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Prevalence of Silver Resistance in Bacteria Isolated from Urinary Tract Infection

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ABSTRACT

Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels and damage to several internal organs. Several bacteria have naturally developed tolerance to a wide range of toxic heavy metals. Some bacteria have also evolved mechanisms to detoxify heavy metals. Among several metals, silver has been reported to possess medicinal property and hence used as an antibacterial agent. Inspite of this few silver resistant bacteria have been reported in clinical and environmental samples. Prevalence of silver resistance among several isolated strains provided the basis of the current investigation to determine the prevalence of silver resistance in clinical isolates. The goals of this study were 1) to isolate the uropathogens 2) to investigate the presence and prevalence of silver resistance of urine isolates taken from urinary tract infected patients and 3) to obtain the antibiogram pattern and 4) to determine the maximum tolerance range of silver by the isolated uropathogens, thereby standardize the concentration of silver as antibacterial agent.

Keywords: heavy metals; silver; uropathogens

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INTRODUCTION

Heavy metals can damage the cell membranes, alter enzymes specificity, disrupt cellular functions and damage the structure of the DNA. Toxicity of these heavy metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions[1]. Also, toxicity can occur as a result of alterations in the conformational structure of the nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance[2]. Due to the selective pressure from the metal in the growth environment, microorganisms have evolved various mechanisms to resist the heavy metal stress. Several metal resistance mechanisms have been identified: exclusion by permeability barrier, intra and extra cellular sequestration, active transport, efflux pumps, enzymatic detoxification, and reduction in the sensitivity of the cellular targets to metal ions[2]. Heavy metal contamination in the environment has become a serious problem due to the increase in the addition of these metals to the environment. Natural sources as well as the anthropogenic sources account for this contamination, which has become a threat to public health. In this perspective many approaches have been used to assess the risk posed by the contaminating metals in soil, water bodies etc. At present the tolerance of soil bacteria to heavy metals has been proposed as an indicator of the potential toxicity of heavy metals to other forms of biota[3]. Therefore, there is a dramatic increase in the interest on studying the interactions of heavy metals with microorganisms. The favoured approach now is selecting the organisms that can be used to develop tools to assess the metal levels in the environment[4]. It is said that metal resistance in a microorganism can facilitate a biotechnological process used in bioleaching and in bioremediation of metal contaminated environment.

MATERIALS AND METHODS

Test Chemicals and Media

Stock solutions of silver salts were prepared by dissolving the respective nitrate salt (Sigma) in MilliQ water. Working test metal solutions were prepared by diluting the concentrated stock solutions as required, and were sterilized by filtration. All glassware was acid washed before use to avoid binding of metal. All the media used in the experiments were dissolved in MilliQ water and sterilized by autoclaving.

Organism collection and identification. The micro-organisms used in this study were routinely isolated from the urinary tract infected patients attending a local hospital in Tiruchirappalli, South India. All micro-organisms were cultured and identified at Microbiology lab using standard protocols. All bacteria were screened for resistance to silver salt.

Determination of the Effect of Metals on Bacterial Growth

Toxicity of the selected metal to the bacterial isolates was determined using 10ug/ml concentration of the metal. 48 well sterile polystyrene microplates was used in this study as growth vessels. Sterile NB was amended with heavy metal and inoculated with exponentially growing cultures (24 h old, optical density of 0.090 at 600 nm) of bacterial isolates prepared in the same medium. Medium without metal but the bacterial inoculum (bacterial growth control) and medium with metal but without bacteria (abiotic control) served as controls. All the experiments were conducted in triplicates. Microplates were then closed with aluminum foil and sealed using additional laboratory film (Parafilm® M). The test microplates were incubated at 25ºC on an orbital shaker at 100 rpm. Bacterial growth was measured in terms of optical density at 600 nm at 0hr, 24 hrs and 48 hrs respectively.

Maximum tolerable concentrations

MTC of heavy metals

To determine the Maximum Tolerable Concentration (MTC) of the metal, several dilutions of the metal salt was prepared based on preliminary screening (1µg, 5 µ, 10 µg , 25 µg , 50 µg , 75 µg , 100 µg). To each set a bacterial culture was inoculated and plates were incubated and observed for growth by streaks on Nutrient agar plates. Strains that could not grow on 1.0 µg were termed as sensitive to the metal, while that which grew in 50 µg were further tested for higher concentration.

Antibiogram Pattern of metal treated organisms

Such metal treated bacteria were tested for antibiotic resistance following Bauer method[5]. The zones of inhibition were compared with that of metal untreated bacteria.

RESULT & DISCUSSION

Isolation and identification of bacteria

The collected bacteria were cultured and identified at Microbiology lab using standard protocols. The isolates obtained for this study were identified as *E.coli, Staphylococcus aureus* and *Pseudomonas aeruginosa sp.* based on standard biochemical tests.

