and (ii) bioaccumu-lation: energy-dependent process of metal uptake into the cells (Nies, 1999; Al-Shahwani et al., 1984; Vesper et al., 1996; Volesky, 1994; Karna et al., 1996; Li et al., 2004).

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Resistance to antibiotic is achieved through four main strategies: (i) reduction of membrane permeability to antibiotics; (ii) drug inactivation; (iii) rapid efflux of the antibiotic and (iv) mutation of cellular target(s). Biofilm production also has potential contribution in metal and antibiotic tolerance (Krulwich, 2005; Baker-Austin et al., 2006; Thakur et al., 2007).

Among bacteria, Gram-negative species in general, appear to be more metal tolerant than Gram-positive ones (Duxbury, 1981; Abbas and Edwards, 1989). Pseudomonad represent a versatile group of Gram-negative motile, rod bacteria of the genus *Pseudomonas* and considered to be cosmopolitan in the environment (Johansen et al., 1996) having multiple metal and antibiotic resistance (Timoney et al., 1978; Calomiris et al., 1984; Appanna et al., 1996; Nostrand et al., 2007; Oyetibo et al., 2009). However, in this review, we focus on the metal and antibiotic resistance mechanisms prevalent in pseudomonads.

DUAL RESISTANCE IN PSEUDOMONADS

Various studies have reported dual resistance in this bacterial group from different environments (Timoney et al., 1978; Marques et al., 1979; Calomiris et al., 1984; De Vicente et al., 1990; Perron et al., 2005; Akinbowale et al., 2007; Kumar et al., 2008; Oyetibo et al., 2009; Sarma et al., 2009; Matyar et al., 2010). Being Gram-negative bacteria, pseudomonad cells have an intrinsic capacity to restrict the entry of small molecules whose outer membrane provide an effective barrier and constitute a first line defence against any antimicrobial challenge. Gram- positive organisms lack the outer membrane and hence lack this front-line defence. Besides this, several chromosome- and plasmid-encoded metal and antibiotic resistance genetic systems have been studied in *Pseudomonas* and related bacteria belonging to pseudomonads. Marques et al. (1979) reported antibiotic and heavy metal resistance of *Pseudomonas aeruginosa* from soils of Catalonia (Spain). Matyar et al. (2010), in their study with 6 heavy metals and 15 antibiotics, reported metal and antibiotic resistant Pseudomonas from Iskenderun Bay, Turkey, Northeast Mediterranean Sea. The Bay received domestic wastes, including hospital wastes in addition to factories, like iron and steel, a fertiliser factory, a refinery and a coal- fired power plant which discharged a high amount of processed and unprocessed wastes.

De Vicente et al. (1990) studied the resistance patterns of *P. aeruginosa* strains isolated from freshwater and seawater to different antimicrobial agents and heavy metals. They found that a close relationship occurs between the degree of pollution and the frequency of heavy metal resistant strains of *P. aeruginosa*. The highest frequencies of resistance to mercury and arsenic were obtained from marine environments with little faecal pollution, where the highest incidence of multi-resistant strains was also observed. Oyetibo et al. (2009) in their study found different pseudomonads showing dual resistance to metal and antibiotics. Heavy metals can be taken up into foods, particularly fish and shellfish, and these contaminants may be introduced into the aquaculture system through use of fish-based meal that can bring in fat-soluble contaminants such as heavy metals and polychlorinated biphenyls into the aquaculture diet (Erickson, 2002). Though different studies have reported the incidence of occurrence of antibiotic and heavy metal resistant bacteria in fish from various metal-contaminated environments (Miranda et al., 1998; Karbasizaed et al., 2003; Akinbowale et al., 2007) isolated dual resistant *Pseudomonas* sp. from commercial aquaculture systems. To study the association of metal and antibiotic resistance in bacteria and to reveal the hypothesis that bacteria are more tolerant to metal and antibiotic found in metal exposed environment than those isolated from metal unexposed environment, Calomiris et al. (1984) did an experiment on bacterial populations isolated from drinking water system and from raw water system. In their study along with other bacterial groups, a group of *Pseudomonas alcaligenes* bacteria were found to be associated with multiple metal and antibiotic resistance.

