Antibacterial Effect of Different Concentrations of Zinc Sulfate on Multidrug Resistant Pathogenic Bacteria

Revised: 05.02.2020

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Article History:

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Submitted: 09.01.2020

ABSTRACT Background: The most important reason for the appearance and distribution of strains of bacteria with multi-drug resistant is the overuse of antibiotics which lead to search for agents that might have antibacterial properties.

Objective: The present study designed to investigate the growth inhibitory effect of different concentrations of zinc sulfate against multidrug resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, Proteus spp., Klebsiella spp., *Pseudomonas aeruginosa* and Enterobacter spp.

Method: The agar well diffusion technique was used to establish the antibacterial action of zinc sulfate by measuring the zone of inhibition diameter.

Results: All concentrations of zinc sulfate showed antibacterial effect. The maximum antibacterial effect of zinc sulfate had shown with (10mg/ml) concentration for *Staph.aureus*, *Staph.epidermidis* and

klebsiella whereas the maximum antibacterial effect of zinc sulfate had shown with (14mg/ml) concentration for *E.coli, Enterobacter, proteus and pseudomonas.* Conclusion: Different concentrations of zinc sulfate were found to have antibacterial effect against multidrug resistant *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,* Proteus spp., Klebsiella spp., *Pseudomonas aeruginosa* and Enterobacter spp.
Key words: zinc sulfate, multidrug resistant
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Accepted: 05.03.2020

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DOI: <u>10.5530/srp.2020.3.32</u> @Advanced Scientific Research. All rights reserved

INTRODUCTION

Zinc is a vital biological trace element and it is an essential constituent of every day food intake. Zinc deficiency may cause numerous pathological symptoms ⁽¹⁾. Dietary phytate, iron and oxalate and certain drugs including sodium valproate and penicillamine inhibit the absorption of zinc from the diet ⁽²⁾. Zinc supplements are usually indicated to alleviate some conditions, such as zinc-deficient states, upper respiratory infection, diarrhea, wound healing and human immunodeficiency virus ⁽³⁾. Zinc considered as an environmentally friendly substance ⁽⁴⁾. It is found in the dental plaque and in saliva ⁽⁵⁾. Because of the antibacterial action, zinc successfully and frequently intended for dermatological uses in lotions and ointments ⁽⁴⁾.

The useful outcome of zinc in acute infantile diarrhea, management of common cold and acrodermatitis enteropathica has been reported ⁽⁶⁾. Zinc lack, which considered common in kids in developing countries, make them at high possibility of infectious diseases ⁽⁷⁾.

There are different mechanisms by which zinc ion can affect bacterial cells for instance inhibition of glycolysis and increasing proton permeabilities to membranes of the bacterial cells ⁽⁸⁾.

AIM OF STUDY

The aim of the present study was to establish the *in vitro* antibacterial effect of different concentrations of zinc sulfate against multidrug resistant *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,* Proteus spp., Klebsiella spp., *Pseudomonas aeruginosa* and Enterobacter spp.

MATERIALS AND METHODS

The bacterial strains which used in the current study were clinical strains, they are: Staphylococcus aureus, Staphylococcus epidermidis, Proteus spp., Escherichia coli, Pseudomonas aeruginosa, Klebsiella spp. and Enterobacter spp. Zinc sulfate was solubilized in distilled water to obtain the final concentrations. Five concentrations used (2mg/ml, 6mg/ml, 10mg/ml, 14mg/ml, and 20mg/ml). The agar well diffusion technique was used to establish the antibacterial action of zinc sulfate ⁽⁹⁾ in which the diameters of the zone of inhibition were considered ⁽¹⁰⁾. Bacterial suspension of approximately 1.0× 10⁶ cell/ml in sterile normal saline was prepared as described by Forbes ⁽¹¹⁾. The culture medium was inoculated with every one of the tested bacteria. Six millimeter diameter wells were punched into the agar and filled with 0.1 ml of each concentration. Erythromycin (15 µg / disc), cephalexin (30 μg /disc), clindamycin (2 μg /disc) and Tetracycline (30 μg /disc) were used as positive control. The plates were then incubated at 37°c for 24 hrs. After incubation, the antibacterial action of zinc sulfate was established by measuring the zone of inhibition diameter in millimeter; the sample was tested in duplicate.

RESULTS

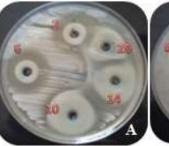
The seven types of bacteria examined in the study found to be resistant to Erythromycin (15 μ g / disc), cephalexin (30 μ g /disc), clindamycin (2 μ g /disc) and Tetracycline (30 μ g /disc).

