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Potential use of copper surfaces to reduce survival of epidemic meticillin-resistant *Staphylococcus aureus* in the healthcare environment

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Summary Epidemic meticillin-resistant *Staphylococcus aureus* (EMRSA) emerged in the early 1980s with EMRSA-15 and -16 being the most prevalent strains within the UK. MRSA transmission between patients is largely via the hands of healthcare workers, and contamination of the hospital environment may occur. The objective of this study was to evaluate the effectiveness of copper and brass to reduce the viability of air-dried deposits of three MRSA strains [MRSA (NCTC 10442), EMRSA-1 (NCTC 11939) and EMRSA-16 (NCTC 13143)] compared with stainless steel. MRSA and EMRSA [10^7 colony-forming units (CFU)] were inoculated on to coupons (1 cm × 1 cm) of copper, brass or stainless steel and incubated at either 22 °C or 4 °C for various time periods. Viability was determined by resuspending removed CFUs and plating out on tryptone soy agar plates in addition to staining with the respiratory indicator fluorochrome 5-cyano-2,3-ditolyl tetrazolium. On pure copper surfaces, 10^7 MRSA, EMRSA-1 and EMRSA-16 were completely killed after 45, 60 and 90 min, respectively, at 22 °C. In contrast, viable organisms for all three strains were detected on stainless steel (grade 304) after 72 h at 22 °C. At 4 °C, complete kill was achieved on copper for all three strains within 6 h. The results demonstrate an antimicrobial effect of copper on MRSA, EMRSA-1 and -16 in contrast to stainless steel. Consequently, the contemporary application of stainless

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steel in hospital environments for work surfaces and door furniture is not recommended.

Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) is resistant to all β-lactam antibiotics due to possession of the *mecA* gene encoding the low-affinity penicillin-binding protein.[1,2] The first reported MRSA strain was isolated in 1961 in England (NCTC 10442), and although its prevalence declined in the 1970s, it re-emerged in the 1980s in the form of epidemic MRSA (EMRSA), defined as MRSA isolated from two or more patients in at least two hospitals.[3,4] This first epidemic strain, designated EMRSA-1, was recognized in 1981 and continued to cause outbreaks in hospitals until the late 1980s.[5,6] EMRSA strains initially seemed to be confined to outbreaks in one region, but the isolates that emerged in the 1990s (EMRSA-15 and -16) have caused outbreaks of infection and colonization in hospitals in more than one region.[7] EMRSA-15 and -16 have proven highly transmissible and durable, and have consequently gained a reputation as 'super' EMRSA.[8] To date, EMRSA-15 and -16 are the most prevalent strains found in the UK and have also been found in a number of European countries and the USA.[9] The most notable method of MRSA transmission between patients is on the hands of healthcare workers (HCWs).[10] Consequently, hand hygiene, either by washing or disinfection, remains the single most effective strategy for preventing cross-contamination.[11] Compliance among HCWs remains poor, resulting in the inevitable contamination of the surrounding hospital environment.[12,13] Door handles, in particular, may be important secondary reservoirs for cross-contamination between HCWs and patients.[14–16]

Twenty years ago, Kuhn implemented a study to assess the growth rates of streptococci and staphylococci on stainless steel and brass.[17] She observed heavy growth on stainless steel, but only sparse growth on brass doorknobs. In light of these findings, Kuhn suggested that hospitals should retain and maintain their old brassware. If hospitals have steel doorknobs and push plates, the author suggested that they should be plated with brass or disinfected every day to prevent the spread of contaminants. The results of this study are not unexpected as the antimicrobial effects of copper are well documented.[18–22] However the ability of copper or brass to control EMRSA has, as yet, remained untested. With this in mind, we wished to establish the ability of copper and brass to reduce the viability of air-dried deposits of the standard MRSA strain, EMRSA-1 and -16 with regards to both temperature and exposure time. The survival on copper and its alloys was compared with stainless steel (grade 304), a material commonly used for work surfaces and door fittings such as handles and push plates.

