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Review

Selection of resistance by antimicrobial coatings in the healthcare setting

F. Pietsch [a, 1], A.J. O'Neill [b, 1], A. Ivask [c, d, 1], H. Jenssen [e], J. Inkinen [f], A. Kahru [c], M. Ahonen [g, **, 1], F. Schreiber [a, *, 1]

[a] Federal Institute for Materials Research and Testing, Department of Materials and Environment, Division of Biodeterioration and Reference Organisms, Berlin, Germany
[b] School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK
[c] Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia
[d] Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia
[e] Department of Science and Environment, Roskilde University, Roskilde, Denmark
[f] Finnish Institute for Health and Welfare, Department of Health Security, Helsinki, Finland
[g] Satakunta University of Applied Sciences, Faculty of Technology, WANDER Nordic Water and Materials Institute, Rauma, Finland

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SUMMARY

Antimicrobial touch surfaces have been introduced in healthcare settings with the aim of supporting existing hygiene procedures, and to help combat the increasing threat of antimicrobial resistance. However, concerns have been raised over the potential selection pressure exerted by such surfaces, which may drive the evolution and spread of antimicrobial resistance. This review highlights studies that indicate risks associated with resistance on antimicrobial surfaces by different processes, including evolution by de-novo mutation and horizontal gene transfer, and species sorting of inherently resistant bacteria dispersed on to antimicrobial surfaces. The review focuses on antimicrobial surfaces made of copper, silver and antimicrobial peptides because of the practical application of copper and silver, and the promising characteristics of antimicrobial peptides. The available data point to a potential for resistance selection and a subsequent increase in resistant strains via cross-resistance and co-resistance conferred by metal and antibiotic resistance traits. However, translational studies describing the development of resistance to antimicrobial touch surfaces in healthcare-related environments are rare, and will be needed to assess whether and how antimicrobial surfaces lead to resistance selection in these settings. Such studies will need to consider numerous variables, including the antimicrobial concentrations present in coatings, the occurrence of biofilms on surfaces, and the humidity relevant to dry-surface environments. On-site tests on the efficacy of antimicrobial coatings should routinely evaluate the risk of selection associated with their use.

* Corresponding author. Address: Federal Institute for Materials Research and Testing, Department of Materials and Environment, Division of Biodeterioration and Reference Organisms, Unter den Eichen 87, 12205 Berlin, Germany. Tel.: +49 30 8104 1414.
** Corresponding author. Address: Satakunta University of Applied Sciences, WANDER Nordic Water and Materials Institute, PL 211, P.O. Box 211, Suojantie 2, FI-26101 Rauma, Finland. Tel.: +358 44 710 3061.
E-mail addresses: merja.ahonen@samk.fi (M. Ahonen), frank.schreiber@bam.de (F. Schreiber).
[1] AJO, AI, FP, MA and FS contributed equally.

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Introduction

Infections caused by antibiotic-resistant bacteria are among the most severe healthcare problems, and are associated with a heavy economic burden. It has been estimated that antimicrobial resistance (AMR) causes 33,000 deaths and costs 1.5 billion EUR per annum in Europe alone [1—3]. High-touch surfaces in near-patient areas are linked to healthcare-associated infections (HAIs) [4] by acting as vectors for spreading infectious agents [5], including AMR microbes. An increasingly popular strategy to mitigate this problem is to coat these surfaces with antimicrobial compounds. In combination with traditional infection prevention and control procedures, such as proper hand hygiene, efficient cleaning and disinfection, and appropriate usage of antibiotics, antimicrobial coatings (AMCs) have the potential to reduce the development and transmission of AMR [6]. However, exposure to AMCs may also harbour the potential to drive the selection and spread of AMR bacteria [7,8].

The aim of this review is to explore studies that indicate risks associated with evolution and dissemination of AMR due to the use of AMCs, and the translation of such studies to healthcare environments. First, the general mechanisms by which AMR can evolve and spread are reviewed. Next, the usage, mode of action, efficacy and possible resistance mechanisms to antimicrobial substances used as AMCs are evaluated, with an emphasis on copper, silver and antimicrobial peptides (AMPs). While the efficacy of AMCs [9] and the potential for selection of antibiotic resistance by metals [10] has been covered previously in excellent reviews, the aim of this review is to provide a link between these topics and its relevance for healthcare settings. Finally, this review will provide a preliminary risk evaluation, and highlight open questions that need to be addressed to improve risk assessment.