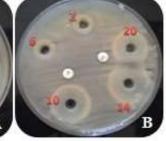
All concentrations of zinc sulfate showed antibacterial effect. A summarized information about the zone of inhibition by using the agar well diffusion technique were provided in table (1). The maximum antibacterial effect of zinc sulfate had shown with (10mg/ml) concentration for *Staph.aureus*, *Staph.epidermidis* and *klebsiella* whereas the maximum antibacterial effect of zinc sulfate had shown

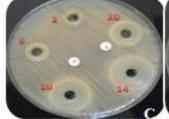
with (14mg/ml) concentration for *E.coli*, *Enterobacter*, *proteus and pseudomonas*.

| Bacteria | Mean of Zone of inhibition | | | | |
|----------------------------|----------------------------|-----------------|------------------|------------------|------------------|
| | Conc. 2mg/ml | Conc. 6mg/ml | Conc. 10mg/ml | Conc. 14mg/ml | Conc. 20mg/ml |
| Staphylococcus.aureus | 28 | 25 | 38 | 31.5 | 26.5 |
| Staphylococcus.epidermidis | 14.5 | 13 | 25.5 | 22 | 21 |
| klebsiella | 14 | 13 | 25.5 | 25 | 21.5 |
| E.coli | 16.5 | 15.5 | 22.5 | 27 | 23.5 |
| Enterobacter | 13.5 | 13.5 | 26 | 26.5 | 22.5 |
| proteus | 15 | 13.5 | 24.5 | 26.5 | 22.5 |
| pseudomonas | 13.5 | 12 | 24 | 28 | 20.5 |

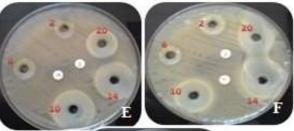
Table 1: Antibacterial activity of Zinc sulfate on tested bacteria











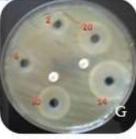


Figure 1: Effect of Zinc sulfate on Mean Zone of Inhibition of (A): *Stap. Aureus* (B): *Stap. Epidermidis* (C): *Klebsiella* (D): *E.coli* (E): *Enterobacter* (F): *Proteus* (G): *Pseudomonas aeruginosa*

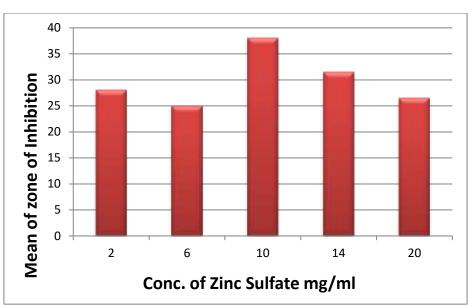


Figure 2: Effect of Zinc Sulfate on Mean Zone of Inhibition of Staph. Aureus

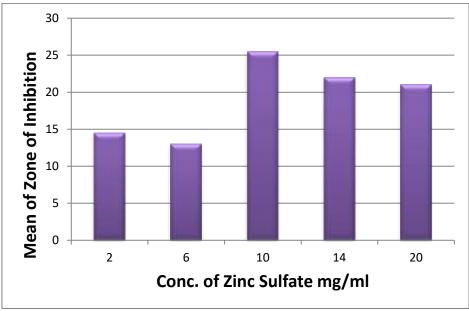


Figure 3: Effect of Zinc Sulfate on Mean of Zone of Inhibition of Staph. epidermidis

