IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 7 Ver. 9 (July. 2018), PP 22-26
www.iosrjournals.org

In vitro antibacterial analysis of copper solution (microparticle) – a novel anti-infective molecule for wound management

Pramila M[1], Meenakshisundaram M[1], Prabhusaran N[2*], Lalithambigai R[2], Karthik P[3]

[1]Department of Biotechnology, Nehru Memorial College, Tiruchirapalli, India
[2]Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre, Tiruchirapalli, India (Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai)
[3]Department of Surgery, Trichy SRM Medical College Hospital and Research Centre, Tiruchirapalli, India (Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai)
Correspondence author- Prabhusaran N (E. mail- leptoprabhu@gmail.com)

Abstract: *Now a days the emergence of antibiotic resistant bacterial pathogens are considered as the major alarm, there is a need to concentrate on the synthesis of new antibacterial agents. Most of the public getting aware about the beneficial role of conventional methods like usage of copper and silver vessels to act against bacterial pathogens. Copper is a trace element metal particle that is highly useful to inhibit microbial growth in media by means of disrupting membrane permeability leads to cellular damage and cytolysis. To keep all these in mind an objective with the study of in vitro antibacterial analysis of copper in solution to bacterial pathogens isolated from the wounds. Battery of seven bacterial isolates for wound and six laboratory bacterial strains were included in this investigation. As a result, among the wound isolates, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa inhibited at the maximum concentration of 4% copper solution with 20mm zone of inhibition each; whereas among laboratory isolates Serratia sp and Providencia rettigeri were shown with 20 and 18mm inhibition. Thus from this study, it was clearly identified that the copper may be useful as external antibactieral solution for wound care and avoiding infections.*

Keywords: *Wound, bacteria, copper, bacteriolysis*

Date of Submission: 03-07-2018 Date of acceptance: 21-07-2018

I. Introduction

Prevention of Hospital Acquired Infection (HAI) is a major issue in the hospital environment[1]. A huge emergence of different antimicrobial resistant microbial strain is leads to the development of effective antibiotics[2]. Recent, study reported that, nearly 3.2 million HAI was recorded between 2011- 2012 in the European country. To overcome this issue, new antimicrobial materials are highly needed. In this situation, metals and its derivatives have the ability to resist against bacteria, since ancient times[3]. HAI increased the severity of the infection, hospitals may also increase and screening of antibiotic is highly complicated[4].

Despite the technical advancement in the surgical procedure HAI is still a major problem. Hospital acquired infection is patient and surgical procedure related, it's varied from hospital to hospital[5,6]. After the discovery of the relationship between the pathogens and its virulencity, antibiotics were arising. Continuous usage of antibiotics against specific pathogens may leads to the development of Multi-drug resistant (MDR). In the healthcare industry, this is the crucial situations to develop a new, effective and environment friendly antibiotic to treat the MDR[7].

Now the researchers are use metal and its derivatives to treat MDR in a better way. Recent studies revealed that the metals and metallic derivatives have an ability to work against Multi- Drug Resistant (MDR) in an effective way[8]. Antimicrobial activity of metal ions was well established from ancient days also[9] which are used to treat various infections and disease[10]. Copper is used as disinfectant[11] and copper based utensils, drinking water containers and water pipes are used in ancient days, this avoids the spread of microbial infections[12].

Copper is an essential metal for most of the organisms, thus helps to their growth and functions[13]. In and around the 19th century the use of copper in various countries like Greeks, Romans, Aztecs and others, used to treat headache, burns, intestinal worms, ear infection and also for general hygiene. The US Environmental Protection Agency (EPA) in 2008, officially reported that, the copper and its alloys as the foremost effective metallic antimicrobial agent. Copper alone have an ability to kill 99.9% pathogens within 2 hour and its quick progress done by "contact killing", thus allows rapidly eliminate the pathogens. The pathogenic killing mechanism takes place at a rate of at least 7-8 folds per hour and there is no sufficient environment available for microbial growth[14,15].

