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The Efficacy of Zinc and Manganese in Controlling Methicillin-Resistant Staphylococcus aureus Wound Infections in vitro

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Chapter I: Introduction

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common nosocomial and community acquired bacterium resistant to beta-lactam antibiotics such as penicillin and its derivatives. *Staphylococcus aureus* infections were successfully treated with penicillin until the emergence of antibiotic resistant strains of the bacterium in the late 1940s. The symptoms of MRSA are similar to *S. aureus* infections, including various skin infections, toxic shock syndrome, and necrotizing pneumonia. The majority of MRSA infections start in wounds and other breaches to the integrity of the skin usually caused by trauma, surgery, or medical devices. An average of two in one hundred people are carriers of MRSA, and it is a leading cause of hospital-acquired infections. MRSA derives its resistance by producing a penicillin-binding protein (PBP2a) that binds with a lower affinity to beta-lactam antibiotics. In addition, there are a variety of other proteins and genetic elements that increase the pathogen's resistance to commonly used antibiotics. The ability of MRSA to form a biofilm is a concern further enhancing resistance. Because of the difficulty in treating MRSA, alternative methods are important in combating this pathogen. Historically, metals have been used for thousands of years in medicine. It is known that copper and silver possess antimicrobial properties, however there is less knowledge on the antimicrobial effects of zinc and manganese. This project investigates the efficacy of natural dietary minerals, namely zinc (Zn) and manganese (Mn) in treating wound infections of MRSA *in vitro*.

Experiments were conducted to establish the sub inhibitory concentration (SIC) and minimum inhibitory concentration (MIC) of Zn and Mn on three strains of MRSA. In addition, the effect of Zn and Mn on increasing MRSA sensitivity to oxacillin was

159 determined. Finally, the effect of Zn and Mn on MRSA adhesion and invasion on human
160 keratinocytes was studied *in vitro*. Future applications of this study include the use of Zn
161 and Mn in treating wound infections of MRSA with or without antibiotics. This study is
162 expected to contribute to the development of a topical ointment, gel, or patch that could
163 potentially be used for treating and controlling wounds infected with MRSA.
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Chapter II: Literature Review

History

Staphylococcus aureus, first discovered in the 1880s, was a significant nosocomial and community pathogen that led to fatal infections (23). The introduction of penicillin in the 1940s lead to successful treatment and control of the pathogen (20). However, by the late 1940s *S. aureus* strains resistant to penicillin emerged (20). Subsequently, methicillin with beta-lactamase-resistant properties was introduced in 1959 (17), however, S. *aureus* developed resistance to methicillin with the first case of Methicillin Resistant *Staphylococcus Aureus* (MRSA) reported in the United States in 1968 (20). Vancomycin is the current antibiotic of choice used to treat MRSA infections, however there are growing concerns of Vancomycin Resistant *Staphylococcus aureus* (17).

Biology

MRSA are gram-positive cocci, catalase positive, non-motile, non-spore forming, salt tolerant facultative anaerobes (10). MRSA viewed under scanning electron microscopy appears as characteristic grape-like clusters. MRSA is a misnomer as strains of MRSA are resistant to several beta lactam antibiotics, including penicillin, amoxicillin, oxacillin, methicillin, cephalosporin, and carbapenem (15). MRSA can be tested through broth microdilution testing, cefoxitin disk screen test, latex agglutination test for PBP2a, or plating with oxacillin (15). Warren et al. (30) reported that PCR detection for the *mecA* gene had a 91.7% sensitivity compared to traditional culture methods and took significantly less time for confirmation of MRSA.

Prevalence and Transmission

S. aureus is commonly found on skin surfaces, including the respiratory tract and nasal passageway. The pathogen has the ability to form biofilms, which presents a significant issue during cleaning of healthcare settings. The epidemiology of MRSA is commonly split into healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA). HA-MRSA is defined as MRSA infections that occur following healthcare setting stays or within a 12-month timeframe of a medical procedure. In HA-MRSA, transmission is common via contaminated hands and hospital surfaces. In a study at Grady Memorial Hospital in Atlanta Georgia, researchers found that 7.3% of patients had nares culture positive for MRSA (14). In addition, the National Nosocomial Infections Surveillance System over a period of 12 years reported a MRSA prevalence of 60% in the intensive care unit (2). It is also known that MRSA is the leading cause of surgical site infections in community hospitals (2).

Clinical Presentation and Wound Infections

Symptoms of MRSA are similar to *S. aureus* infections, including various skin infections such as folliculitis, cellulitis, impetigo, abscess, and boils (2). The majority of MRSA infections start in wounds and other breaches to the integrity of the skin usually caused by trauma, surgery, or medical devices. Normally, *S. aureus* are found in one-third of the population posing no health risk to healthy individuals even if small wounds or cuts get infected (9). However, MRSA infections of wounds can lead to more serious health implications, including necrotizing fasciitis, necrotizing pneumonia, and infective endocarditis (2). The treatment for wounds infected with MRSA is on a case-by-case basis

based on a variety of factors, including the level of intrusion into soft tissue. Treatment typically involves a combination of incision, drainage and prescription of an antibiotic. The antibiotics of choice include vancomycin, clindamycin, trimethoprim-sulfamethoxazole, tetracylcines, and oxazolidinones (17). However according to Daum et al. (7), the data comparing the effectiveness of antimicrobial agents against MRSA is lacking. Specialized treatments against MRSA include hyperbaric oxygen therapy where increased oxygen levels help promote tissue healing.