Growth of bacteria with metal induction curves

To find out whether metal tolerance mechanism was inducible or not, growth curves of the chosen bacterial cultures were studied. Overnight cultures were inoculated in nutrient broth containing 10µg/ml of silver nitrate. Growth curves with respect to OD600 were obtained. It was observed that there was a steady increase in growth in *E.coli* even up to 48 hrs (Table 1), whereas in Pseudomonas *sp* and *S.aureus*, there was an initial growth up to 24hrs, followed by a dip at 48hrs.

Table: 1 Growth of bacteria treated with silver salt at 10µg/ml

Organism	OD at 600nm		
	0hr	24hr	48hr
Pseudomonas sp.	0.276	0.728	0.176
Staphylococcus aureus	0.442	2.321	0.176
E.coli	0.51	1.153	1.548

Maximum tolerable concentrations

MTC of heavy metals

All the three chosen isolates, (*S.aureus, E.coli and Pseudomonas aeruginosa*) were seen to grow upto 50 µg, while *E.coli* could tolerate metal upto 75 µg, and *S.aureus* was seen to grow even in 100 µg concentration (Table 2).

Table 2: MTC of heavy metals

Con. of metal	*S.aureus*	*E.coli*	*Pseudomonas sp.*
1µg	+	+	+
5µg	+	+	+
10µg	+	+	+
25µg	+	+	+
50µg	+	+	+
75µg	+	+	-
100µg	+	-	-

+indicates presence of growth, - indicates absence of growth

Antibiogram Pattern of metal treated organisms

Such metal treated bacteria when tested for antibiotic resistance, most of the bacteria demonstrated zones of inhibition greater than the metal untreated bacteria as shown in the table (Table 3).

Table:3 Comparison of the zones of inhibition of metal treated and untreated bacteria

Organisms	Zones of inhibition (mm)			
	Nx-10	IPM -10	AMC-30	Va-30
Pseudomonas sp.	15	20	7	7
Pseudomonas sp.-metal treated	17	21	7	7
Staphylococcus aureus	7	15	10	6
S. aureus- metal treated	9	17	15	7
E.coli	8	10	6	3
E.coli -metal treated	5	14	40	-

Heavy metals are found naturally in some environments at high concentrations[6]. Sometimes even long term contact with a few heavy metals can cause cancer and also reduced quality of life.

Silver is a precious metal with medicinal use as antimicrobial agent[7]. Inspite of these silver resistant bacteria have evolved[7]. Silver-resistant organisms have been reported in clinical[8-11] and environmental[12,13] samples. The genetic basis for silver resistance was first reported by McHugh[14] underscoring that silver resistance was plasmid-encoded and hence can be transported from one bacterium to another[15].This conclusion has been confirmed by others[16]. The physiological, biochemical, genetic, and structural studies of the silver-resistant determinant plasmid pMG101 established the molecular basis of silver resistance[17]. Prevalence of silver resistance among several isolated strains provided the basis of the current investigation to determine the prevalence of silver resistance in clinical isolates. Among the bacteria, the gram negative is more widespread in metal contaminated regions than gram positive. In general it is thought that metal tolerance is inducible. Bacterial resistance to antibiotics is a growing problem today. Antibiotic resistance has increased rapidly during the last decade, creating a serious threat to the treatment of infectious diseases. Drug resistance is one of the most serious global threats to the treatment of infectious diseases[18]. In addition to resulting in significant increases in costs and toxicity of newer drugs, antibiotic resistance is eroding our therapeutic armamentarium. Drug resistance is one of the most serious global threats to the treatment of infectious diseases[19].Metal tolerance and antibiotic tolerance behavior have revealed a very interesting pattern, where the strains that showed tolerance against the metal demonstrated tolerance against the antibiotics. This signifies an important observation regarding correlation of metal tolerance with antibiotic resistance. Products such as sterilants, disinfectants, heavy metals used in industry along with antibiotics create a selective pressure in the environment leads to mutations that allow them to better survive in the environment[20]. Thus in an environment with multiple stresses, it is important for the organism to acquire multiple resistance for its better survival. Calomeris[21] similarly reported multiple resistance of bacteria isolated from drinking water.

CONCLUSION

In our experiment, *E.coli* showed an increase at 24hrs and 48hrs in different concentrations in metal treated, but a decrease after initial increase in untreated. This explains that the tolerance mechanism may be inducible, where the bacteria could have oxidized or utilized the metal for its survival. The growth pattern in *Pseudomonas sp.* and *S.aureus* was different, where there was an initial increase, followed by a decrease in both metal treated and untreated, where the tolerance mechanism is rather constitutive and not inducible. Hence, in short there are prospects for future research on heavy metal tolerance in bacteria.

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