Potential use of Dry Metallic Copper and Colloidal silver solution to reduce survival of *Pseudomonas aeruginosa* isolates from healthcare environment

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Abstract

The objective of this study was to evaluate the activity of dry metallic copper and colloidal silver solution to reduce the viability of *P.aeruginosa* isolates compared with stainless steel as a control. Three clinical isolates of *P.aeruginosa* (108, 110 and 111) which were multi antibiotics resistant tested by inoculating 10^7 CFU/ml on to coupons(1cm x 1cm) of copper and stainless steel and incubated at room temperature for various time periods ranging from 30minutes up to 180 minutes .Bacterial viability was determined by plate viable count CFU/ml. The results on copper coupons shows complete killing of isolates after 120 min in contrast to stainless steel, viable organisms were detected after 180 min, indicating a significant *P* value (P <0.0001) . To the evaluation of colloidal silver activity on *P.aeruginosa* isolates, 10^7 CFU/ml of the resistant bacterial isolates were tested against 5ppm and 10 ppm of colloidal silver solution at room temperature for various time periods ranging from 30min up to 180 min also by using agar dilution method and killing rate technique .The results shows that bacterial isolates were sensitive to colloidal silver in both concentrations and complete killing of microorganisms achieved using 10 ppm concentration after 180 min with a significant *P* value (P <0.0001) .

Keyword: antimicrobial copper, colloidal silver, *Pseudomonas aeruginosa*

استعمال النحاس المعدني ومحلول الفضة الغروي في تقليل تواجد بكتريا *Pseudomonas aeruginosa* في بيئة الرعاية الصحية

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الخلاصة:

هدفت الدراسة الحالية إلى تقييم مدى فعالية النحاس ومحلول الفضة الغروي في تقليل حيوية بكتريا *P.aeruginosa* مقارنة مع الفولاذ المقاوم للصدأ كنموذج سيطرة . تم اختبار ثلاث عزلات سريريه متعددة المقاومة للمضادات من بكتريا(108، 110 و 111)*P.aeruginosa* وذلك بحقن لقاح بتركيز 10^7 خلية/مل على صفائح بمساحة (1 سم x 1 سم) لكل من النحاس و الفولاذ المقاوم للصدأ وتم حضنها بدرجة حرارة الغرفة لمدد زمنية تتراوح من 30 دقيقة إلى 180 دقيقة . تم بعدها تحديد الأعداد الحية للبكتريا بطريقة حساب العدد الحي للبكتريا من الطبق، حيث أظهرت النتائج انعدام البكتريا وقتلها بالكامل على صفائح النحاس بعد مرور 120 دقيقة بينما أظهرت النتائج وجود أعداد من البكتريا على صفائح الفولاذ المقاوم للصدأ بعد مرور 180 دقيقة بقيمة احتمالية (p < 0.0001). لتحديد فعالية محلول الفضة الغروي أخذت العزلات السريرية نفسها من بكتريا *P.aeruginosa* وذلك بحقن لقاح بتركيز 10^7 خلية/مليليتر في محلول الفضة الغروي بتركيز 5 جزء من المليون و 10 جزء من المليون على التوالي ودراسة التأثير في البكتريا بطريقة

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الانتشار من الحفر و تحديد مستوى القتل بحساب العدد الحي للبكتريا بعد حضنها بدرجة حرارة الغرفة لمدد زمنية مختلفة، أظهرت النتائج حساسية العزلات البكتيرية لمحلول الفضة الغروي بكلا التركيزين المستخدمين ونتائج حساب العدد الحي للبكتريا أظهرت حدوث القتل التام للبكتريا بمحلول الفضة الغروي ذو تركيز 10 جزء من المليون بعد مرور 180 دقيقة .

Introduction

Nosocomial infections cause a substantial burden for health and economics worldwide [1] . Of the most common nosocomial infectious agents is *Pseudomonas aeruginosa* with additional concern is the emergence and dissemination of organisms with increased resistance to antimicrobial agents [2 - 3] as this bacteria is inherently resistant to many antibiotics and have the ability to acquire resistance to other effective antimicrobials that are commonly used in hospitals [4].

