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Effect of Copper concentration on the local pathogenic bacteria

Raneen Khaleel Tawfeeq
Biology Division – Anatomy depart. – Medical College- Baghdad University.

Abstract: *Metals and metalloids have a long empirical history of human usage in medicine and agriculture. copper or copper compounds being used as astringents, antiseptics and antifungals, to treat wounds, to purify and sterilize drinking water, and in contraceptive intrauterine devices, Inorganic and organic copper compounds have been used to treat a variety of skin diseases, syphilis, tuberculosis and anaemia, amongst other maladies. There is also interest in copper-containing wound/ulcer dressings that have been trialled and reported to be effective. Most recently, the use of copper antimicrobial solid surfaces to reduce microbial contamination and transmission of hospital-acquired infections has progressed to clinical trials, with the installation of copper-containing surfaces and fixtures in wards and clinics. The aim of this research is : to clarify the role of copper's antibacterial activity against four local pathogenic bacteria species (Gram positive and Gram negative) could be utilized in health care facilities and in food processing plants to reduce the bio-burden, which would increase protection for susceptible members of the community.*

Key words: *copper, Copper compounds, Klebselia pneumonia, Staphlococcus aureus, E.coli.*

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I. Introduction

Copper is an essential metal to aerobic forms of life, which being involved in donating or accepting electrons in redox-active enzymes, or in the electron transport chain (Solioz *et al.*, 2010). It is also toxic to prokaryotes and eukaryotes at higher cellular concentrations (Gaetke & Chow, 2003). Copper is one of the most abundant heavy metals present in swine manure(Yin *et al*.2017).

Copper compounds are used as wood preservatives, in antifouling paints and as molluscicides (Borkow & Gabbay, 2009). In agriculture, copper compounds have been used as antimicrobial, algicidal, pesticidal and antifungal agents, and as animal feed additives. Copper sulphate solutions were used as an antifungal treatment of seed grains in the eighteenth century. In the late nineteenth century, Bordeaux mixture (copper sulphate and calcium hydroxide) and Burgundy mixture (copper sulphate and sodium carbonate) were widely used to control mildew on grape vines, and to control fungal and bacterial diseases of seeds or plants (Russell, 2005).

Copper carries out an essential role as an electron donor/ acceptor in many enzymes, but copper can also take part in Fenton-like reactions leading to the generation of hydroxyl radicals, hydrogen peroxide and superoxide, which can cause cellular damage (reviewed by Grass *et al.*, 2011). This has been generally accepted as the major mechanism for copper toxicity. However, recent experimental evidence from experiments in liquid culture shows that coppermediated ROS generation occurs largely in the periplasm of *E. coli*, so the importance of ROS generation by copper as a cellular toxicity mechanism has been under debate (Macomber *et al.*, 2007).

The aim of this research is : to clarify the role of copper's antibacterial activity against bacterial species could be utilized in health care facilities and in food processing plants.

II. Material and methods

1- Bacteria:

The bacterial strains which used were :

A. *Klebselia pneumonia* isolated from sputum.
B. Staphlococcus aureus isolated from body fluid.
C. *Staphlococcus aureus* isolated from ear.
D. *E.coli* isolated from urine.

The bacteria were from Teaching laboratory / Medical city of Baghdad. All bacteria were grown on brain heart infusion agar and Blood agar and BHI broth for for 24h in 37c˚ with shaking at 180 rev min^{-1}.
$CUSO_4. 5H_2O$ was used in all experiment in 0.0392 ppm .

2- Evaluation of Copper:

Liquid culture method for Copper tests were conducted and four bacteria (*Klebselia pneumonia*, two *Staphlococcus aureus* from different sources isolated and *E.coli*) were used. After stationary phase, bacterial cell density was adjusted to an optical density (O.D.=600) of 0.3 cell density was measured using a

spectrophotometer and samples were diluted ten-folds. After (24h) of incubation. The cell density was measured using spectrophotometer. The concentration of Copper (392, 39.2, 3.92, 0.392, 0.0392 & 0.00392 ppm) were used and growth curves of the bacteria were obtained.

III. Results

Fig-1 shown the growth curves and the concentration of Copper in four local pathogenes bacteria.

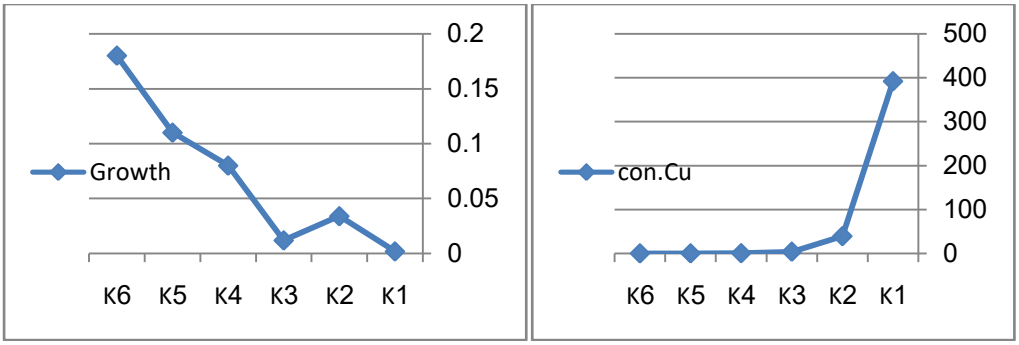

Fig-1-a) the growth curve and the concentration of Copper in *Klebselia pneumonia* isolated from sputum.

