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# Antimicrobial coatings for environmental surfaces in hospitals: a potential new pillar for prevention strategies in hygiene

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## ABSTRACT

Recent reports provide evidence that contaminated healthcare environments represent major sources for the acquisition and transmission of pathogens. Antimicrobial coatings (AMC) may permanently and autonomously reduce the contamination of such environmental surfaces complementing standard hygiene procedures. This review provides an overview of the current status of AMC and the demands to enable a rational application of AMC in health care settings. Firstly, a suitable laboratory test norm is required that adequately quantifies the efficacy of AMC. In particular, the frequently used wet testing (e.g. ISO 22196) must be replaced by testing under realistic, dry surface conditions. Secondly, field studies should be mandatory to provide evidence for antimicrobial efficacy under real-life conditions. The antimicrobial efficacy should be correlated to the rate of nosocomial transmission at least. Thirdly, the respective AMC technology should not add additional bacterial resistance development induced by the biocidal agents and co- or cross-resistance with antibiotic substances. Lastly, the biocidal substances used in AMC should be safe for humans and the environment. These measures should help to achieve a broader acceptance for AMC in healthcare settings and beyond. Technologies like the photodynamic approach already fulfil most of these AMC requirements.

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## 1. Introduction

Pathogenic microorganisms and viruses (MoV) represent a major threat to human health because they may cause severe and life-threatening infections when entering the human body via different pathways (Turner et al. 2019; Cevik et al. 2020; Pierson and Diamond 2020). One starting point is the colonization of the skin surface with MoV, which then could be transferred by the own hands to the mucosa of the mouth or nose, wounds, or surfaces of any devices. This entails the risk of skin, mucosa and bloodstream infections (Uehara et al. 2000; van Duijkeren et al. 2005; Folgari et al. 2018; Lee et al. 2018; McNeil and Fritz 2019). Besides the skin contact with other persons, the colonization of the skin may also occur when touching any environmental (inanimate) surface that is already contaminated with MoV (Fujikura et al. 2019; van Doremalen et al. 2020). The transmission of MoV through such inanimate surfaces can occur by direct or indirect contact (Otter et al. 2011). It is meanwhile accepted that healthcare-associated infections (HAIs)

are connected to contamination of environmental surfaces (Kanamori et al. 2017; Suleyman et al. 2018; Fujikura et al. 2019).

MoV may keep their hazard potential on surfaces for a time span that ranges from hours to months and even years (Kramer et al. 2006; van Doremalen et al. 2020). Bacteria like *Acinetobacter* spp., *Enterococcus* spp., and *Pseudomonas aeruginosa* can survive on such surfaces from days to months, *Candida albicans* from 1 to 120 days (Kramer and Assadian 2014). Most viruses show a shorter survival time ranging from a few hours up to a few weeks (Kramer and Assadian 2014; Thompson and Bennett 2017; Kampf et al. 2020; Ong et al. 2020; van Doremalen et al. 2020). For example, Influenza A virus (H1N1) remained infectious for a time of a few hours up to a few days depending on the respective surface material (Oxford et al. 2014). Thus, inanimate surfaces are potential reservoirs for MoV allowing their transmission from one individual to the next. Transmission of pathogens occurs not only in health care settings but also in the food production industry and public areas.

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Various measures are applied to reduce the number of MoV on surfaces like surface cleaning as well as physical and chemical disinfection procedures (Rutala and Weber 2019; Song et al. 2019). However, these procedures reduce the number of MoV only at the time of its use and the reduction efficacy depends largely upon its correct execution (Goodman et al. 2008; Doidge et al. 2010). At any time after disinfection, recontamination inevitably occurs. In particular, on frequently touched surfaces, MoV may accumulate again on a surface until the next disinfection procedure occurs (Dancer 2014). According to many routine cleaning schedules of hospitals, the majority of patient-near surfaces are decontaminated once a day only which further underscores the relevance of inanimate surfaces for transmission of nosocomial pathogens (Weber et al. 2019).

AMC technology may help to reduce MoV contamination on inanimate surfaces, which is often due to inadequate disinfection procedures. But one has to admit that AMC technologies are still considered undefined, mysterious, and incomprehensible as recently expressed by some hygiene professionals in the international consortium on Anti-Microbial Coating Innovations (AMICI) (Dunne et al. 2020). Questions regarding AMC include its interaction with routine cleaning and monitoring of AMC efficacy. For regulators, credible blinded, controlled proof of use *in situ* (field studies) is scarce and the impact (positive and negative) on antimicrobial resistance (AMR) remains undefined (Dunne et al. 2020). To achieve a broader acceptance for AMC, the consortium concluded that all these questions and uncertainties should be addressed, in particular a clear approach for AMC technologies concerning laboratory testing under real-life conditions (e.g. field studies) (Dunne et al. 2020).

Thus, the present review describes the size of the problems with nosocomial transmission of MoV through inanimate surfaces, the problems with testing procedures of AMC in laboratories, the small number of field studies so far, the potential concerns regarding AMR, and the impact for the safety of humans and environment. The review also provides recommendations, which should lead to more acceptance of AMC in health care settings and beyond.

## 2. Environmental surfaces – presence and transmission of MoV

In health care settings, several important healthcare-associated pathogens, including vancomycin-resistant enterococci (VRE), *Clostridium difficile*, *Acinetobacter* spp., and methicillin-resistant *Staphylococcus aureus*

(MRSA) are transported from one inanimate surface to the next (Martinez et al. 2003; Dancer 2014; Anderson et al. 2017; Correa-Martinez et al. 2020). The extent of pathogen transmission clearly correlates to the colonization of patients and contamination of patient-near surfaces including medical equipment (Weber et al. 2010; Kanamori et al. 2017; Adams and Dancer 2020), which was confirmed by Whole-Genome-Sequencing (Fujikura et al. 2019; Correa-Martinez et al. 2020).

Patient-near surfaces in hospitals may be reservoirs for various bacteria with numbers ranging from a few colony-forming units per cm<sup>2</sup> (cfu/cm<sup>2</sup>) up to hundreds of cfu/cm<sup>2</sup>. Unfortunately, only a limited number of such cfu/cm<sup>2</sup> values are available for health care units (Table 1) (Shams et al. 2016; Souli et al. 2017; Casini et al. 2018; Costa et al. 2019; Eichner et al. 2020). The presence and transmission of MoV play a role also in other fields like food processing (Gogliettino et al. 2019; Ma et al. 2019; Xing et al. 2019) and highly frequented public areas, in which many people alternately touch surfaces like door handles (Shams et al. 2016; Thapaliya et al. 2017), mobile phones (Kirkby and Biggs 2016; Katsuse Kanayama et al. 2017), banknotes (Vriesekoop et al. 2010; Angelakis et al. 2014), and other items (Table 1) (Ijaz et al. 2016; Carrascosa et al. 2019; Qi et al. 2019; Zou et al. 2019).