P.aeruginosa is a major cause of nosocomial infections in immuncompromised and debitated patients and may cause severe cases of nosocomial pneumonia (45-70%)[5]. *P.aeruginosa* is also the third leading cause with 12% of hospital acquired urinary tract infections [6] and may cause other nosocomial infections like septicemia and chronic lung infections [7]. The contribution of contaminated hard surfaces such as bed rails, bed side tables and door knobs consider to be a major cause of nosocomial infections as the microbes can survive on hard surfaces for months [8 -9], with the presence of bacterial resistance to most conventional antibiotics this led to search for possible alternative antimicrobial agents that can destroy the resistance bacteria without side effects and lower coast [10]. Many studies shows that copper surfaces may help diminish surfaces related hygiene problems, copper surfaces proved to have a great killing efficiency against a wide range of microbes [11-12], as copper ions accumulate inside the cell and prevent reproduction by damaging cell membrane and proteins which cause cell death [13]. Colloidal silver which is a suspension of submicroscopic metallic silver particles in a colloidal base, possesses very high broad spectrum antimicrobial activity by causing deactivation of enzymes responsible for their respiration, multiplication and metabolism [14] . Gould *et al*., [15] reported in his study of antimicrobial properties of copper coupons compared with stainless steel coupons on various pathogens that the majority of *P.aeruginosa* isolates were killed within 60 minutes, while Iroha *et al*., [14] reported in his study of colloidal silver antimicrobial effect on *P.aeruginosa* isolates that the majority of these isolates were killed within 90 minutes. In this study, the evaluation of the effect of metallic copper and different concentration of colloidal silver on the viability of clinical isolates of *P.aeruginosa* were determined in different time periods.

Material s and methods

-Identification of *P.aeruginosa* isolates

The three selected isolates (108, 110 and 111) were identified depending on their morphological and biochemical tests compared with identification scheme described by Holt *et a., l* and Collee *et al*, [16-17]. The susceptibility results of the three selected isolates were shown in table 1.

Table 1-The susceptibility results of the three selected *P.aeruginosa* isolates (108, 110 and 111)

Antimicrobials	*P.aeruginosa* isolates		
	108	110	111
Ampicillin	R	R	R
Amoxicillin / Cavulanic acid	R	R	R
Pipracillin / Tazobactam	S	R	S
Cefazolin	R	R	R
Ceftriaxone	R	R	R
Cefepime	S	R	S
Imipenem	S	R	S
Meropenem	S	R	S
Amikacin	R	I	R
Gentamicin	S	I	S
Ciprofloxacin	R	R	R
Levofloxacin	S	R	S
Tetracycline	R	R	R
Nitrofurantion	R	R	R
Trimethoprim / sulfamethoxazole	R	R	R

*R= resistant, * S=sensitive, * I= intermediate

-Preparation of *P.aeruginosa* inoculum

Twelve bacterial isolates of *P.aeruginosa* were collected from different sources (sputum, pus, wounds swab). Selection of multi antibiotics resistant *P.aeruginosa* isolates in this study were depending on the susceptibility results of VITEK2 compact system as shown in

Table 1- The most resistant isolates (108, 110, 111) were selected and cultured on brain-heart infusion agar medium, incubated at 37 c^0 for 18-24 hours. Several colonies from the selected isolates were taken by sterile loop and placed in a tube containing 10 ml normal saline, mixed with the vortex . Several dilutions were made from this tube by culturing on nutrient agar and the colonies were counted, the tube which indicate approximately 10^7 CFU/ml was selected [18] .

-Preparation of copper and stainless steel coupon samples

Sample sheets(0.5 mm thickness) from each of copper and stainless steel (Produits dentaires S.A.vevey .Suisse) were sectioned into small coupons (1 cm x 1cm).Then coupons were degreased and cleaned by vortexing for 30 minutes in 10 ml of acetone containing approximately 30 glass beads of 2mm diameter . After cleaning, coupons were sterilized by ethanol and flaming, then all coupons transferred to a lidded plastic container before inoculation to prevent contamination. Coupons remained in the container during each experiment [18].

-Metal copper coupon samples testing with bacterial inoculum

Each coupon sample was aseptically inoculated with20 µL of 10^7CFU/ml from *Pseudomonas aeruginosa* (108, 110 and 111) and incubated at room temperature in closed sterile container, for varying time periods ranging from 30 minutes to 180 minutes . Post inoculation**,** organisms were removed from the coupons by vortexing for 30 minutes in 10 ml sterile phosphate buffer saline (PBS), PH- 7.2 containing approximately 20 glass beads of 2mm diameter . To ascertain the number of viable organisms removed from the coupons, 100 µL of (PBS) was removed and serially diluted to 10^{-4} in sterile (PBS)

Nutrient agar plates were inoculated with 50 µL of each dilution which was spread over the agar with sterile L-shaped spreader, Duplicates were done for each diluents, then all plates were incubated at 37 °C for 18-14 hours. Then the number of viable count for each dilution was counted and the mean of each dilution was calculated to estimate the number of viable CFU/coupons. Control coupons (stainless steel) were removed immediately after inoculation at time zero to determine the initial number of viable bacteria [18].