Methods

This review was initiated by the EU COST action CA15114 AMiCI 'Anti-microbial coating innovations to prevent infectious diseases' where the safety analysis of application of AMCs on frequently touched surfaces in healthcare settings was one of the central tasks. One important safety aspect of AMCs is the development of AMR that must be considered and evaluated for a risk-benefit analysis of the use of these novel coatings [7,8,11]. Initial analyses within the COST consortium showed that silver- and copper-based coatings are most relevant to current use and development [7,12]. Surfaces coated with AMPs were also included as an example of an emergent technology. Next, a group of scientific experts who investigate evolution and mechanisms of resistance to antimicrobials, including biocides and surface coatings of silver, copper and AMPs, was assembled. The most important general mechanisms for resistance evolution and spread that may be underpinned by AMCs were defined, namely de-novo evolution, horizontal gene transfer and species sorting. To put resistance to the AMC into a general context, information was included for each active substance on use, efficacy, mode of action and resistance by the three general mechanisms. Each expert performed searches in electronic databases (PubMed or Web of Science) using the terms 'antimicrobial surface', 'resistance' and 'copper', 'silver' or 'antimicrobial peptides'. The abstracts and results sections of the scientific publications were evaluated to identify those publications that provided evidence for each aspect (use, efficacy, mode of action, resistance) of the reviewed active substance. Publications that were known to the authors as cornerstone studies in the field but were not identified in the search were also included.

Results

General mechanisms of AMR selection

There are three main mechanisms of resistance against antimicrobial compounds: (i) reducing intracellular concentrations; (ii) target alteration, modification or protection; and (iii) enzymatic transformation of the antimicrobial agent [13]. Resistance to antimicrobials can evolve and spread in a population by two principal mechanisms: de-novo mutation and horizontal gene transfer (HGT). De-novo mutations occur randomly, and a subset of these will improve the growth or survival of these mutants in the presence of an antimicrobial compound. This selective benefit of the mutant leads to a relative increase of its progeny in the population, whereby the mutations are transmitted vertically across generations. Alternatively, resistance can be acquired horizontally from resistant cells by the processes of conjugation, transduction or transformation. DNA, newly acquired through HGT, can subsequently be transmitted vertically to the progeny. Lastly, species sorting of inherently resistant populations is a potentially important process for the transmission of AMR. The chronic presence of an antimicrobial compound can affect microbial community assembly, whereby populations with inherent or acquired resistance that are dispersed into the treated environment increase relative to non-resistant populations without the need for genetic changes [14].

Two phenomena — cross-resistance and co-resistance — are key to understanding the risk of AMCs in driving the emergence and spread of AMR in healthcare settings. Cross-resistance describes a phenomenon whereby a single molecular mechanism is capable of mediating resistance to different toxic substances [15—18]. If resistance evolves against an antimicrobial used as an AMC, and the same resistance mechanism decreases the sensitivity of bacteria to antibiotics, AMCs would contribute to the problem of AMR through the selection of cross-resistance. For example, cells that evolve decreased porin expression in the presence of silver may also exhibit decreased susceptibility to antibiotics [17]. Co-resistance is observed if different resistance mechanisms are genetically linked on the same genetic element. The selective pressure on one mechanism is sufficient to ensure retention of all of these within the population [16,18]. This type of co-selection is commonly related to mobile genetic elements, such as plasmids, that harbour different resistance mechanisms which — due to their physical association — are all maintained in the presence of a single antimicrobial [16,19,20]; for example, certain plasmids harbour genes conferring resistance to silver and antibiotics [17,20,21]. The presence of silver-coated surfaces could potentially co-select for the maintenance or spread

of AMR genes because the plasmid confers a fitness advantage through carriage of silver resistance genes.

