Review

Application of copper to prevent and control infection. Where are we now?

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ARTICLE INFO

Article history:
Received 18 February 2012
Accepted 21 May 2012
Available online xxx

Keywords:
Copper
Environmental contamination
Healthcare-associated infection
Hygiene
Infection prevention and control

SUMMARY

Background: The antimicrobial effect of copper has long been recognized and has a potential application in the healthcare setting as a mechanism to reduce environmental contamination and thus prevent healthcare-associated infection (HCAI).

Aim: To review the rationale for copper use, the mechanism of its antimicrobial effect, and the evidence for its efficacy.

Methods: A PubMed search of the published literature was performed.

Findings: Extensive laboratory investigations have been carried out to investigate the biocidal activity of copper incorporated into contact surfaces and when impregnated into textiles and liquids. A limited number of clinical trials have been performed, which, although promising, leave significant questions unanswered. In particular there is a lack of consensus on minimum percentage copper alloys required for effectiveness, the impact of organic soiling on the biocidal effect of copper, and the best approach to routine cleaning of such surfaces. Limited information is available on the ability of copper surfaces to eradicate spores of Clostridium difficile.

Conclusion: Additional studies to demonstrate that installing copper surfaces reduces the incidence of HCAI are required and the cost-effectiveness of such intervention needs to be assessed. Further research in a number of key areas is required before the potential benefits of using copper routinely in the clinical setting to prevent and control infection can be confirmed and recommended.

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Introduction

Copper, a metal utilized by human civilization for more than 10,000 years, has become the focus of renewed scientific interest for its antimicrobial properties and potential application in the healthcare setting. Although the exact mechanisms by which this metal exerts its biocidal effect are not fully understood, its benefits have long been recognized. An Egyptian papyrus written between 2600 and 2200 BC describes the application of copper to sterilize chest wounds and to purify drinking water. Later, Hippocrates recommended the topical application of copper to treat leg ulcers, and, in the pre-antibiotic era of the nineteenth and twentieth centuries, copper preparations were widely used in the treatment of skin conditions, syphilis and tuberculosis.1

In the modern healthcare setting one of the most widespread and successful applications of the antimicrobial effect of copper is in the control of legionella and other bacteria in hospital water distribution systems using the method of copper and silver ionization.2 However, recent research into the antimicrobial effects of copper has focused on the mechanism by...
which there is ‘contact killing’ of microbes on exposure to copper surfaces and the impact this may have on reducing environmental contamination. In 2008 commercial interest in this potential application of copper increased due to the decision of the US Environmental Protection Agency (EPA) to grant recognition to copper surfaces as having antimicrobial efficacy. Copper is the first metal to be awarded such a status and to date almost 300 copper and copper alloy surfaces have demonstrated their biocidal effect against five strains of bacteria when tested according to US EPA protocols. In addition to its use as a material for contact surfaces, the biocidal effects of a wide variety of copper-impregnated textiles and liquids have been reported, with particular speculation about their potential to reduce healthcare-associated infection (HCAI).

This article reviews the rationale, mechanism of antimicrobial effect, efficacy and clinical studies on copper to reduce the microbial load on contact surfaces. The addition of copper in water systems to prevent legionella is well established and represents its use to prevent a specific waterborne pathogen; this aspect is not addressed here. A PubMed search of the available literature was conducted using such terms as ‘copper’, ‘antimicrobial copper’, ‘copper-based biocide’, ‘copper resistance’, ‘hospital acquired infection’, ‘infection prevention and control’, ‘hygiene’, ‘cleaning’ and ‘environmental contamination’. The search was limited to articles published in English. References from bibliographies of articles included in the search were also assessed.

Rationale for using copper surfaces in the healthcare setting

Environmental surfaces are a likely reservoir for potential pathogens and play a role in the acquisition of healthcare infection. Studies have demonstrated that hard surfaces can be contaminated with isolates such as meticillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE) and spores of Clostridium difficile which can remain viable for several weeks to months. These microorganisms from contaminated surfaces may then be transmitted via hands to other inanimate objects or to patients. To standardize the assessment for monitoring hospital cleanliness, benchmarks for assessing hygiene have recently been updated. The original quantitative standard stated that aerobic colony counts (ACC) on hand-touch sites should not exceed 5 cfu/cm² but this has since been reduced to 2.5 cfu/cm². Achieving such a target may be challenging, especially as there is considerable variation in standards and methods of cleaning. In one US study a fluorescent marker solution was employed to determine cleaning efficacy of more than 13,000 surfaces in 23 hospitals. Terminal room cleaning after patient discharge decontaminated a mean of only 49% of the standardized surfaces including <30% of toilet handholds, bedpan cleaners, room doorknobs and bathroom light switches. It is clear that in addition to routine cleaning, additional strategies to reduce microbial contamination should be considered.