Antibiotic Resistance and Virulence

Staphylococcus aureus strains are considered methicillin resistant if they contain the *mec* gene and have an oxacillin MIC $\geq$ 4 μg/mL (17). MRSA have the mobile genetic element, staphylococcal cassette chromosome, that at minimum contains the *mec* gene (21). Resistance occurs when a penicillin-binding protein (PBP2a) encoded on the *mec* gene binds with a lower affinity to beta-lactam antibiotics allowing transpeptidase activity to continue as normal for cell wall assembly (15). In addition, two regulatory components, namely *mecR1-mecI* and beta-lactamase genes (*blaI, blaRI, and blaZ)* act as negative regulators of *mecA* transcription leading to varied resistance levels (17). Auxiliary genes like *fem* affect the level of resistance by building pentaglycine cross-bridges that link glycan chains enhancing the PBP2a protein (28). Salt concentration, pH, temperature, and osmolarity can affect resistance levels (28).

MRSA possesses many virulence factors that enhance its ability to colonize and proliferate in host cells. Notably, Panton-Valentine leukocidin (PVL) is a cytotoxin that causes cell destruction and tissue death (17). In a study conducted in a Veterans Medical

Center, researchers found that 60% of all MRSA isolates contained the genes for the PVL toxin (12). Other virulence factors include alpha-hemolysin toxin, phenol soluble modulins, and arginine catabolic mobile element (17).

Biofilm Formation in Wound Infections

MRSA has the ability to form biofilms that increase its resistance to antibiotics, the ability to evade host immune response, and causes prolonged healing times. Biofilms form when bacteria adhere to a surface and embed themselves in a matrix of extracellular polymeric substances (EPS). The sequence of biofilm development consists of attachment to a surface, EPS production and growth of microbes, and finally maturation and dissemination (24). The development of biofilms includes quorum sensing between microorganisms and water channels that serve for delivery of nutrients and excretion of wastes (24). The ability to form biofilms is a major factor that increases the virulence of MRSA, especially in wound infections and its persistence on medical devices.

The molecular mechanism of biofilm formation of MRSA is not fully understood. In one study, 48 genes were found to be induced and 84 genes repressed during biofilm formation compared to planktonic conditions indicating that biofilm formation requires an adaptive response (6). Atshan et al. (5) using qPCR demonstrated the role of genes encoding fibronectin binding protein A and B, clumping factor B, elastin binding proteins, and intracellular adhesion protein C during specific stages of biofilm formation. The study showed that biofilm formation is a complex interplay of activation of different genes in a temporal organization. In another study, Ando et al. (3) observed that MRSA isolates from patients with urinary tract infections from inserted catheters expressed higher *hla*, *hlb*, and

fnbA gene products compared to catheter-unrelated cases. Roche et al. (27) created a murine wound biofilm model to test the efficacy of antimicrobial agents on biofilms, and found that the antimicrobial agents had reduced effectiveness 24 hours after *S.* aureus inoculation compared to 4 hours after inoculation.

Natural Antimicrobials

Emerging antibiotic resistance in pathogens is a significant and growing public health issue that directly impacts patient care. It is estimated that 63,000 patients in the United States die from hospital-acquired bacterial infections per year (1). The need for novel treatments is critical to control antibiotic resistant bacteria. A majority of the antimicrobials used in the clinical settings are derived from the golden era of antibiotic discovery from limited ecological niches and taxonomic groups. To increase diversity of compounds, research needs to be conducted for identifying potential antimicrobials from marine environments, plants, and fungi. This research focuses on the potential therapeutic application of two natural essential minerals, namely Zn and Mn in wounds infected with MRSA. Traditionally transition metals have been used for thousands of years in medicine. Egyptians used copper as an astringent, silver was used to prevent infection of surgical wounds, and mercury salts were used to treat diseases such as leprosy and tuberculosis (16). Silver is a well-known transition metal that has gained traction as an antimicrobial agent in the healthcare field for coating coat surgical tools, catheters, and furniture among other fomites. The proposed mechanism is that silver disrupts chemical bonds in bacteria cells leading to cell death (28). Commercial products like Silvazine showed zones of inhibition against all 200 tested *S. aureus*, *staphylococci* and *pseudomonas aeruginosa*

isolates (11). In addition, Lemire et al. (16) described the various antimicrobial mechanisms of metals, including generating reactive oxygen species, antioxidant depletion, disrupted membranes, and genotoxicity. This research will determine the antimicrobial efficacy of Zn and Mn for potential application in treating wounds infected with MRSA.

Zinc and Manganese

Both Zn and Mn are naturally occurring essential microelements recommended for daily intake by the United States Food and Drug Administration. These minerals are present in a wide range of foods in addition to being used as dietary supplements. Zn is an essential mineral involved in the catalytic activity of about 100 enzymes besides playing roles in immune function, protein synthesis, DNA synthesis and cell division (21). The recommended dietary allowance for Zn for an average adult is between 8 mg for females and 11mg for males (21). Zn is known to exert antimicrobial properties against *S. aureus*, *S. epidermis*, and *P. aeruginosa* (4). Xie et al. (31) showed that zinc oxide had bactericidal effects on *Campylobacter jejuni*. McDevitt et al. (19) reported that a high Zn to Mn ratio caused increased sensitivity of *Streptococcus pneumoniae* to oxidative stress and polymorphonuclear leucocyte killing. Zn complexes utilized as antimicrobial wound dressings showed partial killing of *S. aureus* (25). Similarly, Mn is an essential trace mineral that plays a role in the development of connective tissue and hormones, and is necessary for fat and carbohydrate metabolism, blood sugar regulation, nerve function, and antioxidant production (18). The recommended daily dietary allowance of Mn is 1.8 mg in females and 2.3 mg in males. Compared to silver and Zn, Mn is not as well studied with

regards to its antimicrobial properties. Rahman et al. (26) found that the concomitant use of Mn salt with a variety of antibiotics increased the activity of the antibiotic. However, the majority of the literature on Mn is based on its use in metal complexes. To date there is no research on Mn for controlling wound infections.

