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Short Communication

Resistance of heavy metals on some pathogenic bacterial species

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Microorganisms are known to be highly sensitive to the presence of heavy metals and some of the early attempts to control microorganisms had used copper sulphate as plant fungicide and mercury salts for some infectious diseases; but, the order of toxicity varies among different organisms and in general mercury and silver are more toxic than manganese and zinc. It has been seen that responses of organisms to heavy metal occur at concentration considerably below those at which they response to alkali and alkaline earth metal occur. Here an attempt has been made to study the susceptibility and resistance pattern of three common pathogenic bacteria, *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus* against heavy metals. The inhibitory effect of different concentrations of five metal salts, namely chromium, nickel, iron, cobalt and zinc on microbial growth were studied using gel diffusion method. Results show that all three study organisms were completely resistant for all concentrations of chromium and iron salts. *E. coli* and *S. aureus* were most susceptible for zinc and nickel salts as compared to *K. pneumonia*. In all salts, zones of inhibition were increased along with increasing concentrations of salts and maximum inhibition was seen at 150 mM concentration. All the three microbes were highly susceptible for zinc.

Key words: Chromium, iron, metal salts, resistant.

INTRODUCTION

Microorganisms are ubiquitous in nature and involved in almost all biological processes of life. Heavy metals have been found in increasing proportions in microbial habitats because of rapid urbanization and natural processes (Issazadeh et al., 2013). Metals like nickel, copper (Clausen, 2000), cobalt and zinc have been playing a major role both directly or indirectly in almost all metabolic processes, growth and development of microorganisms (Tamer et al., 2013). However, increasing concentrations of metals beyond tolerance levels have forced these organisms to adapt to various biological mechanisms to cope with this condition (Nikaido, 2009). Some mechanisms like metal efflux systems, complexation, reduction of metal ions or utilization of the metal as a terminal electron acceptor in anaerobic respiration helps microbes to tolerate heavy metal accumulation (Nageswaran et al., 2012). Bacteria that is resistant to such heavy metals

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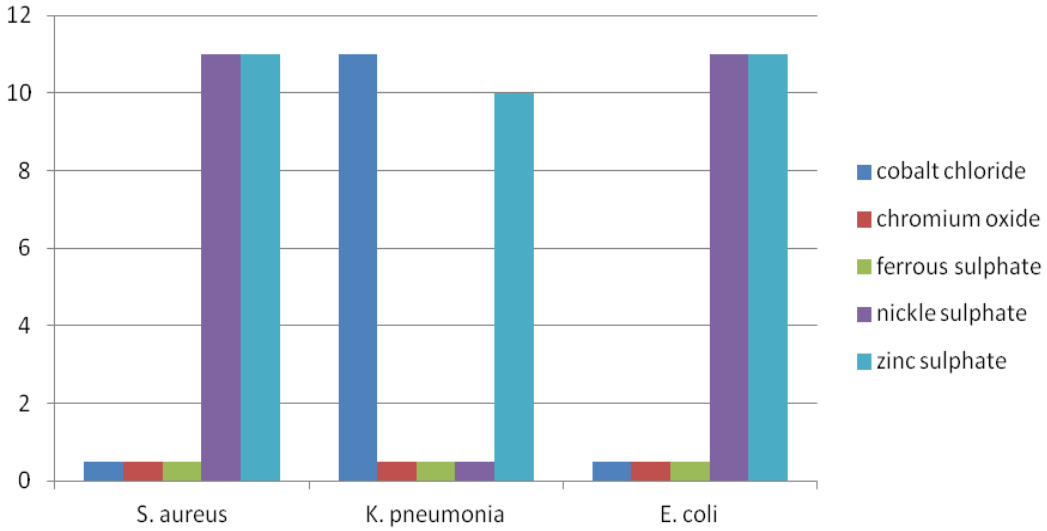

Figure 1. Inhibition of test organisms by metal salts at 10mM concentration. The X-axis represents the test organisms and the Y-axis represents the diameter of Zone of inhibition, measured in mm. Total resistance of organisms towards chromium and ferrous was observed, whereas only Klebsiella was inhibited by 10 mM $CoCl_2$).