A new awareness of copper's medical potency was spawned by the observation that copper workers appeared to be immune to cholera in the 1832 and subsequent outbreaks in Paris, France[3,16]. The mechanism of copper on microbes were done by ways: the presence of Cu^{+} ions on the surface of the microbes damage the integrity of cell membranes, then directly damage the bacterial proteins, finally induces the formation of highly damaging hydroxyl radicals which can able to damage the entire cell[17,18]. The administration of copper ions induces the inactivation of enzymes[7,19]. Copper containing materials for surfaces in the hospital environment may be a valuable adjunct for the prevention of healthcare-associated infections[20]. This study is designed in such a way to determine the *in vitro* antibacterial activity of various concentrations of copper solution against bacterial isolates from wound and laboratory bacterial strains.

II. Materials And Methods

Sample and bacterial culturing

Sterile swab specimen from pus and wound samples were collected and aseptically dispensed in sterile nutrient broth in bedside itself and transported to the laboratory for further macroscopic, microscopic and cultural analysis. The pre-enriched swab specimens were inoculated into the Nutrient agar, MacConkey agar and Blood agar plates and were incubated at 37°C for 24 hours. The macroscopic analysis like colony morphology, color and shapes were recorded. Microscopic examination, including gram's staining, motility and special staining were done according to the suspected bacterial colonies. Futher, the bacterial strains were confirmed at genus and species level by standard and routine biochemical tests.

In vitro antibacterial activity of copper solution

Various concentrations of copper solutions from 1 to 4% were prepared for analyzing the antibacterial potential. Muller Hinton Agar plates were prepared and checked for its sterility. The bacterial isolates from wound specimen and laboratory cultures were included in this study. Each bacterial isolates were seeded on MHA plates separately. Four wells were prepared using agar puncher and 100µl of all four concentrations of copper solutions were loaded and noted. All the processed plates were incubated at 37°C for 24 hours for the determination of zone of minimum and maximum inhibition.

III. Results

The bacteria isolated and identified from this study were *Proteus* sp, *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus* sp *and Micrococcus* sp. Further, the abovesaid wound based isolates and laboratory isolates including *Salmonella typhi*, *Providencia rettigeri, Klebsiella oxytoca, Serritia sp* and *Salmonella para* B were analyzed for its in vitro antibacterial activity. Among the wound isolates, *S. aureus*, *K. Pneumoniae* and *P. aeruginosa* showed maimum zone of inhibition (20 mm) at the maximum concentration of 4% whereas *Proteus* sp (18 mm), *E. coli* (16 mm) and *Flavobacter* sp (16 mm) showed moderate level of inhibition and Enterococcus sp (11 mm) showed least inhibition rate at all the concentrations (Table 1 and Figure 1).

Table 1: *In vitro* antibacterial activity of copper solutions

No.	Bacterial pathogens	Concentration of Cu solution verses zone of inhibition in mm			
		1%	2%	3%	4%
Wound pathogens					
1	*Proteus* sp	15	16	18	18
2	*Staphylococcus aureus*	10	14	16	20
3	*Klebsiella pneumoniae*	10	13	17	20
4	*Escherichia coli*	10	12	14	16
5	*Pseudomonas aeruginosa*	17	20	20	20
6	*Enterococcus* sp	-	5	8	11
7	*Flavobacter* sp	10	12	14	16
Laboratory isolates					
8	*Salmonella typhi*	5	10	13	16
9	*Providencia rettigeri*	10	12	15	18
10	*Shigella sonnei*	10	12	13	13
11	*Klebsiella oxytoca*	6	10	12	15
12	*Serratia sp*	13	15	20	20
13	*Salmonella para* B	-	-	7	10

Among the laboratory isolates, maximum zone of inhibition observed in *Serratia* sp (20 mm) and *P. rettigeri* (18 mm); moderate inhibition in *S. typhi* (16 mm) and *K. oxytoca* (15 mm) and least inhibition observed in *S. sonnei* (13 mm) and *S. paratyphi* B (10 mm) (Table 1 and Figure 1). The result confirmed that the copper has an ability to act against bacterial pathogens. While increasing the concentration of the solution, the inhibition rate was also proportionally increased.