Summary

The objective of this study was to investigate the antimicrobial properties of Zn and Mn on MRSA for future applications in wound infection treatments. The specific objectives included determining:

1. The sub inhibitory concentration (SIC) and minimum inhibitory concentration (MIC) of Zn and Mn on three strains of MRSA.
2. The effect of SIC and MIC of Zn and Mn on increasing MRSA sensitivity to oxacillin.
3. The effect of Zn and Mn on MRSA adhesion to and invasion of human keratinocytes.

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Chapter III: Efficacy of Zn and Mn in Controlling Methicillin-Resistant *Staphylococcus aureus* Wound Infections *in vitro*

ABSTRACT

The emergence of Methicillin-Resistant *Stapylococcus aureus* has triggered an increased interest in finding alternative natural antimicrobials to control the pathogen and combat growing antibiotic resistance. This study investigated the antimicrobial effect of two naturally occurring essential minerals, zinc (Zn) and manganese (Mn) on MRSA for potential application in wound infections. The sub inhibitory concentration (SIC) and minimum inhibitory concentration (MIC) of Zn and Mn against MRSA were determined. The effect of MIC and 2x MIC of Zn and Mn in increasing MRSA susceptibility to oxacillin, and the effect of SIC and MIC of these minerals on MRSA cell adherence and invasion of human keratinocytes were investigated. The SIC and MIC of Zn and Mn against MRSA were 0.4 mM and 2.5 mM, and 1.6 mM 4.7 mM, respectively. Both metals in combination with oxacillin were more effective in reducing MRSA than oxacillin alone. In addition, both Zn and Mn significantly reduced MRSA adhesion and invasion of human skin cells. Results indicate that the aforementioned antimicrobials can be potentially used to control MRSA wound infections, however further studies are warranted.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nosocomial and community acquired pathogen, which is one of the common causes of bacterial infection in humans (2). According to the Emerging Infections Program and National Healthcare Safety Network, it is estimated that a total of 75,000 MRSA infections occur every year (15). In addition, two in 100 people are carriers of MRSA (15). An estimated 63,000 patients in the United States die each year from hospital-acquired bacterial infections (1). The symptoms of MRSA are similar to other *S. aureus* infections, which include impetigo, folliculitis, abscesses, pneumonia, endocarditis, and sepsis (21). MRSA infections are attributed to increased mortality rates and hospital costs contributing to a total estimate of $1.87 billion in additional costs of treating hospital-acquired infections (6). MRSA is a misnomer as strains of MRSA are resistant to a number of beta lactam antibiotics, including penicillin, amoxicillin, oxacillin, methicillin, cephalosporin, and carbapenem (3).

The majority of MRSA infections start in wounds and other breaches to the integrity of the skin usually caused by trauma, surgery, or medical devices. Approaches to treatment of skin and soft tissue infections include a combination of incision, drainage and antimicrobial therapy (12). Vancomycin is the antibiotic of choice against MRSA although there are growing concerns of vancomycin resistance (22). Changes in MRSA vancomycin susceptibility have been associated with increasing minimum inhibitory concentrations, increasing frequency of hetero-resistant vancomycin-intermediate *S. aureus* and adverse clinical outcomes (21). Alternatives to vancomycin include, clindamycin, tetracylines, tigecycline, linezolid, and daptomycin (4). Simor et al. (22)

found there was no significant difference in clinical outcomes of MRSA infections when comparing vancomycin to other antimicrobial agents, namely teicoplanin, trimethoprim-sulphamethoxazole, and linezolid. It raises significant concern that MRSA is becoming increasingly drug resistant even to newer antimicrobial agents such as linezolid, vancomycin, teicoplanin, and daptomycin (10). *S. aureus* has the unique ability to respond to new antibiotics with the development of resistance mechanisms such as decreased affinity for beta-lactam antibiotics via penicillin-binding protein 2a, decreased affinity for vancomycin via a D-Ala-D-lac substitution in peptidoglycan formation, and efflux pumps that work against fluoroquinolones and tetracylines (18).

Metals have been used since antiquity as antimicrobials in medicine and agriculture (11). The use of metals in healthcare settings is well known through metal-impregnated dressings and antimicrobial metal nanoparticles (11). Metals such as silver have gained attraction as a viable antimicrobial in the healthcare field with their use to coat surgical tools, catheters, and furniture. Topical silver has broad-spectrum antimicrobial activity that is used in wound dressings. Commercial products like Silvazine showed inhibition against 200 tested S. aureus, staphylococci and *P. aeruginosa* isolates (7).

