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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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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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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

BHI and incubated at 37 °C overnight to yield $\sim 5 \times 10^8$ cfu/ml.

Each coupon was inoculated with 20 μl of culture (2.5×10^7 cfu) and spread using the tip of a sterile pipette. The coupons were then incubated at either room temperature or at 4 °C for the designated exposure period (i.e. 15, 30, 45, 60, 75, 90, 180, 270 and 360 min). After incubation, the coupons were placed in sterile disposable screw-capped bottles containing 10 ml sterile phosphate-buffered saline (PBS) with ~20 glass beads (2 mm in diameter), and centrifuged for 30 s at 300 *g*. One hundred microlitres were removed and serially diluted through four 10-fold dilutions in sterile PBS. Nutrient agar plates were inoculated with 100 μl of each dilution and spread evenly over the surface of the agar with a sterile glass rod. After incubation at 37 °C for 18 h the counts from each coupon were recorded. Each set of coupons was tested in triplicate from each dilution at both temperatures; the mean was taken as the final result. The controls were sampled at time zero and the initial number of viable cells determined.

MTB strains

All mycobacterial work was carried out in a Level III Biosafety laboratory. MTB strains were grown on Lowenstein—Jensen culture medium. After incubation at 37 °C for 21 days, purity of the MTB strains was ascertained by acid-fast staining and then suspended in 7H9 mycobacterial growth medium. The bacterial suspension was added to a Bactec vial containing the radiolabelled Bactec 12B growth medium (Becton Dickinson, Franklin Lakes NJ, USA). To obtain an approximately standard inoculum, vials were checked every 24 h until a growth inhibition (GI) index of 300—500 was achieved; this index is a quantitative determination of radioactive CO_2 on a scale from 0 to 999. The bacterial suspension was added to a Bactec vial containing Bactec 12B. Growth was monitored every 24 h until a GI-value of 300—500 was reached; 0.1 ml of this culture was added to a new Bactec vial and the growth monitored every 24 h until a GI-value of 500 was reached. This primary culture was used for testing bacterial viability against a variety of metal alloys. The metal coupons were aseptically arranged in a sterile plastic 150 mm Petri dish which contained a 2 mm layer of sterile 7H11 mycobacterial agar to prevent drying and disc movement during handling. One hundred microlitres of the primary culture was added to each disc using a 1 ml tuberculin syringe. Each 100 μl aliquot was gently agitated to eventually form an 8 mm circular spread on the disc. The Petri dishes were covered and sealed.

After 72 h at 37 °C (three doubling times) cultures were aspirated from the individual coupons and added to a fresh Bactec vial which was then incubated at 37 °C and the GI index monitored every 24 h for up to 15 days.

The inhibitory effect of the coupons on mycobacterial growth was evaluated by the time taken for the culture to become detectable by Bactec with a growth index of >10 [time to positive (TTP)].

Results

Killing curves showing the antimicrobial activity of the metal alloys at room temperature are illustrated while the activity at 4 °C is described in the text. Overall, better antimicrobial activity was noted for all the metal alloys at room temperature than 4 °C.

Candida albicans

The activity of Cu and related alloys was better at room temperature (Figure 1) than at 4 °C. Cu was equally active against C. albicans at both temperatures, resulting in a reduction from 10^7 colony-forming units (cfu)/ml to zero at 60 min. The antifungal activity of NiAg improved from 360 min at 4 °C to 60 min at room temperature. Other alloys such as DZR, Brass 70/30 and CuNi showed a 100-fold reduction at 4 °C after 260 min and total killing at 60 min and 90 min respectively at room temperature (Figure 1). It was noteworthy that at 360 min C. albicans was not isolated from SS; a 100-fold reduction was noted with PVC.

Klebsiella pneumoniae

This was completely inhibited by Cu at both temperatures by 60 min. A 100-fold reduction was noted for DZR and NiAg at 4 °C after 360 min. At room temperature the antibacterial activity of Cu, DZR and Brass 70/30 against *K. pneumoniae* was complete within 60 min and with NiAg total inhibition was evident at 270 min (Figure 2). Neither SS nor PVC demonstrated a lethal effect on *K. pneumoniae* at either temperature.

Pseudomonas aeruginosa

Copper and its alloys showed little or no effect against *P. aeruginosa* at 4 °C; however, at room temperature, total inhibition was achieved with Brass 70/30 and NiAg at 180 min and with Cu and DZR at 270 min (Figure 3). A 100-fold reduction was noted with CuNi but not with either SS or PVC.

