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The antimicrobial properties of copper surfaces against a range of important nosocomial pathogens

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Abstract - Hospital acquired infections (HAI) are a major problem worldwide and controlling the spread of these infections within a hospital is a constant challenge. Recent studies have highlighted the antimicrobial properties of copper and its alloys against a range of different bacteria. The objective of this study was to evaluate the antimicrobial properties of copper compared to stainless steel against a range of clinically important pathogens. These pathogens consisted of five isolates of each of the following organisms; meticillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Escherichia coli,* vancomycin-resistant Enterococci (VRE) and Panton-Valentine Leukocidin positive community acquired-MSSA (PVL positive CA-MSSA). MRSA, *P. aeruginosa, E. coli,* and CA-MSSA isolates were not detectable after a median time of 60 minutes. No detectable levels for all VRE isolates were determined after a median time of 40 minutes. However, for all isolates tested the stainless steel had no effect on the survival of the bacteria and levels remained similar to the time zero count. The results of this study demonstrate that copper has a strong antimicrobial effect against a range of clinically important pathogens compared to stainless steel and potentially could be employed to aid the control HAI.

Key words: nosocomial pathogens, copper, infection, antimicrobial.

INTRODUCTION

Nosocomial infections due to both Gram positive and negative organisms are a major problem in hospitals worldwide. This is further intensified by these organisms developing resistance to conventional antibiotics (Lautenbach and Polk, 2007). Bacteria of particular concern include meticillin resistant *Staphylococcus aureus,* vancomycin resistant Enterococci, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Mycobaterium tuberculosis*.

The sanitizing properties of copper have been known for millennia (Dollwet and Sorenson, 2001); the earliest recorded use of copper appears in the Smith papyrus (*circa* 2400 BC), which mentions how the ancient Egyptians used copper to sanitize drinking water and wounds (Dollwet and Sorenson, 2001). A later papyrus (Ebers papyrus *circa* 1500 BC) highlights how the same culture used copper as a remedy for headaches, "trembling of the limbs", burns, and itching. Later cultures including the Greeks, Celts, Hinus and American pioneers used copper for treating sores and skin infections, an approach which is still used in Africa and Asia (Dollwet and Sorenson, 2001). A number of recent studies have explored the potential benefits of using copper in place of stainless steel on surfaces in a number of settings including hospitals and the food industry (Faúndez *et al.*, 2004; Noyce *et al.*, 2006a, 2006b; Airey and Verran, 2007; Mehtar *et al.*, 2008). Both Noyce *et al.* (2006a) and Methar *et al.* (2008) have demonstrated that copper surfaces reduce bacterial loads from 10^7 CFU/ml to below detectable limits within 180 minutes and in some cases, with meticillin resistant *Staphylococcus aureus* (MRSA), in as little as 60 minutes. The former report focuses on the use of copper to reduce *E. coli* O157 contamination during food processing and the latter reports reduction of nosocomial organisms in healthcare facilities.

It is notable that the majority of studies reported to date utilise clean copper surfaces which may not reflect the reality of healthcare facilities or food processors during use. Airey and Verran (2007) demonstrated that even after the repeated cleaning regimes found in hospitals, considerable contamination of the copper surfaces was found using albumin in a model study. A variety of approaches are under development to overcome issues of biofouling of copper surfaces with concomitant reduction in antimicrobial properties. These include insertion of copper compounds in chitosan and glass fibres, paints, varnish and stainless steel (during manufacture) along with the development of copper based nanoparticles and surface coating of silicone rubbers with copper (Cooney, 1995; Abou Neel *et al.*, 2005; Song *et al.*, 2005; Baena *et al.*, 2006; Qin *et al.*, 2007; Ruparelia *et al.*, 2008; Thneibat *et al.*, 2008; Wheeldon *et al.*, 2008). Multiple metal

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coatings have been assessed for antimicrobial activities on catheter materials with Cu-Ag coatings being most effective (Mclean *et al.*, 1993). A recent study has demonstrated the biocidal activities of copper oxide coated glass against Gram negative *Escherichia coli* and Gram positive *Staphylococcus epidermidis* (Yates *et al.*, 2008).