Zn is an essential mineral involved in the catalytic activity of about 100 enzymes in addition to playing roles in immune function, protein synthesis, DNA synthesis and cell division (17). Mn is an essential trace mineral that plays a role in the development of connective tissue and hormones, and is necessary for fat and carbohydrate metabolism, blood sugar regulation, nerve function, and antioxidants (5). The objective of this study was to evaluate the efficacy of Zn and Mn as potential antimicrobials against wounds

infected with MRSA. The specific objectives included establishing the SIC and MIC of Zn and Mn against MRSA, determining the effect of Zn and Mn on increasing MRSA sensitivity to oxacillin, and determining the effect of Zn and Mn on MRSA cell adhesion and invasion onto human keratinocytes.

MATERIALS AND METHODS

Bacterial Strains

Three clinical strains of MRSA were used in this study (US 384, US 192, and US 194). All bacteriological media used in the study was purchased form Difco (Sparks, MD, USA). For the preparation of inoculum, each strain was grown separately in 10 mL of sterile tryptic soy broth (TSB) at 37°C for 24 hours. The cells were separated by centrifugation (3,600 x g for 10 min at 4°C) and resuspended in phosphate buffered saline (PBS, pH 7.2). Subsequently 0.1 mL from each strain was added to a tube of 9.9 mL of PBS. The bacterial population in each culture was determined by serial dilution and plating of 0.1 mL aliquots on tryptic soy agar (TSA) plates and incubating the plates at 37°C for 24 hours.

Determination of Sub Inhibitory Concentration (SIC) and Minimum Inhibitory Concentration (MIC)

Mn and Zn were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Mn was in the form $MnCl_2$ and Zn was in the form $ZnSO_4$. The SIC and MIC of Mn and Zn against each MRSA strain were determined as previously published (8). Twenty-four well plates containing sterile TSB was separately inoculated with each strain

of MRSA (~ 4.5 log CFU/mL) with varying concentrations of each mineral. The plates were then incubated at 37°C for 24 hours. After incubation each well was mixed thoroughly, serially diluted, plated on TSA, and incubated at 37°C for 24 hours. Duplicate samples were included for each treatment at each concentration for each strain, and the whole study was replicated three times. The SIC was taken as the highest concentration of Zn or Mn that did not inhibit MRSA after 24 hours of incubation. The MIC of Zn or Mn that inhibited visible growth of the bacteria after 24 hours incubation was taken as the MIC.

Effect of Mn and Zn on Increasing MRSA Sensitivity to Oxacillin

Methicillin is no longer commercially available in the United States and oxacillin is more stable during storage (3). Therefore oxacillin was used as the antibiotic in this study. Twenty-four well plates containing sterile TSB were inoculated with each strain of MRSA (~4.5 log CFU/mL) followed by the addition of the break point concentration of oxacillin against MRSA(4 µg/mL) in combination with MIC and SIC of each metal. The plates were incubated at 37°C for 24 hours. After incubation each well was thoroughly mixed, serially diluted, plated, and incubated at 37°C for 24 hours.

Keratinocyte Cell Culture

MRSA cell adhesion and invasion were determined by a previous protocol (9). Human skin keratinocyte, HEK001 (ATCC CRL-2404) was obtained from the American Culture Collection (Manassas, Virginia). HEK001 cells were grown in a 25 cm^2 cell culture flask containing keratinocyte serum free (KSFM) supplemented with human

recombinant epidermal growth factor (Invitrogen, Carlsbad, CA, USA) at 37°C for 24-48 hours in an aerobic incubator containing 5% CO_2.

Adhesion and Invasion Assay

The effect of MIC of Zn and Mn on MRSA adhesion and invasion of HEK001 keratinocyte cells was determined as previously described (16). Twenty four-well tissue culture plates (BD, Franklin Lakes, NJ) was seeded with 10^5 cells/well, and incubated at 37°C for 24 hours in a 5% CO_2 incubator to form a monolayer. MRSA was grown to mid log phase at 37°C, washed and re-suspended in KSFM with MIC of Zn and Mn. Bacteria suspended in KSFM was used as control. Aliquots of 100 μL of the bacterial suspension containing ~ 6 log CFU/ well (MOI 1:10) were inoculated in duplicates into the HEK001 monolayer, and incubated at 37°C in 5% CO_2 incubator for 2 hours. For adhesion assay, the infected monolayer after incubation was washed three times with PBS, and the cells were lysed using 0.1% Triton X-100 (Invitrogen, City, state). The number of viable adhered bacteria was enumerated by serial dilution and culturing on TSA plates. For the invasion assay, the HEK001 monolayer was washed three times with PBS, followed by incubation for 2 hours in KSFM containing gentamicin (100 microgram/ml) (Invitrogen) in order to kill the extracellular bacteria. Subsequently, the wells were washed three times with PBS and the cells were lysed using 0.1% Triton X-100 to release the intracellular bacteria. The number of invaded bacteria was enumerated by serial dilution in PBS and culturing on TSA plates. Both adhesion and invasion assays were done in duplicates and the experiment was repeated three times.

Statistical Analysis

All experiments were a completely randomized design. The data from independent replicate trials of each experiment were averaged and analyzed using Proc GENMOD, SAS 9.4 version (SAS Institute, Cary, NC) Variations among replicates were used as the error term. Data was expressed as least squares means and differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The emergence of antibiotic resistance in pathogenic microorganisms, including MRSA has triggered investigations to identify novel antimicrobials. In study, two essential minerals, namely Zn and Mn were investigated for their potential to control MRSA, especially for wound infections.