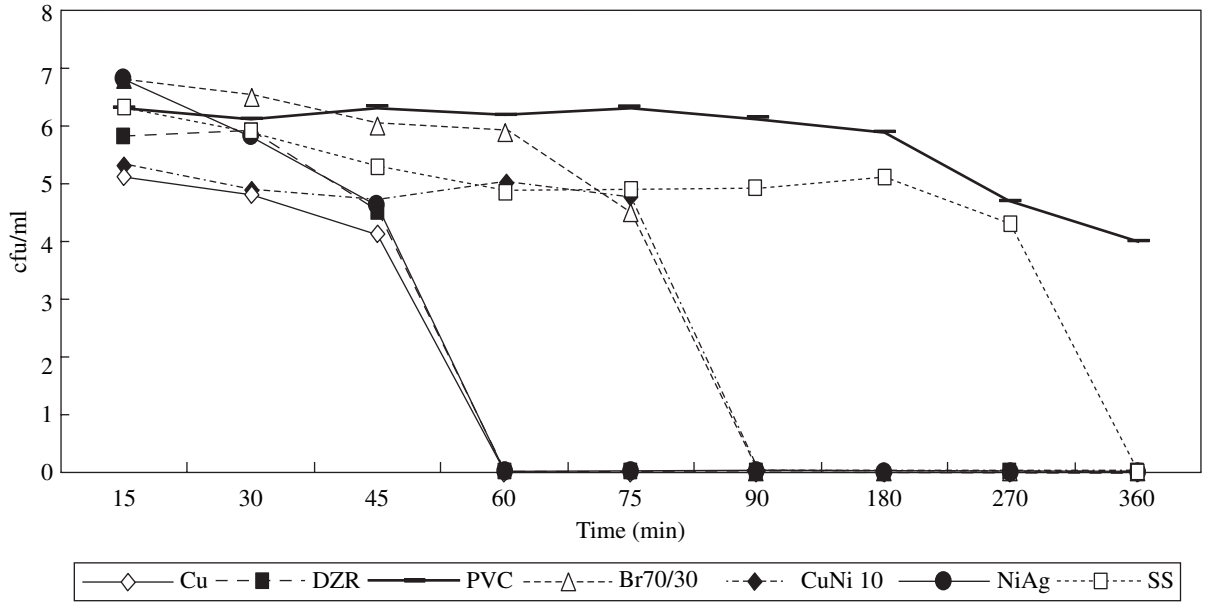

Figure 1 Killing curves for C. albicans (intensive care unit, room temperature). *C. albicans* was killed after 60 min exposure to copper (Cu), DZR Brass, copper nickel (CuNi) and at 90 min with Brass (Br) 70/30 and nickel silver (NiAg). PVC, polyvinylchloride; SS, stainless steel.

Acinetobacter baumannii

Copper and all its alloys inhibited *A. baumannii* after 180 min at room temperature (Figure 4); at 4 °C DZR demonstrated total inhibition of the strain at 180 min, whereas the other metals demonstrated the same effect only after 360 min. There was no effect with either SS or PVC.

Meticillin-resistant *Staphylococcus aureus* (MRSA)

There was little or no inhibition of MRSA at 4 °C when exposed to any of the metal alloys. However, at room temperature, inhibition was found with NiAg and Brass 70/30 at 180 min and with Cu and DZR at 270 min (Figure 5). A 100-fold reduction