Polymicrobial biofilms are well-known in medicine and can be also found on surfaces, for example, catheters (Gaston et al. 2020) and medical implants (Weaver et al. 2019). On inanimate surfaces, species interactions within polymicrobial biofilms can have adverse effects on cleaning and disinfection. For example, *Acinetobacter* spp. and *E. coli* have both been shown to enhance the production of other species' biofilm mass when co-cultured (Habimana et al. 2010). Additionally, polymicrobial biofilms are even more resistant to disinfectants than mono-species biofilms (Burmolle et al. 2006).

Noteworthy, scientific publications mostly report on bacteria on inanimate surfaces, whereas reports on viruses are clearly less frequent (Table 2). Nevertheless, contamination of surfaces with viruses is well known and likewise offers a potential risk of its transmission, for instance for Adenovirus (D'Arcy et al. 2014; Ganime et al. 2016). Torque-teno virus (D'Arcy et al. 2014), Rhinovirus (Phan et al. 2020), Influenzavirus (Rule et al. 2018; Phan et al. 2020), Human Papillomavirus (Gallay et al. 2016), Rotavirus A (Ganime et al. 2016), Norovirus (Morter et al. 2011; Pankhurst et al. 2014; Cui et al. 2017), Para-Influenzavirus (hPIV-3) (Kim et al. 2017), or the thrombocytopenia syndrome virus (SFTS-V) (Ryu et al. 2018).

**Table 1.** Bacteria on inanimate surfaces.

cfu/cm <sup>2</sup>	Details	Sites of detection	MoV*	Reference
6	Emergency rooms, ward, and outpatient departments, hospitals, Germany	Tables, door handles, pc mouse and keyboard, over bed table	<i>M. luteus</i> , <i>S. epidermidis</i> , <i>S. hominis</i> , <i>S. lugdunensis</i> , <i>S. warneri</i> , <i>S. pasteurii</i> , <i>S. caprae</i> , <i>Bacillus</i> spp., <i>Paenibacillus</i> spp., <i>S. aureus</i> , <i>E. faecium</i>	Eichner et al. (2020)
16–54	Hospitals, USA	Bed rails, television remote, call button, telephone; overbed table, door handle, portable commode, bedpan, toilet flush handle	MRSA, VRE, <i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>C. difficile</i>	Shams et al. (2016)
5–30	medical intensive care unit, USA	Bed rails, bed control panels, bed lift, bed footboard	Not specified	Schmidt et al. (2019)
1–7	Sick- and Well-Child Waiting Rooms in Paediatric Outpatient Facilities, USA	Seats, tables, children's tables, children's seats, magazines and books	<i>S. pyogenes</i> , <i>S. paucimobilis</i> , <i>E. faecium</i> , <i>P. aeruginosa</i> , <i>C. sakazakii</i>	Gudakova et al. (2017)
25–32	Wound care ambulatory clinic and a diabetology ambulatory care visit, hospital, Italy	Not specified	Not specified	Casini et al. (2018)
1–54	Intensive care units, Germany	Keyboards, mobil phones	coag.-neg. staphylococci and <i>Micrococcus</i> spp., <i>S. aureus</i> , <i>Streptococcus</i> spp., <i>Bacillus</i> spp., <i>Lactococcus</i> spp., <i>Corynebacterium</i> spp.	Frickmann et al. (2018)
54–106	Intensive care units, Greece	Hospital beds and accessories, nurse's cupboards	coag.-neg. staphylococci, <i>K. pneumoniae</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>Acinetobacter</i> spp.	Souli et al. (2017)
5–52	Acuity paediatric units, Chile	Bed rails, bed rail levers, intravenous poles, faucet handles, the surface of health care workstation	Not specified	Schmidt et al. (2016)
1–2	Hospital, UK	Floor, beds, panels, tables, handles	<i>C. difficile</i> , <i>C. difficile</i> spores	Ali et al. (2015)
1–5	hospital, Canada	Curtains, beds	MRSA	Shek et al. (2018)
127	Hospitals, USA	Stethoscopes	Mannitol-fermenting microbes	Schmidt et al. (2017)
up to $1.8 \times 10^5$	Intensive care units, Brazil	Telephones, keyboards, container for newborn feeding bottle	VRE, ESBL-producing <i>Klebsiella</i> , ESBL-producing <i>Proteus</i> spp.	Costa et al. (2019)
2–150	banknotes, world-wide	Banknotes of 11 different countries	<i>E. coli</i> , <i>S. aureus</i> , <i>B. cereus</i> , <i>Salmonella</i> spp.	Vriesekoop et al. (2010)
100	Restaurants, USA	Menu cards	<i>E. coli</i> , <i>Salmonella</i> spp.	Sirsat et al. (2013)
753	Gran Canaria, Spain	Shopping trolley handles and baskets in different supermarkets	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>C. freundii</i> , <i>P. rhodesiae</i> , <i>P. fluorescens</i>	Carrascosa et al. (2019)
$10^7$ – $10^8$	Railway station, UK	Different surfaces in 17 railway stations	<i>Pseudomonas</i> , <i>Acinetobacter</i> , <i>Bacillus</i> spp., <i>Micrococcus</i> , <i>Staphylococcus</i> spp., <i>Fusarium</i> , <i>Penicillium</i> , <i>Aspergillus</i> , <i>Eurotium</i> spp., <i>Cladosporium</i> ,	Patel et al. (2018)

Note. Hygiene failures were defined as aerobic colony counts of  $>2.5$  cfu/cm<sup>2</sup> and/or the presence of *S. aureus* on hand touch sites (Dancer 2014).

\*The listing of MoV might be incomplete.

**Table 2.** Viruses on inanimate surfaces.

pfu/cm <sup>2</sup>	Details	Sites of detection	MoV	Reference
Inanimate surfaces in hospitals and experimental studies 0–2500	Hospital ward, USA	Tray table, monitor, bed rail, computer keyboard, and computer mouse	Influenza virus, Rhinoviruses, other viruses	Phan et al. (2020)
0.3–6.1	Adult emergency department, USA	Common high-contact, non-porous hard surfaces	Influenza virus	Rule et al. (2018)
Inanimate surfaces – experimental studies 0.3–166	Virus dissemination in restrooms	Toilet bowl rim, toilet seat top, and toilet seat	Coliphage MS2	Sassi et al. (2018)
$3.93$ – $8.07 \times 10^5$	Virus dissemination on gloves	Health care workers, disposal of gloves	Bacteriophage PR772	Munoz-Gutierrez et al. (2019)

For instance, *Sassi et al.* detected the Ebola virus on the inanimate surface in toilets. The authors attribute the colonization of surfaces to small droplets, which were caused by toilet flushing (up to  $4.22 \log_{10}$  pfu/100 cm<sup>2</sup>) (*Sassi et al. 2018*). In a field study, the Ebola virus was detected in the close vicinity of patients and on personal protective equipment of Health Care workers (*Palich et al. 2017*). Similar problems need to be faced concerning norovirus, as it is also distributed by vomiting and can be transferred easily from inanimate surfaces to humans and vice versa (*Weber et al. 2010*). Additionally, norovirus can survive harsh treatments and are responsible for sudden, far-reaching outbreaks in the hospital environment (*Wu et al. 2005*).