Materials and methods

Preparation of MRSA, EMRSA-1 and EMRSA-16 colony-forming units

MRSA (NCTC 10442) was supplied by TCS Biosciences, (Buckingham, UK: Code MM37) and maintained on glycerol protect beads (Fisher Scientific, Loughborough, UK) at −80 °C. EMRSA-1 (NCTC 11939) and EMRSA-16 (NCTC 13143) were supplied by the National Collection of Type Cultures (Health Protection Agency, London, UK) and maintained on glycerol protect beads at −80 °C. For experimental tests, 15 mL of tryptone soy broth was aseptically inoculated with a single bead and incubated at 37 °C for 16 h. After this incubation period, MRSA, EMRSA-1 and EMRSA-16 cultures contained approximately 5×10^8, 9.5×10^8 and 7.5×10^8 colony-forming units (CFU)/mL, respectively. Unless otherwise stated, media were obtained from Oxoid (Basingstoke, UK).

Preparation of sample metal coupons

Table I lists the compositions of the alloys tested during this study. Sample sheets (0.5-mm thickness) of each metal type (provided by the Copper Development Association, New York, USA) were sectioned into small coupons (1 cm × 1 cm). Prior to testing, coupons were degreased and cleaned by vortexing for 30 s in 10 mL of acetone containing approximately 30 glass beads of 2-mm diameter (Merck, West Drayton, UK). After cleaning, coupons were sterilized by ethanol emersion and Bunsen flaming, and were then transferred to a lidded plastic container prior to inoculation to

Table I Metal samples and their constituent components

Metal type	UNS code	Composition	
		%Cu	%Zn
Copper	C19700	100	—
Brass	C24000	80	20
Zinc		—	100
Stainless steel	S30400	Fe — 65.45%, C — 0.8%, Cr — 20%, Ni — 10%, Mn — 2%, Si — 1%, P — 0.45%, S — 0.3%	

UNS, unified numbering system.

prevent contamination. Coupons remained in the container during each experiment.

Sample metal testing

Coupons were aseptically inoculated with 20 μL of either MRSA NCTC 10442 (approximately 10^7 CFU), EMRSA-1 (approximately 1.9×10^7 CFU) or EMRSA-16 (approximately 1.5×10^7 CFU) culture and incubated at either room temperature (22 ± 2 °C) or 4 °C (to represent cold storage areas), for varying time periods ranging from 15 min to 6 h. Relative humidity in the laboratory was monitored and recorded ($50 \pm 10\%$). The effect of desiccation on the viability of each MRSA strain was investigated and no effect was seen (data not shown). Post incubation, organisms were removed from the coupons by vortexing for 30 s in 10 mL of sterile phosphate-buffered saline (PBS) containing approximately 20 glass beads of 2-mm diameter. The effect of copper release into the PBS on the viability of recovered organisms was investigated by the addition of ethylenediaminetetraacetic acid (EDTA, 20 mM) which readily complexes free copper.[23] No significant difference was seen (data not shown) between samples recovered into PBS or PBS with EDTA. Thorough analysis of coupons by episcopic differential interference contrast (EDIC) microscopy revealed no attached organisms after washing.[24] To ascertain the number of viable organisms removed from the coupons, 100 μL was removed and serially diluted to 10^{-4} in sterile PBS. Nutrient agar plates were then inoculated with 50 μL of each dilution, which was spread evenly over the surface of the agar with a sterile, glass spreader. Post inoculation, plates were incubated at 37 °C for 18 h and the number of CFU was counted and used to calculate the number of viable CFU/coupon. Three plates were completed for each dilution and the mean was calculated. Experiments were repeated in triplicate. Control coupons for stainless steel, copper and brass were removed immediately after inoculation at time zero to determine the initial number of viable bacteria.

Reduced inoculum testing

MRSA contamination of door handles in a hospital environment has been estimated at between 1 and 6×10^3 CFU.[15] To determine the effect of inoculum size on the time required for total kill on copper C19700, the number of EMRSA-16 CFU inoculated on to sample coupons was reduced by serially diluting the original culture. Five 1:10 dilutions were performed and sample coupons were inoculated with 20 μL of a chosen dilution. Coupons were tested with inocula ranging from 10^2 to 10^7 CFU. Tests were conducted at room temperature (22 ± 2 °C) and exposure periods ranged from 15 min to 1 h. Post exposure, coupons were transferred to tubes containing 2 mL of sterile PBS with glass beads and treated as described previously.