The SIC and MIC of Zn and Mn against MRSA were 0.4 mM and 2.5 mM, and 1.6 mM 4.7 mM, respectively. The effect of MIC and 2x MIC of Zn and Mn on increasing MRSA sensitivity to oxacillin is depicted in **Fig. 1** and **2**. The 2x MIC was used in lieu of comparing SIC and MIC of the metals because preliminary studies showed SIC of both metals did not increase the sensitivity of MRSA to oxacillin. In control tubes, all three strains grew to an average of 8.5 log CFU/mL. In the presence of breakpoint concentration of oxacillin, as expected the MRSA strains being resistant to the antibiotic grew by ~ 2 to 3 log CFU/mL. In addition, in the presence of the MIC of Zn and Mn, bacterial count after 24 h did not change significantly from the inoculation of level of 4.5 log CFU/mL. However, when MRSA was grown in the presence of antibiotic and the MIC or 2x MIC of Zn or Mn, its growth after 24 h was significantly decreased in

comparison to that in tubes containing oxacillin alone ($P < 0.05$). For example, the MRSA counts recovered from tubes containing oxacillin and Zn or Mn were ~ 3 to 4 log CFU/mL as against ~ 6 to 7log CFU/mL in samples containing only oxacillin. The trend was observed in all three MRSA strains. Except in strain USA 384, there was no difference in bacterial reductions between samples containing the MIC and 2x MIC Zn or Mn with oxacillin

The effect of SIC and MIC of Zn and Mn on MRSA adhesion to human keratinocytes is depicted in **Fig. 3** and **4**. Compared to control samples where ~ 6 to 7 log CFU/mL of MRSA attached to the keratinocytes, the SIC of Zn brought about ~ 2.5 log CFU/mL reduction in bacterial count across all three strains ($P<0.05$). Similarly, the MIC of Zn reduced the attachment of MRSA onto skin cells by 3.0 log CFU/ml in all three strains ($P<0.05$). Similar results were observed in the experiments with Mn, where the SIC of Mn reduced the attached MRSA by about 2.4 log CFU/mL in all three strains. The MIC of Mn on the other hand brought about a 3.0 log CFU/mL reduction in attached bacterial.

The effect of SIC and MIC of Zn and Mn on MRSA invasion of human keratinocytes is depicted in Fig. 5 and 6. In control wells with no Zn and Mn, ~ 4 to 4.5 log CFU/mL of MRSA attached the cells. However, the SIC and MIC of Zn decreased the invaded bacteria by ~1.7 log CFU/mL 2.1 log CFU/mL, respectively. A similar magnitude of reduction in the invaded MRSA counts was observed in Mn-treated cells.

The aforementioned results indicate that Zn and Mn were effective in increasing the sensitivity of MRSA to oxacillin when the antibiotic was combined with the minerals.

In addition, it was found both minerals were effective in reducing MRSA attachment and invasion of human keratinocytes.

Although the exact mechanism behind the anti-MRSA effect of Zn and Mn is not clear, the proposed antimicrobial mechanisms of action of metals include creation of reactive oxygen species, antioxidant depletion, oxidation of side chains leading to protein dysfunction, interference with nutrient assimilation, and genotoxicity in microbes (11).

The recommended daily dietary allowance of Zn is 8 mg for females and 11 mg for males with a tolerable upper intake level of 40 mg (17). The recommended daily dietary allowance of Mn is 1.8 for females and 2.3 mg for males with a tolerable upper intake level of 11 mg (14). Although the concentrations of minerals used in this experiment are at or above oral intake levels, the route of intended application is topical on wound infections rather than oral. To conclude, the results of this study indicate that Zn and Mn have the potential to be used as effective and safe antimicrobials to combat wound infections of MRSA. However, follow up studies in an *in vivo* a wound model are necessary in addition to experiments for delineating the antimicrobial mechanisms of Zn and Mn.

CONCLUSION

Results of this study indicate that Zn and Mn increased the sensitivity of MRSA to oxacillin. In addition, Zn and Mn reduce the amount of bacteria that both adhere and invade onto human keratinocytes. Therefore, these metals show potential as antimicrobials used to combat wounds infected with MRSA.

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MRSA Sensitivity to Oxacillin Study