Sundin et al. (1994) reported natural population of *Pseudomonas syringae* showing resistance to copper and streptomycin. Raja and Selvum (2006) isolated a *P. aeruginosa* strain from treated oil mill industry effluent wastewater samples, showing resistance towards heavy metals such as cadmium, chromium, nickel and lead as well as towards antibiotics *viz.* ampicillin, tetracycline, streptomycin, chloramphenicol, kanamycin and erythromycin . Dual tolerant pseudomonads were isolated from soil and water samples of uranium rich Domiasiat area of Meghalaya, India by Kumar et al. (2008) and Sarma et al. (2009), respectively. In both the studies metal enriched plates were used to isolate metal tolerant bacteria and the isolates were found to be multiple antibiotic resistant showing resistance to different common antibiotics *viz.* ampicillin, kanamycin, chloramphenicol, aztreonam, tetracycline, piperacillin, amikacin etc. Though various reports of dual tolerant pseudomonads from different environments are there, only few studied the actual mechanisms behind this phenomenon. Different shared functional characteristics of metal and antibiotic resistance systems in pseudomonads and other prokaryotes are shown in Table 1.

DUAL RESISTANCE MECHANISMS IN PSEUDOMONADS

Inheritable resistance mechanisms in pseudomonads

Inheritable resistance to antibiotics and metals can be conferred by chromosomal or mobile genetic elements

Table 1. Different inheritable shared mechanisms of metal and antibiotic resistance systems in pseudomonads and other prokaryotes.

Resistance mechanisms	Metals	Antibiotics	References
Alteration of cellular target(s) to lower its sensitivity to drug and metal.	Hg, Zn, Cu	Carbapenem, ciprofloxacin, ß-lactams, trimethoprim, rifampicin	Conejo et al. (2003), Barkay et al. (2003), Roberts (2005).
Rapid efflux of drug and metal from cell.	Zn, Co, Cd, Cu, Ni, As	Ipipenem, tetracycline chloramphenicol, ß-lactams	Levy (2002), Nies (2003), Perron et al. (2004)
Alteration of drug and metal by enzymatic degradation or modification.	As, Hg	Gentamicin, ß-lactams	Jacoby (1974), Mukhopadhyay and Rosen (2002), Wright (2005)
Reduction in membrane permeability to metal and antibiotic	Co, Ni, Cu, Zn, Al, Pb	Cefsulodin, cefpirome, cefozopran, aztreonam, imipenem, panipenem, meropenem, ofloxacin, tetracycline, chloramphenicol, gentamicin	Angus et al. (1982), Silver (1996).

viz., plasmids, transposons etc. (Krulwich et al., 2005). Nickel-resistant microorganisms are often tolerant to other metals as well, either because the Ni resistance mechanism confers resistance to other metals or because the resistance determinants are linked. Stoppel and Schlegel (1995) found that tolerance to Co, Zn, and Cd was frequently observed with Ni tolerance for a variety of microorganisms, including strains of *Cupriavidus* (*Alcaligenes*) sp., *Burkholderia* sp., *Acinetobacter* sp., *Pseudomonas* sp. and *Arthrobacter* sp., isolated from sites containing high concentrations of Ni and/or other metals. Various studies have revealed from plasmid curing experiments, that metal and antibiotic resistance are inherited with extra-chromosomal factors (Mergeay et al., 1978; Raja and Selvam, 2009).

According to Lawrence (2000), in discussion of the 'selfish operon theory', clustering of genes on a plasmid, if both or all genes clustered are useful to the organism, is beneficial to the survival of that organism and its species because those genes are more likely to be transferred together in the event of conjugation. Thus, in an environment with multiple stresses, for example, antibiotics and heavy metals, it would be more ecologically favourable, in terms of survival, for a bacterium to acquire resistance to both stresses. If the resistance is plasmid mediated, those bacteria with clustered resistance genes are more likely to simultaneously pass on those genes to other bacteria, and those bacteria would then have a better chance of survival. In such a situation, one may suggest an association with antibiotic resistance and metal tolerance. From available literature, there are three general conditions of occurrence of dual resistance towards metal and antibiotic by inheritance viz., co-resistance, cross-resistance and co-regulation.