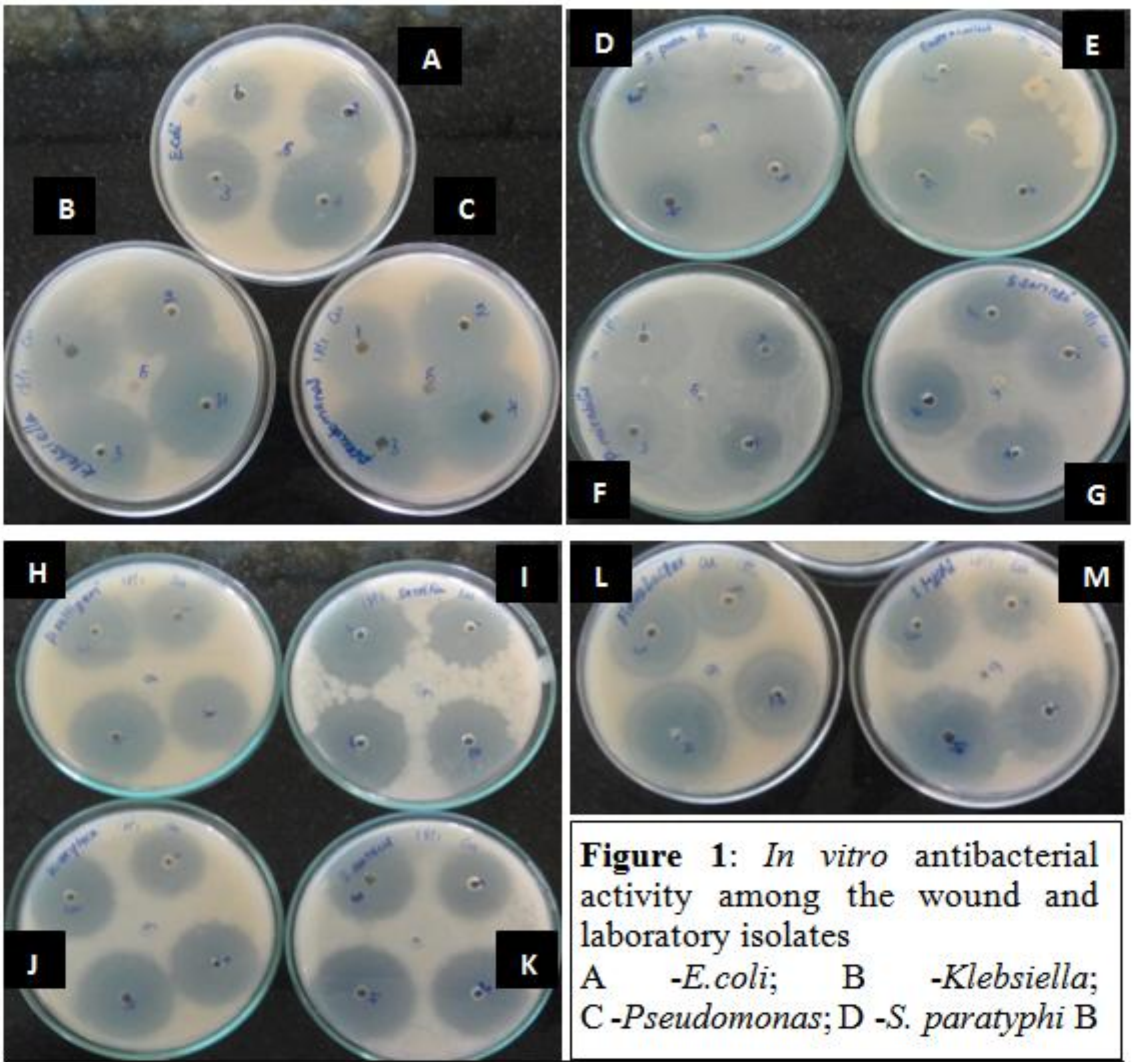

Figure 1: *In vitro* antibacterial activity among the wound and laboratory isolates A -*E.coli*; B -*Klebsiella*; C -*Pseudomonas*; D -*S. paratyphi* B E –*Enterococcus*; F –*Proteus* sp; G –*S. sonnei*; H –*P. rettigeri*; I – *Serratia*; J –*K. oxytoca*; K –*S. aureus*; L –*Flavobacter* sp and M – *S. typhi*

IV. Discussion

Several studies reported that the metal stress on bacteria may reduce the bacterial function and activity. Long term association and concentration of copper solution may enhance the bactericidal nature of the metal and avoid the presence and survival of such bacterial community environment[6,21]. Another study describe that the bactericidal effect of copper is better than ampicillin and streptomycin[22]. The antibacterial activity of copper was well studied against *P. aeruginosa* when compare with *S. aureus* and *Sarcina lutea* were recorded[2,14,23], but in this study *P. aeruginosa, S. aureus* and *Serritia* sp showed maximum zone against copper solution. A study stated that the synergistic effect of copper and lactic acid also showed the better inhibition in *E. coli*[17,24] but in this study the copper inhibit *E. coli* cells in observable manner.