When detecting the colonization of surfaces with the Middle East Respiratory Syndrome coronavirus (MERS-CoV) in a hospital, the authors concluded that surfaces might contribute to its transmission (*Bin et al. 2016*; *Khan et al. 2016*; *Kim et al. 2016*). Also, SARS CoV and SARS CoV-2 can colonize inanimate surfaces in hospital wards (*Kampf et al. 2020*; *Ong et al. 2020*; *van Doremalen et al. 2020*). Patient-near surfaces could also contribute to the transmission of this new, pandemic virus (*Wu et al. 2020*).

*Candida auris* is a multidrug-resistant fungal pathogen that persistently provokes nosocomial candidemia (*Calvo et al. 2016*; *Schelenz et al. 2016*). A paper reported an ongoing outbreak of 50 cases with *C. auris* in a cardio-thoracic centre, London, UK, which was correlated to an enduring presence of this yeast on inanimate surfaces (*Schelenz et al. 2016*; *Vallabhaneni et al. 2017*). Transmission of *C. auris* may occur again via medical devices like reusable axillary temperature probes (*Eyre et al. 2018*).

### 2.1. Dry biofilms on surfaces

Bacteria within a biofilm on inanimate surfaces are more resistant to desiccation, removal by detergents, and inactivation by disinfectants. Thus, biofilms may contribute to the maintenance of environmental contamination of inanimate surfaces (*Hu et al. 2015*; *Ledwoch et al. 2018*; *Amaeze et al. 2020*; *Ledwoch et al. 2021*). Bacterial biofilms have been identified on many medical devices and are associated with the presence of moisture and/or liquid (*Yin et al. 2021*).

More recently, biofilms have been discovered on dry surfaces, despite effective infection control measures, which are referred to as “dry biofilms” (*Ledwoch et al. 2018*). These dry biofilms, which may also contain viable multi-resistant organisms, occur despite cleaning on clinical surfaces in an intensive care unit (*Vickery et al.*

*2012*). A study investigated surfaces in hospitals and found dry biofilms containing *S. aureus*, *S. saprophyticus*, and *S. epidermidis* as well as *B. licheniformis* and *B. subtilis*, whereas the only Gram-negative bacterial species were *Pseudomonas* spp. (*Ledwoch et al. 2018*). Also the surfaces of keyboards exhibit dry biofilms containing Coliforms and non-lactose fermenting Gram-negative bacterial species and also MDR-*Acinetobacter* spp., VRE, and MRSA (*Ledwoch et al. 2021*). The authors pointed out that standard methods failed to detect bacteria from keyboards, but pathogens were recovered using enrichment culture, water, or NaOCl-soaked wipes (*Ledwoch et al. 2021*). *Hu et al.* reported that over 90% of ICU surfaces contained bacteria in biofilms including clinically important *S. aureus* (*Hu et al. 2015*). These results unfold a new challenge for hygiene measures on inanimate surfaces in health care settings (*Hu et al. 2015*).

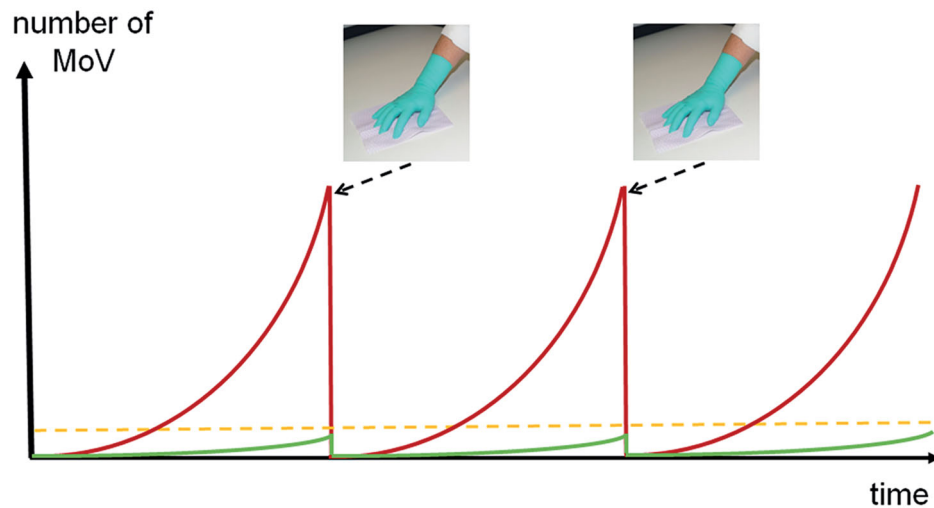
Interactions between *C. albicans* and other microbes (e.g. *S. aureus*, *E. coli*) found on biotic and abiotic substrates are versatile and complex. *C. albicans* can protect anaerobic bacteria from high concentrations of oxygen by providing a hypoxic microenvironment that supports the growth of *Bacteroides fragilis*, *B. vulgatus*, and *Clostridium perfringens* (*Ponde et al. 2021*).

It should be worth investigating in health care settings, whether AMC may also hamper dry biofilm formation on patient-near surfaces by its permanent antimicrobial effect. This would be an additional and important advantage of AMC.

### 3. The role of AMC in hygiene measures

Incorrectly executed hygiene measures associated with problems like shortage of staff, the pressure of time and costs, misunderstanding among staff, and simple neglect further highlight the role of inanimate surfaces for possible MoV transmission. Staff frequently forgets or neglects hand disinfection that was confirmed by a recent review reporting a low mean compliance rate of 41% (*Clancy et al. 2021*). Low hand hygiene rates enable hygiene gaps providing the basis for MoV reservoirs on surfaces (*Goodman et al. 2008*), which could be reduced by the action of AMC technology (*Figure 1*). The compliance for hygiene measures on inanimate surfaces is scarcely reported and may show also a low compliance rate of 48 % (*Carling et al. 2008*).

The efficacy of AMC should be not compared to standard surface disinfectants, because the purposes of both measures are fundamentally different. Surface disinfectants can inactivate MoV by several  $\log_{10}$  steps during a few minutes, however, the disinfectant effects



**Figure 1.** The number of MoV on inanimate surfaces may increase after each disinfection (red line). AMC may keep the mean number of MoV on inanimate surfaces small (green line), possibly below a certain benchmark, for example, 2.5 cfu/cm<sup>2</sup> in case of bacteria (Dancer 2014) (yellow dashed line).



**Figure 2.** The number of MoV are reduced when frequently touched surfaces are equipped with an AMC. Consequently, the transmission of MoV should decrease.

vanish directly after execution (Dancer 2014). Inactivation of MoV by AMC technologies acts slower because its antimicrobial effect is considered permanent (i.e. long-lasting) when compared to the standard consecutive cleaning cycles. AMC acts independently and autonomously without the assistance of the staff, in particular in the time gap of two consecutive standard surface disinfection. That causes a permanent reduction of the mean number of MoV on such coated surfaces and thereby reducing the risk of their transmission (Figure 2) (Weber et al. 2010; Otter et al. 2013; Russotto et al. 2015; Adams and Dancer 2020).

