Journal of Hospital Infection (2008) **68**, 45–51

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The antimicrobial activity of copper and copper alloys against nosocomial pathogens and *Mycobacterium tuberculosis* isolated from healthcare facilities in the Western Cape: an in-vitro study

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Received 6 June 2007; accepted 9 October 2007
Available online 11 December 2007

KEYWORDS
Copper; Alloys; *Mycobacterium tuberculosis*; Bactec 12B growth medium; In-vitro activity; MRSA; *Acinetobacter baumannii*; *Klebsiella pneumoniae*; Candida albicans

Summary Clinical isolates of meticillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, Candida albicans and *Mycobacterium tuberculosis* (MTB) were tested against copper (Cu) and its alloys. Stainless steel and polyvinylchloride (PVC) were used as controls. The amount of Cu required to inhibit test isolates at room temperature (24 °C) and at 4 °C was determined. At room temperature, Cu, DZR Brass (Cu 62%, Pb 2.5%, arsenate 0.13% and Zn 22.5%) and Brass 70/30 (Cu 70% and zinc 30%) inhibited C. albicans and *K. pneumoniae* at 60 min; nickel silver (NiAg) inhibited C. albicans at 60 min and *K. pneumoniae* at 270 min. *P. aeruginosa* was inhibited by Brass 70/30 and nickel silver (NiAg) at 180 min and at 270 min by Cu and DZR. Cu and DZR inhibited *A. baumannii* at 180 min while the other alloys were effective at 360 min. Stainless steel and PVC showed little or no inhibitory activity. Two *M. tuberculosis* strains, one isoniazid resistant (R267) and the other multidrug resistant (R432), demonstrated growth inhibition with Cu of 98% and 88% respectively compared with PVC; the other alloys were less

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0195-6701/$ - see front matter
doi:10.1016/j.jhin.2007.10.009

active. Time to positivity (TTP) for R267 was >15 days with Cu and 11 days for the other alloys; with R432 it was 5 days. Effective inhibition of nosocomial pathogens and MTB by Cu and alloys was best when the Cu content was >55%.

Introduction

An alarming increase in antibiotic resistance among hospital pathogens has revived interest in alternative methods of reducing bioburden in healthcare facilities, focusing on the environment within hospitals.[1]

Copper is known to have activity against bacteria and fungi. Its natural ability to reduce the bioburden of environmental microbes is exploited in water purification, paint and building material, and the textile industry. The activity of Cu against Gram-positive cocci such as meticillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacilli causing food-associated disease, such as *Escherichia coli* O157 *Campylobacter jejuni* and *Salmonella* spp., has been reported.[2] More recently multidrug-resistant (MDR) and extremely drug resistant (XDR) *Mycobacterium tuberculosis* (MTB) in South Africa has drawn attention to the spread of tuberculosis in hospitals.[3,4]

The aim of this study was to establish the in-vitro activity of Cu and its alloys against highly multiple-antibiotic-resistant nosocomial pathogens, yeast and MTB isolated from South African patients. Test strains were selected from the currently prevalent nosocomial isolates in healthcare facilities and clinical isolates of MTB from the Western Cape, and used to establish the minimum quantity of Cu required in an alloy that would produce sterilisation.

One of the drawbacks of Cu is discoloration when exposed to oxygen. This study also aimed to establish the Cu content in alloys with low tarnishing properties which could be applicable to healthcare facilities with sufficient antimicrobial activity to reduce the environmental bioburden.

Methods

Metal alloys

Metal coupons (1 cm × 1 cm) of Cu and its alloys (supplied by the International Copper Association) were tested against multiple-antibiotic-resistant clinical isolates. The coupons were made of deoxided phosphorus high (DPH) Cu containing 99.9% Cu, Brass 70/30 (Cu 70%, zinc 30%), copper nickel (CuNi) (Cu 90% and Ni 10%), nickel silver (NiAg) (Cu 55%, Ni 18%, Zn 27%), dezincification resistant (DZR) Brass (Cu 62%, Pb 2.5%, arsenate 0.13%, Zn 22.5%). Stainless steel (SS) and polyvinylchloride (PVC) were included as controls. Each set of coupons was allocated a code which was broken at the end of the study. The coupons were sterilised by autoclaving followed by flaming with 70% ethanol, and stored in sterile Petri dishes; the difference in the inhibitory effect of Cu and its alloys by the two methods of sterilisation was noted.

Bacterial and fungal strains

Candida albicans, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and meticillin-resistant *Staphylococcus aureus* (MRSA) were isolated from blood cultures, wounds and endotracheal sites of patients admitted to the intensive care unit. The strain of *Acinetobacter baumannii* used was a multiple-antibiotic-resistant isolate from a patient in the burns unit. Two clinical strains of MTB, Strain R267 resistant to isoniazid alone (>0.1 μg/ml) and Strain R432 multidrug resistant (isoniazid > 0.1 μg/ml, rifampicin > 2.0 μg/ml, streptomycin > 2.0 μg/ml, and ethambutol > 2.5 μg/ml), were tested against Cu and its alloys, stainless steel (SS) and PVC (control) at both room temperature (−25 °C) and 4 °C.

Killing curves

Non-MTB strains[2]

A single colony of each test strain was transferred to Sabouraud agar for C. albicans, and Brain Heart Infusion (BHI, Biolab, South Africa) for *K. pneumoniae*, *S. aureus*, *P. aeruginosa* and *A. baumannii* and incubated at 37 °C overnight. After purity had been checked by Gram stain, 0.1 ml of *C. albicans* was inoculated into 15 ml of Sabouraud and similarly *K. pneumoniae*, *P. aeruginosa*, *A. baumannii* and *S. aureus* were inoculated in 15 ml of