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ANTIBACTERIAL ACTIVITY OF ZINC SULPHATE AND SILVER NITRATE AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Metals enroutes various metabolic pathways forming the enzymatic suffixes, permeability indices or chelates of plasma. Through these entities either they promote or decline the metabolic activity depending upon the metal and targeted organism. In the present study the antimicrobial activity of zinc sulphate and silver nitrate was evaluated against human pathogenic *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli*. The antimicrobial assay was performed by agar well diffusion method. The minimum inhibitory concentration (MIC) of zinc sulphate was observed to be the lowest for *Escherichia coli* i.e. 1.2±0.035mg/ml and of silver nitrate was lowest against *Staphylococcus aureus* i.e.0.05±0.035. Both metal salts exhibited good antimicrobial performances. The bactericidal effect and a broad activity spectrum of these compounds reveal their scope as suitable candidates for the higher step of drugs fabrication.

KEYWORDS: Zinc Sulphate, Silver Nitrate, *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli*, MIC.

INTRODUCTION

Since ages, metal has been deployed for their antimicrobial activities in ethnic literature. These metals were utilised in the form of vessels or additive coins or as an ornamental junction. Silver and copper vessels are the two examples of widely used metals of this category which were used undoubtedly among various cultures. Although in lag back,

awareness about pathogenic microbial source was not adequate, but practice to eradicate the source of infection and contamination through metallic sources were commonly practiced.

Antimicrobial metals have a rich history of use in medicine as well. The use of silver nitrate ($AgNO_3$) to prevent gonorrhoeal eye infections in newborns[1] and a range of wound dressings with slow-release Ag compounds including Acticoat, Actisorb Silver and Silverlon are used to prevent infection of surgical wounds.[2] Over the past two centuries, physicians have also used Te, Mg and As oxides, as well as Cu and Hg salts, to treat diseases such as leprosy, tuberculosis, gonorrhoea and syphilis.[3,7] The medicinal use of metals was prevalent until the discovery of antibiotics by Nobel laureate Sir Alexander Fleming in the 1920s, at which point these applications rapidly diminished. Now, at the beginning of the twenty-first century, with the burgeoning of multidrug resistance and the dearth of new antibiotics in the pipeline, the use of antimicrobial metals is undergoing a renaissance.

Zinc is an essential element required for life and found in many enzymes; zinc ions can be effective as antimicrobials even at low concentration. Zinc compounds have been described since at least Roman times as an ancient ingredient in eye disease treatment and zinc tablets were found in a small medical container dating back to 140–130 BC retrieved from a Roman shipwreck.[8]

There is no known beneficial role for silver in metabolism and it is highly toxic to bacteria.[9] We have not been able to find any evidence in the literature for the use of silver compounds as antimicrobials in agriculture, except for the use of silver iodide (AgI) in cloud seeding. The use of silver as an antibacterial agent was first reported to have occurred over 2000 years ago in drinking water containers[2] and silver is still widely used in water filters and in other treatments for potable water, or as an algicide for swimming pools.

In present study two metal salts, viz. $ZnSO_4$ and $AgNO_3$ were characterized for the antibacterial activity against certain human pathogenic organism. The study is based on zinc (Zn) and silver (Ag) compounds against pathogenic microorganism like *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli*.

MATERIALS AND METHODS

Materials

Metal salts Zinc sulphate ($ZnSO_4.7H_2O$) (Sigma) and Silver nitrate ($AgNO_3$) (Sigma) were purchased from market.

Test microorganisms and growth media

Clinical isolates of the test organisms *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* were obtained from RNT Medical College, Udaipur (Rajasthan). Cultures were authenticated by biochemical characterization.

Preparation of Zinc Sulphate solution

A stock solution of Zinc Sulphate was prepared by dissolving 1 g of $ZnSO_4$.$7H_2O$ in 10 ml of distilled water to obtain a concentration of 100 mg/ml. The solution was sterilized by filtration, using a 0.2µ Millipore filter. The zinc sulphate solution ranging from 10 to 100 mg/ml concentration were prepared by diluting stock solution by using autoclaved distilled water.