Thus, standard hygiene disinfections and AMC are not competitors but are complementary measures and thereby improve the hygiene conditions on inanimate surfaces, in particular in health care settings (Dancer 2014; Adlhart et al. 2018). Noteworthy, the efficacy of an AMC technology may decrease over time to sub-inhibitory levels due to mechanical abrasion or eluting of the respective biocidal substances. This is another important reason that the respective technology should

not contribute to AMR (see also section: Risks of antimicrobial resistance emergence).

#### 4. AMC – frequently investigated technologies

A recent review listed comprehensible demands for antimicrobial coatings in health care units, primarily under real-life conditions. Besides the reduction of the microbial burden on frequently touched surfaces, also other properties of the coating should be considered like stability, (eco)-toxicological hazards, and the risks of antimicrobial resistance emergence together with an affordable and easy implementation, ideally on-site (Adlhart et al. 2018).

In 2019, Rosenberg and co-authors searched the Scopus database and found metal toxicity as the most frequently published technology for AMC. Among metals, silver (ions, nanoparticles) is most frequently used followed by copper, titanium (titanium dioxide), and zinc (zinc oxide). Chitosan, peptides, and quaternary ammonium compounds (QACs) are less mentioned

(Rosenberg et al. 2019). However, when looking into the growing market of AMC, the use of QACs is frequently advertised (Rosenberg et al. 2019; Lucintel 2021; Vereshchagin et al. 2021).

In 2017, Ahonen and co-authors found in ISI Web of Science 3455 papers on antimicrobial coatings and quantified the different technologies to be silver (30%), chitosan (17%), shortcut titan (14%), copper (5%), zinc (4%), and others (27 %) (Ahonen et al. 2017). As a synopsis of both surveys, AMC predominantly use metals and heavy metals like silver, copper, zinc, and titanium as biocidal substance. The mechanisms of action of those metals are its toxicity by releasing ions to MoV, contact killing by affecting the cell membrane integrity, or generation of reactive oxygen species (ROS), partially supported by exposure to radiation in the ultraviolet spectrum (Nakamura et al. 2020). The details are provided in the following section.

#### 4.1. AMC – mechanisms of action

Most AMC technologies are based on the release of the active biocidal agent from the coating to kill microorganisms on top of the coated surface (Ahonen et al. 2017). Other technologies may use anti-adhesive surfaces, contact-active surfaces, or light-activated molecules to counteract MoV on environmental surfaces (Seil and Webster 2012; Ahonen et al. 2017; Adlhart et al. 2018; Rosenberg et al. 2019). The most frequent technologies together with the biocidal substances used are listed in Table 3.

##### 4.1.1. Anti-adhesive action

Anti-adhesive surfaces can reduce the adhesion force between MoV and a solid surface to reduce MoV attachment to surfaces. Among others, super-hydrophobic surfaces and zwitterionic polymer brushes may delay or

even prevent microbial attachment to a surface (Adlhart et al. 2018). Polymer-functionalized substrates could reduce bacterial adhesion, when compared to the control surface, with viable adherent fractions of *E. coli* cells to 4–10% of control (Yang et al. 2013). Antibacterial fluorinated silica colloid super-hydrophobic surfaces reduced the adhesion of *S. aureus* and *P. aeruginosa* by  $2.08 \pm 0.25$  and  $1.76 \pm 0.12$  log over controls, respectively (Privett et al. 2011). Such antimicrobial efficacies are inferior to other technologies (Table 6).

##### 4.1.2. Contact-active action

Contact-active surfaces provide their antimicrobial activity without releasing biocidal substances (Adlhart et al. 2018). For example, water-soluble antimicrobial polymers, chemically bound to a surface, kill microbes without releasing biocides. The working mechanism for these surfaces has been discussed as the polymeric spacer effect (Siedenbiedel and Tiller 2012). Recent simulations confirm that the congregation of negatively charged lipids within the membrane towards the QACs is an important and quantitative component in the overall mechanism of membrane destabilization (Alkhalifa et al. 2020).

In AMC, QACs are usually covalently bound to surface materials. Surface coatings with well-defined densities of quaternary ammonium functions showed antimicrobial activity against *S. aureus* of greater than 3 log<sub>10</sub> steps (Bieser and Tiller 2011). QACs were frequently tested for AMC on medical implants or catheters, which are in close contact with the tissue being equivalent to wet conditions (Pant et al. 2017; Adlhart et al. 2018; Wang et al. 2019). Laboratory experiments are available for QACs coatings that can be used on environmental surfaces in healthcare settings or other areas. These studies showed antimicrobial efficacy

**Table 3.** AMC technologies.

AMC technology	Mechanisms of action	Biocidal substance
Anti-adhesive	Reduced adhesion of microorganisms to surface	none (Privett et al. 2011)
Contact-active	Perforation and/or depolarisation of cellular membranes	Copper (Warnes et al. 2012) QACs (covalently bound) (Bieser and Tiller 2011)
Release of substances	Biocidal substances reach the microorganisms via diffusion	Silver (Varghese et al. 2013; Scuri et al. 2019) copper (Thukkaram et al. 2021) zinc (Pintaric et al. 2020) QACs (not covalently bound) (Druvari et al. 2016) zinc-pyrrithione (Pittol et al. 2017) iodocarb (Zhang et al. 2020) bronopol (Wu et al. 2011) isothiazolinone (Peng et al. 2018) diuron (Fay et al. 2007)
Photocatalytic action	Different reactive oxygen species are generated by TiO <sub>2</sub> under UV exposure	Oxygen radicals, hydrogen peroxide (Nakano et al. 2013; Fisher et al. 2014; Li et al. 2018)
Photodynamic action	Gaseous singlet oxygen is generated by photosensitizer molecules under visible light exposure	Exclusively singlet oxygen (Eichner et al. 2020)

against various bacterial species, whereas the reduction ranged from 1 to 8 log<sub>10</sub> steps depending on the QACs and the bacteria applied. However, the laboratory tests were performed only under wet and clean surface conditions (Koufakis et al. 2020; Lee and Pascall 2020; Song et al. 2020).

In the case of copper (Cu), the antimicrobial effect is linked to the fact whether Cu is present as ions or nanoparticles. Also the Cu oxidation state, the concentration of Cu in contact with the microbes, the proximity of microbes to Cu-containing surfaces, and the form of application (wet or dry) may play an important role (Mitra et al. 2020). The main mechanism of Cu is attributed to the depolarisation of bacterial outer membranes for both Gram-positive and Gram-negative bacteria, whereas the kinetics and extent of membrane damage are governed by the cell type. The action of copper is sometimes described as contact killing (Mitra et al. 2020). It is assumed that bacteria cells are facing a high amount of copper ions when getting in close contact with copper surfaces leading to cell wall destruction and loss of membrane potential. However, the authors stated that this contact killing requires a permanently clean surface of the copper coating, free of oxide, wax, or other coating agents, which would hamper the biocidal effect of copper (Grass et al. 2011). Nevertheless, the antimicrobial use of copper alloys was registered by the Environmental Protection Agency (US-EPA, EPA Registration Nos. 82012-1 through -6). Dry copper surfaces showed an antimicrobial effect of up to 7 log<sub>10</sub> steps against several types of MoV in laboratory experiments (Molteni et al. 2010; Warnes et al. 2012; Bleichert et al. 2014).