(Narasimhulu et al., 2010) and have the ability to grow in high concentrations of these metals play an important role in their biological cycling which has great potential in bioremediation of poorly cultivable soil high in heavy metal content (Nyamboya et al., 2013). The present study is formulated to evaluate the effect of increasing metal salt concentration on growth of bacteria. Five metals such as cobalt chloride, zinc sulphate, chromium oxide, ferric chloride and nickel sulphate were used for metal tolerance tests against three strains (*K. pneumonia, E. coli* and *S. aureus*) in which *K. pneumonia* and *E. coli* are Gram negative whereas *S.aureus* is Gram positive.

MATERIALS AND METHODS

Test organisms

Three common organisms *S. aureus* (ATCC25923), *E. coli* (ATCC25922) and *K. pneumonia* (ATCC700603) were included in the study. The organisms were made as stock by mixing 100 µl of suspension in 10 ml of sterile nutrient broth and grown overnight. The organisms were maintained by subculturing them on nutrient agar at regular intervals and used throughout the study.

Preparation of metal salts

Five metal salts such as cobalt chloride, zinc sulphate, chromium oxide, ferric chloride and nickel sulphate were used in this study. One molar stock solution of metal salts was prepared, from which dilutions of different molarities (10, 50, 100 and 150 mM) were prepared.

Testing of microbial susceptibility to metal salts

The microbial susceptibility test was done by gel diffusion method. After preparing Nutrient Agar plates, four wells of 0.5 mm width and 0.5 mm depth were made at equal distance on each plate aseptically. Separate plates were inoculated with 50 µl of *E. coli*, *K. pneumonia* and *S. aureus*. For each test organism, 50 µl of salt solutions of different molarities was put in the wells and plates were incubated at 37°C for 24 and 48 h. Antimicrobial activity was expressed in terms of zone of inhibition (mm). Each experiment was repeated thrice and average was taken.

RESULTS AND DISCUSSION

Though many metals are essential for growth, some can be harmful for living organisms (Reilly, 1991). This is mainly due to the fact that heavy metals form complexes with protein molecules and inactivate them (Shanker et al., 2004). Three different microbes were taken in this study and their susceptibility patterns were studied against heavy metal salts. The organisms responded in a variety of patterns. Zones of inhibition were observed at different concentrations of salts and the results are demonstrated in Figure 1.

For cobalt chloride, *K. pneumonia* was found to be more susceptible than the other two. Figure 1 shows that $CoCl_2$ at 10 mM concentration was highly susceptible for *K. pneumonia* strain while the other microbes have demonstrated resistance against the provided concentration of metals. At increasing concentrations, all three organisms were inhibited and showed no resistance.

There was no inhibition on growth for any of the test organisms with CrO_2 and $FeSO_4$ salt solution and all microbes showed complete resistance till the highest concentration used; that is, 150 mM.

The results for nickel sulphate ($NiSO_4$) demonstrated that *S. aureus* and *E. coli* had a high susceptibility and

less resistance to the metal. *K. pneumonia* was resistant at the lowest used concentration; that is, 10 mM; however it was inhibited by increasing the concentration of $NiSO_4$. *E. coli* has shown largest zone of inhibition at all concentrations of $NiSO_4$, indicating more susceptibility for the salt when compared with the other two.

All the test organisms showed a strong susceptibility towards the $ZnSO_4$ salt and growth was inhibited for all the test bacteria with *K. pneumonia* being least affected.

A number of studies have shown that microorganisms have the capacity to resist antibiotics and heavy metals, which may be extremely harmful to human being and animals (Samanta et al., 2012; Monchy et al., 2003; Silver, 1996). The current study also demonstrates effective inhibition of growth of the microbes by salts. In future, the work can be extended to some other salts, some other strains of bacteria and at lesser and higher concentrations. Much more studies in this regard are required to be done. The metal tolerant nature of bacteria has tremendous potential in the bioremediation of heavy metal accumulation in soil and water (Karthikeyan and Kulakow, 2003) and also in the treatment of sewage and toxic wastes (McIntyre, 2003).

Conflict of interests

The authors did not declare any conflict of interest.

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