Mechanism by which copper exerts its antimicrobial effect

Copper is an essential trace element involved in numerous physiological and metabolic processes. Although toxicity in humans can occur at high concentrations, in general exposure to copper is considered safe, as is evidenced by the widespread use of copper intrauterine devices and the documented low risk of adverse reactions due to dermal contact with copper. The low sensitivity of human tissue to copper can be contrasted with micro-organisms which are extremely sensitive to its toxic effects.

The exact mechanisms by which copper exerts its biocidal effect is a source of ongoing investigation. It is thought that the cause of cell death is multifactorial rather than the result of a single universal mechanism. A key property of copper which significantly contributes to its toxic effect is its ability to accept and donate single electrons as it changes oxidation state between Cu²⁺ and Cu³⁺ (+ Cu(I) and Cu(II)). This allows copper to act as a catalyst for the generation of reactive oxygen species (ROS) such as hydroxyl radicals and superoxide anions. These ROS have the potential to cause oxidative damage to vital cell constituents such as proteins, nucleic acids and lipids (including those in the cell membrane). Free copper ions may compete with zinc or other metal ions for important binding sites on proteins, leading to conformational change and the loss of protein function. Copper ions can also inactivate proteins by damaging Fe–S clusters in cytoplasmic enzymes needed to make branched-chain amino acids.

Recent research into the biocidal properties of copper surfaces has focused on establishing the primary mechanisms which result in cell death, and on the effect of copper on bacterial DNA. One set of studies, involving enterococci (including VRE) exposed to copper alloys, reported that cell death results from the action of released copper ionic species and the generation of superoxide, leading to arrested respiration with the substantial disintegration of both plasmid and genomic DNA as a primary effect. Research from this group also suggests that different mechanisms of toxicity are observed in Gram-negative bacteria such as Escherichia coli and Salmonella spp. with depolarization of the cytoplasmic membrane playing a key role and DNA degradation occurring at a slower rate. By contrast, other studies have proposed that depolarization of the cytoplasmic membrane is the main target for the antimicrobial effect of copper and that degradation of genomic material only occurs subsequent to cell death. Although investigations into the exact biocidal effects of copper toxicity are ongoing, the consensus that degradation of DNA occurs at some point is noteworthy. Compromising DNA in this way has a role to play in preventing resistance mutations and inhibiting the potential transmission of toxin, virulence and antibiotic resistance genes.

In vitro evidence for biocidal efficacy of copper surfaces

The use of copper materials in contact surfaces to reduce environmental contamination was first postulated almost 30 years ago. During a training session to promote hygiene awareness, cleaning staff in a US hospital were asked to take environmental swabs from a variety of locations and it was noted that brass doorknobs (an alloy of typically 67% copper and 33% zinc) had very sparse bacterial growth in comparison with swabs from doorknobs of stainless steel. Initial laboratory protocols to investigate this phenomenon in a standardized way were derived from a testing method developed in...
Japan: JIS Z 2801 (Japanese Industrial Standards Association, 2000). However, this method is not representative of actual surface contamination events in a hospital setting, since it involves applying a dilute liquid inoculum to the surface area, which is maintained at a relative humidity of >90% for a period of 24 h, and incubated at a higher than ambient temperature of 35°C.21

In an attempt to replicate in vivo situations, two main experimental techniques have since been described, a moist inoculation technique and a dry inoculation technique. Incubation temperatures and relative humidity in both methods are also modified to more accurately reflect indoor settings. In studies using a moist inoculation technique, small volumes of liquid suspensions of bacteria are applied to metal plates (coupins) and can take >30 min to dry.22 It has been suggested that the aqueous nature of the contaminating inoculum may have an impact on the toxicity of the copper surface, and this technique, which mimics a wet contamination incident such as a sneeze or a wipe, does not reflect the contamination of dry surfaces encountered in healthcare settings.15 In an attempt to address this issue, a second method has been developed which involves the application of liquid cell suspensions to metal plates using a cotton swab. This provides a higher concentration of inoculum in the form of a thin film of liquid which evaporates within seconds and may more accurately reflect the clinical scenario.22