-Determining the effect of different concentrations of colloidal silver solution on bacterial isolates by using agar well diffusion technique

Bacterial inoculum (10^7 CFU/ml) of three selected *Pseudomonas aerugiosa* was streaked on Mueller-hinton agar plates, then an 8mm well was aseptically bored on the Petri dishes containing the agar by using a sterile cork borer. Colloidal silver solution in different concentration (5ppm and 10 ppm) was used to fill the holes in the agar plates, then the plates were incubated at 37 °C for 18-14 hours. The susceptibility of bacterial isolates to colloidal silver solution determined by measuring the inhibition zone diameter (IZDmm) around the hole that contain the colloidal silver solution [14]. Plates without colloidal silver solution served as control .

-Determination of the killing rates of different colloidal silver solution concentration

All *Pseudomonas aerugiosa* isolates in this study was cultured on brain –heart infusion agar medium and incubated at 37 °C for 18-14 hours, then, an inoculum of 10^7 CFU/ml of the test organisms were prepared as mentioned in preparation of inoculums . A volume 0.5ml of The 10^7 inoculum were added into 3 ml of colloidal silver solution in different test tubes containing (5ppm and 10 ppm) colloidal silver solution, incubated at room temperature, for varying periods ranging from 30 minutes to 120 minutes [10]. To determine the number of viable organisms after each incubation period, 100 µL of colloidal silver solution in both concentration (5 ppm and 10 ppm) was removed and serially diluted to 10^{-3} in sterile normal saline solution .Nutrient agar plates were inoculated with 50 µL of each dilution which was spread over the agar with sterile L-shaped spreader, Duplicates were done for each diluent, then all plates were incubated at 37 °C for 18-14 hours. Then the number of viable count for each dilution was counted and the mean of each dilution was calculated to estimate the number of viable CFU/ml [18]. The viable count of the tubes containing only the test organisms without colloidal silver served as control.

-Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-20 (Statistical Packages for Social Sciences-version 20), while different dependent means were tested using Student –t-test. The

significance of difference of different means was tested using analysis of variance (ANOVA), the statistical difference was determined as P<0.0001

-Results and Discussion

The results of exposure to copper produced significant reduction (*P* <0.0001) in the viability of all the three *P. aeroginosa* isolates . On copper, complete kill of the bacterial inoculum was achieved within 60 minutes as shown in figure 1. The results shows that the significant reduction in viability produced from exposure to 10ppm colloidal silver solution is very similar to copper effect (*P* <0.0001) except that the complete kill of bacterial inoculums by colloidal silver solution achieved within 180 minutes, ndicating greater tolerance to antimicrobial effect of copper .