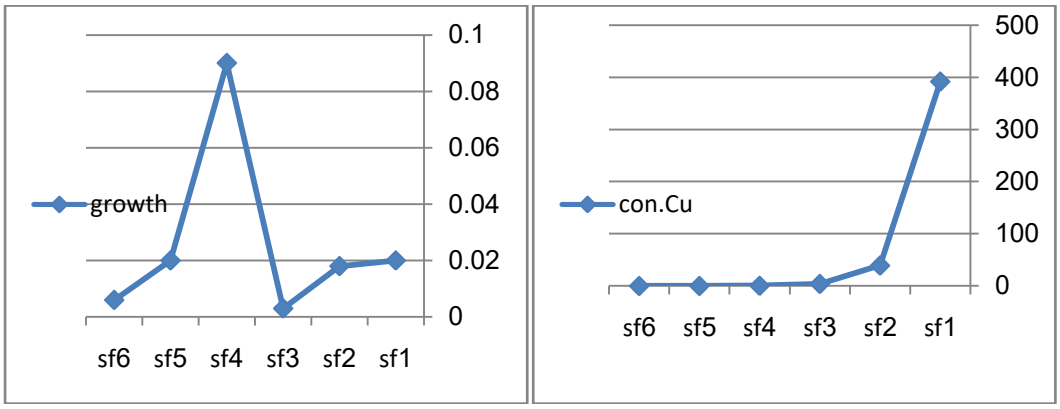

Fig-1-b) the growth curve and the concentration of Copper in *Staphlococcus aureus* isolated from body fluid.

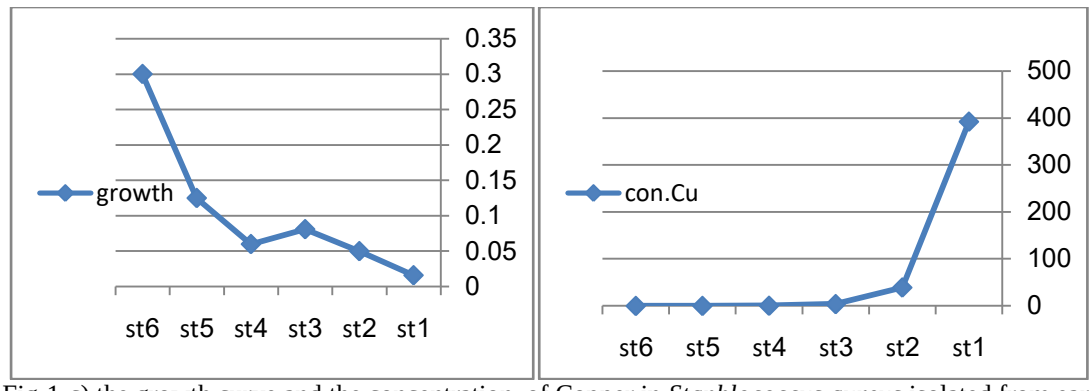

Fig-1-c) the growth curve and the concentration of Copper in *Staphlococcus aureus* isolated from ear.

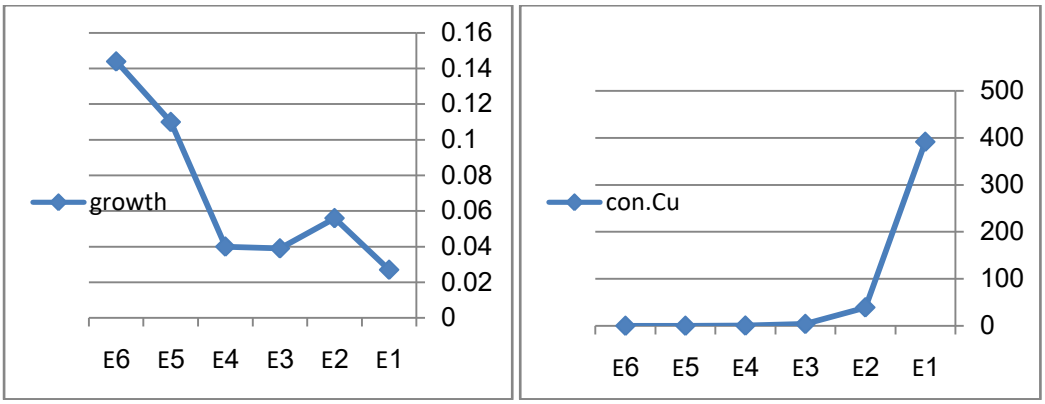

Fig-1-d) the growth curve and the concentration of Copper in *E.coli* isolated from urine.

Fig-1 (a,b,c,d) the growth curve and the concentration of Copper of *Klebselia pneumonia (K)*, two *Staphlococcus aureus(SF,St)* from different sources isolated and *E.coli*(E).