EDIC and epifluorescent microscopy analysis

In order to confirm the results obtained from the direct culturing of organisms recovered from sample coupons and also to investigate the possibility of sublethally damaged or viable but non-culturable cells, images were taken of inoculated coupons using both EDIC and epifluorescent microscopy. For the epifluorescent analysis, MRSA and EMRSA CFU on inoculated coupons were stained with 5-cyano-2,3-ditolyl tetrazolium (CTC) which detects actively respiring bacteria.[25] Coupons were sterilized and inoculated with 20 μL of culture as described in the alloy testing protocol. Based on findings from the previous tests, metal sample C19700 was analysed after an exposure period of 45, 60 and 90 min for MRSA (10442), EMRSA-1 and EMRSA-16, respectively. For stainless steel, sample coupons were analysed after an exposure period of 6 h, and then after 24, 48 and 72 h. After the exposure period, coupons were transferred to 55-mm Petri dishes, 50 μL of 10 mM CTC was added to the surface, and samples were incubated in the dark for 4 h. After incubation, the coupons were examined thoroughly using an EDIC/epifluorescent microscope (Nikon Eclipse Model ME600, Best Scientific, Swindon, UK) equipped with a 40× objective with epifluorescent

filters appropriate for CTC. For each coupon tested, representative EDIC and epifluorescent pictures were taken using a digital camera (Model CoolSnap CF, Roper Industries, UK) connected to a personal computer with digital image analysis software (Image-Pro Plus, version 4.5.1.22, Media Cybernetics, UK).

Statistical analysis

Data were expressed as the mean and standard error of the mean (SEM). For group comparison, Mann Whitney U-test was used. Statistical significance was defined as $P < 0.05$. Statistical procedures were performed using SigmaStat version 2.03, and graphical analyses were performed with SigmaPlot version 8.0.

Results

MRSA, EMRSA-1 and EMRSA-16 viability on copper, brass or stainless steel

The effect of exposure to stainless steel (S30400), copper (C19700) or brass (C24000) at either 22 °C or 4 °C on MRSA, EMRSA-1 or EMRSA-16 viability can be seen in Figures 1 and 2, respectively. Comparison of the data for the three MRSA strains regarding exposure to stainless steel revealed no significant difference. Thus, for clarity in the figures, only the data for MRSA (10442) are shown. It can be seen that exposure to stainless steel grade 304 at 22 °C had no significant effect ($P > 0.05$) on the mean number of viable CFU/coupon for MRSA, with 6.2×10^6 CFU remaining after 6 h compared with 1.1×10^7 CFU at time zero.

In comparison, exposure on both copper and brass at 22 °C produced significant reductions ($P < 0.05$) in the viability of all three MRSA strains tested. On copper, complete kill of the inoculum was achieved in 45, 60 and 90 min for MRSA, EMRSA-1 and EMRSA-16, respectively. For MRSA and EMRSA-1, significant reductions in viability were achieved after only 30 min on C19700, with the number of organisms being reduced 10^4-fold and 100-fold, respectively. For EMRSA-16, 45 min was required before a 100-fold reduction was observed. Figure 1 shows that the viability curve on copper over time for MRSA is very similar to the curves for EMRSA-1 and -16, except that their respective curves have moved to the right indicating greater tolerance to the antimicrobial effects of copper exposure compared with the standard NCTC strain.

Results for brass (80% Cu) at 22 °C are still significant with regards to viability reduction, although not to the same extent as those for pure copper. Longer exposure periods were required before significant ($P < 0.05$) reductions were achieved, notably 3 h for all three MRSA strains compared with previous results on copper of 30–45 min. The mean number of viable CFU remaining on