In addition, growth as surface-associated biofilms is known to affect intrinsic susceptibility to killing by antimicrobials, as well as increasing the appearance and transfer of genetically determined AMR. This is relevant for the consideration of AMR risks of AMCs because cells are surface-associated when in contact with AMCs. Eradication of cells growing in a biofilm by antimicrobials is hindered by the slow growth of cells embedded in the biofilm compared with genetically identical planktonic cells (a feature that makes them intrinsically far less susceptible to bactericidal action), the active expression of stress tolerance genes and the protective extracellular matrix of the biofilm [22]. Moreover, several studies have demonstrated that de-novo mutation and HGT in pathogenic bacteria occur at greater frequency in biofilms. For example, the frequency of conjugative transfer of plasmids increases 1000-fold in *Escherichia coli* biofilms [23] and up to 16,000-fold in *Staphylococcus aureus* biofilms [24]. In bacteria such as *Streptococcus pneumoniae* that are capable of undergoing transformation through uptake of naked DNA from their environment, transformation is often only observed during biofilm growth [25]. The biofilm is also associated with accelerated evolution of AMR through de-novo mutation, as has been described in diverse human pathogens including *S. pneumoniae*, *Pseudomonas aeruginosa* and *S. aureus* [26—28]. It should be noted that surface biofilms on dry AMCs in healthcare settings are formed by deposition of bacteria (e.g. from body fluids or contact transmission by touching). This process likely differs considerably from the developmental formation process of submersed biofilms that is more commonly studied. In the context of hospital surfaces, studies on the characteristics of deposited, dry biofilms and how their characteristics relate to AMCs are currently lacking.

Copper

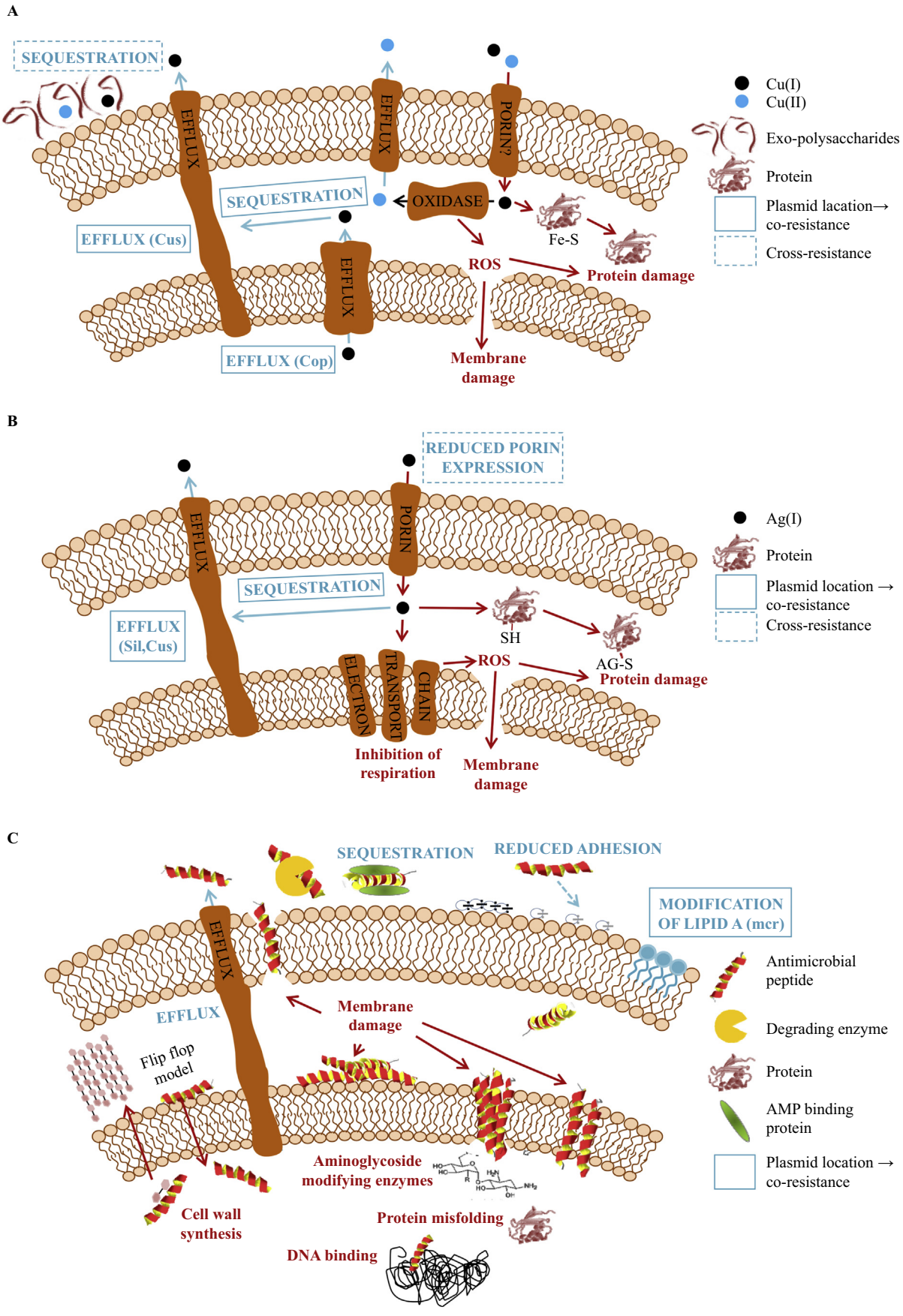
Copper is the most common metal used as an AMC, with well-established efficacy and broad application in healthcare settings [29]. Copper has been incorporated in the form of pure copper, copper alloys, copper composites or nanoparticles in hard non-porous products (e.g. door handles, handrails) as well as in porous products (e.g. textiles) [30,31].