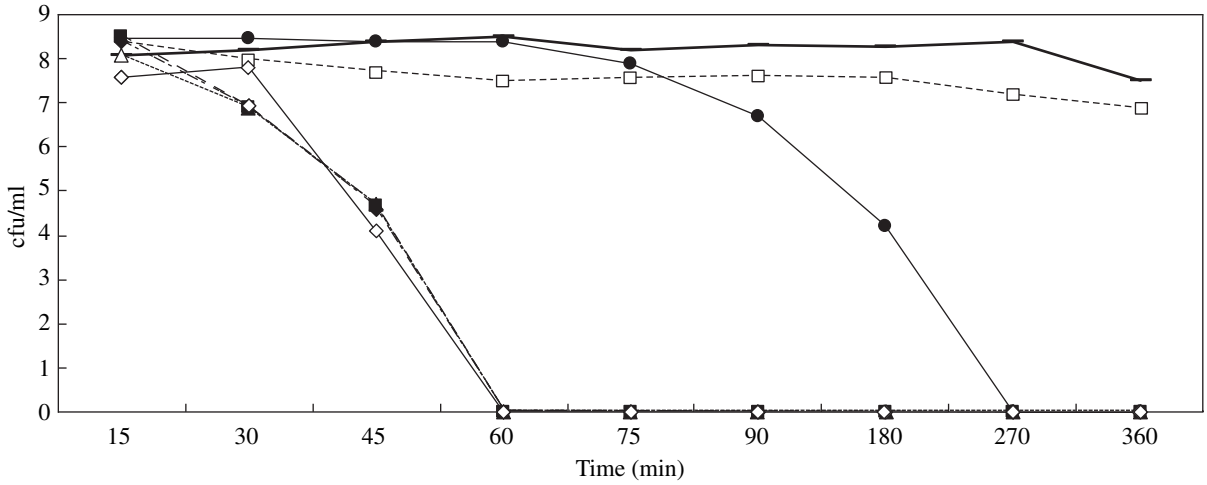

Figure 2 Killing curves for *K. pneumoniae* (intensive care unit, room temperature). *K. pneumoniae* was killed after 60 min exposure to copper and its alloys except NiAg (270 min). No killing effect was noted by polyvinylchloride (PVC) or stainless steel (SS) on prolonged exposure. For key, see Figure 1.

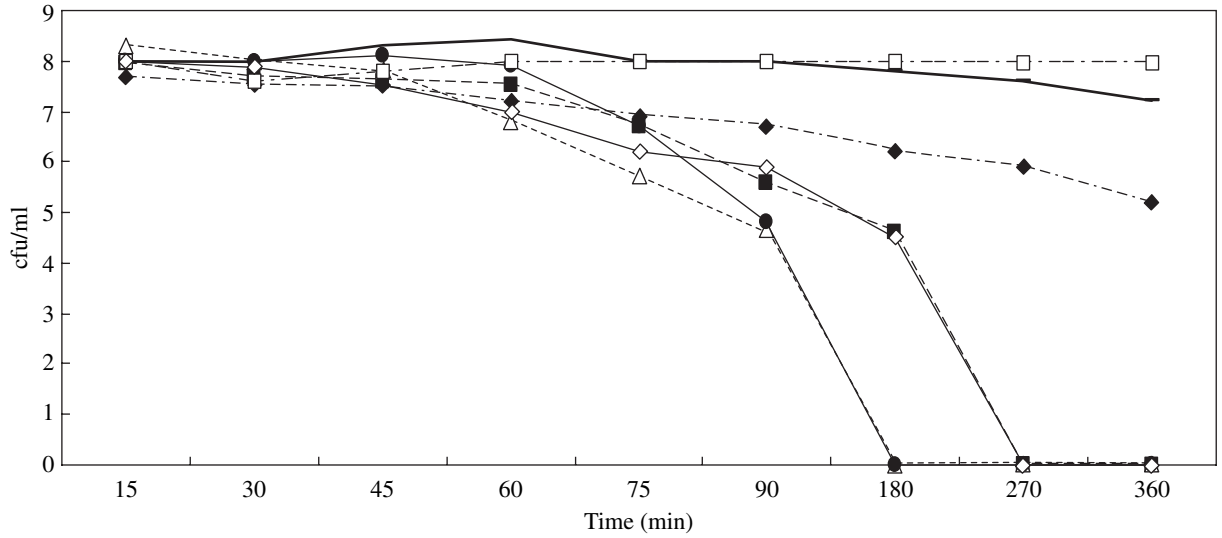

Figure 3 Killing curves for *P. aeruginosa* (burns unit, room temperature). *P. aeruginosa* was killed by between 180 and 270 min after exposure to copper and its alloys; no killing effect was noted with stainless steel (SS) or polyvinylchloride (PVC). For key, see Figure 1.

was noted with CuNi at room temperature; neither PVC nor SS demonstrated any antimicrobial effect.

Overall, the antimicrobial activity of Cu and its alloys was much less at 4 °C than at room temperature except for Cu against C. albicans and *K. pneumoniae*; at room temperature, total killing within the 360 min of the experiment was noted against all strains tested except MRSA (Figures 1—5). DZR and NiAg were equally active against the various test isolates, except *K. pneumoniae* for which DZR was better. Brass 70/30 was active against *P. aeruginosa*, C. albicans, *K. pneumoniae* and MRSA.

Mycobacterium tuberculosis

Two clinical strains of MTB, one resistant to isoniazid alone (R267) and the other multidrug resistant (R432), were tested against Cu and its alloys for inhibitory activity.

Growth inhibition

Growth inhibition, relative to the control, was calculated when both cultures reached GI > 300. Strain R267 was inhibited by Cu (98% inhibition) and remained so after 15 days of incubation. Compared with the control (PVC), growth inhibition by DZR (78%), Brass 70/30 (83%), CuNi (81%) and NiAg (64%) was superior (Table I).