Although these studies demonstrate the potential applications of copper based biocidal surfaces, several further considerations are warranted. Many of the studies have been conducted on clean surfaces under laboratory conditions using type strains of the organisms. The copper coating processes and copper doped stainless steel approaches require significant technological involvement and considerable expense which may limit their widespread use. The overall aim of this project is to develop an economic and feasible copper based biocidal coating for application in the hospital environment. A key stage is to determine the effectiveness of copper surfaces against isolates of hospital acquired infections from UK. This is the first report of the antimicrobial properties of copper coupons (Cu) compared to stainless steel coupons (SS) against a range of five key nosocomial pathogens isolated from patients.

MATERIALS AND METHODS

Bacterial strains. A total of 25 isolates (consisting of five isolates per bacterial species) were collected from two London based hospitals. The bacteria collected included clinical isolates of: meticillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Escherichia coli* and vancomycin-resistant Enterococci (VRE) and Panton-Valentine Leukocidin positive community acquired-meticillin sensitive *Staphylococcus aureus* (PVL CA-MSSA).

Antibiotic sensitivity testing. The five groups of bacteria were tested against a specific panel of antibiotics for that group using standard operating procedures defined by the British Society for Antimicrobial Chemotherapy (Andrews, 2007). The MRSA isolates were tested against cefoxtin (10 µg), penicillin (1 unit), erythromycin (5 µg), gentamcin (10 µg), rifampicin (2 µg), cancomycin (5 µg), mupirocin (5 µg) and cefuroxime (5 µg). The PVL CA-MSSA isolates were tested against penicillin (1 unit) and meticillin (5 µg). The *P. aeruginosa* isolates were tested against gentamycin (10 µg), ceftazidime (30 µg), ciprofloxacin (1 µg), tazocin (85 µg), amikacin (30 µg), colistin (25 µg) and meropenem (10 µg). The *E. coli* isolates were tested against: ampicillin (10 µg), ceftazidime (30 µg), ciprofloxacin (1 µg), tazocin (85 µg), gentamycin (10 µg), cefuroxime (30 µg), amikacin (30 µg), cefotaxime (30 µg), meropenem (10 µg), colistin (25 µg), ertapenem (10 µg), and tigarcillin (15 µg). The VRE isolates were tested against: ampicillin (10 µg), tetracycline (10 µg), teicoplanin (30 µg), gentamycin (200 µg), vancomycin (5 µg) and linezolid (10 µg).

Preparation of sample metal coupons. Copper foil (0.5 mm thick; purity 99.98%, Oxoid) and stainless steel samples were first cut into 1 cm^2 coupons. Coupons were cleaned before testing using a modified method as described by Noyce *et al.* (2006b). The coupons were vortexed in approximately 5 ml of a commercially available cleaner containing sulfamic acid and phosphoric acid, to remove any tarnishing and subsequently washed thoroughly with water. After drying the coupons were individually degreased and cleaned by vortexing in 10 ml of acetone containing approximately 30 glass beads of 2 mm diameter (BDH). After cleaning, the coupons were sterilized by being dipped in ethanol, the ethanol ignited and the residual alcohol allowed to burn off. Sterilized coupons were then placed in a sterile plastic Petri dish prior to use.

Inoculation of bacteria onto copper and stainless steel coupons. Antimicrobial properties of the metals were tested using a modified method as described by Noyce *et al.* (2006b). Overnight starter cultures were inoculated into 15 ml of Nutrient broth (Oxoid) and grown for 16 h at 37 °C. Aliquots of culture broth (20 µl) were spotted onto the centre of either copper (Cu) or stainless steel (SS) coupons and incubated at room temperature for 2 h. Every 20 min a set of Cu and SS coupons were aseptically removed and placed into 10 ml aliquots of sterile phosphate buffered saline (PBS, Oxoid), containing approximately 20 glass beads and vortexed for 30 s to remove bacteria from the coupon. Viable counts were determined by removing 100 µl and serially diluting to 10^{-4} in PBS, 50 µl of each dilution was then spread evenly over a nutrient agar plate and incubated aerobically at 37 °C for 24 h. Post incubation colony forming units per coupon were determined for each time point from zero to two hours. Bacterial density of the starter culture was determined as above. All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Antibiotic sensitivity testing

Antibiotic sensitivity profiles were first determined for clinical isolates of five different groups of bacteria (Table 1), of these only the *P. aeruginosa* isolates were determined to be totally sensitive to the panel of seven antibiotics tested against them. The MRSA isolates were shown to be resistant on average to half the antibiotics in the panel test (four of eight drugs). The CA-MSSA isolates were only resistant to penicillin. The *E. coli* isolates showed resistance to, on average, five of the twelve antibiotics in the panel. Whilst the VRE isolates were shown to be resistant to a mean of three of six antibiotics, with all isolates resistant to vancomycin and ampicillin. The relationship between antimicrobial resistance patterns and resistance to copper exposure is discussed in the following paragraphs.