Co- resistance

Co-resistance is a potential mechanism of dual resistance that occurs when the genes specifying resistant phenotypes are located together on the same genetic element such as a plasmid, transposon or integron (Chapman, 2003). This physical linkage results in the co-selection for other genes located on the same element. It has been known for several decades that metal- and antibiotic resistance genes are linked, particularly on plasmids, because the evidence for co-resistance as a mechanism of antibiotic-metal co-selection came from studies that used transformation, plasmid curing and plasmid sequencing approaches (Novick and Roth, 1968; Foster, 1983). Jacoby (1974) studied properties of R-plasmids determining gentamicin resistance by acetylation in *P. aeruginosa*. They found that plasmids also determine a number of other properties not previously known to be associated with *Pseudomonas* R-factors, such as resistance to ultraviolet (UV) light, to Hg^{2+}, and to organic mercurials. Mindlin et al. (2005) suggested that the emergence of integron-carrying transposons that contain both antibiotic-resistance and mercury-resistance determinants is a relatively recent phenomenon because permafrost-derived *Pseudomonas* (15000–40000 years old) contained closely related transposons found in many present-day bacteria, albeit devoid of antibiotic-resistance cassettes. Ryan et al. (2005) isolated multiple metal resistant pseudomonads from metal contaminated soil that were able to transfer its resistance. Raja and Selvam(2009) carried out plasmid curing experiment on a multiple metal and antibiotic resistant *P. aeruginosa* strain and found that Ni and ampicilin resistance factors were linked on the plasmid pBC15 (Figure 1).

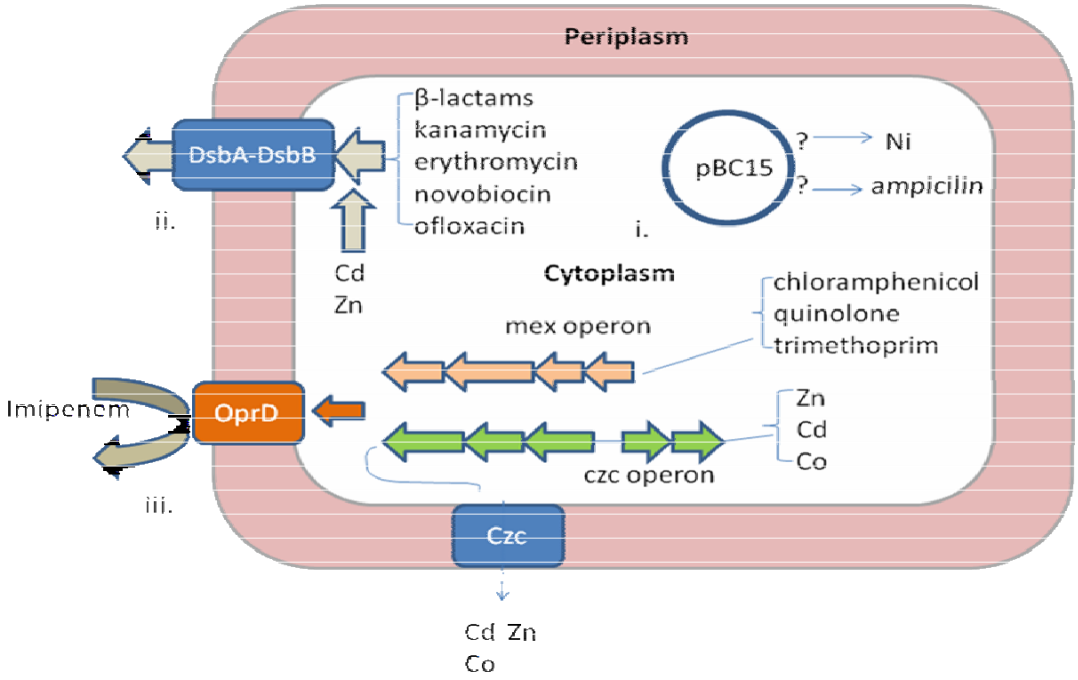

Figure 1. Model showing examples of three major molecular mechanisms responsible for dual tolerance in pseudomonads. (i) Co-resistance towards Ni and ampicilin due to physical linkage of resistance determinants for the two antimicrobial factors on pBC15 plasmid (Raja and Selvam, 2009). (ii) Cross-resistance due to presence of a membrane-bound DsbA–DsbB disulfide bond formation system that confers resistance to five antibiotics accompanied by two metals (Hayashi et al., 2000), (iii) Co- regulatory resistance due to the linkage of mex and czc operons leads to expression of metal efflux and imipenem resistance (Perron et al., 2004).

Cross- resistance

Cross-resistance is another mechanism involved in the selection and proliferation of antibiotic resistance and it can occur when different antimicrobial agents attack the same target, initiate a common pathway to cell death or share a common route of access to their respective targets. The end result is the same, the development of resistance to one antibacterial agent is accompanied by resistance to another agent (Chapman, 2003). Because of the importance of this association, large-scale genomic sequencing of these mobile genetic systems involved in horizontal gene transfer is necessary and should provide further insights into the ubiquity of environmental metal–antibiotic co-selection (Baker-Austin et al., 2006). Multidrug resistance pumps (MDRs) are responsible for the extrusion of chemically unrelated antimicrobials from the bacterial cell (Brooun et al., 2000) and cross resistances are mainly gained through efflux pumps where irrespective of the structure of the compounds, bacteria can efflux out non-related chemicals from cell (Aendekerk et al., 2002).