Studies have been published demonstrating the ability of copper to inactivate a multitude of bacteria, fungi and viruses in the laboratory setting. These include MRSA, enterococci, Pseudomonas spp., Acinetobacter spp., Klebsiella spp., Escherichia coli, Listeria spp., Campylobacter spp., Salmonella spp., Staphylococcus warnerii, influenza A, Mycobacterium tuberculosis and Candida spp.13,15,21–26 The majority of these laboratory studies have been carried out using a ‘wet inoculation’ technique and there is wide variation in incubation temperatures, relative humidity and copper content of the alloys tested. Nonetheless a number of consistent findings are reported. In general, micro-organisms are inactivated within hours although the greatest efficiency is seen in alloys with higher copper content. The percentage copper required for bacterial killing more rapidly than moist ones, though the mechanism for this is as yet unclear.14,27

In vivo evidence for biocidal efficacy of copper surfaces

The efficacy of copper in the contact killing of microbes has been the subject of extensive laboratory investigation. However, in vivo studies are limited and to date there have been only five reports published in the literature.

The first study was a 10-week trial in a busy acute medical ward of a UK hospital.28 A plastic toilet seat, a chrome set of taps handles and an aluminium ward entrance door push plate were replaced by equivalent items containing a minimum of 60% copper. The items were installed six months prior to the study to facilitate ageing and for staff to become accustomed to them. To further reduce bias the study was designed as a cross-over trial with the copper- and non-copper-containing controls interchanged after five weeks. Items were sampled on a weekly basis for the presence of micro-organisms. A benchmark value for all bacteria of <5 colony-forming units per cm² (cfu/cm²) was used in line with standards which had been proposed at the time.5 The results of the study showed that, based on median total aerobic cfu counts, 5/10 controls and 0/10 copper sample points failed the proposed benchmark value of <5 cfu/cm². Although this benchmark value has subsequently been lowered to 2.5 cfu/cm² the overall findings that median numbers of micro-organisms harbourd by the copper-containing items were between 90% and 100% lower than their control equivalents remain significant. An additional finding of the 10-week study was that although no isolates of MRSA and C. difficile were isolated from either type of surface, meticillin-susceptible Staphylococcus aureus ( MSSA), VRE and E. coli were found only on the control surfaces.

A second extended phase of this hospital trial was carried out over a six-month period.29 Fourteen types of frequently touched items made of copper alloy were installed in an acute medical care ward three months prior to the study. These included door handles, push plates, toilet seats and flush handles, grab rails, light switches, pull-cord toggles, sockets, overbed tables, dressing trolleys, commodes, taps, and sink fittings. The percentage copper content of the alloys used ranged from 58% to 99.95%. After 12 weeks the copper and standard items were switched over. Weekly sampling was carried out for 24 weeks. The study found that 8/14 item types demonstrated significantly lower cfu counts on the copper surfaces than on the standard materials with the other six types showing reduced microbial numbers on the copper surfaces but the difference did not reach statistical significance. The study also assessed the presence of five indicator organisms MRSA, MSSA, VRE, C. difficile and coliforms. All five bacteria were recovered from both control and copper-containing surfaces. However, significantly fewer copper surfaces were contaminated with VRE, MSSA and coliforms than were the controls.29

A third trial was conducted in the consulting rooms of a walk-in primary care clinic in South Africa. Contact surfaces such as a desk, trolleys, the top of a cupboard and windowsills were covered with copper sheets (99.9% copper alloy). Over six months the surfaces were sampled every six weeks for a 4.5-day period with multiple samplings per day. An overall 71% reduction in the bacterial load on the copper surfaces was observed compared with that of the control surfaces. Comparable numbers of bacteria were counted when surfaces remained untouched for 71 h over the weekends but this was not investigated further.30

A fourth study was carried out on medical wards of a German hospital.31 Touch surfaces such as push plates, doorknobs and light switches were replaced with new copper-containing alloys (percentage of copper alloy not stated). The trial was carried out over 32 weeks equally divided between summer and winter. The number of aerobic heterotrophic cfu on the surfaces was determined once or twice per week and the presence of ciprofloxacin-resistant S. aureus (CRSA) was chosen as an indicator organism for the presence of resistant nosocomial bacteria. The study found that the total number of cfu on metallic copper surfaces was 63% of that on control surfaces with statistically significant differences noted between door
knobs. No significant difference in survival of CRSA on copper surfaces versus controls was noted. Following initial sampling each morning all surfaces were cleaned with disinfectant. It was noted that surfaces repopulated at different rates, 12.4 cfu/h for copper surfaces and 22.5 cfu/h on other surfaces.