Inoculation of bacteria onto copper and stainless steel coupons

Many inanimate surfaces found within hospitals, such as door handles, knobs, push plates and taps are made from stainless steel. The choice of stainless steel over other material results from its durability, ease in cleaning, as well as the lack of tarnishing that may make the surface appear unclean. However, a recent review of current evidence concluded that important organisms, both Gram positive (MRSA, VRE) and negative (*Acinetobacter* spp., *E. coli*, *P. aeruginosa*), had been found to survive on stainless steel surfaces for prolonged periods of time (Kramer *et al.*, 2006). These surfaces can potentially act as a reservoir for the transfer of these organisms to health care workers (HCW), patients and family members; ultimately leading to the potential transfer from HCW to patient (Oie *et al.*, 2002). Cross transmission of infection from patient to patient, via a HCW remains one of the main routes of transfer in the hospital environment (Halwani *et al.*, 2006; Vonberg *et al.*, 2007). A number of recent studies have examined the potential antimicrobial use of copper compared to stainless steel (Faúndez *et al.*, 2004; Noyce *et al.*, 2006a, 2006b; Airey and Verran, 2007; Mehtar *et al.*, 2008; Weaver *et al.*, 2008). These studies demonstrated that when

TABLE 1 - Bacterial samples, source and the antibiotic sensitivity profiles for all 25 isolates

Isolates	Source*	Antibiotics to which the isolates were resistant**
Meticillin resistant *Staphylococcus aureus* 1	BW	Fluxclox/FOX, Pen, CXM
Meticillin resistant *Staphylococcus aureus* 2	WS	Fluxclox/FOX, Pen, Ery, CXM
Meticillin resistant *Staphylococcus aureus* 3	NS	Fluxclox/FOX, Pen, Ery, Gent, CXM, MUP
Meticillin resistant *Staphylococcus aureus* 4	SPT	Fluxclox/FOX, Pen, Ery, CXM
Meticillin resistant *Staphylococcus aureus* 5	NS	Fluxclox/FOX, Pen, Ery, CXM
Community acquired MSSA 1	WS	Pen
Community acquired MSSA 2	WS	Pen
Community acquired MSSA 3	WS	Pen
Community acquired MSSA 4	WS	Pen
Community acquired MSSA 5	WS	Pen
Pseudomonas aeruginosa 1	SPT	No resistance
Pseudomonas aeruginosa 2	SPT	No resistance
Pseudomonas aeruginosa 3	U	No resistance
Pseudomonas aeruginosa 4	U	No resistance
Pseudomonas aeruginosa 5	EAR	No resistance
Escherichia coli 1	IAA	Amp, Cip, TZP, CXM, CT
Escherichia coli 2	U	Amp, Caz, Cip, Gent, CXM, CT
Escherichia coli 3	PL.FLU	Amp, Caz, TZP, Gent, CXM, CT
Escherichia coli 4	U	Amp, Caz, Cip, CXM, CT
Escherichia coli 5	U	Amp, Cip, Gent, CXM
Vancomycin resistant Enterococci 1	Stool	Amp, Tec, Va
Vancomycin resistant Enterococci 2	Stool	Amp, Va
Vancomycin resistant Enterococci 3	Stool	Amp, Tet, Tec, Gent, Va
Vancomycin resistant Enterococci 4	U	Amp, Va
Vancomycin resistant Enterococci 5	U	Amp, Tec, Va

* BW: breast wound, WS: wound swab, NS: nose swab, SPT: sputum, U: urine: EAR: ear, IAA: intra abdominal abscess, PL.FLU: pulmunary fluid.