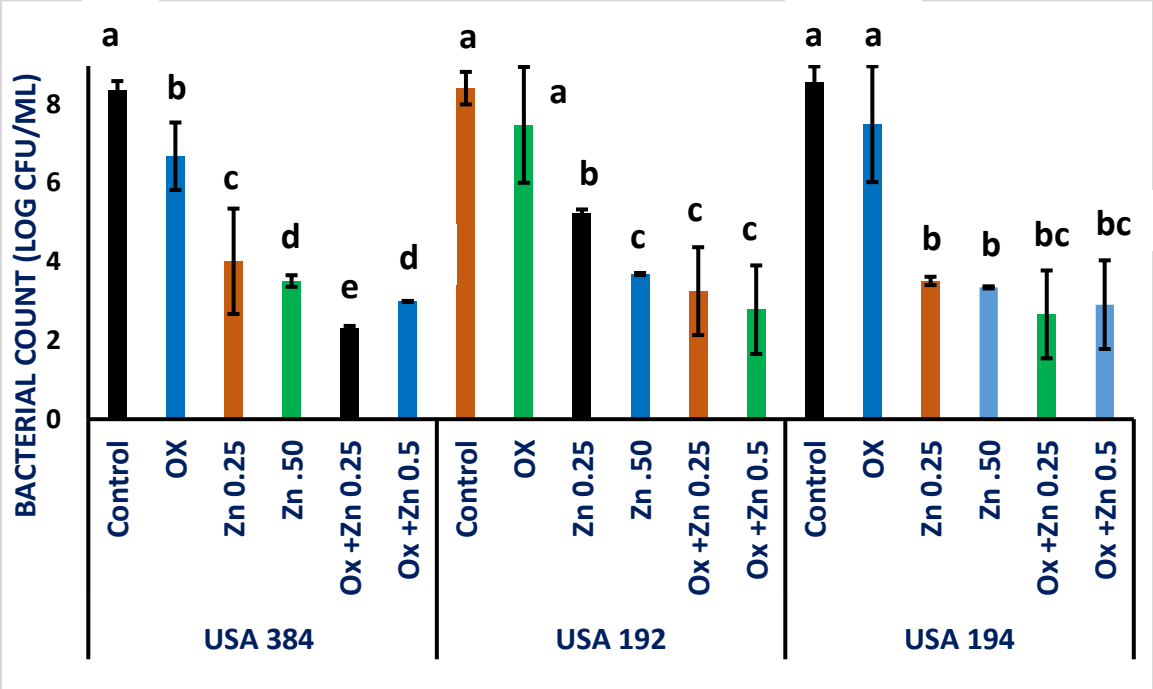

Figure 1 Effect of MIC and 2x MIC of Zn on increasing MRSA sensitivity to oxacillin in 3 strains. Bars with different letters are significantly different from each other (P<0.05)

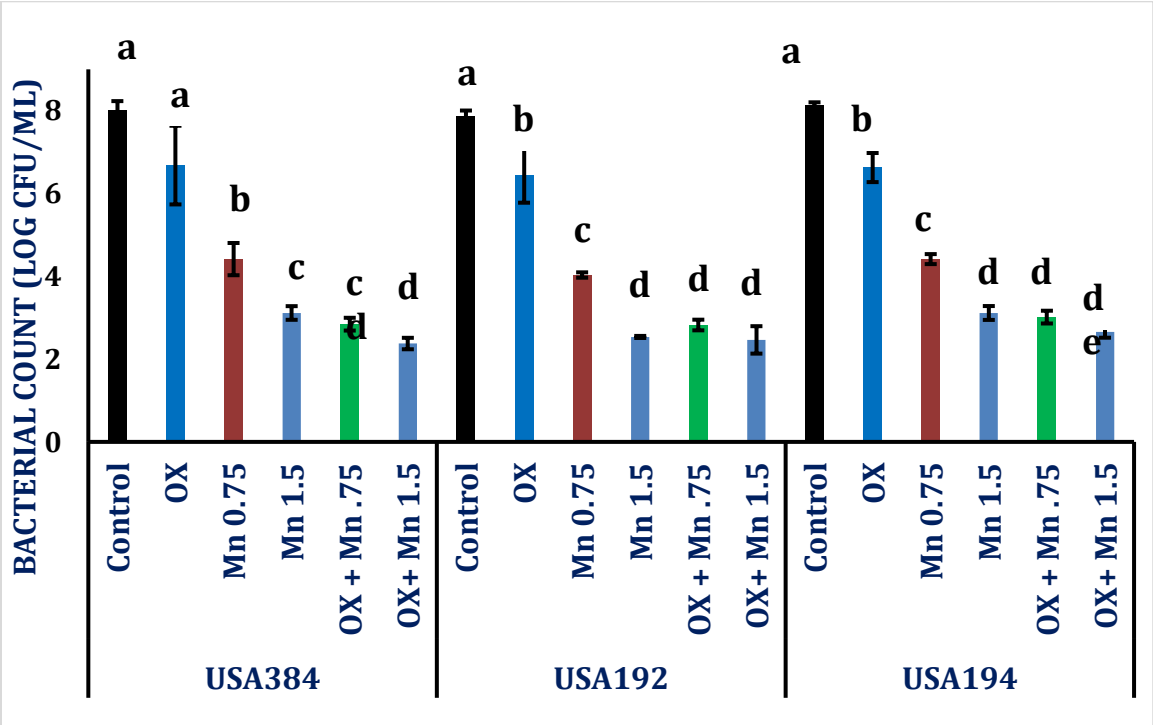

Figure 2 Effect of MIC and 2x MIC of Mn on increasing MRSA sensitivity to oxacillin in 3 strains. Bars with different letters are significantly different from each other (P<0.05)