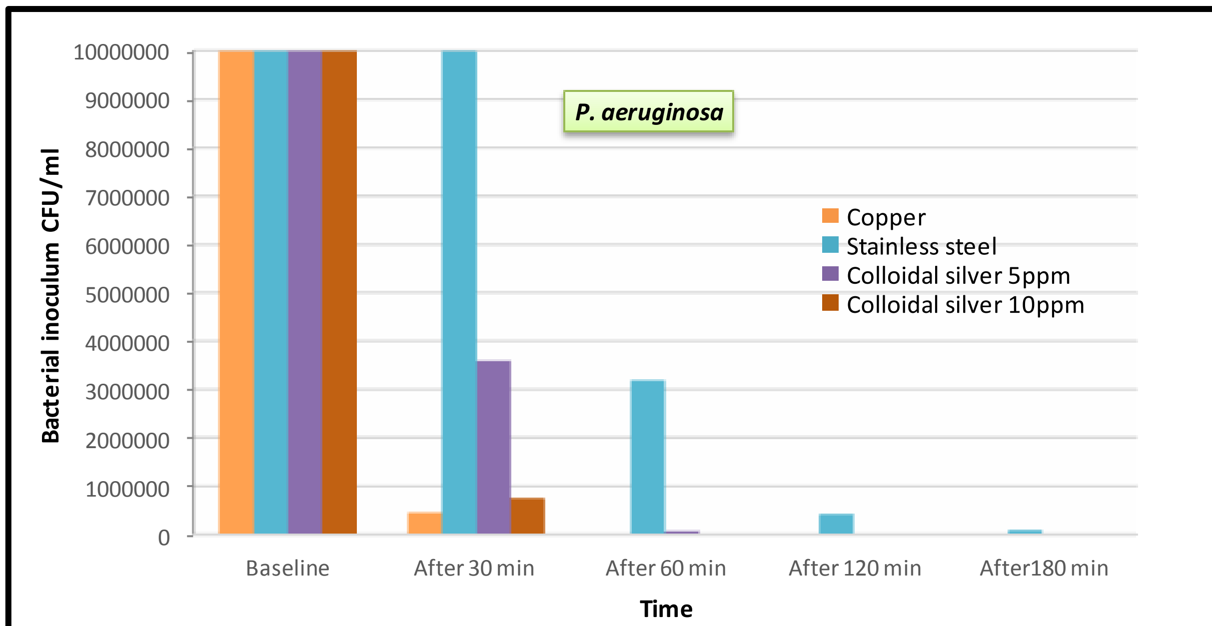


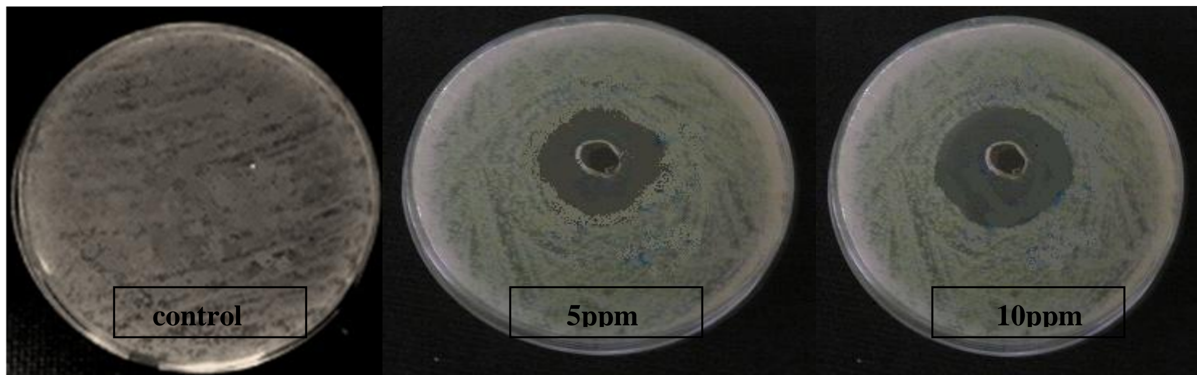
Figure 1- The effect of exposure to copper, colloidal silver and stainless steel at room temperature on *P.aeruginosa* isolates (108, 110 and 111)

Comparison of the data for *P.aeruginosa* isolates regarding exposure to Aluminum, stainless steel and 5 ppm colloidal silver are still significant (*P* <0.001) with regards to viability reduction, although not the same extent as those for copper and 10 ppm colloidal silver. Longer exposure periods were required before complete killing was achieved, notably 180 minutes for *P.aeruginosa* isolates compared with 60-120 minutes for copper exposure . The mean number of viable Cfu remained on stainless steel coupons and 5 ppm colloidal silver after 180 minutes were $88x10^3$, 103 CFU/ml respectively, while complete bacterial kill achieved with copper and 10 ppm colloidal silver within 120 minutes of exposure compared with 10^7 CFU/ml at time zero.

The results of sensitivity studies for colloidal silver in both concentrations 5 ppm and 10 ppm were shown in table 2. The obtained results shows that *P.aeruginosa* isolates were susceptible to antimicrobial action of colloidal silver with greater effect for 10 ppm concentration as shown in figure 2.

Table 2- Susceptibility of *P.aeruginosa* isolates to colloidal silver solution

Tested organisms	5ppm Colloidal silver Inhibition zone diameter (IZD mm)	10 ppm Colloidal silver Inhibition zone diameter (IZD mm)
P.aeruginosa 108	35.00	40.00
P.aeruginosa 110	32.00	38.00
P.aeruginosa 111	29.00	35.00



Figuer 2 - Inhibition zone diameter of *P.aeruginosa* 108 for Colloidal silver at 5ppm and 10 ppm

The results of this study shows high significant difference between exposure of bacteria to metallic copper and stainless steel, this difference may refer to the reason that cells on dry copper are not in an environment that promote growth, the elevated copper concentrations could led to rapid decline in cell membrane integrity [19] and this will cause alteration in its functions including permeability, transport of protein activity [20] . The accesses of copper ions interact with cell proteins and alter their functions [21-22]. The copper effect in killing bacteria have important implications for hospital environment where stainless steel is a common material used for work surfaces, consequently the incorporation of copper into work surfaces could significantly reduce the cross contamination between patients and hospital work surfaces which led to reduce hospital acquired infections [18].

The significant effect of 10 ppm colloidal silver may refer to its quick and fast penetration of minute silver particles into bacterial cell wall and works by inhibiting of oxygen metabolism enzymes that cause cell death in short periods of time [14].This observation suggests that colloidal silver may be effectively used as alternative antimicrobial agent for treatment of bacterial infections.

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