** Fluclox/FOX: cefoxtin, Pen: penicillin, CXM: cefuroxime, Ery: erythromycin, Gent: gentamicin, MUP: muprocin, Amp: ampicillin, Cip: ciprofloxacin, TZP: tazocin, CT: colistin, Caz: ceftazidime, Tec: teicoplanin, Va: vancomycin, Tet: tetracycline.

cultures were applied to both metals, stainless steel was shown to have no antimicrobial effect over a six hour period. Whereas antimicrobial affects were seen with the copper coupons, with no detectable growth of the starter culture within approximately sixty minutes. However, to date, these studies are limited to only one or two clinical isolates and have relied mainly on testing its affect against typed laboratory strains.

In the current study a range of important clinical isolates (25 isolates in total), were exposed to copper coupons and it was found that the majority of isolates were killed after 60 min, with time ranging from as little as 40 min up to 100 min. However, it should be noted that survival to 100 min was only achieved by one MRSA isolate. The antimicrobial resistance patterns of the isolates did not appear to have any effect on the antimicrobial properties of copper. No antimicrobial effects were seen with the cultures inoculated onto the stainless steel coupons.

The majority of the MRSA isolates (three of the five isolates) were killed within 60 min (Fig. 1); two remaining isolates demonstrated no detectable growth after 80 and 100 minutes respectively. No CA-MSSA could be detected after 80 mins of incubation on the copper coupons (Fig. 2). The majority of the isolates (four of five the isolates) were killed within 40 to 60 min. The majority of the *P. aeruginosa* isolates (four of five the isolates) were killed within 60 min (Fig. 3); however, one isolate had a slightly longer survival time of 80 min. When the *E. coli* were spotted onto the Cu coupons no detectable levels of bacteria were isolated after 80 min (Fig. 4). The majority of the isolates (three of the five isolates) were killed within 60 min, one isolate showed a faster rate of killing with no detectable growth after just 40 min. After 60 min, no detectable levels of VRE were isolated from the copper coupons (Fig. 5). Three of the five isolates were killed within 40 min contact with the Cu coupon, with the remaining isolates demonstrating no detectable growth after 60 min. These results concur with current data (Faúndez *et al.*, 2004; Noyce *et al.*, 2006a, 2006b; Mehtar *et al.*, 2008; Weaver *et al.*, 2008); however, this is the first time that copper surfaces have been tested against a large number of clinical isolates of important organisms as apposed to laboratory strains. In addition it is the first time it has been shown to be effective against multi drug resistant *E. coli* and VRE.

Many of the previous studies have examined pure copper as well as copper based alloys (Noyce *et al.*, 2006a; Mehtar *et al.*, 2008; Weaver *et al.*, 2008) and in all of these studies the pure copper was shown to be the most effective contact antimicrobial. Some copper based alloys show antibacterial properties although the survival times of the bacteria on these metals were found to be longer than that on pure copper. Noyce *et al*. (2006a; 2006b) investigated pure copper and copper based alloys in a number of studies using different organisms. On pure copper no detectable growth of organisms was seen between 50 to 80 min; however, on the alloy of 80% copper the time had increased to approximately 265 min. This extended period is on average four times longer than on pure copper and raises a question about which form of copper should be used, pure versus alloy. Copper in its pure form is a soft metal and therefore likely to wear faster than a copper alloy, such as brass, which is a hard and more durable metal. However, current research has shown that pure copper decreases the bacterial load four times faster than alloys and hence pure copper was focused upon in this current study.