Bacterial killing rates on copper surfaces can reach 7—8 logs per hour in laboratory settings, with no microbes recoverable from the surface after longer incubation times [32]. Copper surfaces were the first to be approved as AMCs by the US Environmental Protection Agency (EPA) in 2008. According to EPA guidelines [33,34], such surfaces can be registered as antibacterial if they reduce bacterial counts by 99.9% in 2 h. Based on this criterion, EPA-registered copper alloys with at least 62% copper content have been shown to be effective against a range of pathogens including *S. aureus* [including meticillin-resistant *S. aureus* (MRSA)], vancomycin-resistant enterococci, *E. coli* O157: H7, *P. aeruginosa* and *Enterobacter aerogenes* [33]. Moreover, studies using a more stringent activity threshold (i.e. 99.99% kill) showed that copper materials with ≥62% copper content kill *S. aureus* cells within 10 min [35], *Acinetobacter* spp. within 240 min [36] and *E. coli* O157 within 350 min [37]. Generally, laboratory studies report higher antimicrobial efficacy with increasing copper content. However, copper content alone is not sufficient to explain the antimicrobial efficacy of the studied surfaces [32,38]. Copper-containing surfaces generally exhibit more rapid bacterial killing (measured in seconds to a few minutes) in dry conditions than when wet, where exposure of minutes to a few hours is needed to achieve a biocidal effect for most microbes [32]. This property is in strong contrast to most other AMCs, and is beneficial, given that most real-life scenarios for AMCs involve prolonged dry periods. Additionally, surfaces that are made entirely of copper are, in principle, able to elaborate antimicrobial ions continuously, while microbicide-releasing coatings that are intended to leach an antimicrobial agent must be considered to be non-permanent [6].

In hospital settings, pure copper or copper alloy items have been tested in multiple touch surface types in patient rooms (i.e. furniture and other permanent indoor interior designs). A reduction in total bacterial counts of 63—100% compared with control surfaces has been observed in different studies over periods of 10 weeks to 9 months [39—44]. Copper surfaces, compared with control surfaces, also delayed repopulation with bacteria [44], and decreased the number of bacterial spores [45,46], which are otherwise difficult to eradicate. Copper surfaces have also been shown to affect biofilm formation under submersed conditions, although these studies were conducted outside the healthcare environment. Compared with no copper surfaces and surfaces with low amounts (0.1 mol%) of copper, 5 mol% copper surfaces inhibited marine biofilm formation significantly [47]. Similarly, the formation of *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia* biofilms over 24 h and 48 h was inhibited by copper alloys that contained 57—96% of copper [48]. The antimicrobial effect of copper surfaces appears to translate into a reduced prevalence of HAIs in patient rooms with copper-containing hard-touch surfaces [43,49] or copper-containing linens [30,50—53]. However, assessing the impact of such interventions on the rates of HAIs is challenging [49], and despite the modest microbial reduction confirmed in a systematic review [9], uncertainties about the efficacy of copper surfaces for the prevention of HAIs still remain.

The antimicrobial mode of action of copper is attributed to its redox properties and the tendency to transition between cuprous [Cu(I)] and cupric [Cu(II)] oxidation states (Figure 1A) [54]. Cu(I) ions are believed to trigger the production of hydrogen peroxide and further hydroxyl radicals, which, in turn, cause damage to various cellular structures [32]. Copper ions also compete with iron in Fe—S clusters, as well as with zinc in the active sites of proteins [32]. Thus, the overall effect of copper surfaces on bacteria is a combination of the damage inferred by Cu(I) ions and reactive oxygen species (ROS), leading to lipid peroxidation, loss of membrane integrity and cell death [32,55]. Furthermore, induction of oxidative DNA damage has been observed [32], although this is unlikely to represent a primary cause of copper-mediated surface killing [38,55].