The final in vivo study of the contact killing effect of copper surfaces was carried out in a critical care unit.32 This small trial compared the contamination of copper versus stainless steel pens after use over a 12 h clinical shift. In total 25 pens of each type were examined. A lower total number of cfu was found on copper pens sampled immediately after collection but this did not reach statistical significance. When pens were left in storage for 11 h (reflecting the time lapse between shifts) significantly fewer copper-containing pens were contaminated compared with stainless steel pens. A summary of each in vivo assessment and its findings is outlined in Table I.

**Copper-impregnated textiles and liquids**

In addition to its use as a contact surface, the antimicrobial effect of copper is being exploited in a number of other settings. This has been facilitated by the development of a technique for the mass production of copper oxide-impregnated textiles, latex and other polymer products.4 In the area of personal protective equipment, for example, the addition of copper oxide into respiratory protective face masks has been shown to have anti-influenza biocidal effects without altering the physical barrier properties of the material.33 A role for copper oxide-impregnated wound dressings has also been investigated with preliminary results from animal models demonstrating a strong biocidal effect with no adverse reactions in closed skin wounds.34 Furthermore a novel clinical study assessing the impact of copper-impregnated socks demonstrated improvement in the symptoms of fungal foot infections.35

Although it has been postulated that there may be a role for making hospital soft surfaces such as sheets and clothing from copper-impregnated biocidal textiles, there are no clinical data to support the efficacy of such an intervention in reducing HCAI and questions remain unanswered about issues such as cleaning and the decontamination of such materials.36 The biocidal effects of liquid formulations containing copper have also been assessed; a number of laboratory studies have postulated a role for copper-based hand rubs and cleaning products as effective infection prevention and control interventions.37,38 Furthermore, a clinical study assessing the performance of ultramicrofibre cleaning technology with the addition of a copper-based biocide (CuWB50) demonstrated a significant reduction in total viable count in the hospital environment when compared with ultramicrofibre mops and cloths moistened with water alone.39

**Resistance to copper**

As copper is an essential micronutrient but toxic at elevated concentrations, micro-organisms have developed complex systems to maintain precise intracellular levels. In addition to specific uptake and efflux pumps, other mechanisms of tolerance include exclusion by a permeability barrier, intra- and extracellular sequestration, enzymatic detoxification and reduction in the sensitivity of cellular targets to copper ions.40 Although the genes responsible for such processes can be encoded by transmissible plasmids, the potential emergence of widespread bacterial strains resistant to copper surfaces appears unlikely given the rapid rate of contact killing and the complete degradation of DNA known to occur.22

Studies to investigate this issue have focused on known plasmid-borne copper resistance mechanisms. One such target is the tcrB gene identified in certain strains of *E. faecium* and *E. faecalis*. This gene encodes for a membrane-bound protein involved in copper homeostasis and is thought to originate from pigs fed with copper sulphate-supplemented food.41 Although isolates containing the tcrB gene exhibit growth on brain—heart infusion agar plates containing high concentration of copper sulphate, it is thought that their resistance mechanism is not sufficient to prevent cell death when exposed to copper surfaces.41,42 Other studies of *E. coli* strains containing a resistance plasmid PCo demonstrated a decreased killing rate when exposed to copper surfaces but did not prevent cell death.27 Efforts to assess the potential for resistance to develop in bacteria in continual contact with copper led to a novel investigation of isolates colonizing European 50 cent coins. Although coins have not been confirmed to have antimicrobial efficacy as defined by EPA standards, the authors of this study postulated that 50 cent pieces (89% copper alloy) may be ideal surfaces to give rise to natural selection of metallic copper-resistant bacteria.42 In total, 294 strains of bacteria (the majority being Gram-positive cocci) were recovered from an international sample of coins and tested for survival on a pure copper surface (99% Cu). The survival of the isolates was compared to matched type-specific control strains. Although some isolates demonstrated prolonged survival on dry surfaces compared with their controls, no significant copper-resistant bacteria were identified. *Staphylococcus* spp. isolated from coins did not have antibiotic resistance profiles more extensive than their matching control strains, arguing against the co-selection of copper surface resistance traits.42