Characterization of the MexGHI–OpmD efflux pump of *P. aeruginosa* showed that the presence of the entire pump operon *in trans* resulted in increased resistance to vanadium, ticarcillin and clavulanic acid compared with mutants that lack MexGHI–OpmD (Aendekerk et al., 2002). Hayashi et al. (2000) reported in *Burkholderia cepacia* by mutational analysis of a membrane-bound DsbA–DsbB disulfide bond formation system, followed by phenotypic analysis and suggested that the DsbA–DsbB system is involved in the formation of a metal-efflux system and a multi-drug resistance system with some other activities (Figure 1).

Co-regulation

Recent studies have provided insights into co-regulated resistance to metal and antibiotics in bacteria like pseudomonads (Conejo et al., 2003; Perron et al., 2004). Perron et al. (2004) tackled the case of metal and antibiotic co-regulatory resistance in *P. aeruginosa*. Isolates exposed to zinc were also found to be resistant to other heavy metals (cadmium and cobalt) and the carbapenem- class antibiotic imipenem. Analysis of the mechanisms that underline cross-resistance to both zinc and imipenem revealed co-regulation of imipenem influx with heavy metal efflux. They correlated heavy metal resistance in the mutants by quantitative real time polymerase chain reaction (PCR) with increased expression of the heavy metal efflux pump CzcCBA and

its cognate two-component regulator genes *czcR-czcS*. The *P. aeruginosa* of two component sensor protein CzcS was subsequently found to be responsible for resistance to both zinc and Imipenem (Figure 1). Another study in *P. aeruginosa* by Conejo et al. (2003) showed that zinc eluted from silicone latex urinary catheters exerted a negative effect on the expression of OprD2, a membrane porin responsible for carbapenem resistance, which subsequently increased the overall resistance to this class of antibiotic.

NON-INHERITABLE DUAL RESISTANCE IN PSEUDOMONADS

There are some physiological states that render bacteria insensitive to antibiotics and other stress factors like metals. In general, slow growing or non-growing bacteria are less sensitive to antibiotics than actively growing cells. This property has been called drug indifference. It is a property shown by the whole population and so far no specific mechanism has been attributed to it. However, there are some specific physiological states in which bacteria show high resistance towards antibiotics and metals. As these states are transient, reversible and non-heritable, it is not correct to describe such states as resistance; instead, it would be more appropriate to call them stress -tolerant states. Three such stress-tolerant states have been described in previous studies (Brooun et al., 2000; Hayashi et al., 2000; Stewart and Costerton, 2001; Lewis, 2008; Jayaraman, 2008) namely biofilms, persistence and swarming.

Biofilm

Some bacteria have the ability to encase themselves in a hydrated matrix of polysaccharide and protein, forming a slimy layer known as a biofilm. *P. aeruginosa* is one of the best studied and well known biofilm producing organisms (Stewart and Costerton, 2001). Nichols et al. (1989) gave the opinion that cells of mucoid and non-mucoid *P. aeruginosa* in colonies were at least one thousand fold less sensitive to the antibiotics tobramycin or cefsulodin than were cells of the same bacteria in dispersed suspension. According to the CDC, 65% of all infections in developed countries are caused by biofilms, bacterial communities that settle on a surface and are covered by an exopolymer matrix (Hall-Stoodley et al., 2004). These include common diseases such as childhood middle ear infection and gingivitis; infections of all known indwelling devices such as catheters, orthopedic prostheses, and heart valves; and the incurable disease of cystic fibrosis. Biofilms are produced by most, if not all pathogens.

P. aeruginosa is reported to cause an incurable infection in cystic fibrosis patients (Singh et al., 2000). Stewart and Costerton (2001) in their study on contribution of the MDR-mediated efflux to antibiotic resistance of *P. aeruginosa* biofilms by using strains over expressing and lacking the MexAB-OprM pump, observed that though MDRs are responsible for the extrusion of chemically unrelated antimicrobials from the bacterial cell, *P. aeruginosa* biofilm resistance to ciprofloxacin, a substrate of MexAB-OprM pump, did not depend on the presence of this pump. Resistance of *P. aeruginosa* biofilms to ofloxacin was dependent on the expression of MexAB-OprM but only in the low concentration range. Unexpectedly dose-dependent killing indicated the presence of a small “super-resistant” cell fraction. This fraction was primarily responsible for very high resistance of *P. aeruginosa* biofilms to quinolones. Biofilms are considered to be the predominant growth phenotype of bacteria in industrial, clinical and environmental ecosystems and account for a number of recalcitrant infections in clinical settings, including *P. aeruginosa* (Costerton et al., 2003).

