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Antibacterial Properties of Some Metals on Isolated Drinking Water Bacteria (*E. coli* and *S. aureus*)

Raj Kumar[1*], **Dr. Subhash Chander Dhuria**[2]
[1]Phd Student, Department of Microbiology, Tantia University, Sri ganganagar, Rajasthan, India
[2]Assistant professor, Department of Microbiology, Tantia University, Sri ganganagar, Rajasthan, Indaia

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***Corresponding author**
Raj Kumar

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Abstract: The drinking water (potable water) is that water which is safe for human consumption .The analysis of drinking water determines the identification of bacteria and the effect of some other metals on them. The work was carried out in Sri Ganganagar zone. The samples were collected from 15 to 35 km. area of Sri Ganganagar,Rajasthan. The work was completed in the department of microbiology of Tantia University, Sri Ganganagar. Isolation and cultivation of *Escherichia coli* (*E.coli*) and *Staphylococcus aureus*(*S.aureus*) was done by using varies isolation techniques like serial dilution method, spreading etc. and applied different media like nutrient agar, Eosin Methylene Blue , MacConkey agar and special media and various biochemical reactions. Therefore, *S.aureus* and *E.coli* strains were selected for this antibacterial study. Both gram positive and gram negative microorganisms were used for the test and Al, Zn, Mn, and Fe metals were selected for study of antimicrobial effects. Prepared the cultured plate of *E.coli* and *S.aureus* and add the metals in the middle of the Petri plate and after overnight incubation obsevered the effect of metals. The result shown that Mn has no antimicrobial effect but Al, Fe and Zn has antimicrobial properties. Fe shown strong effect as compared to Al and Zn. The object of this study to investigate the antimicrobial effect of metals such as Mn, Al, Zn and Fe on drinking water bacteria such as *E.coli* and *S.aureus*. Antimicrobial properties of metals will be use for purification of drinking water and remove the bacterial contamination from the drinking water with the help of metals.

Keywords: *Staphylococcus aureus*, *Escherichia coli*, Zinc, Aluminium, Manganese, Iron and Drinking water.

INTRODUCTION

Natural resources are the important wealth of our country, water is one of them. Water is a wander of the nature. "No life without water '' is a common saying depending upon the fact that water is the one of the naturally occurring essential requirement of all life supporting activities [1] . It is an important natural resource on earth and necessary for all living organisms, ecological system, human health, food production and economic development [2]**.**Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, rain, springs, and wells [3]**.** People obtain their consumable water from surface and ground water [2]**.** In India, most of the population is dependent on surface water (damp water) as the only source of drinking water supply. People usually collect drinking water from surface sources like ponds, wells, streams, municipal pipes, stored water from tanks or storage level itself. Water may become contaminated at any point between collection, storage, serving at homes [4-6]**.**

Water related diseases continued to be one the major health problems globally [7-9]**.** According to World Health Organization [10] each year 3.4 million people, mostly children, die from water-related diseases [10] According to United Nations Children's Fund [11] assessment, 4000 children die each day as a result of contaminated water[11]**.**Ideally drinking water should be free from pathogenic microorganism and free from bacteria indicative of fecal pollution [12]**.**The General Assembly of United Nations [13] has declared the access to clean water, a basic human right. Unfortunately many developing countries are still facing the problem of water related diseases due to the consumption of contaminated drinking water containing pathogens.

Several metal oxides in form of nanoparticles have been reported to exhibit marked antibacterial activity allowing efficient eradication of various bacterial strains. This fact has attracted significant interest of environmental, agricultural and health care industries that are searching for newer and better agents to control or prevent bacterial infections. Many studies have been undertaken to explain the efficacy and mechanisms of antibacterial action of metal oxide nanoparticles but the existent literature is still controversial and incomplete. Metal oxide nanoparticles may show bacteriostatic or bactericidal effect. In case of

bacteriostatic effect, treated bacteria do not die but stop to reproduce or grow.