Bacteria have evolved mechanisms to protect themselves from the toxic effects of copper, including extracellular sequestration of copper ions, low permeability of outer and inner membranes of copper ions, active efflux of copper ions from the cell, and the presence of copper-scavenging proteins (Figure 1A) [32,55]. An important example of efflux-mediated resistance is the Cus system present in *E. coli*, which actively pumps out copper ions (Figure 1A). Analogous mechanisms are described for most other Gram-negative bacteria [32,55—57].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Flip flop model
Aminoglycoside modifying enzymes
Cell wall synthesis
Protein misfolding
DNA binding
Antimicrobial peptide
Degrading enzyme
Protein
AMP binding protein
Plasmid location → co-resistance

While there is ample knowledge on native cellular systems that mediate copper resistance, the authors are not aware of studies that have successfully evolved copper resistance by de-novo mutation in the laboratory. For *P. aeruginosa*, it has been shown that copper exposure can trigger an upregulation of copper efflux systems, and simultaneously mediate cross-resistance to the carbapenem drug imipenem via down-regulation of a porin that allows uptake of carbapenems [58,59]. Moreover, copper ions can interfere directly with antibiotics, and either diminish (e.g. by binding to the antibiotic molecule and decreasing its potency) or enhance (due to synergistic effects between the metal ion and the antibiotic) their single effect [60–62]. Thus, interactions between the metal and the antibiotic drug may also play a role in the selection of resistance.

Copper resistance can spread via HGT. A number of conjugative plasmids harbouring copper resistance genes have been described [20,21,63], which poses the risk of co-selecting antibiotic resistance genes upon copper usage. Initial evidence for potential co-resistance has been provided by Yang *et al.* [20], who reported that multi-drug-resistant Enterobacterales (see https://jb.asm.org/content/jb/55/3/287.full.pdf) carried copper (and other metal) resistance genes up to seven times more frequently than antibiotic-sensitive strains. Moreover, horizontal transfer of copper resistance genes along with various antibiotic resistance genes has been observed in MRSA [64], *Salmonella typhimurium* [65] and enterococci [66–68]. Touati *et al.* (2010) detected copper resistance in all 16 extended-spectrum beta-lactamase-producing Enterobacterales strains (N=62) isolated from hospital surface environments [69]. Furthermore, clinical isolates of *Klebsiella pneumoniae* harbouring large multi-drug-resistant plasmids with both copper and antibiotic resistance genes have been described [21,62]. Recent but not yet peer-reviewed research suggests that copper stress can increase plasmid uptake [70], which may therefore also increase the potential for spread of antibiotic resistance genes and co-selection.

Species sorting of AMR microbes by copper has been observed in soils [71–74], aquaculture [74], wastewater environments [75] and drinking water networks [76]. Moreover, the use of copper in animal feed has led to increased levels of antibiotic-resistant *Salmonella* spp. and enterococci in swine [18,77]. Despite these known correlations between copper and antibiotic resistance in environments with constant high copper ion exposure, a causal relationship between copper-containing AMCs and selection of AMR in real-life conditions has yet to be proven [57]. However, it can be speculated that species sorting could be an important mechanism for AMR selection in this context because copper-sensitive strains landing on a copper surface will have only limited chance to become resistant by de-novo mutations or HGT before they are killed. In contrast, intrinsically resistant strains will become enriched on a selective AMC because the resistance mechanism (potentially conferring co- or cross-resistance to antibiotics) provides a benefit over the sensitive population. The resulting shift in microbial community composition will be of clinical relevance if the resistant strains are pathogens, have means to transfer resistance genes to pathogens, are co- or cross-resistant to antibiotics, or can protect pathogens by secreting factors that diminish the toxicity of AMCs.

Silver

The principal use of silver in healthcare settings is in the prevention of bacterial infections in wounds and burns, although it is also used to coat medical devices (e.g. catheters) to reduce device-associated infections [78]. Use of silver as a dry AMC is less common than for copper, and when employed in this context, it is typically used in combination with other biocidal substances (see below) [79–81]. Nevertheless, silver is increasingly marketed as an AMC for dry surfaces including panels, paints and textiles [82].