The degree of antibacterial potential against both gram positive (*S. aureus*) and gram negative (*P. aeruginosa*) bacteria revealed considerable detection of cytolysis thereby increasing the amount of liquid peroxidation and genomic DNA degradation happen while using copper as antibacterials. The antibacterial resistance against the conventional antibiotics is not active mainly due to the misuse, abuse and overuse of such antibiotics when not in use as it is or concentrations. Thus the study of alternative for antibacterial innovations may rise now a days; positively traditional methods like metal microbe interactions and inhibitions may be much evoluable[5,25].

A study suggested the usage of copper related surfaces in hospital and other health care environment for the creation of antimicrobial surface[26]. Due to the poor understanding of the activity of copper, fear on systemic toxicity and other mythological thoughts, the research on copper is not much evoked. But the theory suggested that the copper particles have the high degree of antibacterial action by means of its oxidation potentiality of the surface, constant release of electrons to the host cell wall where lipolysis and proteolysis takes place leads to drastic decrease in the cellular integrity[26,27].

The mechanistric action of the copper have certain experimental observations like less oxygen tension in the supportive media and cellular damage of the bacterial cells. Due to the high oxidative nature of the copper, the release active principles by the copper in the environment and interact with the bacterial cells are high[28,29], thus the aerobic environment dominates in its action leads to strong and concrete bound to organic materials. Currently lot of interest evolved of using natural remedies for various ailments likely the usage of copper in form of plates, jugs, glasses, water bottles and other vessels that are largely useful as day to day consumables. The usage of such metal in solution may be largely useful in medical, industrial, food and other industries where the microbial contamination, spoilage and deposits increased.

From this study, we come to conclusion that the prompt usage of such copper based microparticles against bacterial pathogens may be much useful based on its thermal conductivity and elevated temperature in the environment. We further suggested the usage of copper in solution for various antibacterial activities in routine, medical, industrial and other environment wherever necessity arised.

References

[1]. Allegranzi B, Storr J, Dziekan G, Leotsakos A, Donaldson L, Pittet D. The first global patient safety challenge clean care is safer care: from launch to current progress and achievements. Journal of Hospital Infections 2007;65(2):115–123.

[2]. Rakhmetova AA, Alekseeva TP, Bogoslovskaya OA, Leipunskii IO, Olkhovskaya IP, Zhigach AN and Glushchenko NN. Wound healing properties of copper nanoparticles as a function of physicochemical parameters. Nanotechnologies in Russia 2010;5(3):271–276.

[3]. Dollwet HHA, Sorenson JRJ. Historic uses of copper compounds in medicine.Trace Elemental Medicine 1985;2(2):80–87.

[4]. Owens CD, Stoessel K. Surgical site infections: epidemiology, microbiology and prevention. Journal of Hospital Infection 2008;70(2):3-10.

[5]. Pradhan GB, Agrawal J. Comparative study of post-operative wound infection following emergency lower segment caesarean section with and without the topical use offusidic acid. Nepal Medical College Journal 2009;11(3):189-191.

[6]. Vikrant N, Shekhar P, Deepak J, Munesh kumar S, Neelam S. Bacteriological profile of surgical site infections and their antibiogram: a study from resource constrained rural setting of Uttarakhand state, India. Journal of Clinical and Diagnostic Research 2015;9(10):17-20.

[7]. Hassan IA, Parkin IP, Nair SP, Carmalt CJ. Antimicrobial activity of copper and copper (I) oxide thin films deposited via aerosol-assisted CVD. Journal of Material Chemistry B 2014;2(2):2855–2860.

[8]. Chatterjee AK, Chakraborty R, Basu T. Mechanism of antibacterial activity of copper nanoparticles. Nanotechnology 2014;25(13):5101-5112.

[9]. Sondi I, Salopek SB. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. Journal of Colloidal Interface Science 2004;275(1):177–182.

[10]. Moghimi SM. Nanomedicine: prospective diagnostic and therapeutic potential. Asia Pacific Biotech News 2005;9(1):1072-1077.