In 2016, Muller reviewed available field studies on the antimicrobial efficacy of different AMC technologies (mainly copper) under real-life conditions. The use of copper surfaces yielded modest reductions in microbial contamination with a high risk of study bias (Muller et al. 2016). In 2018, a review on copper AMC in health care settings concluded that copper AMC shows antimicrobial activity but its importance in healthcare settings remains unclear, especially regarding healthcare-associated infections (Chyderiotis et al. 2018). In another clinical study with copper AMC, different surfaces were sampled once a week for 10 weeks. When comparing the microbial burden of coated and uncoated surfaces, the results showed a reduced efficacy of up to 73%, whereas the authors admitted that some results showed no statistical significance (Palza et al. 2018).

Chitosan is also used as an antimicrobial material and its antimicrobial efficacy is shown in different

applications like foods and leather surfaces (Vasconez et al. 2009; Alvarez et al. 2013; Fernandes et al. 2013). A chitosan coating was proven to be effective against *S. aureus* and *E. coli* when applied on titanium alloy (D'Almeida et al. 2017). It seems that there is quite a strong influence of especially the positively charged amino groups in its antimicrobial efficacy (Yang et al. 2016). This substance is also used as a carrier material for other antimicrobial substances (Ahonen et al. 2017; Adlhart et al. 2018; Rosenberg et al. 2019; Song et al. 2020).

As a critical remark, contact-active technologies usually need close contact of the surface and MoV. Any soiling on the surface, which should be the case under real-life conditions, may critically hamper the efficacy requiring recurrent and well-executed cleaning (Chyderiotis et al. 2018).

#### 4.1.3. Release of substances

Silver is one of the most prominent examples concerning the release of a biocidal substance. Liao and co-authors recently stated that there is an ongoing debate over whether silver nanoparticles (Ag-NPs) or Ag-ions are responsible for the antimicrobial effects (Liao et al. 2019). The mechanisms responsible for the bactericidal effect of Ag-NPs are not fully elucidated yet (Rosenberg et al. 2019). On one hand, direct contact of Ag-NPs with large surface areas on a bacterial cell wall could lead to membrane damage. On the other hand, there is strong evidence that the antimicrobial effect of Ag-NPs may result from the continuous oxidative dissolution of Ag-ions from Ag-NPs (Gilabert-Porres et al. 2016; Liao et al. 2019). Most of the papers in a survey reported a release of Ag-ions (Rosenberg et al. 2019). Once released, Ag-ions can react with bisulphides of proteins, disturbing the respiratory chain of bacteria or destroying the bacterial membrane (Jung et al. 2008). Ag-ions can penetrate the outer cell membrane of bacteria that leads to degradation of the chromosomal DNA but may also affect thiol groups of cytoplasm proteins (Hsueh et al. 2015).

It is obvious that Ag-NPs or Ag-ions need transportation by any fluid on the coated surface (wet conditions) in order to show its antimicrobial effect (Deshmukh et al. 2019). A polyethylene (PE) doped with AgNO<sub>3</sub> showed 8 log<sub>10</sub> steps reduction for *S. aureus* and more than 5 log<sub>10</sub> steps for different bacteria on Ag-SiO<sub>2</sub> coatings, however, both studies used wet conditions (ISO 22196) (Varghese et al. 2013; Scuri et al. 2019). In some clinical studies, a mixture of Ag-ions technology and zinc-pyrithione is applied and thus it remains unclear which biocidal substance is responsible for the



effect and to which extent (Orti-Lucas and Munoz-Miguel 2017). Field tests of silver AMC in health care settings, especially on patient-near surfaces, were not published (peer-reviewed) to the best of our knowledge.

When copper is released from AMC, CuO particles or Cu ions may enter the bacteria via passive diffusion of copper transporters (Tamayo et al. 2016; Giachino and Waldron 2020). In general, copper plays an essential role for various enzymes in prokaryotic cells by donating or accepting charges ( $\text{Cu}^+$  versus  $\text{Cu}^{2+}$ ). Copper involves the generation of ROS for bacteria-killing that more likely occurs when Cu-nanoparticles are internalized into bacterial cells (Slavin et al. 2017). An increase in intracellular ROS, mediated by copper, is mainly attributed to its ability to catalyze Fenton chemistry and the production of hydroxyl radicals from hydrogen peroxide. Also, intermediate sulphur radical chemistry may contribute to ROS production (Mitra et al. 2020). Intracellular ROS damage various biomolecules including DNA. Copper ions may also deplete sulfhydryl groups like in cysteines or glutathione (Grass et al. 2011). In addition, copper may affect the peptidoglycan maturation and displace other metals like iron from its protein bindings sites (Giachino and Waldron 2020). When titanium discs were coated with amorphous hydrocarbon film containing copper nanoparticles, the experiments yielded up to 4  $\log_{10}$  steps reduction of *E. coli* and *S. aureus* after 24 h incubation (Thukkaram et al. 2021). Regardless of the respective mechanism, it was observed that continuous copper ion release is essential for the efficacy of copper surfaces and cleaning protocols must be able to remove any substances on coated surfaces that may chelate released ions (Warnes et al. 2012).

Also, the release of QACs from AMC was reported when bound electrostatically to a surface, included in paints or degradable polymers, or released as QACs-containing nanoparticles (Rosenberg et al. 2019). ZnO shows antimicrobial efficacy against various Gram-positive bacteria. The antimicrobial mechanisms of ZnO nanoparticles in bacteria seem to be complex (Seil and Webster 2012). As for other metal nanoparticles, the production of reactive oxygen species and the disruption of cell membranes caused by ZnO nanoparticles may cause the bactericidal effect. An antimicrobial textile surface containing ZnO nanoparticles showed about 7  $\log_{10}$  step reduction for *E. coli* and *S. aureus* under wet conditions (Pintaric et al. 2020). Other biocidal substances can be released from AMC like zinc-pyrithione, iodocarb, bronopol, isothiazolinone, and diuron, which have a potential risk concerning humans and the

environment (see Section 7) (Fay et al. 2007; Wu et al. 2011; Popelka et al. 2015; Pittol et al. 2017; Vallieres et al. 2021). AMC with these biocidal substances are meanwhile already on the coating market. Field tests of QACs or ZnO AMC in health care settings, especially on patient-near surfaces, were not published (peer-reviewed) to the best of our knowledge.

As a critical remark for the release of biocidal substances, its transportation requires transportation media (wet surface conditions) and efficacy should be hampered on surfaces, which are usually dry under real-life conditions (Eichner et al. 2020).