The MDR clinical isolate of MTB (R432) became culture positive within 24 h of incubation. R432 showed maximum inhibition by Cu alone [88% growth inhibition, followed by DZR (76%)]. Growth inhibition by the other alloys such as Brass 70/30, CuNi and NiAg averaged 29.7% inhibition. Compared with R267 where the average growth inhibition was 76.1% for the above alloys, R432 demonstrated less growth inhibition. Stainless steel did not affect the growth rate of strain R432 (3%).

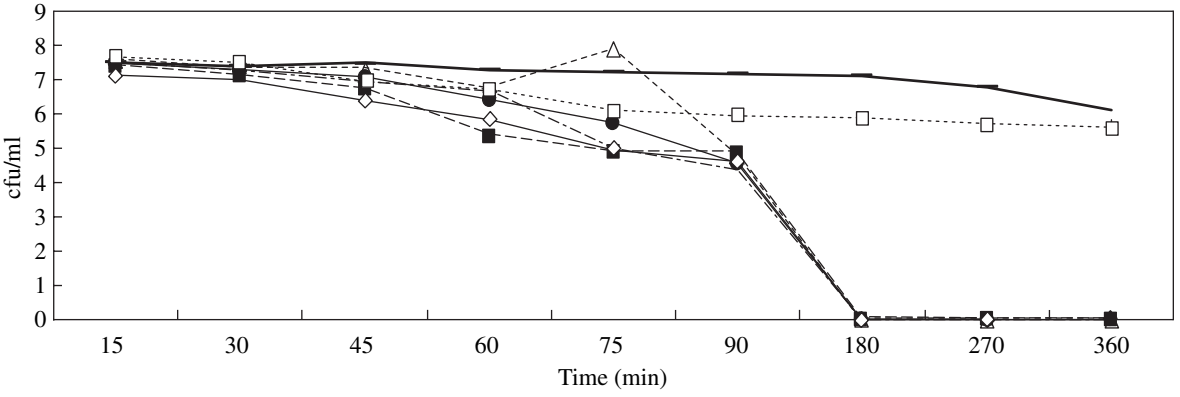

Figure 4 Killing curves for *A. baumannii* (burns unit, room temperature). *A. baumannii* was killed by all concentrations of Cu when exposed for 180 min. PVC, polyvinylchloride; SS, stainless steel. For key, see Figure 1.

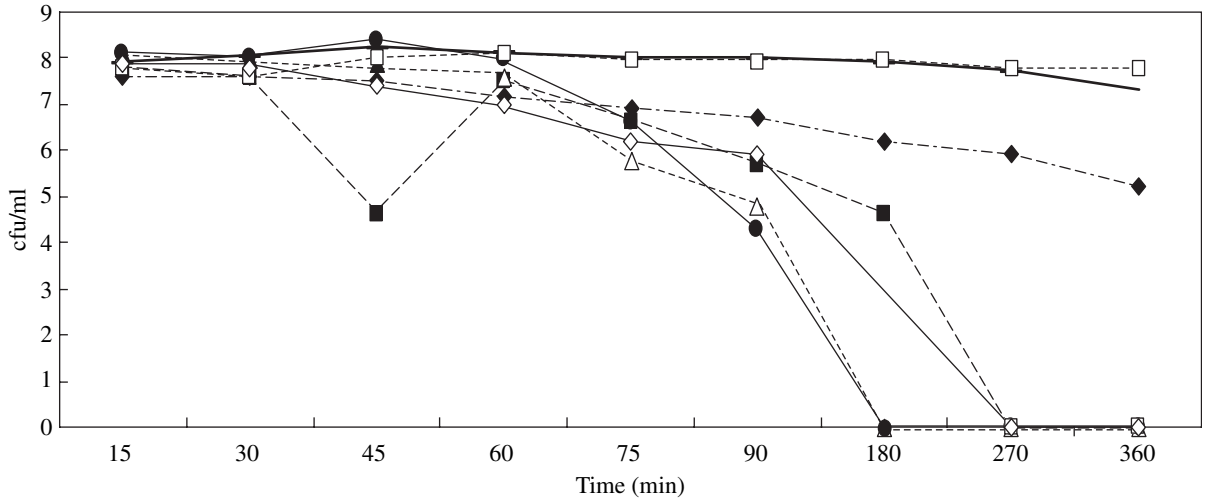

Figure 5 Killing curves for meticillin-resistant *Staphylococcus aureus* (MRSA; intensive care unit, room temperature). MRSA was killed within 180 min of exposure to NiAg and Brass 70/30, and at 270 min of exposure to Cu and DZR. For key, see Figure 1.