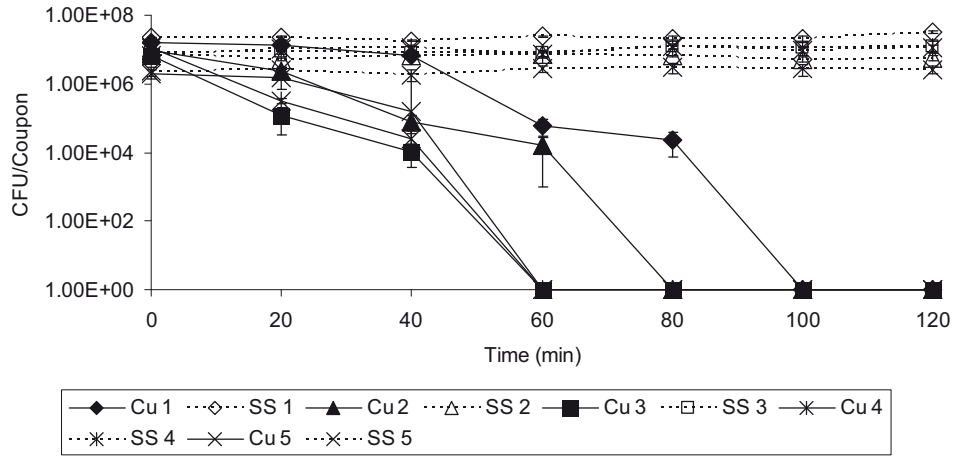

FIG. 1 - Survival curves for the five isolates of meticillin resistant *Staphylococcus aureus* on copper (Cu) and stainless steel (SS) coupons. Error bars represent standard error of the mean.

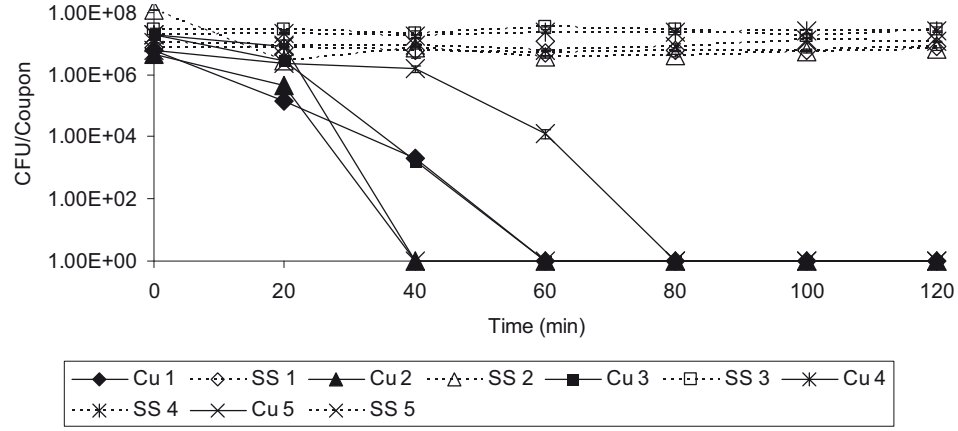

FIG. 2 - Survival curves for the five isolates of community acquired MSSA on copper (Cu) and stainless steel (SS) coupons. Error bars represent standard error of the mean.

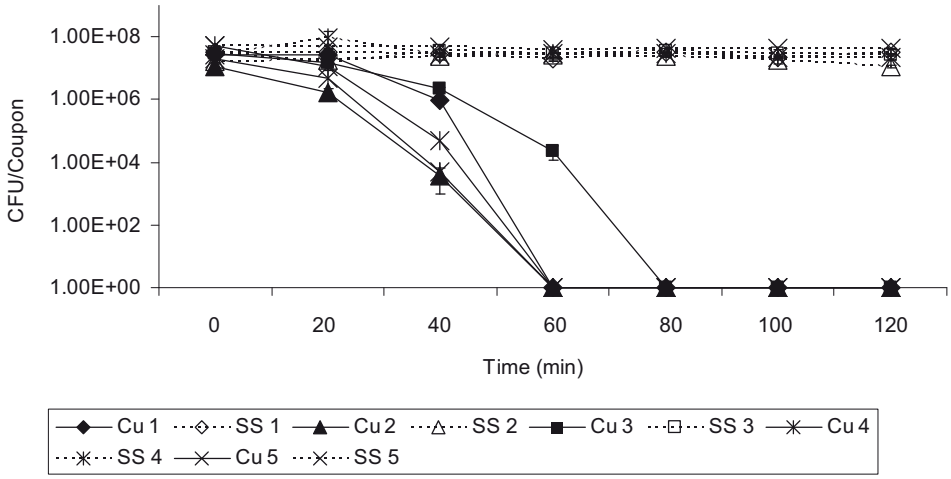

FIG. 3 - Survival curves for the five isolates of *Pseudomonas aeruginosa* on copper (Cu) and stainless steel (SS) coupons. Error bars represent standard error of the mean.