IV. Discussion

The concentration of Copper (392, 39.2, 3.92, 0.392, 0.0392 & 0.00392 ppm) were used and growth curves of the bacteria were obtained, liquid culture method for Copper tests were conducted and four bacteria (*Klebselia pneumonia*, two *Staphlococcus aureus* from different sources isolated and *E.coli*) were used.In Fig-1 (a,b,c,d) the growth curve and the concentration of Copper of *Klebselia pneumonia (K)*, two *Staphlococcus aureus(SF,St)* from different sources isolated and *E.coli* (E), the results shown when decrease the concentration of Copper(from 392 to 0.00392 ppm), the growth increase in four bacteria which are local strains pathogenic bacteria, which means there is an inverse relationship between the copper concentration and growth of bacteria. These findings suggest that copper's antibacterial activity against bacterial species could be utilized in health care facilities and in food processing plants to reduce the bio-burden, which would increase protection for susceptible members of the community.

The results were agreement with Tian *et al.*, Copper yielded a significant decrease in the viable bacterial counts at 2 h exposure and a highly significant decrease at 4 h. Loss of cell integrity and a significantly higher influx of copper into bacterial cells exposed to copper surfaces, as compared to those exposed to the controls, were documented. There was no increase in mutation rate and DNA damage indicating that copper contributes to bacterial killing by adversely affecting cellular structure without directly targeting the genomic DNA (Tian *et al.*,2012).

The importance of ROS generation by copper as a cellular toxicity mechanism has been under debate (Macomber et al., 2007). Gram-positive bacteria lack a periplasm, and although many are tolerant to hydrogen peroxide (Solioz *et al.*, 2010), recent evidence from *Staphylococcus aureus* shows oxidative stress resistance and protein misfolding repair transcriptional responses, and hydrogen peroxide scavenging defence (Baker *et al.*, 2010). According to the Irving–Williams series, copper has a higher affinity than other first-row transition metals for ligands, and displacement of iron from iron–sulphur clusters by copper in liquid culture experiments has been reported to be an important mechanism of copper toxicity (Macomber & Imlay, 2009). There is also a role for copper and ROS in phagosome killing of bacteria (reviewed by German *et al.*, 2013). The rapid killing of bacteria on solid copper surfaces is thought to be due to cellular damage caused by very high local concentrations of copper dissolving from the surface, which causes membrane rupture, coupled with ROS generation leading to further cellular destruction, including degradation of plasmid and chromosomal DNA (Grass *et al.*, 2011).Various laboratory and clinical studies have confirmed that solid copper/copper alloy surfaces promote rapid killing of Gram-negative and Gram-positive bacteria(Hobman& Crossman , 2014).

As conclusion: Copper and Copper compounds could be utilized in health care facilities and in food processing plants to reduce the bio-burden, which would increase protection for susceptible members of the community.

References

[1] Baker, J., Sitthisak, S., Sengupta, M., Johnson, M., Jayaswal, R. K. & Morrissey, J. A. (2010). Copper stress induces a global stress response in Staphylococcus aureus and represses sae and agr expression and biofilm formation. Appl Environ Microbiol 76, 150–160.

[2] Borkow, G. & Gabbay, J. (2009). Copper, an ancient remedy returning to fight microbial, fungal and viral infections. Curr Chem Biol 3, 272–278.

[3] Gaetke, L. M. & Chow, C. K. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology 189, 147–163.

[4] German, N., Doyscher, D. & Rensing, C. (2013). Bacterial killing in macrophages and amoeba: do they all use a brass dagger? Future Microbiol 8, 1257–1264.

[5] Grass, G., Rensing, C. & Solioz, M. (2011). Metallic copper as an antimicrobial surface. Appl Environ Microbiol 77, 1541–1547.

[6] Hobman, J.L. and Crossman ,L. C.. (2014). Bacterial antimicrobial metal ion resistance.Journal of Medical Microbiology 64, 471–497.
[7] Macomber, L. & Imlay, J. A. (2009). The iron–sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. Proc Natl Acad Sci U S A 106, 8344–8349.
[8] Macomber, L., Rensing, C. & Imlay, J. A. (2007). Intracellular copper does not catalyze the formation of oxidative DNA damage in Escherichia coli. J Bacteriol 189, 1616–1626.
[9] Russell, P. E. (2005). A century of fungicide evolution. J Agric Sci 143, 11–25.
[10] Solioz, M., Abicht, H. K., Mermod, M. & Mancini, S. (2010). Response of Gram-positive bacteria to copper stress. J Biol Inorg Chem 15, 3–14.
[11] Tian WX, Yu S, Ibrahim M, et al.(2012).Copper as an antimicrobial agent against opportunistic pathogenic and multidrug resistant Enterobacter bacteria. J Microbiol. 2012 Aug;50(4):586-93.
[12] Yin Y, Gu J , Wang X, Song W, Zhang K, Sun W, Zhang X, Zhang Y, and Li, H.(2017). Effects of Copper Addition on Copper Resistance, Antibiotic Resistance Genes, and *intl1* during Swine Manure Composting. Front Microbiol. 8: 344.

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