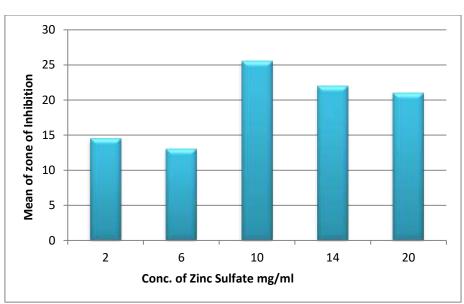


Figure 4: Effect of Zinc Sulfate on Mean of Zone of Inhibition of Klebsiella

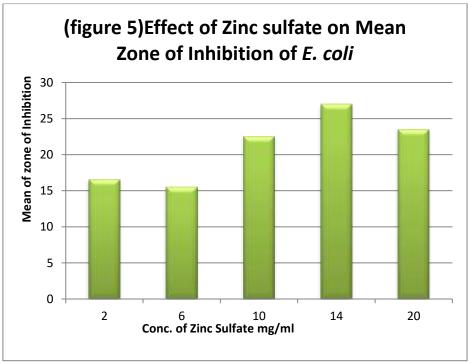


Figure 5: Effect of Zinc Sulfate on Mean of Zone of Inhibition of E. coli

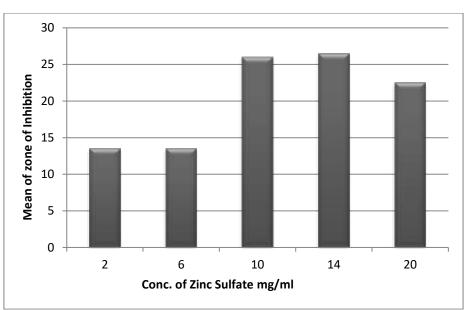


Figure 6: Effect of Zinc Sulfate on Mean of Zone of Inhibition of Enterobacter

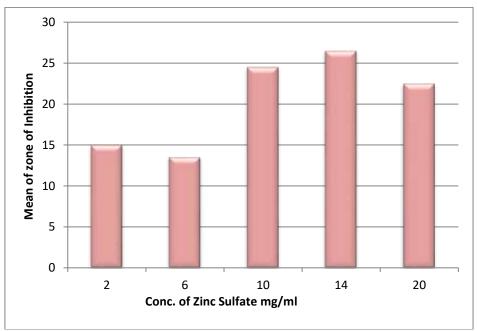


Figure 7: Effect of Zinc Sulfate on Mean of Zone of Inhibition of Proteus

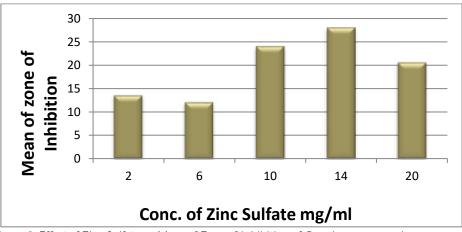


Figure 8: Effect of Zinc Sulfate on Mean of Zone of Inhibition of Pseudomonas aeruginosa

DISCUSSION

The study was considered to explore the possible antibacterial effect of zinc sulfate on different types of bacteria. Zinc used in five concentrations and the mean of zone of inhibition was determined for the seven types of bacteria used. All concentrations of zinc sulfate which were used give antibacterial effect (in all concentrations there were zone of inhibition).

Zinc salts found to have in vitro action on different pathogens, including human immunodeficiency virus, herpes simplex virus and Chlamydia trachomatis (12 ^{,13,14,15,16,17)}. There was a significant decrease in the incidence of pneumonia in children in developing countries by administering zinc supplementation ^(18, 19). Zinc have shown antiviral effects against certain viruses. Clinical researches showed that zinc considerably decreased the length of symptoms during rhinovirus infection ^(20, 21). Zinc enhances the human response to numerous infections and has vital function in the homeostasis of the immune system ⁽²²⁾. The dose of zinc demonstrated in previous study was 20 mg/day that equivalent to 88 mg zinc sulfate (23), approximately 5-26% of the zinc sulfate will be absorbed ⁽²⁴⁾. Zinc administration showed a decrease in length and severity of diarrhea in infants and children (25, 26). The possible mechanisms for the effect of zinc sulfate on diarrhea included enhanced the intestinal absorption of water and electrolytes, renewal of intestinal epithelium and the return of its function, improved levels of enterocyte brush border enzymes and immunological mechanisms for elimination of infection ^(27, 28).

In 2003, Surjawidjaja *et al* ⁽²⁹⁾ established that zinc showed an antimicrobial action on enteric pathogens. So, zinc has a dual effect, physiological therapeutic effect and an antimicrobial action.

In double-blind placebo-controlled trials of zinc supplementation, zinc decreased the occurrence and the period of both acute and chronic diarrhea. Zinc also reduced the incidence and duration of acute lower respiratory tract infections in infants and children ^(30, 31). Zinc administration for patients of sickle cell anemia resulted in lowering the occurrence of staphylococcus aureus pneumonia, streptococcus pneumonia tonsillitis, and *E. coli* urinary tract infections ⁽³²⁾.

CONCLUSION

Different concentrations of zinc sulfate were found to have antibacterial effect against multidrug resistant *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,* Proteus spp., Klebsiella spp., *Pseudomonas aeruginosa* and Enterobacter spp.

ACKNOWLEDGEMENT

The authors are grateful to college of pharmacy/ Mustansiriya University for performing the analytical methods in this study.

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