MRSA Cell Adhesion onto Human Keratinocytes

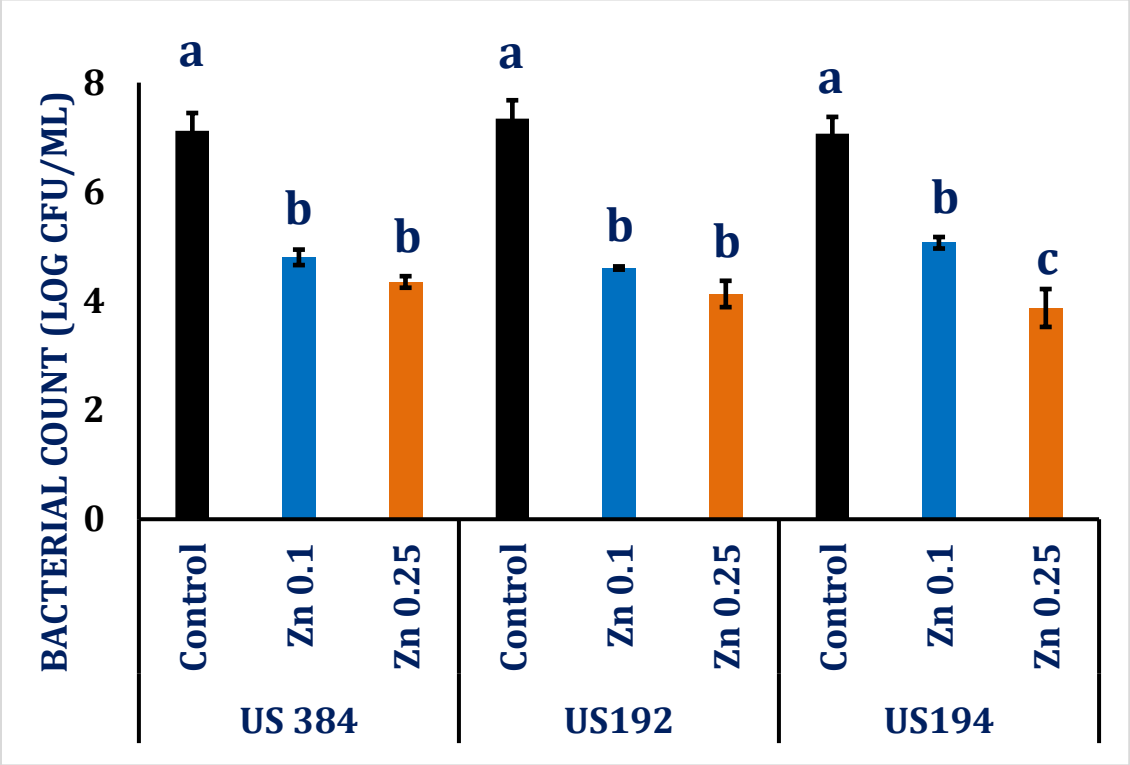

Figure 3: Effect of SIC and MIC of Zn on MRSA adhesion to human keratinocytes. Bars with different letters are significantly different from each other (P<0.05)

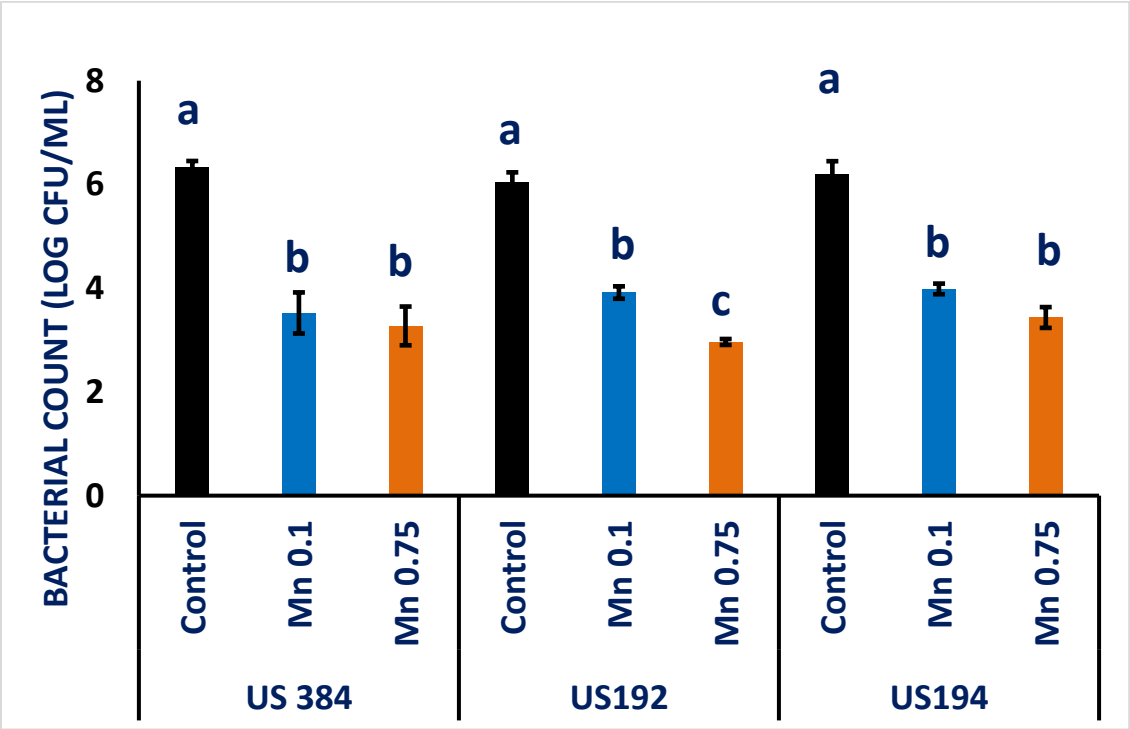

Figure 4: Effect of SIC and MIC of Mn on MRSA adhesion to human keratinocytes. Bars with different letters are significantly different from each other (P<0.05)