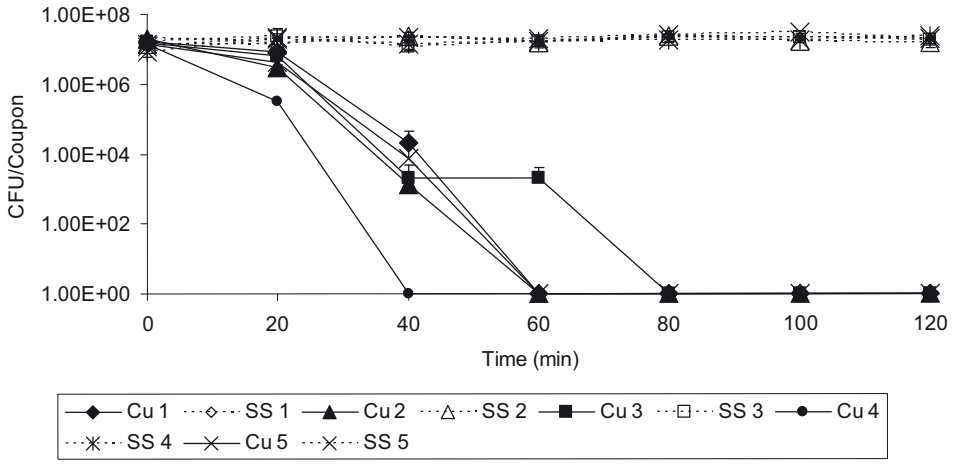

FIG. 4 - Survival curves for *Escherichia coli* on copper (Cu) and stainless steel (SS) coupons. Error bars represent standard error of the mean.

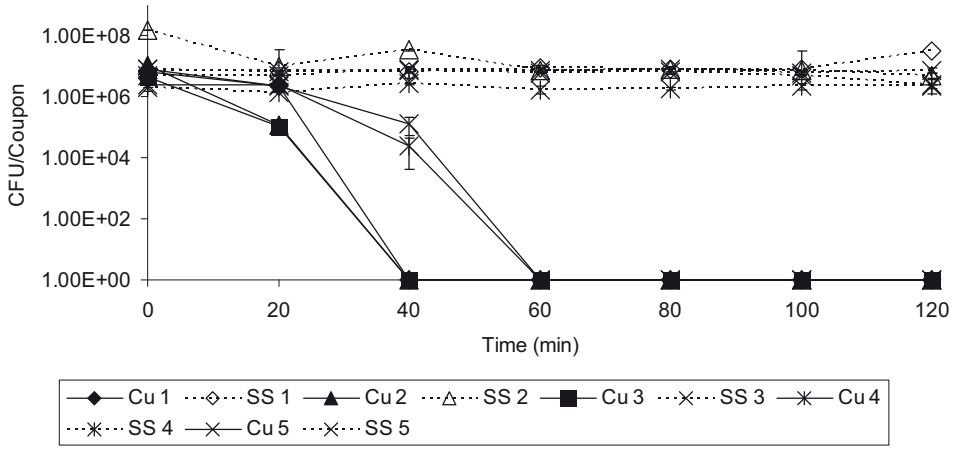

FIG. 5 - Survival curves for the five isolates of vancomycin resistant Enterococci on copper (Cu) and stainless steel (SS) coupons. Error bars represent standard error of the mean.

In conclusion, the aim of this study was to investigate the antimicrobial properties of copper versus stainless steel against a range of important clinical isolates. This study has shown for the first time that copper surfaces can kill clinical isolates of multi drug resistant *E. coli* and VRE as well as isolates of MRSA, CA-MSSA and *P. aeruginosa* in an average time scale of 60 min.

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References

Abou Neel E.A., Ahmed I., Pratten J., Pratten J., Nazhat S.N., Knowles J.C. (2005). Characterisation of antibacterial copper releasing degradable phosphate glass fibres. Biomaterials, 26: 2247-2254.

Airey P., Verran J. (2007). Potential use of copper as a hygienic surface; problems associated with cumulative soiling and cleaning. J. Hosp. Infect., 67: 271-277.

Andrews J.M. for the BSAC Working Party on Susceptibility Testing (2007). BSAC standardized disc susceptibility testing method (version 6). J. Antimicrob. Chemother., 60: 20-41.

Baena M.I., Marquez M.C., Matres V., Botella J., Ventosa A. (2006). Bactericidal activity of copper and niobium-alloyed austenitic stainless steel. Curr. Microbiol., 53: 491-495.