#### 4.1.4. Photocatalytic action

Besides the chemical generation of ROS (e.g. Cu ions), the absorption of electromagnetic radiation in special molecules (photo-catalyst) can also generate ROS. The major photo-catalytically active substance in AMC is titanium dioxide ( $\text{TiO}_2$ ) that can be applied as a self-disinfecting material on surfaces (Nakano et al. 2013). However, the absorption of electromagnetic radiation in titanium dioxide is a rather complex issue (Baldini et al. 2017). It absorbs UV radiation from about 400 to 250 nm and reacts with water and oxygen on its surface to generate mainly hydroxyl and superoxide radicals (Nakano et al. 2013; Liao et al. 2020). Due to the very short lifetime of oxygen radicals and, hence, their short range of oxidative damage, it is very important that MoV and  $\text{TiO}_2$  photoactivated surfaces are in close contact (Jalvo et al. 2017). The photocatalytic process with  $\text{TiO}_2$  coatings should be fostered by the presence of a sufficient extent of water (wet surface). This might explain the differences in antibacterial effects measured *in vitro* (wet) and under real-life conditions (dry). For example, *in vitro* experiments under wet conditions with  $\text{TiO}_2$  surfaces yielded an up to 5  $\log_{10}$  steps reduction of different bacteria and viruses exposure to UVA radiation (Nakano et al. 2013).

In contrast to outdoor, the amount of UV radiation indoors is reduced because usual light sources indoors emit almost no UV radiation. The UV source indoor should be only the part of solar radiation that enters a room through window glass (Mohelníková and Altan 2009). Such glass partially blocks the UV-A (320–400 nm) and completely blocks UV-B (280–320 nm) and shorter wavelengths (UV-C). However, activation of photocatalytic coatings with substances like pure titanium dioxide needs ultraviolet radiation, a clear limitation when used indoor (Nakano et al. 2013). Any laboratory tests with titanium dioxide AMC should be performed under realistic UV conditions, which are prevailing indoor. Furthermore, the

application of ultraviolet radiation in combination with TiO<sub>2</sub> needs proper controls in order to differentiate between cell death induced by radiation and cell death induced by the AMC itself.

To overcome that limitation with UV radiation, titanium dioxide can be doped with small amounts of different metals such as silver, copper, iron, or manganese. In addition, non-metals are commonly used for doping like carbon or nitrogen (Liao et al. 2020). Leyland et al showed a 4.2 log<sub>10</sub> reduction of *S. aureus* when using T5 light bulb spectrum (~380–730 nm), however, the testing was performed again under wet conditions (Leyland et al. 2016). Comparable to TiO<sub>2</sub>, ZnO can be used as a photocatalyst to produce ROS when exposed to UV radiation. Photoactive coatings of sol-gel ZnO suspensions were electro-sprayed on glass substrates to produce self-cleaning antimicrobial functionalized surfaces, which yielded a reduction of >99.5 % of *S. aureus*, again under wet conditions (Valenzuela et al. 2019).

Studies using titanium dioxide AMC in health care environments provide contradictory results showing moderate or no efficacy (de Jong et al. 2018; Reid et al. 2018).

#### 4.1.5. Photodynamic action

The use of visible light for the generation of ROS would facilitate the application of AMC indoor, in particular in health care settings. The photodynamic mechanism requires a special molecule (photosensitizer), which absorbs light in the visible part of the spectrum. The excited photosensitizer transfers the absorbed energy via its triplet state to adjacent molecules, in particular molecular oxygen leading to the generation of ROS. The generation of ROS in photodynamic therapy of tumours and other diseases has been used widely in medicine for decades (Hu et al. 2021).

Although being a member of ROS, singlet oxygen plays a specific role in AMC. Singlet oxygen is the first excited state of molecular oxygen lying 0.98 eV above the oxygen ground state (Maisch et al. 2007; Wainwright et al. 2017). In the case of AMC, photo dynamically generated singlet oxygen offers three major advantages. Firstly, the generation of singlet oxygen is feasible using visible light. Secondly, the gaseous singlet oxygen molecule can leave the AMC via diffusion and can easily reach microorganisms on the coated surface (Felgentrager et al. 2014; Eichner et al. 2020). Thirdly, the collision of singlet oxygen with air molecules limits its presence to a thin layer above the coated surface of a few millimetres only (Felgentrager et al. 2014; Wang et al. 2020). Being a gaseous

molecule, singlet oxygen needs no transport medium and therefore efficiently acts on normal dry surfaces.

Using a photodynamic AMC, a recent study in two hospitals showed a significant reduction of the microbial burden on patient-near surfaces during the whole study time (Eichner et al. 2020). This field study recurrently measured cfu/cm<sup>2</sup> on patient-near surfaces and on uncoated control surfaces during 6 months. The uncoated surface showed mean bacterial values of 6.1 ± 24.7 cfu/cm<sup>2</sup>, whereas the mean value on the photodynamic AMC was significantly lower (1.9 ± 2.8 cfu/cm<sup>2</sup>, *p* < 0.001). The latter value is below the benchmark for hygiene failures of 2.5 cfu/cm<sup>2</sup> (Eichner et al. 2020). The reduction of the standard deviation from 24.7 to 2.8 additionally indicated a significantly smaller frequency of high cfu/cm<sup>2</sup> values on the AMC surfaces.

## 5. Testing the efficacy of AMC – the different roles of laboratory and field studies

AMC technologies are usually tested under laboratory conditions using different test procedures. AMC are predominantly tested against bacteria and tests against viruses or fungi are significantly less published (Rosenberg et al. 2019).

After a first successful test in the laboratory (laboratory test), it is essential that the same AMC technology undergo a second test, now in the field of the application under real-life conditions (field test) (Ahonen et al. 2017; Adlhart et al. 2018). However, contrarily to the high number of laboratory tests, only a small number of such field studies are published (Muller et al. 2016; de Jong et al. 2018; Albarqouni et al. 2020; Eichner et al. 2020; Dauvergne and Mullie 2021). On one hand, such a field test is mandatory to prove efficacy beyond laboratory experiments, which can be considered innately artificial. On the other hand, prove of AMC efficacy under real-life conditions would enhance acceptance of such new technology in hygiene measures.

### 5.1. Test procedures in laboratories

Depending on its place of action, AMC can be applied on various surfaces like metals, glass, hard plastic materials, plastic films, paper, or cardboard (carrier material). These surfaces can be already equipped with other coatings like lacquers, varnishes, or wall paints. To perform a realistic test, the antimicrobial efficacy of AMC should be evaluated when the antimicrobial substance is coated on its carrier material (Adlhart et al. 2018).

AMC and its carrier material may appear as solid or soft samples being porous or non-porous (Redfern et al.

**Table 4.** Potential test procedures for AMC.

Test norm	Test object	Exposure time	Test conditions	Weakness
ISO 20645	For textiles, leaching antimicrobial agents (agar diffusion plate test)	24 h	Dipped into agar	<ul style="list-style-type: none"> <li>• The extent of inhibition zone is no quantitative evaluation</li> <li>• For leaching agents only</li> </ul>
ASTM E 2149	For different types of treated substrates with immobilised antimicrobial agents	24 h	Immersed and shaken	<ul style="list-style-type: none"> <li>• Test criteria show a wide scope</li> <li>• Tested at low concentrations of bacteria</li> <li>• Low sensitivity for non-leaching agents</li> </ul>
ASTM E 2180	For polymeric or hydrophobic materials with incorporated antimicrobial agents	24 h	Dipped into agar	<ul style="list-style-type: none"> <li>• Test criteria show a wide scope</li> <li>• Agar dipping is an unrealistic condition</li> </ul>
AATCC TM 100	For textiles with antibacterial finishes	24 h	Continuously wet	<ul style="list-style-type: none"> <li>• Difficult for hydrophobic surfaces</li> <li>• Vague and biased success criteria</li> </ul>
ISO 20743	For textile products	24 h	Continuously wet	<ul style="list-style-type: none"> <li>• Difficult for hydrophobic surfaces</li> <li>• Sponsor determines the criteria for antimicrobial success</li> <li>• Not accepted by US-EPA for so-called health claims</li> </ul>
ISO 22196	For plastics and other non-porous surfaces	24 h	Continuously wet	<ul style="list-style-type: none"> <li>• Difficult for hydrophobic surfaces</li> <li>• Low sensitivity</li> <li>• May show false positive results for some agents</li> </ul>

2018). Thus, out of the different test procedures available, the appropriate one must be selected. Once the test procedure is selected, one should bear in mind that all these procedures have limitations, and the results of the testing should be carefully interpreted (Microchemlab 2021) (Table 4).