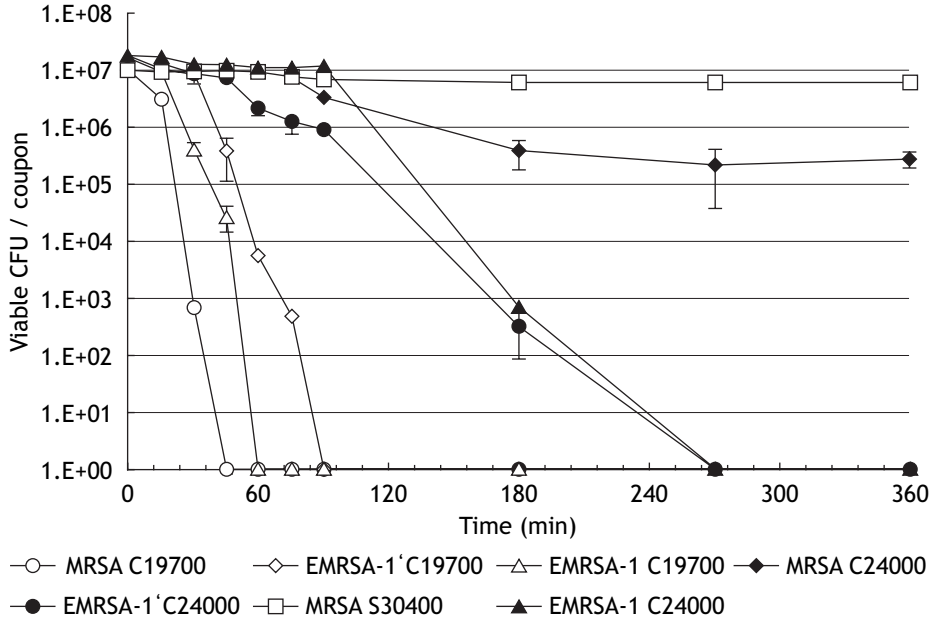

Figure 1 Effect on meticillin-resistant *Staphylococcus aureus* (NCTC 10442), epidemic MRSA-1 (EMRSA-1) and EMRSA-16 viability of a 6-h exposure to either stainless steel (S30400), copper (C19700) or brass (C24000) at 22 °C. Points represent the mean ($N = 3$) ± standard error of the mean.

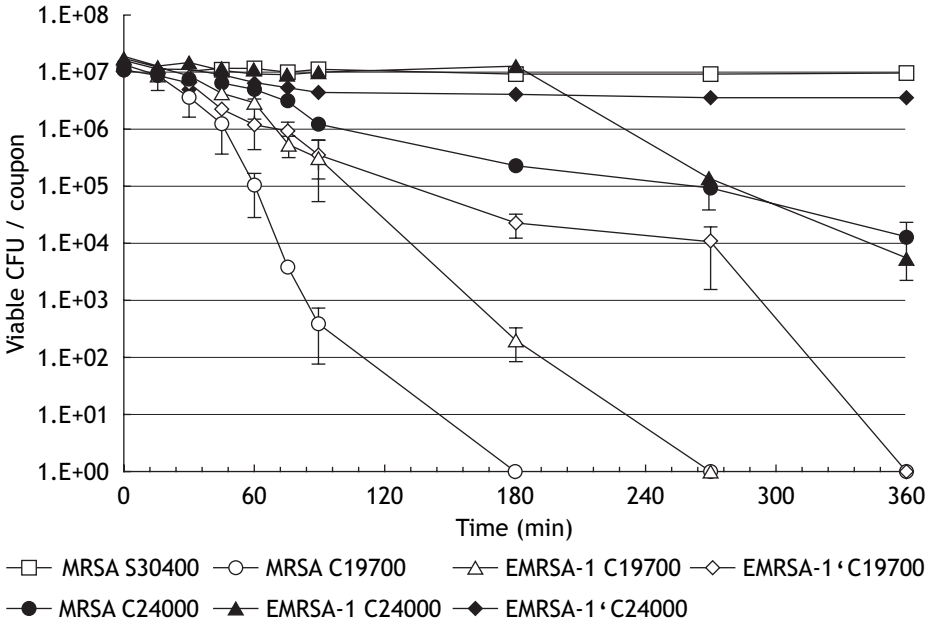

Figure 2 Effect on meticillin-resistant *Staphylococcus aureus* (MRSA), epidemic MRSA-1 (EMRSA-1) and EMRSA-16 viability of a 6-h exposure to either stainless steel (S30400), copper (C19700) or brass (C24000) at 4 °C. Points represent the mean ($N = 3$) ± standard error of the mean.

brass coupons after 3 h for MRSA, EMRSA-1 and EMRSA-16 were 333, 733 and 3.9×10^5 CFU, respectively. Complete kills were achieved for MRSA and EMRSA-1 after 4.5 h of exposure, with both strains producing similar viability curves over time. However, the effect on EMRSA-16 viability, although significant, was less than a 100-fold reduction over 6 h, with 2.8×10^5 CFU remaining after the exposure period. This indicates that this particular strain of MRSA possesses increased tolerance to copper. The effect of zinc on the viability of each strain (data not shown) over 6 h at either 22 °C or 4 °C was not significant ($P < 0.05$), indicating no contributory effect towards MRSA death for brass C24000.