Although evaluated extensively as an AMC in the context of medical devices, the utility of silver as an AMC for healthcare settings is less well studied. The standard tests for assessing the antimicrobial properties of hard, non-porous surfaces *in vitro* are the efficacy testing methods JIS Z 2801 and ISO 22196. These protocols specify a relative humidity of >90% over the period in which bacteria are in contact with the test surface. Under such conditions, surfaces containing silver exhibit comparable antibacterial potency to those of copper [83]. However, the antibacterial efficacy of silver-containing surfaces appears to be critically dependent on high levels of hydration [83], and this assay therefore fails to reflect the low moisture levels that many surfaces in healthcare settings experience in use. Studies conducted at relative humidity representative of indoor environments (<20%) suggest that surfaces containing silver are devoid of antibacterial activity [83,84]. This reflects the fact that silver metal is, under ambient conditions, less susceptible than copper to the surface oxidation required to produce the ionic species responsible for the antibacterial effect. Common coating materials include

Figure 1. Antibacterial modes of action of, and bacterial resistance towards, copper, silver and antimicrobial peptides. (A) Antimicrobial action of, and resistance to, copper. Copper can mediate both indirect and direct damage (dark red arrows and text), mainly through reactive oxygen species (ROS) or by direct binding to proteins. Resistance to copper (light blue) includes modification and sequestration of copper in the periplasm or extracellularly, or export through transmembrane proteins. (B) Antimicrobial action of, and resistance to, silver. Similarly to copper, silver causes indirect and direct cellular damage (dark red arrows and text). An important and early consequence of this damage is loss of integrity and function of the cytoplasmic membrane. While silver has been shown to have direct effects on electron transport chain proteins, this effect may also be anticipated for copper, but the authors are not aware of evidence for such direct interactions. Resistance to silver (light blue) predominantly results from efflux, either in combination with decreased influx through the outer membrane or sequestration in the periplasm. (C) Antibacterial mode of action of antimicrobial peptides (AMPs) and bacterial resistance mechanisms. The naturally occurring short (12–50 residues), cationic (+2–8) and hydrophobic (~50% of the residues) sequences interact and destabilize negatively charged bacterial membranes (red). The intracellular presence of AMPs may lead to inhibition of cell wall synthesis, binding to nucleic acids, protein misfolding and regulation of transcription/translation. Resistance mechanisms (light blue) cover changes in bacterial surface charge, increased efflux activity, degradation by secreted proteases and/or sequestration by secreted protein binders.

silver salts (frequently, silver oxide) or silver nanoparticles. Whilst the latter are comprised of the elemental metal, their large surface-to-bulk ratio means that they contain considerable quantities of silver oxide that provide a source of silver ions [85]. Nanoparticulate silver often exhibits greater antibacterial potency *in vitro* than an equivalent concentration of silver salts, an effect that is likely the result of increasing silver availability rather than because of any 'particle-specific' toxic effects [86]. It has been reported that coatings containing silver can reduce microbial contamination of surfaces in healthcare settings; however, in most cases, the coatings evaluated also contained other antimicrobial agents (e.g. quaternary ammonium compounds or zinc pyrithione) in addition to silver, thereby preventing an assessment of the contribution made by silver [79—81]. In one case where the coating apparently contained silver ions as the sole antibacterial compound, a 10-fold reduction in colony-forming units was reported across different treated vs untreated surfaces [87]. Further studies are therefore required to establish whether silver represents a useful AMC in healthcare settings.

The antibacterial effect of silver ions derives, in part, from their ability to bind Fe—S clusters in iron-containing enzymes, thereby inhibiting crucial cellular functions including the electron transport chain, and, in turn, driving the formation of ROS, predominantly via the Fenton reaction [88—90]. In addition, binding of silver ions to thiol groups inhibits disulphide bond formation in proteins, preventing correct folding and inducing aggregation [89]. When these latter effects impact membrane proteins, this results in destabilization and loss of membrane integrity [89,91].