A first and simple test is a suspension test, in which the antimicrobial substance or AMC is added to a suspension containing an aqueous medium, and bacteria. When using a control suspension without adding the antimicrobial substance, such an experiment shows the possible reduction of viability of bacteria. Norms provided by the International Organisation for Standardisation (ISO) ISO 20743 or by the American Association of Textile Chemists and Colourists (AATCC) AATCC TM 100 quantify the antibacterial activity of textile products or antibacterial finishes on textile materials, respectively (Table 4) (ISO 20743 2013; AATCC 2019).

A piece of the AMC can be applied on an agar plate in the so-called agar disc-diffusion testing (e.g. DIN EN ISO 20645) (Bulman et al. 2017). In case the biocidal substance leaves the AMC, it inactivates the test microorganisms in the direct surrounding of the AMC leading to a zone of growth inhibition. This zone semi-quantitatively shows the extent of antimicrobial activity depending on the concentration of the biocidal substance and its diffusion properties. Clinical and Laboratory Standards Institute (CLSI) published many accepted and approved test standards (Balouiri et al. 2016).

ASTM E 2149 test can be applied when the antimicrobial agents are chemically bonded to the surface (ASTM International 2020). This test method

must ensure sufficient adsorption of bacteria and the treated fibre, fabric, or other substrates. The test specimen is then constantly shaken in the respective suspension. The corresponding antiviral test is ASTM E 1053 (ASTM International 2021).

In the case of hard, non-porous coatings, many researchers used a test procedure that is rather similar to ISO 22196 test (22196 2020). That norm specifies a method of evaluating the antibacterial activity of antibacterial-treated plastics and other non-porous surfaces of products including intermediate products. The norm dictates that the inoculated control and antimicrobial test surfaces are covered by a thin foil to keep the bacteria wet for the time span of testing (24 h) using a temperature of 37 °C (Microchemlab 2021). ISO 22196 can be modified to be used as an antiviral test or the comparable test ISO 21702 is applied against different enveloped or non-enveloped viruses (International Organization for Standardization 2021).

In light of the increasing number of AMC technologies and applications, a more realistic procedure should be developed to prove the efficacy of AMC and to address all the weakness issues as listed in Table 4 at least. The AMC technology should be applied to adequately sized carrier materials. One group of the carriers should be coated with the antimicrobial substance included (active coating). Another group of carriers should be coated with the same material but without the antimicrobial agent (control coating). The parallel test of both types of carriers allows the quantification of the efficacy of the respective AMC technology.

To quantify the efficacy, the most common procedure is the plating of microorganisms on appropriate agar plates and counting the colony-forming units (cfu)

(Warnes et al. 2012; Varghese et al. 2013; Eichner et al. 2020), whereas a future application might be growth curves of bacteria allowing a high throughput of samples in solution (Eckl et al. 2020) or even of biomaterials itself in the form of so-called proliferation assays (Bechert et al. 2000). For the two mentioned unconventional methods, a bacterial culture with known cell density is applied to an AMC surface and a reference surface. After the appropriate exposure time, the bacteria can either be recovered or subjected to growth curves or the whole sample carrier can be used for proliferation assays. The efficacy of the AMC can then be calculated as logarithmic reduction. Flow cytometry is another method applicable in the laboratory for a known bacterial composition on the test subject (Bankier et al. 2018). Prior to the flow cytometry itself, standardized commercial live dead staining protocols should be applied in order to differentiate living and dead microorganisms.

The microbial load can be also quantified by using DNA extraction and qPCR (Bankier et al. 2018). This method requires differentiation between live and dead organisms that can be achieved by using propidium monoazide assay (Nocker et al. 2006). By choosing appropriate primers for the qPCR reaction, the number of surviving organisms can be quantified. However, the investigated gene sequence should be chosen with care, as for example, the 16S rRNA gene might have multiple copies in the genome, therefore possibly causing bias and errors in the quantification (Hong et al. 2009; Kembel et al. 2012). A further possibility to quantify a bacterial population was recently developed showing that RNA-based qPCR methods might prove to be a powerful tool in the future (Magalhaes et al. 2019).

Being no live organism, AMC testing against viruses is a more complex procedure. The number of infectious virus particles is determined indirectly by infecting eukaryotic cells with the respective virus, before and after exposure of the virus to the AMC. Values like the 50% tissue-culture infectious dose [TCID<sub>50</sub>] per millilitre allow the quantification of the AMC efficacy (van Doremalen et al. 2020).

Each testing of AMC should include different controls. Firstly, the initial MoV concentration per millilitre is quantified without getting into contact with AMC, which solely reveals the amount of MoV in the current testing. Secondly, the number of MoV deposited as inoculum on the control coating (initial value) is determined, whereas that inoculum is kept on the control coating for exactly the same time as for inoculum on the AMC. The initial value serves as a reference to

calculate the reduction of viable or infectious MoV as log<sub>10</sub> steps of reduction.

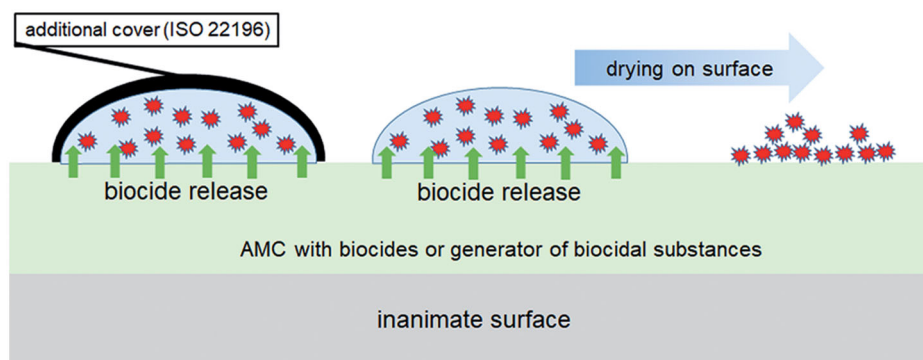
## 5.2. Dry or wet AMC surface

Surfaces may be assigned to two categories: critical and non-critical surfaces (Dancer 2014). In health care settings, noncritical surfaces are sites with non-frequent touch such as floors, walls, and furniture (Dancer 2014). Critical surfaces are surfaces, which are frequently touched by patients and/or staff especially patient-near surfaces as well as clinical equipment like electrocardiogram machines, blood pressure cuffs, or stethoscopes (Hong et al. 2009). Outside health care settings, inanimate surfaces may play a role for MoV transmission like frequently touched surfaces in any public areas (Vriesekoop et al. 2010; Sirsat et al. 2013; Patel et al. 2018; Carrascosa et al. 2019).