ZnO nanoparticles showed bactericidal effects on Gram-positive and Gram-negative bacteria as well as the spores which are resistant to high temperature and high pressure [14, 15]**.** reported comparative investigation of antimicrobial activity of ZnO, CuO, and Fe_2O_3 nanoparticles against Gram-negative (*E.coli* and *Pseudomonas aerogenosa* (*P. aeruginosa*)) and Gram-positive (*S. aureus* and *Bacillus subtilis* (*B.subtilis*) bacteria. According to their results, the most bactericidal activity was reported for the ZnO nanoparticles while Fe_2O_3 nanoparticles exhibited the least] antibacterial effect [15). In particular, ZnO reduces the bacteria viability. However, the exact mechanism of its antibacterial activity has not been well understood so far. One proposed possibility is the generation of hydrogen peroxide as a main factor of the antibacterial activity. It is also believed that, the accumulation of the particles on the bacteria surface due to the electro-static forces could be another mechanism of the antibacterial effect of ZnO particles [16]. ZnO nanoparticles in higher concentrations and larger surface area displayed better antibacterial activity [17]. Small size of ZnO particles can easily penetrate into bacteria cells and may release toxic metal ions upon dissolution. Smaller particles are usually the most efficient antibacterial agents [18].The antibacterial activity of zinc oxide nanoparticles towards *E.coli* was studied by [19] and the oxide nanoparticle was observed to disrupt the membrane structure of the gram negative organism. Zinc oxide nanoparticles are commonly used in antibacterial formulations. But there are instances in which the zinc oxide nanoparticles in aqueous media tend to aggregate into large flocculates due to their hydrophobic nature thus inefficiently interacting with the microorganism.The antibacterial activity of the Aluminium oxide nanoparticle against bacterial strains *Klebsiella Pneumonia, Salmonella typhi, and Vibrio cholera* was determined by zone of inhibition

The Indian Ayurveda describes storing water in a copper vessel overnight and drinking it in the mornings for many health benefits. Storing water in copper and silver pots finds mention in ancient texts of Ayurveda for purification of water [20-22]**.** Copper is known for its antimicrobial effect [21]**.** Combination of metals is more effective antibacterial activity such as Fe- doped ZnO nanoparticles efficiently inhibit *E.coli* bacterial growth without being toxic to mammalian cells [23,14] Combined zinc oxide with iron oxide to produce magnetic composite nanoparticles with improved colloidal stability and effective antibacterial activity against *Staphylococcus aureus* (*S.aureus*) and *E. coli*. Cu and Zn also inhibit the growth of some gram positive and gram negative bacteria [24]Also, ZnO nanoparticles doped with both Mn and Fe ions (10 % molar ratio) exhibited higher antibacterial activities a compared to pure ZnO when incubated with *S.aureus*, *E.coli*, *K.pneumoniae*, *S.typhi*, *P.aeru ginosa* and *B. subtilis* [25]**.**Thus combination of metals shown higher efficiency against Gram-negative than against Gram-positive bacteria.

In the past, there have been several reports on the use of antibacterial property of metals such as copper, silver and their alloys for water purification. Generally, this technique is used in large scale, commercial water disinfection units in which metal ions are generated by electrolytic or chemical means, and the technique is found to be quite effective [26, 27] have reported that the water stored in the copper and silver vessels have antimicrobial, anti- inflammatory, antioxidant and anti-carcinogenic activities. So Inorganic metal oxide nanoparticles can be used as effective disinfectants in view of their non-toxic profile, stability and antibacterial properties

OBJECTIVES

- Investigate the antimicrobial properties of Al, Mn, Zn and Fe on drinking water bacteria such as *E.coli* and S. aureus.
- Antimicrobial properties of the metals use for purification of drinking water.
- To develop a new biological method for purification of drinking water.

MATERIALS AND METHODS

The organisms were identified from the drinking water on the basis of cultural and Biochemical activities. Fresh drinking water Samples were collected 15 to 35 km area of Sri Ganganagar, Rajasthan. Cultural characteristics include Gram reaction, morphological characteristics and Catalase activity. This forms the basis of primary identification of bacteria. It was carried out on various agars like (Nutrient agar) (Himedia laboratories), MCA (MacConkey Agar) (Himedia laboratories) and EMB (Eosine Methylene Blue Agar) (Himedia laboratories) were used. Also special media like HiCrome Aureus Agar (Himedia laboratories), Enterococcus Agar (Himedia laboratories) and HiCrome UTI Agar were used respectively .Growth characteristics on specific media are studied for quick identification of bacteria. Organisms have given different colour on specific media due to the presence of specific chromogen. While Biochemical activities include a series of test specially IMViC, which forms the basis of secondary identification. The presence or absence of enzyme is detected by following these biochemical tests. Also, amylase production and fermentation of carbohydrates is also carried out by using starch and TSIA media. *E.coli* and *S.aureus* were isolated from the water sample and were used for check the antimicrobial effect of metal on these bacteria.

Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* were widely used to bacterial experiment. *S. aureus* and *E. coli* live on drinking water and sometimes occur infection to them.

Furthermore, they show their unique cell envelope structure of Gram positive and Gram negative bacteria. Therefore, *S. aureus* and *E.coli* strains were selected for this antibacterial study both gram positive and gram negative microorganisms were used for the test. The gram positive organism includes S. *aureus*, and gram negative bacteria include *E. coli*. Bacterial strains were maintained on freshly prepared nutrient agar media. Metals such as Zn, Mn, Fe, and Al were selected for study of antimicrobial effect. All the metals were pure. All metals were in the form of palates. Metal pieces were cleaned using abrasive, washed in distilled water, and finally dried in hot air oven. For antimicrobial effect prepared the nutrient agar media and pour into the Petri plate and settled down the media. Take 1 ml culture of *E.coli* and *S. aureus* and spared on different Petri plate with the help of spreader total 5 Petri plate were prepared for *E.coli* and 5 Petri plate for *S.aureus*. Out of these one Petri plate was used for control .with the help of puncher prepared the wells in the middle of each Petri plate except controls. Add appropriate quantity of metals such as Fe, Mn, Zn and Al on different cultured plate of *E.coli* and *S. aureus*. After addition of metals Petri plates were incubated at 37^{o} c for 24 hr to 48 hrs. After 24hrs check the antimicrobial effect of metal on *E.coli* and *S. aureus* cultured plates and measured the diameter of zone.

RESULTS AND DISCUSSIONS

Table-1: result effect of different metals on Identified Organisms

S. No.	Isolates From drinking water	Metals			
		Mn	Al	Zn	Fe
1	*E. coli*	G +	G – (1.8 mm)	G – (0.8mm)	G – (3.1mm)
2	*Staphylococcus aureus*	G +	G – (1.8mm)	G – (1.9mm)	G – (3.8mm)