At temperatures of 4 °C (Figure 2), viability on stainless steel was virtually identical for the three MRSA strains to that seen for exposures at room

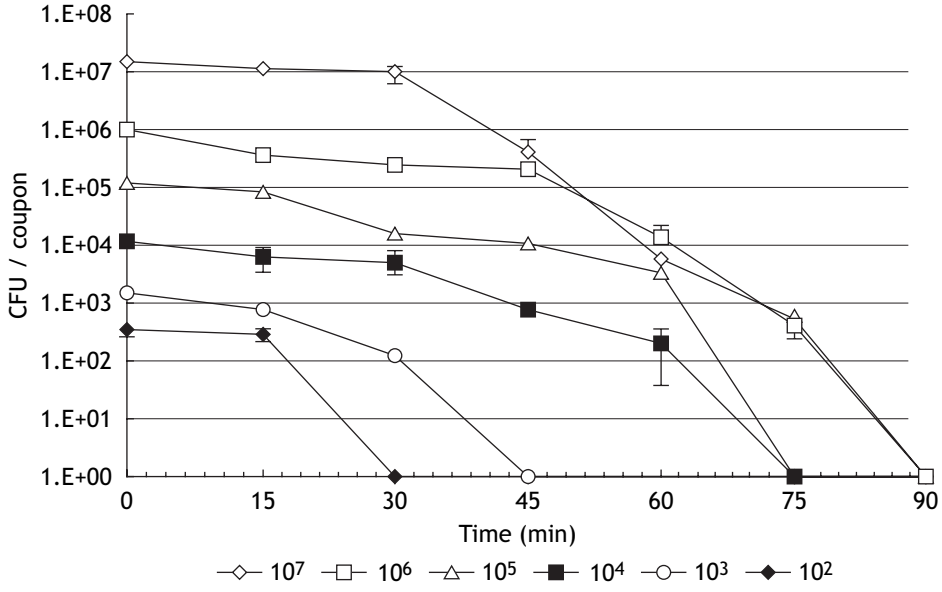

Figure 3 Effect of epidemic meticillin-resistant *Staphylococcus aureus*-16 (EMRSA-16) inoculum size on time for total kill when exposed to copper (C19700). Points represent the mean ($N = 3$) ± standard error of the mean.