Teitzel and Parsek (2003) studied on biofilm and planktonic *P. aeruginosa* to examine the effects of the heavy metals copper, lead, and zinc. It was determined that biofilms were 2 to 600 times more resistant to heavy metal stress than free-swimming cells. Harrison et al. (2005) studied planktonic cells as well as biofilms of different bacteria including a *Pseudomonas* type strain. Six metals- Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+} and Pb^{2+}- were tested at different exposure time to biofilm and planktonic cultures grown in rich or minimal media. During their experiment they found that at 2 or 4 h of exposure, biofilms were approximately 2-25 times more tolerant to killing by metal cations than the corresponding planktonic cultures. Pseudomonad biofilms are also known for their ability to trap charged compounds in their extracellular polymeric substance matrix (Harrison et al., 2005). Harrison et al. (2007) also proposed a multi-factorial model by which biofilm populations can withstand metal toxicity by a process of cellular diversification.

Persistence

Bigger (1944) was the first to note that a culture of growing bacteria cannot be ‘sterilized’ by penicillin and named the surviving cells ‘persisters’. Little is known about persister bacteria (Lewis, 2000; 2001) and the underlying molecular mechanisms are still obscure. Antibiotic-sensitive bacterial populations have a small fraction ($\sim 10^{-6}$) of slow or non-growing, antibiotic-tolerant cells called persisters. How antibiotic-tolerant persisters arise only in a small fraction of the population while the majority of cells remain antibiotic- sensitive has been the subject of intense investigation for the last few decades (Jayaraman, 2009). While measuring a dose response of a *P. aeruginosa* biofilm to ofloxacin. Brooun et al. (2000) noticed that a small fraction of cells was not eliminated

even by very high levels of the antibiotic. Bagge et al. (2000) inferred that conventional antibiotic resistance can develop in biofilms treated repeatedly or for a long time. Stable de-repression of chromosomal -lactamase contributes to the persistence of *P. aeruginosa* biofilm infections. Harrison et al. (2005) suggested that the tolerance of *P. aeruginosa* to six metal cations *viz.* Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+} and Pb^{2+} was due to the presence of a small population of 'persister' cells along with metal sequestration in the biofilm matrix.

Swarming

Swarming is a state of multi-cellularity in many bacteria including pseudomonads, characterized by the migration of highly differentiated cells (swarm cells) on semi-solid surfaces. When swarm cells are subcultured in liquid media, they revert to the planktonic state. Planktonic cells first differentiate into long, multi- flagellated swarm cells, remain in close contact with one another and migrate as a raft (Harshey, 1994; 2003). Recently, Lai et al. (2009) have shown that swarm cells of *Escherichia coli*, *P. aeruginosa*, *Bacillus subtilis*, *Burkholderia thailandensis* and *Serratia marcescens* tolerate exposure to very high concentrations of antimicrobial elements like triclosan, arsenite and 13 different antibiotics.

CONCLUSION

It is well documented that there is a correlation between heavy antimicrobial stresses in an environment and recovery of antimicrobial resistant bacteria from that environment. Due to various anthropogenic activities, different metals are found in bulk in different environments. Pseudomonad is a versatile and cosmopolitan group of bacteria and many species of pseudomonad are clinically important pathogen. If metal gives a selective pressure in bacteria, it will generate dangerous trend because with that selective pressure of metal, antibiotics resistance also will spread in environment and will be a major challenge to medical science.

Though different reports are there in pseudomonads showing the ability of transferring resistance into members of same or other group through R-plasmid, still proper molecular mechanism behind the association is very obscure. Therefore, to understand this complex relationship between the resistance of metal and antibiotic, meticulous study is needed so that the proliferation and persistence of antibiotic resistance in environment can be precisely comprehended.

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REFERENCES

Abbas A, Edwards C (1989). Effects of metals on a range of *Streptomyces* species. Appl. Environ. Microbial., 55: 2030-2035.

Adarsh VK, Mishra M, Chowdhury S, Sudarshan M, Thakur AR, Ray Chaudhuri S (2007). Studies on metal microbe interaction of three bacterial isolates from east Calcutta wetland, OnLine J. Biol. Sci., 7(2): 80-88.