MRSA Cell Invasion onto Human Keratinocytes

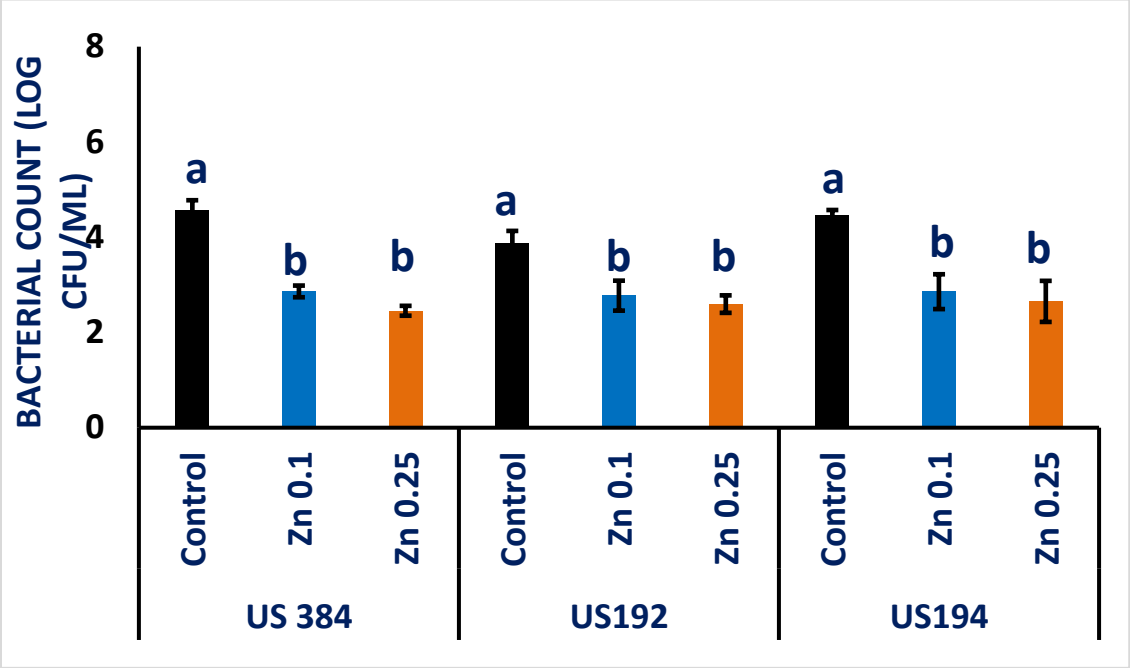

Figure 5: Effect of SIC and MIC of Zn on MRSA invasion of human keratinocytes. Bars with different letters are significantly different from each other (P<0.05)

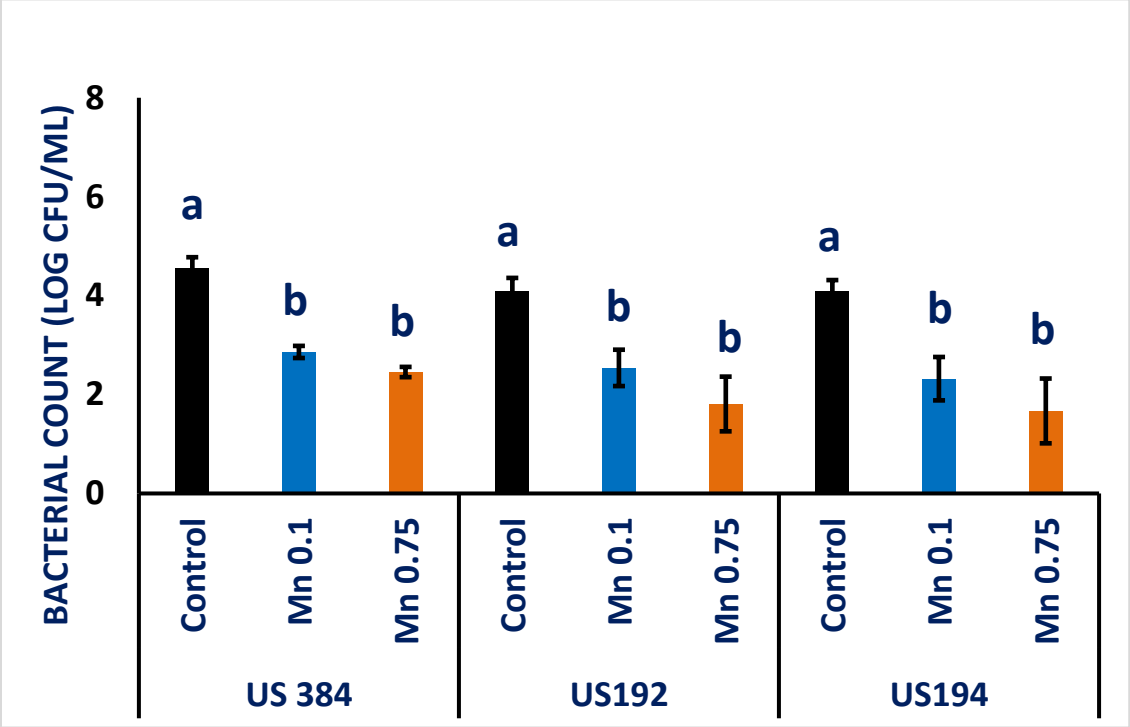

Figure 6 Effect of SIC and MIC of Mn on MRSA invasion of human keratinocytes. Bars with different letters are significantly different from each other (P<0.05)