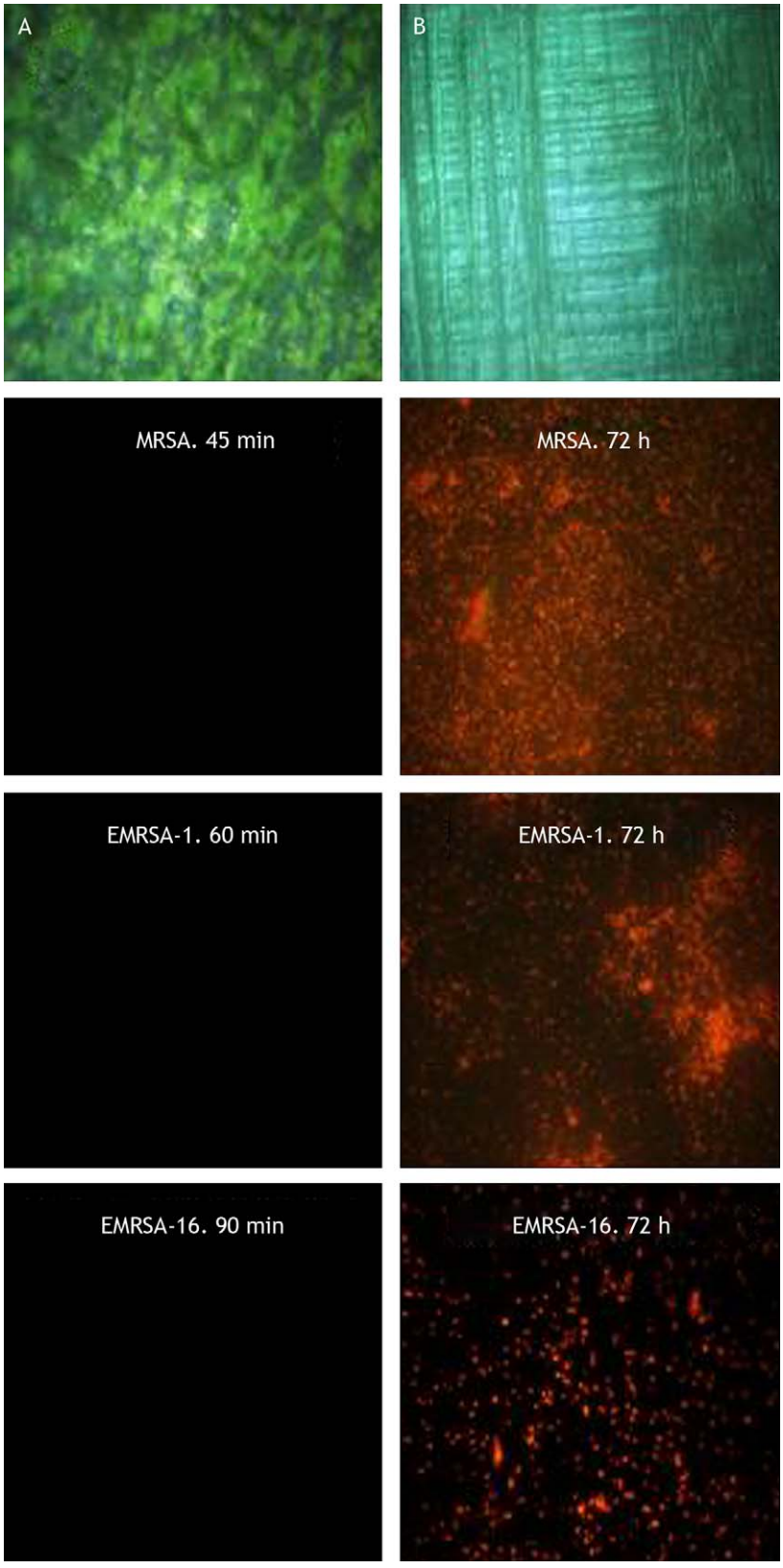

Figure 4 Episcopic differential interference contrast and epifluorescent images of copper (A) and stainless steel (B) inoculated with meticillin-resistant *Staphylococcus aureus* (MRSA), epidemic MRSA-1 (EMRSA-1) and EMRSA-16. Red points represent respiring cells. Original magnification ×400.

temperature. For both pure copper and brass, greater time periods were required to significantly reduce the viability of the initial inoculum. Complete kills were produced on copper for all three strains but only after 3, 4.5 and 6 h, respectively, for MRSA, EMRSA-1 and EMRSA-16. Complete kill was not achieved for any of the strains when exposed to brass at chilled temperatures, although a significant reduction was seen for both MRSA and EMRSA-1 after 6 h. No significant effect was seen on the viability of EMRSA-16 during the experimental exposure.

The effect of reducing the inoculum size of EMRSA-16 on total kill time when exposed to copper can be seen in Figure 3. Reducing the number of CFU effectively moved the viability curve over time to the left, i.e. as the inoculum size was reduced, so was the time to kill those CFU present. However, 90 min was still required to completely kill 10^6 and 10^7 CFU. In addition, 75 min was required for complete elimination of both 10^5 and 10^4 EMRSA-16. For the lowest inocula of 10^3 and 10^2 CFU, 45 and 30 min were required, respectively, for total kill.