To date, silver resistance has not been detected amongst important Gram-positive pathogens such as staphylococci [91]. In contrast, several mechanisms of silver resistance have been detected in laboratory studies with medically relevant Gram-negative bacteria [17,19,92—94]. The extent to which these occur in the healthcare setting, and whether they allow their bacterial hosts to effectively overcome the antibacterial properties of silver-coated surfaces, is, for the most part, unknown. The resistance mechanism most likely to be relevant in this context is the Sil system, which is prevalent amongst clinically important Enterobacterales including *Enterobacter* spp., *E. coli*, *Salmonella enterica* and *Klebsiella* spp. [65,95,96]. The Sil operon often resides on plasmids and can be acquired horizontally [17,19,21,65,97,98]. As these plasmids frequently also encode resistance to multiple, clinically important classes of antibiotics, selection for Sil^+ strains will also co-select for antibiotic resistance [17,21,65]. Sil acts to detoxify silver ions through a combination of sequestration in the periplasm (mediated by the SilE protein) and active efflux (via the SilABC transporter), thereby restricting silver ingress into the cell and providing a profound reduction in silver susceptibility over wild-type strains [17,97]. Although Sil is named to reflect its ability to mediate silver resistance, several lines of evidence suggest that its original evolved role is copper transport [17]. Indeed, this system has been shown to be capable of mediating reduced susceptibility to copper ions under anaerobic conditions [65]. Thus, the presence of Sil in a bacterium has the potential to attenuate the antibacterial efficacy of both silver and copper surfaces concurrently. Furthermore, this ability to mediate cross-resistance to silver and copper implies that either type of metal surface could, in principle, select for bacteria carrying Sil, thereby enriching healthcare settings for micro-organisms that are better able to resist the antimicrobial effect of silver in currently deployed wound dressings and medical devices. Similar effects could potentially also occur via the Cus system, which can actively efflux both copper and silver from the cell [17,92], and upregulation of which is central to the resistance phenotype in laboratory-evolved, silver-resistant *E. coli*. However, there is no evidence, at present, that upregulation of the Cus system plays a role in silver resistance outside the laboratory. A general overview of the resistance mechanisms is given in Figure 1B.

Few studies have investigated the effect of silver on the selection of bacteria inherently resistant to antibiotics by species sorting [99]. Silver nanoparticles have been shown to affect community composition in soil [100]. While studies in wastewater and soil report the tendency for the selection of some antibiotic resistance genes in silver-amended environments [99,101], a study in estuarine sediments did not find an effect [102]. As these studies have been performed in soils, sediments and wastewater, it is not known whether silver-coated surfaces in healthcare settings will affect species sorting of bacteria resistant to antibiotics.

Antimicrobial peptides

AMPs are small, naturally occurring peptides with antimicrobial properties which are considered as promising future AMCs. Due to the lack of experimental evidence, this section serves as an outlook rather than an evaluation of the present-day scenario. In laboratory settings, several materials have been functionalized with AMPs, including titanium [103—105], catheters [103—108] and contact lenses [109]. Diverse linker strategies are utilized [103,104,110—117], also contributing anti-adhesive properties [118] and supporting dip coating [113]. Different coatings have demonstrated stability towards ethylene oxide sterilization [119] and repeated washing with hydrochloric acid, sodium hydroxide and ethanol [120], making them attractive for healthcare settings.

AMPs can exhibit antimicrobial activity against planktonic and biofilm bacteria [121—126], viruses [127] and parasites [128]. AMP tethering to surfaces retains the antimicrobial effect in laboratory experiments and in in-vivo models, resulting in a 5 log reduction of colony-forming units when exposing the functionalized surface to 5×10^5 bacteria/mL over 4 h [103,104]; as discussed above, test conditions are in liquid or under high moisture, thus not reflecting the dry conditions in healthcare settings. The antimicrobial effect of AMPs is usually based on their electrostatic interactions with bacterial membranes [129]. Furthermore, antibiofilm effects of peptide functionalized stainless steel [130,131] or titanium [103] have demonstrated a positive impact on the inhibition of biofilm formation. However, it should be noted that all of these experiments monitored the formation of biofilms in liquid media.

Bacteria have multiple mechanisms that confer resistance against AMPs [132], including degradation by extracellular proteases [133], extracellular sequestration (e.g. by exopolymers [134]), cell surface modifications (e.g. changing the net negative surface charge of bacteria in the direction of more positive values [135,136] or membrane rigidity), cytoplasmic membrane alteration and increased efflux pump activity (Figure 1c) [137]. Studies on the evolution of AMP resistance

have shown that evolution by de-novo mutations is possible in laboratory evolution experiments [138]. High-level AMP resistance in this study depended on strong epistatic interactions between multiple mutations, including those in transcriptional regulators, restricting the evolutionary pathways to resistance. In addition, resistance evolution against AMPs is restricted by their pharmacodynamic properties (i.e. their narrow mutant selection window characterized by a steep dose-response curve [139]). Cross-resistance between AMPs and antibiotics seems likely because increased efflux pump activity has been shown to confer AMP resistance [137]. Contrary to this hypothesis, a large comparative study has demonstrated that cross-resistance is rare while collateral sensitivity is widespread [140]. AMPs are part of the immune system of humans, and several studies have demonstrated that resistance that selects against one type of AMP often results in cross-resistance to other AMPs. Therefore, routine use of AMPs as antibiotics as well as AMCs may potentially select for pathogens capable of better evading the immune system [141].