**Areas for further study**

Much work has been done to investigate the bactericidal efficacy of copper as part of a contact surface, but a number of questions remain unanswered about the benefit of the widespread implementation of copper-based products in the healthcare setting. Although there is increasing evidence of the importance of hand-touch sites in the transmission of pathogens, the clinical trials of copper contact surfaces published to date have not been designed to show a reduction in HCAI rates. Instead a surrogate marker of aerobic cfu compared with control surfaces has been used. The clinical impact of such a reduction is unclear. To address this issue a large-scale, multicentre US trial using HCAI rates as an outcome has recently been completed. Preliminary assessment of these unpublished data suggests that significant reductions in HCAI were observed when copper alloys were used in an ICU setting.43 Hospital trials in Japan, South Africa, Greece and Chile are underway and it is possible that results from these trials may provide further evidence in this regard.22,43 Concerns also arise with regard to the lack of clinical trials assessing the role of copper contact surfaces in eradicating anaerobic spores, especially *C. difficile*. As the...
decontamination of surfaces exposed to *C. difficile* spores is challenging for conventional cleaning methods, the beneficial effects of copper contact surfaces may have a significant impact. One laboratory-based study postulated that the efficacy of contact killing may be improved by the addition of a spore germinant to cleaning solutions used on the copper surfaces. Further research, both into the effect of copper and the potential role for spore germinants, is required.

### Table I

The setting, methods used and findings from five studies in clinical areas of copper-impregnated surfaces

<table>
<thead>
<tr>
<th>Study no. (reference)</th>
<th>Setting</th>
<th>Methods and criteria used</th>
<th>Results</th>
<th>Comment</th>
</tr>
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<tbody>
<tr>
<td>1 (Casey <em>et al.</em> [28])</td>
<td>Acute care medical ward, UK. 10-week study.</td>
<td>Cross-over study. Three existing surfaces replaced with copper alloys (toilet seat, tap handles, door push plate). Total aerobic microbial counts per cm² monitored weekly and compared with benchmark value of &lt;5 cfu/cm². Also evaluated for indicator organisms.</td>
<td>Based on median total aerobic cfu, 5/10 controls and 0/10 copper sample points failed the benchmark value. Median numbers of micro-organisms on copper-containing items were 90–100% lower than their control equivalents.</td>
<td>60–70% copper alloys. Items installed 6 months prior to start of study to allow staff to become accustomed to fixtures and so fixtures ‘aged’.</td>
</tr>
<tr>
<td>2 (Karpanen <em>et al.</em> [29])</td>
<td>Extension of study 1. Acute medical ward, UK. 24-week study.</td>
<td>Cross-over study. Fourteen frequent-touch items replaced with copper alloys.</td>
<td>Total aerobic microbial counts per cm² monitored weekly and compared with benchmark value of &lt;5 cfu/cm². Also evaluated for indicator organisms.</td>
<td>&gt;58% copper alloys.</td>
</tr>
<tr>
<td>3 (Marais <em>et al.</em> [30])</td>
<td>Primary healthcare clinic, Western Cape, South Africa. 24-week study.</td>
<td>Consulting room refitted with copper sheets on touch surfaces (desk and trolleys, top of cupboard, windowsill). Sampled every 6 weeks for 4.5 days. Total aerobic colony count.</td>
<td>Overall 71% reduction in bacterial load of copper surfaces compared with that of control surfaces.</td>
<td>99.9% copper alloys used. Comparable numbers of bacteria counted when surfaces remained untouched over the weekends (71 h).</td>
</tr>
<tr>
<td>4 (Mikolay <em>et al.</em> [31])</td>
<td>Oncology, respiratory and geriatric ward, Germany. 32-week study (summer and winter).</td>
<td>In total 147 push plates, doorknobs, light switches replaced with brass (copper/zinc alloy). Sampled once or twice per week for total aerobic colony count. CRSA as an indicator organism.</td>
<td>Average 63% reduction in bacterial load of copper surfaces compared with controls. Results significant for door handles. No significant difference in survival of CRSA although lower numbers on copper surfaces. Also demonstrated average rate of repopulation of copper surfaces less than half that of controls.</td>
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<tr>
<td>5 (Casey <em>et al.</em> [32])</td>
<td>Intensive care unit, UK. Copper pens used during a 12-h clinical shift.</td>
<td>Comparison of surface microbial contamination associated with pens of copper alloy vs stainless steel (50 pens in total).</td>
<td>Statistical significance only reached when pens left in storage for 11 h. Lower total cfu found on copper pens immediately after shift completed but not significant.</td>
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Cfu, colony-forming units; VRE, vancomycin-resistant enterococci; MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-susceptible *Staphylococcus aureus*; CRSA, ciprofloxacin-resistant *Staphylococcus aureus*.
Assuming that a reduction in healthcare infection rates could be attributed to the use of copper contact surfaces and impregnated materials, issues arise in relation to cost-effectiveness. In international markets the price of copper continues to rise. The cost-benefit analysis of replacing existing surfaces and materials would need to be established and it would be important to ascertain which surface areas should be targeted for maximum impact, if, for cost or other reasons, all surfaces could not be replaced. Establishing the minimum percentage of copper required in alloys for efficacy is also an area of uncertainty with wide variation in surfaces tested in laboratory experiments. Studies suggest that anything from 55% to 100% copper composition are required for biocidal impact. In choosing which alloy to employ there needs to be a balance between efficacy and other considerations such as durability of surfaces and their aesthetic appeal.