Epifluorescent microscopy and digital image analysis

Epifluorescent images of coupons stained with CTC suggest a copper-associated interruption to cellular respiration, with none of the strains tested showing actively respiring cells. Results from the previous tests at room temperature were corroborated on the pure copper coupons with zero fluorescent emissions after 45, 60 and 90 min of exposure for MRSA, EMRSA-1 and EMRSA-16, respectively (Figure 4A). In contrast, images of inoculated stainless steel (Figure 4B) after 72 h of incubation at 22 °C clearly show the presence of respiring cells for all three strains tested, as shown by the numerous points of red emission within the images due to the intracellular reduction of CTC to the water-insoluble fluorescent product, 3-cyan-1,5-di-tolyl-formazan.

Discussion

In the UK and many other countries, MRSA is an important cause of wound infection.[26] Direct contact is the principal method of transmission of MRSA; consequently, effective hand hygiene is the most important method of preventing nosocomial infection.[27] However, compliance amongst HCWs is a problem. MRSA is extremely resilient and can remain viable on a range of surfaces and objects, allowing transfer to people who come into contact with them.[28] In light of this, materials that actively reduce the viability of MRSA without secondary intervention may prevent unwanted contamination.

The data from this study show that viability of MRSA and, more importantly, EMRSA-16 can be significantly affected by the composition of the substratum alloy on which it is placed. At room temperature, MRSA and EMRSA-1 and -16 strains were able to persist and remain viable in dried deposits on stainless steel for periods of up to 72 h, as clearly demonstrated by epifluorescent microscopy and standard culture techniques (data not shown). In contrast, survival on copper (C19700) was significantly less, with complete kill of 1.9×10^7 EMRSA-16 CFU achieved after 90 min. In addition, concentrations of EMRSA-16 that are indicative of MRSA contamination found on door handles, i.e. between 10^2 and $10^3/cm^2$, were killed after exposures of only 30 and 45 min, respectively. When exposed to brass (C24000), MRSA, EMRSA-1 and -16 strains survived for longer periods compared with pure copper. In fact, for EMRSA-16, 10^5 CFU were still viable after 6 h at 22 °C, which suggests that copper alloys of greater than 80% copper should be used. This greater resistance of EMRSA-16 to copper and brass may be indicative of a generally greater resistance to environmental stresses, suggesting the presence of more resilient global stress response regulators. It may be timely to investigate whether such genetic or physiological mechanisms are present, and whether they may be transmissible to less resilient pathogens.

At 4 °C, representing refrigerated storage areas such as cold rooms and mortuaries, the numbers of all three MRSA strains remained unaffected on the stainless steel after 6 h. Total kill of the inoculated CFU was once again achieved on pure copper for MRSA, EMRSA-1 and -16. In the case of EMRSA-16, 6 h was required. Nevertheless, this still represents a significant effect compared with stainless steel. This indicates that passive protection can also be applied to cold storage areas through the use of fittings with a high copper content.

These results have important implications for the hospital environment where stainless steel is a common material used for work surfaces and door fittings, including handles and push/kick plates. Once contaminated, stainless steel surfaces could remain as sites for recurring contamination and possible infection of susceptible hosts. This iron alloy is used for its ability to be regularly cleaned without displaying unwanted corrosion. However, French *et al*. have shown conclusively that terminal

cleaning (defined as environmental cleaning after discharge of an infectious patient) is ineffective in eradicating MRSA, with 74% of environmental swabs yielding MRSA before cleaning and 66% afterwards.[14] Evidence suggests the need for more passive preventative measures with regards to reducing MRSA on commonly touched surfaces.

In contrast to stainless steel at 22 °C, copper (C19700) and brass (C24000) have been shown to be intrinsically antibacterial for MRSA, EMRSA-1 and -16, and to prevent their long-term persistence. Consequently, the incorporation of copper into work surfaces and, in respect of door fittings, the re-introduction of high copper brass door handles could significantly reduce the risk of cross-contamination between HCWs and patients. Previous studies involving copper and copper alloys have demonstrated similar findings against other pathogenic bacteria including *Escherichia coli* O157:H7, which further supports the case for the increased application of copper alloys in the critical care environment.[29] Replacing high contact materials with copper alloys should not be seen as a panacea leading to complacency, however, but part of the hygiene armoury of preventative measures used to minimize the risk of hospital-acquired infection. The importance of regimented cleaning programmes and good hand hygiene cannot be underestimated.

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