Cooney T.E. (1995). Bactericidal, activity of copper and noncopper paints. Infect. Control. Hosp. Epidemiol., 16: 444-450.

Dollwet H.H.A., Sorenson J.R.J. (2001). Historic uses of copper compounds in medicine. J. Trace. Elem. Med. Biol., 2: 80-87.

Faúndez G., Troncoso M., Navarrete P., Figueroa G. (2004). Antimicrobial activity of copper surfaces against suspensions of *Salmonella enterica* and *Campylobacter jejuni*. BMC Microbiol., 4: 19.

Halwani M., Solaymani-Dodaran M., Grundmann H., Coupland C., Slack R.J. (2006). Cross-transmission of nosocomial pathogens in an adult intensive care unit: incidence and risk factors. J. Hosp. Infect., 63: 39-46.

Kramer A., Schwebke I., Kampf G. (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect. Dis., 16: 130.

Lautenbach E., Polk R.E. (2007). Resistant Gram-negative bacilli: A neglected healthcare crisis? Am. J. Health-Syst. Ph., 64: S3-21.

Mclean R.J.C., Hussain A.A., Sayer M., Vincent P.J., Hughes D.J., Smith T.J.N. (1993). Antibacterial activity of multilayer silver copper surface-films on catheter material. Can. J. Microbiol., 39: 895-899.

Mehtar S., Wiid I., Todorov S.D. (2008). The antimicrobial activity of copper and copper alloys against nosocomial pathogens and *Mycobacterium tuberculosis* isolates from healthcare facilities in the Western Cape: an in-vitro study. J. Hosp. Infect., 68: 45-51.

Noyce J.O., Michels H., Keevil C.W. (2006a). Use of copper cast alloys to control *Escherichia coli* O157 cross-contamination during food processing. Appl. Environ. Microbiol., 72: 4239-4244.

Noyce J.O., Michels H., Keevil C.W. (2006b). Potential use of copper surfaces to reduce survival of epidemic meticillin-resistant *Staphylococcus aureus* in the healthcare environment. J. Hosp. Infect., 63: 289-297.

Oie S., Hosokawa I., Kamiya A. (2002). Contamination of room door handles by methicillin-sensitive/ methicillin-resistant *Staphylococcus aureus.* J. Hosp. Infect., 51: 140-143.

Qin Y.M., Zhu C.J., Chen J., Liang D., Wo G.F. (2007). Absorption and release of zinc and copper ions by chitosan fibers. J. Appl. Polym. Sci., 105: 527-532.

Ruparelia J.P., Chatteriee A.K., Duttagupta S.P., Mukherji S. (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater., 4: 707-716.

Song J.S., Lee S., Cha G.C., Jung S.H., Choi S.Y., Kim K.H., Mun M.S. (2005). Surface modification of silicone rubber by ion beam assisted deposition (IBAD) for improved biocompatibility. J. Appl. Polym. Sci., 96: 1095-1101.

Thneibat A., Fontana M., Cochran M.A., Gonzalez-Cabezas C., Moore B.K., Matis B.A., Lund M.R. (2008). Anticariogenic and antibacterial properties of a copper varnish using an *in vitro* microbial caries model. Oper. Dent., 33: 142-148.

Vonberg R.P., Wolter A., Chaberny I.F., Kola A., Ziesing S., Suerbaum S., Gastmeier P. (2007). Epidemiology of multidrug-resistant Gram-negative bacteria: Data from an university hospital over a 36-month period. Int. J. Hyg. Environ. Health., 211: 251-257.

Weaver L., Michels H.T., Keevil C.W. (2008). Survival of *Clostridium difficile* on copper and steel: futuristic options for hospital hygiene. J. Hosp. Infect., 68: 145-151.

Wheeldon L.J., Worthington T., Lambert P.A., Hilton A.C., Lowden C.J., Elliott T.S. (2008). Antimicrobial efficacy of copper surfaces against spores and vegetative cells of *Clostridium difficile*: the germination theory. J. Antimicrob. Chemother., 62: 522-525.

Yates H.M., Brook L.A., Sheel D.W., Ditta I.B., Steele A., Foster H.A. (2008). The growth of copper oxides on glass by flame assisted chemical vapour deposition. Thin Solid Films, doi: 10.1016/j.tsf.2008.06.071