Aendekerk S, Ghysels B, Cornelis P, Baysse C (2002). Characterization of a new efflux pump, MexGHI-OpmD, from *Pseudomonas aeruginosa* that confers resistance to vanadium. Microbiol., 148: 2371–2381.

Akinbowale OL, Peng H, Grant P, Barton MD (2007). Antibiotic and heavy metal resistance in motile aeromonads and pseudomonads from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. Int. J. Antimicrob. Agents., 30: 177–182.

Al-Shahwani MF, Jazrawi SF, Al-Rawi EH, Ayar NS (1984). Growth and heavy metal removal by *Klebsiella aerogenes* at different pH and temperature. J. Environ. Sci. Health, Part A., 19: 445-457.

Angus BL, Carey AM, Caron DA, Kropinski AMB, Hancock REW (1982). Outer membrane permeability in *Pseudomonas aeruginosa*: comparison of a wild-type with an antibiotic-supersusceptible mutant. Antimicrob. Agents Chemother., 21: 299-309.

Appanna VD, Gazso LG, Pierre MS (1996). Multiple-metal tolerance in *Pseudomonas fluorescens* and its biotechnological significance. J. Biotech., 52: 75-80.

Bagge N, Ciofu O, Skovgaard LT, Hoiby N (2000). Rapid development *in vitro* and in vivo of resistance to ceftazidime in biofilm-growing *Pseudomonas aeruginosa* due to chromosomal beta-lactamase. APMIS., 108: 589-600.

Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV (2006). Co-selection of antibiotic and metal resistance. Trends Microbiol., 14(4): 176-182.

Barkay T, Miller SM, Summers AO (2003). Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol. Rev., 27: 355-384.

Bigger JW (1944). Treatment of staphylococcal infections with penicillin intermittent sterilization. Lancet., 244(6320): 497-500.

Brooun A, Liu S, Lewis K (2000). A dose-response study of antibiotic resistance in *Pseudomonas aeruginosa* biofilms. Antimicrob. Agents Chemother*.,* 44: 640-646.

Calomiris JJ, Armstrong JL, Seidler RJ (1984). Association of metal tolerance with multiple antibiotic resistance of bacteria isolated from drinking water. Appl. Environ. Microbial., 47(6): 1238-1242.

Chapman JS (2003). Disinfectant resistance mechanisms, cross resistance, and co-resistance. Int. Biodeterior. Biodegradation., 51: 271–276.

Conejo MC, García I, Martínez-Martínez L, Picabea L, Pascual Á (2003). Zinc eluted from siliconized latex urinary catheters decreases OprD expression, causing carbapenem resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother., 47(7): 2313–2315.

Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G (2003). The application of biofilm science to the study and control of chronic bacterial infections. J. Clin. Invest., 112: 1466-1477.

de Vicente A, Avilés M, Codina JC, Borrego JJ, Romero P (1990). Resistance to antibiotics and heavy metals of *Pseudomonas aeruginosa* isolated from natural waters. J. Appl. Bacteriol., 68(6): 625-632.

Duxbury T (1981). Toxicity of heavy metals to soil bacteria. FEMS Microbiol. Lett., 11: 217-220.

Erickson BE (2002). More than just dioxins in food. Environ. Sci. Technol., 36: 267A–268A.

Foster TJ (1983). Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbiol. Rev., 47: 361-409.

Hall-Stoodley L, Costerton JW, Stoodley P (2004). Bacterial biofilms:

from the natural environment to infectious diseases. Nat. Rev. Microbiol., 2: 95-108.
Harrison JJ, Ceri H, Turner RJ (2007). Multimetal resistance and tolerance in microbial biofilms. Nat Rev. Microbiol., 5(12): 928-938.
Harrison JJ, Turner RJ, Ceri H (2005). Persister cells, the biofilm matrix and tolerance to metal cations in biofilm and planktonic Pseudomonas aeruginosa. Environ Microbiol., 7: 981-994.
Harshey RM (1994). Bees are not the only ones: swarming in Gram negative bacteria. Mol. Microbiol., 13: 389-394.
Harshey RM (2003). Bacterial motility on a surface: many ways to a common goal. Annu. Rev. Microbiol., 57: 249-273.
Hayashi S, Abe M, Kimoto M, Furukawa S, Nakazawa T (2000). The DsbA-DsbB disulfide bond formation system of *Burkholderia cepacia* is involved in the production of protease and alkaline phosphatase, motility, metal resistance, and multi-drug resistance. Microbiol. Immunol., 44: 41-50.
Jacoby GA (1974). Properties of R plasmids determining gentamicin resistance by acetylation in *Pseudomonas aeruginosa.* Antimicrob. Agents. Chemother., 6(3): 239-252.
Jayaraman R (2008). Bacterial persistence: some new insights into an old phenomenon. J. Biosci., 35: 795-805.
Jayaraman R (2009). Antibiotic resistance: an overview of mechanisms and a paradigm shift. Curr Sci., 96(11): 1475-1484.
Johansen K, Andersen S, Jacobsen CS (1996). Phenotypic and genotypic characterization of phenenthrene-degrading fluorescent *Pseudomonas* biovars. Appl. Environ. Microbiol., 62: 3818-3825.
Karbasizaed V, Badami N, Emtiazi G (2003). Antimicrobial, heavy metal resistance and plasmid profile of coliforms isolated from nosocomial infections in a hospital in Isfahan, Iran. Afr. J. Biotechnol., 2: 379–383.
Karna RR, Sajani LS, Mohan PM (1996). Bioaccumulation and Biosorption of CO2+ by *Neurospora crassa*. Biotechnol Lett., 18: 1205–1208.
Krulwich TA, Lewinson O, Padan E, Bibi E (2005). Do physiological roles foster persistence of drug/multidrug- efflux transporters? A case study. Nat. Rev. Microbiol., 3: 566–572.
Kumar R, Joshi SR, Acharya C (2008). Metal tolerant *Bacillus* and *Pseudomonas* from Uranium rich soils of Meghalaya. Res. J. Biotechnol., Special Issue: pp. 345-350.
Lai S, Tremblay J, Deziel E (2009). Swarming motility: a multicelluar behaviour conferring antimicrobial resistance. Environ. Microbiol., 11: 126–136.
Lawrence JG (2000). Clustering of antibiotic resistance genes: beyond the selfish operon. ASM News, 66(5): 281-286.
Levy SB (2002). Active efflux, a common mechanism for biocide and antibiotic resistance. J. Appl. Microbiol., 92: 65-71.
Lewis K (2000). Programmed death in bacteria. Microbiol. Mol. Biol. Rev., 64: 503-514.
Lewis K (2001). Riddle of biofilm resistance. Antimicrob. Agents Chemother., 45: 999-1007.
Lewis K (2008). Multidrug tolerance of biofilms and persister cells. Curr. Top. Microbiol. Immunol., 322: 107–132.
Li Q, Wu S, Liu G, Liao X, Deng X, Sun D, Hu Y, Huang Y (2004). Simultaneous biosorption of cadmium (II) and lead (II) ions by pretreated biomass of Phanerochaete chrysosporium. Sep. Purif. Technol., 34: 135–142.
Marques AM, Congregado F, Simon-Pujol DM (1979). Antibiotic and heavy metal resistance of *Pseudomonas aeruginosa* isolated from soils. J. Appl. Bacteriol., 41: 341-350.
Matyar F, Akkan T, Uçak Y, Eraslan B (2010). *Aeromonas* and *Pseudomonas*: antibiotic and heavy metal resistance species from Iskenderun Bay, Turkey (northeast Mediterranean Sea). Environ. Monit. Assess., 167: 309-320.
Mergeay M, Houba C, Gerits J (1978). Extrachromosomal inheritance controlling resistance to cadmium, cobalt, copper, and zinc ions: evidence from curing in a Pseudomonas. Arch. Int. Physiol. Biochim., 86: 440-441.
Mindlin S, Minakhin L, Petrova M, Kholodii G, Minakhina S, Gorlenko Z, Nikiforov V (2005). Present-day mercury resistance transposons are common in bacteria preserved in permafrost grounds since the Upper Pleistocene. Res. Microbiol., 156: 994-1004.
Miranda CD, Castillo G (1998). Resistance to antibiotic and heavy metals of motile aeromonads from Chilean freshwater. Sci. Total. Environ., 224: 167-176.
Mukhopadhyay R, Rosen BP (2002). Arsenate reductases in prokaryotes and eukaryotes. Environ. Health Perspect., 110: 745-748.