Preparation of Silver Nitrate solution

A stock of silver nitrate solution was prepared by dissolving 0.1 g of silver nitrate in 10 ml of distilled water to obtain a concentration of 10 mg/ml. The solution was sterilized by filtration, using a 0.2µ Millipore filter. Silver nitrate solutions ranging from 1 to 10 mg/ml were prepared by diluting stock with autoclaved distilled water.

Determination of Antibacterial activity

The antimicrobial activity of zinc sulphate and silver nitrate solution were analyzed through agar well diffusion method according to Murray et al.[10] The Muller-Hinton agar medium was poured onto the petriplates with an inoculums size of 10^6 colony forming units (cfu)/ml of bacteria. The wells (6mm diameter in size) were done by using borer. Metal salt solutions and control solutions dispensed into the wells and were allowed to diffuse for 45 min following incubation at 37°C for 24 h. The tetracycline solution (10mg/ml) and distilled water used as positive and negative control respectively. The analysis carried out in triplicate and the sensitivity of the microbial species to the extract was determined by measuring the diameter of the inhibitory zones.

Determination of Minimum Inhibitory Concentration

Broth micro dilution method: For the broth micro dilution test 50µl of each bacterial suspension in suitable growth medium was added to the wells of sterile 96 well micro titre plate already containing 50 µl of two fold serially diluted metal salt solution in proper growth medium. The final volume in each well was 100µl. Control wells were prepared with culture medium, bacterial suspension only, and metal salts with broth only. The content of each well

were mixed on a micro plate shaker. The MIC was the lowest concentration where no viability was observed after 24 hr on the basis of metabolic activity.[11] To indicate respiratory activity the presence of colour was determined after adding 20µl of TTC (20mg/ml dissolver in distilled water) and incubated under appropriate cultivation condition for 30min in dark.[12] All measurement of MIC values was taken in triplicate.

RESULTS

Antibacterial Activity

The range of concentration of $ZnSO_4$ & $AgNO_3$ evaluated for antibacterial activity. The minimum concentration used for assay was 10mg/ml for $ZnSO_4$ which showed a clear zone of more than 10 mm around the well against all the three pathogenic cultures (Table-1). The maximum of 33±0.2 mm zone of inhibition obtained against *E.coli* at 70 mg/ml to 100 mg/ml concentration. Silver nitrate has also shown a clear zone of inhibition of more than 10 mm around the well against all the three pathogenic culture (Table- 2). The maximum of 15± 0.2 mm zone of inhibition obtained against *S.aureus* at 5mg/ml concentration.

The clear zone of inhibition observed against all the three pathogenic cultures for standard antibiotic tetracycline (positive control) on 10mg/ml is shown in Table-3.

Minimum Inhibitory Concentration (MIC)

In the present investigation MIC values were recorded for $ZnSO_4$ & $AgNO_3$ (Table 4) against all three pathogenic cultures. The lowest MIC value of $ZnSO_4$ was observed against *E.coli* i.e. 1.2±0.035 mg/ml and for $AgNO_3$ was against *S.aureus i.e.*0.05±0.035mg/ml. The highest MIC value observed against *S.aureus and K.pneumoniae* for $ZnSO_4$ and $AgNO_3$ respectively i.e 1.8 ± 0.023 and 0.09±0.032 mg/ml.

Table 1: Antibacterial activity of $ZnSO_4$ at a range of concentrations against pathogenic bacteria is required. For this water by Agar well diffusion Assay.

S.No	Conc. of $ZnSO_4$(mg/ml)	Zone of inhibition (mm)		
		Escherichia coli	*Klebsiella pneumoniae*	*S.aureus*
1	10	18 ±0.2	10±0.3	11±0.15
2	20	23±0.3	12±0.3	15±0.2
3	30	24±0.2	14±0.2	18±0.2
4	40	26±0.3	15±0.3	20±0.2
5	50	27±0.2	18±0.2	21±0.15
6	60	31±0.3	21±0.3	22±0.2
7	70	33±0.2	25±0.3	24±0.3
8	80	33±0.2	27±0.2	25±0.2

9	90	33±0.2	29±0.2	26±0.3
10	100	33±0.2	29±0.2	26±0.2

Table 2: Antibacterial activity of $AgNO_3$ on various concentrations against various pathogenic Bacteria by Agar well diffusion Assay.