Plate analysis

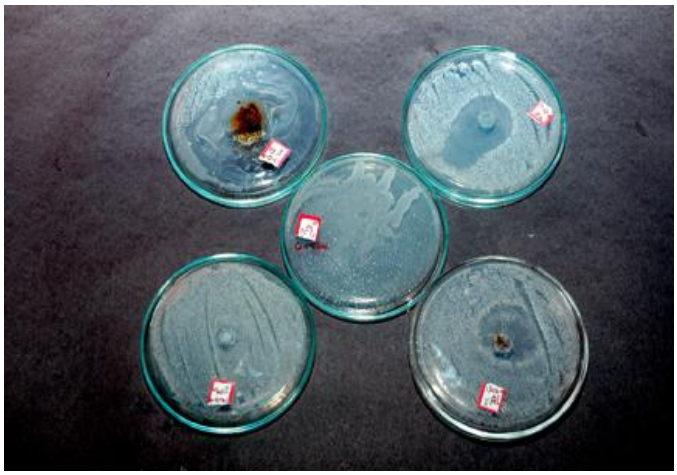

Fig-1: Effect of metals on *E.coli*

Fig-2: Effect of metals on *S. aureus*

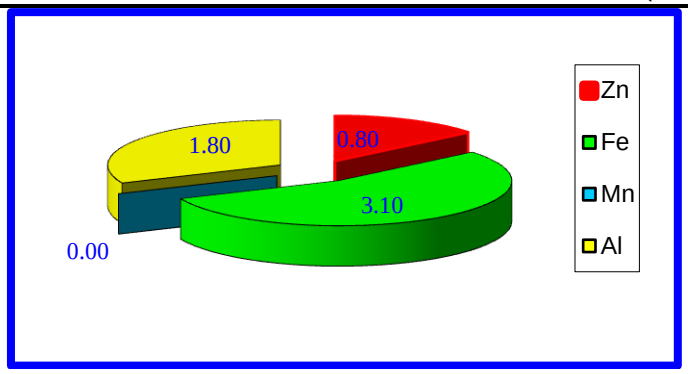

Fig-3: Effect of Different Metals on Eschericia coli

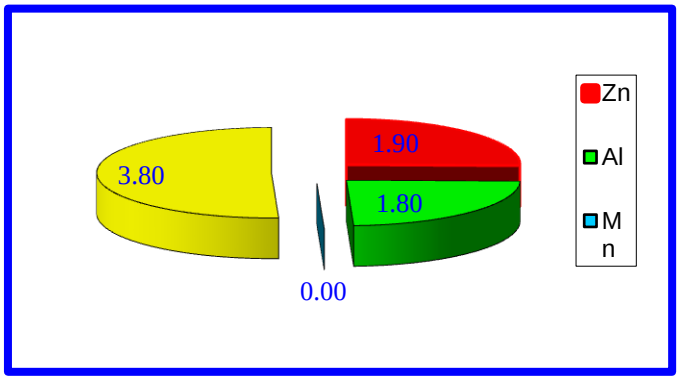

Fig-4: Effect of Different Metals on Staphylococcus aureus

Effect of different metals such as Zn, Fe, Mn, and Al on the drinking water organisms *E.coli* and *S.aureus* have been studied. The results of metal effect are shown in table No.1 Results show that metals have both stimulatory and inhibitory effect. So, different metals have shown different effects on the drinking water organisms. Table no. 1 Show the measurement of Zone, inhibition and enhancement of growth by metal. The variation of response to metal given by *E.coli* is shown graphically in fig. 3 and by *S.aureus* in fig. no.4 and all the response were also shown in plate analysis. Accroding to table no.1 and fig no.3 and fig no.4 metals like Mn have enhanced the growh of *E.coli* and *S.aureus* while Al, Fe, and Zn inhibit the growth of bacteria. The inhibition of growth is shown by the formation of zonation around the metal and enhancement of growth is shown by the presence of microbes around the metal.

Fig. no.3 and table no.1 show the effect of metals on *E.coli*. Fe has highest antimicrobial effect because the inhibition zone of iron was large. The size of inhibition zones that was 3.1mm in diameter and Al was the second largest inhibition zone .the size of inhibition zone in case of Al was 1.8mm in diameter. The effect of Zn on *E.coli* also shows inhibition zone that was 0.8mm in diameter. Fe was most effective antimicrobial agent for *E.coli*, Al and Zn also shown antimicrobial effect. But Mn has no antimicrobial effect they shown enhancement of the growth of *E.coli*.

Fig. no.4 and table no.1 show the effect of metals on *S.aureus*. Fe have highest antimicrobial effect because the inhibition zone of iron was large .The size of inhibition zone that was 3.8 mm in diameter and Zn was the second largest inhibition zone .the size of inhibition zone in case of Zn was 1.9 mm in diameter. The effect of Al on *S.aureus* also shows inhibition zone that was 1.8 mm in diameter. Fe was most effective antimicrobial agent for *S.aureus*, Al and Zn also shown antimicrobial effect. But Mn has no antimicrobial effect they shown enhancement of the growth of *S.aureus*.

The germicidal activity of metals depends on the metals and its property. Previous studies on the antibacterial activity of zinc and aluminum have confirmed that metal ions are responsible for the inactivation of the bacteria However, there seems to be no clear-cut explanation of the inactivation mechanism taking place within the cells of these microorganisms. Some reports suggest that metal ions bind to DNA, enzymes and cellular proteins in the bacteria causing cell damage and death [28].

Other studies shown zinc oxide nanopaticles had antibacterial effects on *E.coli* K88, which is partly in accordance with the reports by [29-32]**.** They reported that zinc oxide or its nanoparticles had antibacterial activity against *S. aureus*, *Streptococcus*, *E. coli* 745 and *E. coli* O157:H7. However, the antibacterial concentrations were inconsistent [29] revealed that 179 and 1790 µg/ml zinc oxide could exhibit a clear antibacterial effect on *S. aureus* [32]**.** The

effect of metal ions on Staphylococcus aureus was done by biochemical and mass spectrometric analyses. [36]**.**

Furthermore, the mode of action underlying their antibacterial effects is not well understood, although a few studies have proposed several mechanisms of antibacterial activity of ZnO nanoparticles , including: (i) the induction of reactive oxygen species including hydrogen peroxide (H_2O_2), which is a strong oxidizing agent harmful to bacterial cells [30**,**33**,** 34**,** 35] (ii) the damage on cell membrane and interaction of intracellular contents with ZnO nanoparticles [19]**.**

CONCLUSIONS

- Some metals such as Fe, Al, and Zn have antimicrobial properties against *E. coli* and *S.aureus* .
- Fe has strong antimicrbial effect as campare to Al and Zn.
- There is no antimicrobial effect of Mn. Mn shown enhancement the growth of E.coli and S.aureus.
- Antimicrobial effect of Fe, Al, and Zn can be used for treatment of drinking water because *E.coli* and *S.aureus* are predominant bacteria of drinking water. so these metals can be used for purification of drinking water instead of chlorine and other chemical substance.

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