Nichols WW, Evans MJ, Slack MPE, Walmsley HL (1989). The Penetration of Antibiotics into Aggregates of Mucoid and Non-mucoid *Pseudomonas aeruginosa.* J. Gen. Microbiol., 135: 1291-1303.
Nies DH (2003). Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microb Rev., 27: 313-339.
Nies DH (1999). Microbial heavy metal resistance. Appl. Microbiol. Biotechnol., 51: 730-750.
Nostrand JDV, Khijniak TV, Gentry TJ, Novak MT, Sowder AG, Zhou JZ, Bertsch PM, Morris PJ (2007). Isolation and characterization of four Gram-positive nickel-tolerant microorganisms from contaminated sediments. Microb. Ecol., 53: 670-682.
Novick RP, Roth C (1968). Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. J. Bacteriol., 95: 1335-1342.
Oyetibo GO, Ilori MO, Adebusoye SA, Obayori OS, Amund OO (2009). Bacteria with dual resistance to elevated concentrations of heavy metals and antibiotics in Nigerian contaminated systems. Environ. Monit. Assess. http://www.springerlink.com /content/5x212710x3114077/.
Perron K, Caille O, Rossier C, Delden CV, Dumas J, Ko¨hler T (2004). CzcR-CzcS, a two-component system involved in heavy metal and carbapenem resistance in *Pseudomonas aeruginosa*. J. Biol. Chem., 279: 8761-8768.
Raja CE, Anbazhagan K, Selvam GS (2006). Isolation and characterization of a metal-resistant *Pseudomonas aeruginosa* strain**.** World J. Microbiol. Biotechnol., 22: 577-585.
Raja CE, Selvam GS (2009). Plasmid profile and curing analysis of *Pseudomonas aeruginosa* as metal resistant. Int. J. Environ. Sci. Tech., 6(2): 259-266.
Roberts MC (2005). Update on acquired tetracycline resistance genes. FEMS Microbiol. Lett., 245: 195-203.
Ryan RP, Ryan DJ, Dowling DN (2005). Multiple metal resistant transferable phenotypes in bacteria as indicators of soil contamination with heavy metals. J. Soils. Sediments., 5(2): 95-100.
Sarma B, Joshi SR, Acharya C (2009). Metal tolerant fluorescent pseudomonads from uranium rich site of Meghalalya. Proceedings of International conference on Emerging Trends in Biotechnology, held at Banaras Hindu University, Varanasi, UP, India, pp. 154-155.
Silver S (1996). Bacterial heavy metal resistance: new surprises. Ann. Rev. Microb., 50: 753-789.
Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP (2000). Quorum sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature, 407: 762-764.
Spain A (2003). Implications of microbial heavy metal tolerance in the environment. Rev. Undergrad. Res., 2: 1-6.
Stewart TS, Costerton JW (2001). Antibiotic resistance of bacteria in biofilms. Lancet., 358: 135–138.
Stoppel RD, Schlegel HG (1995). Nickel-resistant bacteria from anthropogenically nickel-polluted and naturally nickel-percolated ecosystems. Appl. Environ. Microbiol., 61: 2276–2285.
Sundin GW, Demezas DH, Bender CL (1994). Genetic and plasmid diversity within natural populations of *Pseudomonas syningae* with various exposures to copper and streptomycin bactericides. Appl. Environ. Microbiol., 60(12): 4421-4431.
Teitzel GM, Parsek MR (2003). Heavy Metal Resistance of Biofilm and Planktonic *Pseudomonas aeruginosa.* Appl. Environ. Microbial., 69(4): 2313–2320.
Thakur D, Baishya M, Sarma B, Bora TC, Saikia R (2007). Antimicrobial Resistance in Gram Positive and Gram Negative Bacteria: Progress and Challenges. In Saikia et al. (eds) Microbial Biotechnology, New India Publishing Agency, India, pp. 349-375.
Timoney JF, Port J, Giles J, Spanier J (1978). Heavy-metal and antibiotic resistance in the bacterial flora of sediments of New York Bight. Appl. Environ. Microbial., 36(3): 465-472.
Valdman E, Erijman L, Pessoa FLP, Leite SGF (2001). Continuous biosorption of Cu and Zn by immobilized waste biomass *Sargassum* sp. Proc. Biochem., 36: 869-873.

Vesper SJ, Donovan-Brand R, Pete-Paris K, Al-Abed SR, Ryan JA, Davis-Hoover WJ (1996). Microbial removal of lead from solid media and soil. Water, Air Soil Pollut., 86: 207-219.
Volesky B (1994). Advances in biosorption of metals: Selection of biomass types. FEMS Microbiol. Rev., 14: 291-302.
Wright GD (2005). Bacterial resistance to antibiotics: enzymatic degradation and modification. Adv. Drug Deliv. Rev., 57: 1451-1470.