Co-resistance of AMP and antibiotic resistance genes is possible in principle but may be restricted in practice. HGT of resistance to the AMP colistin by the plasmid-associated *mcr-1* gene has been linked with other antibiotic resistance genes [142]. However, studying horizontal transfer of AMP resistance in the human gut microbiome suggested that phylogenetic barriers limit the transfer of single AMP resistance genes [143]. This is in agreement with evolution studies suggesting that multiple mutations need to act in concert to mediate AMP resistance [138].

Discussion

Evaluation of AMR selection risk

Studies on the development of resistance to AMCs such as silver- or copper-coated door handles, sinks and hand railings are rare. Specifically evolution-based AMR studies conducted on such surfaces are not available, but environmental studies have shown that copper-based AMCs can co-select for AMR under submerged conditions [144]. Thus, important data to perform a detailed risk assessment for AMR selection on AMCs in healthcare settings are missing. Although AMCs are meant to act under dry conditions, most experiments have been carried out in solution, and consequently can only suggest the 'potential' of the surfaces for AMR selection and spread. Several cases of reduced susceptibility to antibiotics due to exposure to selective concentrations of metals have been reported [18,63,145]. Environmental copper resistance studies have shown strong evidence for selection of antibiotic resistance due to exposure to subinhibitory copper concentrations [16,73]. As hypothesized by Pal *et al.* [10], the dominant mechanism driving this effect is cross-resistance, often conferred by efflux systems [144]. Important in this context is the example of cross-resistance between copper and silver. Selection for silver resistance can occur via overexpression of components of the Cus system, which is commonly linked to increased copper efflux [17,92]. In addition, expression of the Sil operon will lead to transport of both copper and silver [17,146]. Thus, resistance to one of the metals may also confer resistance to the other via a shared efflux mechanism. In addition, several environmental studies suggest that cross-resistance between antibiotics and metals can facilitate a shift in the community structure towards naturally resistant organisms in high-metal environments, and that metal resistance genes are physically linked with antibiotic resistance genes on plasmids [147]. Therefore, the use of metal surfaces may, instead of eliminating antibiotic-resistant bacteria, constitute a recalcitrant selective environment that potentially contributes to the spread of AMR in healthcare settings via cross-resistance and co-resistance [16,62].

Further studies and the development of standardized laboratory tests are needed to investigate whether the observations made can be transferred to the selection of resistance on copper- or silver-coated surfaces. These studies need to consider that the biofilm mode of growth of bacteria affects sensitivity to antimicrobials and the evolutionary processes by which resistance emerges. In addition, these studies should consider that concentrations employed for antimicrobial copper coatings are usually above the minimum inhibitory concentration, and therefore higher than the selective concentrations present in the above-mentioned studies, and that elemental silver coatings seem to be inert on dry-touch surfaces. Furthermore, these studies should be conducted at relative humidity relevant to a dry-surface environment because different resistance mechanisms may be involved depending on the humidity during selection. Finally, it is important that future clinical trials on the efficacy of AMCs incorporate an evaluation of the risk of resistance selection. To this end, the frequency of strains resistant to the coating and to antibiotics, which were isolated from the AMC compared with control surfaces, should be determined.

In conclusion, considering the rapid increase in mortality caused by infections of antibiotic-resistant bacteria and the resulting urgent need for strict hygiene in clinics, AMCs may be valuable in healthcare settings. It is critically important to demonstrate the efficacy of AMCs under relevant environmental conditions, first in the laboratory and then on site. Copper, silver and AMPs can show strong antibacterial effects *in vitro*. However, only copper has been shown to lead to a moderate reduction in bacterial contamination during on-site tests, and the effect on HAIs is not established solidly. Moreover, mounting evidence suggests that the substances used as AMCs have the potential to promote the selection and spread of antibiotic-resistant bacteria and plasmids via cross-resistance and co-resistance. However, most of the evidence originates from studies in solution or high-moisture environments, which are very different from the environmental conditions found on surfaces in healthcare settings. Thus, the magnitude of the AMR selection risk imposed by AMCs on dry surfaces in healthcare settings cannot be assessed accurately at the present time. Taken together, the benefits of AMCs in healthcare settings may outweigh the risk of selecting AMR. However, further on-site research into the efficacy of AMCs and their potential for AMR selection will be required to provide a clearer answer.

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