Time to positivity (TTP)

When strain R267 was exposed to Cu and its alloys, the time for the culture to become Bactec positive (GI $>$ 10) in the presence of Cu was $>$5 days whereas for other Cu-containing alloys it was $\geq$11 days, Brass 70/30 demonstrating the highest inhibition ($>$11 days). Compared with SS (five days) and PVC (eight days), the antimicrobial activity of Cu and its alloys was at least twice as much (Table I). TTP for R432 was less notable. Cu and its alloys demonstrated inhibition of up to five days compared with 24 h for SS and three days for PVC. Overall, the more sensitive strain of MTB was inhibited by Cu and its alloys compared with R432.

Table I Growth inhibition (GI) index and time to positivity (TTP) for two clinical isolates of *Mycobacterium tuberculosis* (MTB) when exposed to copper (Cu), Cu alloys and stainless steel (SS)

Alloy	MTB strain R432		MTB strain R267	
	% GI	TTP (days)	% GI	TTP (days)
Primary culture		0		4
Copper	87.9	~5	98.0	>15
DZR Brass	75.9	~5	78.5	11
Brass 70/30	37.0	4	83.0	>11
CuNi	27.7	4	80.8	11
NiAg	24.5	4	64.5	11
SS	3	1	0	~5
PVC (control)	0	3	0	8

PVC was used as control with no growth inhibition.
Percentage GI was calculated at the time when the GI of the polyvinylchloride (PVC) culture was >300. At this time the GI values of the inocula from the other materials were calculated as proportions of the PVC culture, and were taken as the growth inhibition. TTP is expressed in days before an MTB strain becomes culture positive in Bactec 12B growth medium (GI $>$ 10).

Discussion

Copper and its alloys demonstrated good antimicrobial activity against multiple-antibiotic-resistant nosocomial bacteria and C. albicans isolated from a Western Cape tertiary hospital. Stainless steel is widely used in the healthcare environment because it is corrosion resistant to most cleaning materials and always appears clean; our studies show that there is no inherent antimicrobial advantage to using this metal. Native PVC is used in the manufacture of most disposable laboratory and hospital plastic ware and has been reported to permit the formation biofilms.[5]

Hand hygiene remains the single most effective strategy for preventing cross-contamination yet compliance among healthcare workers remains poor, resulting in the inevitable contamination of the surrounding hospital environment.[6,7] Door handles, in particular, may be important secondary reservoirs for cross-contamination in healthcare facilities.[8] Boyce *et al.* found that when patients with MRSA in a wound or urine were admitted to the ward, the environmental contamination increased from 27% to 36%. Environmental contamination occurred in the rooms of 73% infected patients and 69% colonised patients.[9] It is well recognised that touch surfaces in a healthcare facility are a potential source of transmission, particularly for MRSA.

K. pneumoniae is well known not only to cause serious nosocomial infections but also as a source

of antibiotic resistance genes including extended-spectrum β-lactamases; these have been identified in South Africa.[10] *P. aeruginosa* has been reported to colonise the environment and equipment surrounding cystic fibrosis patients.[11]

Copper and its alloys showed a marked inhibitory effect on MTB, despite the strains being drug resistant. Growth of both strains showed inhibition by Cu (88—98% inhibition). The monodrug-resistant strain (R267) was inhibited for a longer period (15 days) than the multidrug-resistant strain R432 (~6—7 days). Multidrug resistance in MTB could also reflect reduced susceptibility to other substances such as Cu. It is possible that multidrug resistance in MTB may also involve other genes rendering MTB more resistant to the inhibitory effect of heavy metals such as Cu. This may be an explanation for the difference between R267 and R432 in their response to the alloys in that they have different drug profiles. Copper resistance has been demonstrated in *E. faecium* mediated by the *tcr* genes.[12]

From this study we conclude that the minimum concentration of Cu to be an effective antimicrobial agent is >55% for bacteria excluding MTB and for yeasts. The incorporation of Cu in healthcare facilities may well assist in the reduction of the environmental bioburden and would be a useful adjunct to the current infection prevention and control armamentarium.

The findings for MTB suggest that whereas Cu has activity against the strains tested here, including the MDR-TB strain, higher concentrations of Cu are required to produce a satisfactory outcome. Based on these and other studies, application of Cu touch surfaces in healthcare facilities should be evaluated.

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Conflict of interest statement
None declared.

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