[11]. Huang R, Wallqvist A, Covell DG. Anticancer metal compounds in NCI's tumor-screening database: putative mode of action. Biochemistry and Pharmacology 2005;69(7):1009–1039.

[12]. Seil JT, Webster TJ. Antimicrobial applications of nanotechnology: methods and literature. International Journal of Nanomedicine 2012;7(1):2767-2781.

[13]. Raffi M, Mehrwan S, Bhatti TM, Akhter JI, Hameed A, Yawar W, Hasan MM. Investigations into the antibacterial behavior of copper nanoparticles against *Escherichia coli*. Annals of Microbiology 2010;60(1):75-80.

[14]. Villapun VM, Dover LG, Cross A, Gonzalez S. Antibacterial metallic touch surfaces. Materials 2016;9:736-758.

[15]. Prado V, Vidal R, Duran C. Application of copper bactericidal properties in medical practice. Revista Medica de Chile 2012;140(10): 1325–1332.

[16]. Gregor G, Christopher R, Marc S. Metallic copper as an antimicrobial surface. Applied and Environmental Microbiology 2011;7(5):1541-1547.

[17]. Borkow G, Gabbay J. Copper an ancient remedy returning to fight microbial, fungal and viral infections. Current Chemical Biology 2009;3(3):272–278.

[18]. Huang L, Fozo EM, Zhang T, Liaw PK, He W. Antimicrobial behavior of Cu-bearing Zr-based bulk metallic glasses. Materials Science and Engineering C 2014;39(1):325–329.

[19]. Macomber L, Imlay JA, The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. Proceedings of the National Academy of Sciences of the United States of America 2009;106(20):8344-8349.

[20]. Dancer SJ. The role of environmental cleaning in the control of hospital-acquired infection. 2009;73(4):378-385.

[21]. Turpeinen R, Kairesalo T, Haggblom MM. Microbial community structure and activity in arsenic chromium and copper contaminated soils. FEMS Micobiology Ecology 2004;47(10):39-50.

[22]. Sitalu K, Hari Babu B, Naveena Lavanya Latha J, Lakshmana Rao A. Synthesis, characterization and antimicrobial activities of copper derivatives of NHC- II complexes. Journal of Biological Sciences 2017;20(2):82-91.

[23]. Sanja OPK, Sinisa LM, Ljiljana SV. Physico chemical characterization and antimicrobial activity of copper (II) complexes with 2-amino and 2- methylbenzimidazole derivatives. Acta Periodica Technologica 2014;35(1):247-254.

[24]. Gyawali R, Ibrahim SA, Abu hasfa SH, Smqadri SQ, Haik Y. Antimicrobial activity of copper alone and in combination with lactic acid against *Escherichia coli* O157: H7 in laboratory medium and on the surface of lettuce and tomatoes. Journal of Pathology 2011;2011: 650968.

[25]. Noyce JO, Michels H, Keevil CW. Potential use of copper surfaces to reduce survival of epidemic meticillin-resistant *Staphylococcus aureus* in the healthcare environment. Journal of Hospital Infection 2006;63:289-297.
[26]. Thomas SF, Rooks P, Rudin F, Atkinson S, Goddard P, Bransgrove R, Mason PT, Allen MJ. The Bactericidal Effect of Dendritic Copper Microparticles, Contained in an Alginate Matrix, on *Escherichia coli*. PLoS ONE 2014;9(5):e96225.
[27]. Santo EC, Quaranta D, Grass G. Antimicrobial metallic copper surfaces kill *Staphylococcus haemolyticus* via membrane damage. Microbiology Open 2012;1:46–52.
[28]. Baker J, Sitthisak S, Sengupta M, Johnson M, Jayaswal RK, Morrissey JA. Copper stress induces a global stress response in S*taphylococcus aureu*s and represses *sae* and *agr* expression and biofilm formation. Applied and Environmental Microbiology 2010;76:150-160.
[29]. Rensing C, Fan B, Sharma R, Mitra B, Rosen B. CopA: An *Escherichia coli* Cu(I) translocating P-type ATPase. Proceedings of the National Academy of Sciences of the United States of America 2000;97:652–656.

Prabhusaran N " In vitro antibacterial analysis of copper solution (microparticle) – a novel anti-infective molecule for wound management."IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 7, 2018, pp 22-26.