S.No	Conc. of $AgNO_3$(mg/ml)	Zone of inhibition(mm)		
		Escherichia coli	***Klebsiella pneumoniae***	***S.aureus***
1	1	10±0.3	11±0.1	13±0.2
2	2	12±0.1	13±0.1	12±0.1
3	3	11±0.2	10±0.3	12±0.3
4	4	11±0.3	12±0.2	14±0.2
5	5	11±0.2	12±0.1	15±0.1
6	6	11±0.1	11±0.3	12±0.3
7	7	11±0.1	10±0.1	12±0.1
8	8	13±0.3	10±0.3	12±0.3
9	9	12±0.1	10±0.3	10±0.2
10	10	12±0.2	10±0.1	11±0.1

Table 3: Antibacterial activity of positive and negative control by Agar well diffusion Assay.

Name of pathogenic organism	Zone of inhibition (mm)	
	Tetracyclin (10mg/ml) Positive control	Distilled water Negative control
E. coli	15	nil
K. pneumoneae	12	nil
S. aureus	12	nil

Table 4: Minimum Inhibitory Concentration (MIC) of $AgNO_3$ and $ZnSO_4$ against certain pathogenic bacteria by Broth Dilution Method.

Name of pathogenic organism	Minimum Inhibitory Concentration (mg/ml)	
	$AgNO_3$	$ZnSO_4$
E. coli	0.06±0.025	1.2±0.035
K. pneumoneae	0.09±0.032	1.4±0.023
S. aureus	0.05±0.035	1.8±0.023

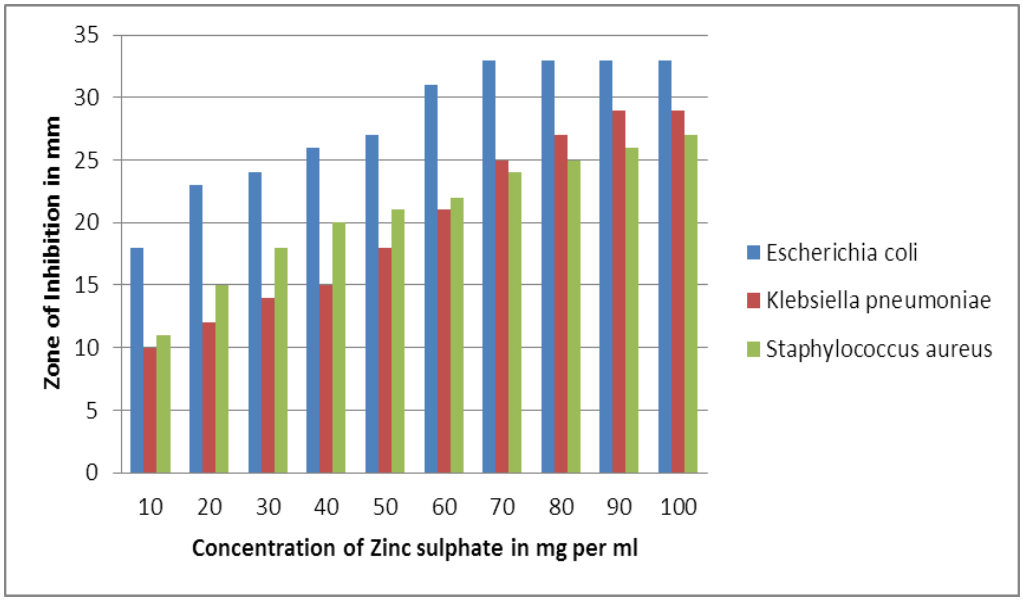

Fig. 1: Anti bacterial activity of zinc sulphate solution against human pathogenic Bacteria.

*Values are mean ±SD of three replication (n=3), SD: standard deviation.