Concerns regarding the impact of soiling and cleaning on the effectiveness of contact killing surfaces also need to be addressed. The effect of soil residue on antimicrobial surfaces has most notably been studied in the area of food handling and preparation. One such laboratory investigation assessed the benefit of using copper alloys to reduce E. coli 0157 cross-contamination and established that the addition of a liquid beef extract mimicking soiling provided a protective matrix for the bacterial cells to ‘hide in’ but significant reductions in viability were still achieved.

Only one laboratory-based study has exclusively addressed the problems associated with cumulative soiling and cleaning on the antimicrobial properties of copper. Test surfaces of copper and copper alloys were soiled with S. aureus suspended in a protein-based organic soil (bovine serum albumin: BSA), dried rapidly and incubated for 24 h. Surfaces were then wiped clean using a standardized wiping procedure with two cleaning agents commonly used in the UK National Health Service (1% sodium hypochlorite and 70% industrial methylated spirit). The soiling/cleaning procedure was carried out daily over five days and after each cycle the amounts of residual soil and live cells were assessed using epifluorescence microscopy. After the second soiling/cleaning cycle it appeared that the application of the cleaning agent caused subsequent layers of the BSA—bacteria soil to bond more strongly to the copper surface, increasing its resistance to cleaning. The stainless steel surfaces by comparison remained highly cleanable. This surface conditioning has also been raised as an issue of concern in one of the clinical trials which noted that the cleaning solution containing glucoprotamin as an active substance may have generated a thin layer between metallic copper surface and the bacteria, reducing the biocidal effect of copper on bacteria.

Conclusion

The biocidal effect of copper as a contact surface has been extensively investigated in a wide variety of laboratory studies and appears to have a potential application in healthcare infection prevention and control efforts. However, it must be acknowledged that further research is required in a number of areas before the widespread implementation of copper contact surfaces could be recommended, including any significant additional costs. In particular, further clinical trials demonstrating a sustained reduction in HCAI rates need to be reported. Minimum percentage copper content and effective cleaning protocols for copper surfaces should be established. It is fitting that the US EPA requires those making public health claims related to the antimicrobial benefit of copper to clearly state that the use of such surfaces is a supplement to, not a substitute for, standard infection prevention and control practices. Effective hand hygiene and the routine cleaning of environmental surfaces remain integral components in reducing HCAI, and the additional routine contribution of copper surfaces, while potentially beneficial, remains to be clearly established.

Conflict of interest statement

H.H. has had recent research collaborations with Steris Corporation, Inov8 Science, Pfizer & Cepheid. He has also recently received lecture and other fees from Novartis, AstraZeneca & Astellas.

Funding sources

None declared.

References