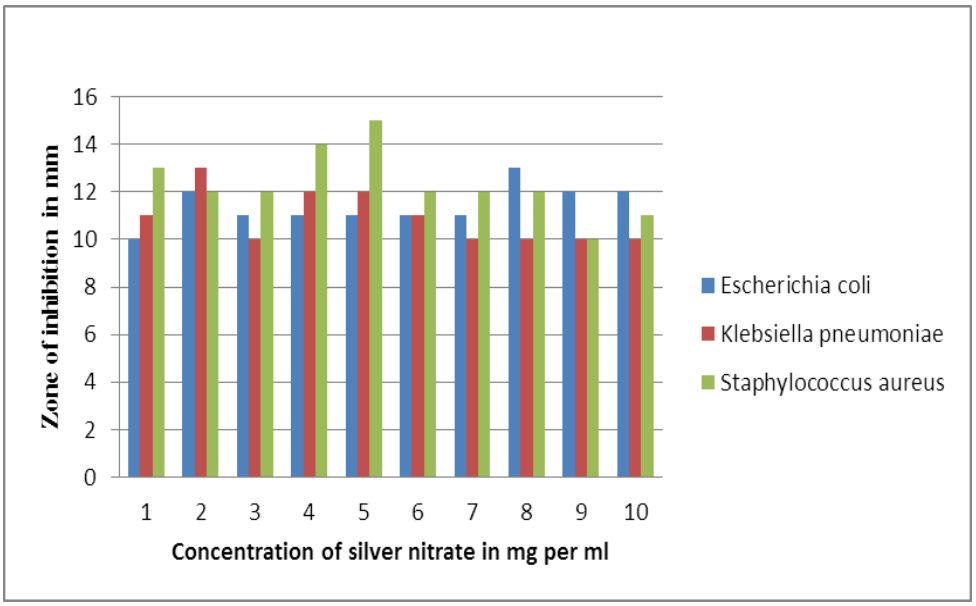

Fig. 2: Anti Bacterial activity of silver nitrate solutions against human Pathogenic Bacteria.

*Values are mean ±SD of three replication (n=3), SD: standard deviation.

DISCUSSION

In view of the issues associated with the use of antibiotics to treat the chronic infection, a search for non-antibiotic antimicrobial agents with strong activity against the pathogenic bacteria. Water soluble salts of silver and zinc were selected for in vitro testing in view of their known antimicrobial properties and low potential for toxicity when delivered locally.

Zinc Sulphate in the form of $ZnSO4.7H_2O$ was used in this study because this agent has been widely administered for supplementation in infectious disease and has proven to be effective in reducing the frequency and severity of infection.[13] An additional advantage is that these compounds lack the side effects and resistance issues associated with conventional antibiotics. Our in vitro study shows that zinc sulphate at concentrations between 1.2 and 1.8 mg/ml inhibited growth of pathogenic bacteria. In contrast silver nitrate at concentrations 0.05 and 0.09 mg/ml inhibited growth of pathogenic bacteria. The antibacterial activity of zinc sulphate was also reported against enteric pathogens by Surjawidjaja et al.[14] While the strong and selective antibacterial activity of silver nitrate reported against periodontal pathogens.[15]

Zinc supplementation has been associated with clinical reduction in duration and severity of diseases in infants and young children.[16,18] The dosage of zinc supplementation used in India as reported by Sazawal et al.[13] was 20 mg daily which is equal to 88 mg zinc sulphate ($ZnSO4\ 7H_2O$) is much higher as zinc sulphate has high potential to inhibit growth against pathogenic organism.

An important consideration with regard to the use of silver as an antimicrobial is the potential for the development of resistance. The effectiveness of silver in the treatment of burns and wounds and the more recent success found with newly developed silver catheters is an indication that induction of silver resistance is uncommon.[19,20] In addition, although cross-resistance between conventional antibiotics is frequently encountered, silver resistance genes afford resistance only to silver and do not alter susceptibility to conventional antibiotics.[20,21]

A clear understanding of the mechanism(s) underlying the antimicrobial activity of silver and copper has yet to be identified.[22] There is, however, evidence to suggest that the antimicrobial activity of silver, copper and zinc may result from their ability to bind to essential enzyme sulfahydrals.[22,23]

CONCLUSION

In conclusion, we have demonstrated through antimicrobial testing, the strong and selective activity of silver nitrate and zinc sulphate against human pathogens. This result, together with its continuing effectiveness in local delivery applications and low potential for toxicity, suggests silver nitrate and zinc sulphate may be a valuable antimicrobial for sustained release local delivery in the treatment of infections.

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