These inanimate surfaces are usually at equilibrium with the temperature and the humidity of the surrounding air being usually dry under normal humidity conditions. Therefore, a test procedure that is performed under permanent wet conditions, where AMC is immersed in a solution with MoV, AMC is dipped into an Agar plate, or AMC is inoculated with MoV suspension (e.g. ISO 22196) for the entire test time cannot reflect reality (Figure 3).

Unfortunately, many studies are performed under wet conditions yet (Dunnill et al. 2011; Varghese et al. 2013; Ebrahimi et al. 2019; Scuri et al. 2019). One reason is easier handling of MoV on wet surfaces as compared to dry ones, for example, keeping control MoV intact during the test procedure. Another reason is the fact that most biocidal substances like metal ions necessitate liquids for their transportation to MoV, especially when organisms are stacked on the surface. For example, a silver doped surface exhibited >5 log<sub>10</sub> reduction in MRSA viability under wet conditions after 24 h. At normal ambient conditions with a relative humidity of 22% (dry conditions) on the surface, no bacterial reduction was detected (Michels et al. 2009). Thus, MoV should dry on AMCs during testing, as it occurs when contaminated hands or items get into contact with inanimate surfaces under normal conditions (Santo et al. 2008; Quaranta et al. 2011; Warnes et al. 2012; Eichner et al. 2020).

The drying process depends on the respective surface material and the MoV applied. The drying may require seconds or minutes without being a standardized process with a clear endpoint (Quaranta et al. 2011; Bleichert et al. 2014; Eichner et al. 2020). Any new test norm should provide either a drying time or a method that delivers such an endpoint of drying. In



**Figure 3.** Frequently, the MoV are kept wet during the entire time of testing. For ISO 22196, the inoculum is additionally covered with a foil to avoid drying (left). As long as the inoculum is wet, biocidal substances can enter the liquid and reach the MoV via diffusion (middle). In contrast, inanimate surfaces are rather dry under normal humidity conditions and any contamination should rapidly dry possibly embedded in any soiling material like fatty acids from skin (right).

**Table 5.** Attachment of MoV to surfaces.

MoV	Contact mechanism	Specific comments
Bacteria	Flagella	Hydrophobic nature, comparable to most of the surfaces (Van Houdt and Michiels 2005; Bruzaud et al. 2015)
	Pili	Type I pili, Curli pili, and type IV pili in <i>E. coli</i> (Pratt and Kolter 1998; Niba et al. 2008; Xicohtencatl-Cortes et al. 2009)
	Membrane vesicles	Cup pili or Tad pili in <i>P. aeruginosa</i> (Ruer et al. 2007; Hug et al. 2017)
	Whole cell body	for example, <i>Pseudomonas putida</i> , within 10 min in the presence of toxic concentrations of long-chain alcohols or under osmotic stress (Krasowska and Sigler 2014)
Fungi	Ligands hydrophobic cell surface	Van der Waals force, electrostatic interactions (Jucker et al. 1996)
Viruses	Electrostatic interactions	Gram-negative bacteria O-specific antigen of the lipopolysaccharide (Jucker et al. 1997)
		<i>P. aeruginosa</i> : flat orientation on the surface producing polysaccharides (Cooley et al. 2013)
		<i>C. albicans</i> , adherence to plastic temperature dependent (Blanco et al. 1997)
		Differences for enveloped and non-enveloped viruses (Vasickova et al. 2010; Armanious et al. 2016)

case of a long drying time, a large inoculum droplet may mimic again wet conditions leading to a potential release of biocidal substances out of the coating. This could be avoided by applying the inoculum as many tiny droplets or by spreading the inoculum as thin liquid film.

When MoV contaminate inanimate surfaces, also soil may be present at the same time potentially shielding the MoV from AMC action. Besides the presence of dust and dirt, touching inanimate surfaces with hands should mainly leave human skin constituents behind like fatty acids, amino acids, or corneocytes (Croxtton et al. 2010; Girod and Weyermann 2014). Thus, the typical use of serum albumin and erythrocytes as soiling in test norms (Table 4) is not recommended. Also any constituents of cell processing procedures, for example, cell culture medium, should be not present on the AMC while testing because this is also unnatural soiling. In conclusion, it is important to develop a procedure that sufficiently reflects the contamination of real inanimate surfaces with MoV.

In addition, the attachments processes described below and hence changes of microbial cells should not be the same when using dry or wet surface conditions. This again increases the artificial character of wet surface conditions.

### 5.3. Attachment of MoV to inanimate surfaces

It is an important issue to understand the attachment of MoV to inanimate surfaces (Cheng et al. 2019). To quantify AMC efficacy in laboratory experiments, the reverse process is necessary by which MoV should be quantitatively detached from AMC surface yielding a sufficiently high recovery. Detachment is also important for field studies outside laboratories (Eichner et al. 2020). To gain realistic data, MoV should be detached from environmental surfaces as completely as possible and regardless of the kind of surfaces and nature of MoV. The contact of MoV to inanimate surfaces is a rather complex process that depends on the different MoV and the different surface features

**Table 6.** Selection of laboratory studies on AMC.

AMC technology	MoV	Reduction efficacy	Test condition	Investigations concerning resistance development	Reference
Copper alloys (60–99.9% Cu)	<i>S. enterica</i>	1–7 log <sub>10</sub>	Dry + wet	Resistant strains (pco) showed prolonged survival, especially under wet conditions	Zhu et al. (2012)
Copper alloys (70–99.9% Cu)	<i>E. coli</i> , <i>E. faecium</i>	Up to 7 log <sub>10</sub>	Wet	Copper resistant strains showed partly prolonged survival on copper alloys compared to non-resistant counterparts	Elgundi et al. (2011)
Copper alloys (70–99.9% Cu)	<i>P. aeruginosa</i>	Up to 6 log <sub>10</sub>	Dry	The presence of cinR, cinA and cinQ genes lead to prolonged survival on tested surfaces	Elgundi et al. (2009)
Copper alloy (99.9% Cu)	<i>E. coli</i> , <i>S. aureus</i>	~2 log <sub>10</sub>	Dry	Artificial microevolution (strains were called mCu60) can lead to tolerance to copper surfaces, mutant strains were genetically similar to parent strains, resistance might be mediated by alterations in expression levels	Bleichert et al. (2021)
Copper alloy (99.9% Cu)	23 strains of various bacteria	Up to 6 log <sub>10</sub>	Dry + wet	Several bacterial strains have at least a certain natural tolerance towards copper surfaces	Santo et al. (2010)
Copper alloys (62–99.9% Cu)	<i>E. coli</i>	Up to 6 log <sub>10</sub>	Dry	copA, cusCFBA and cueO confer a certain tolerance, pco did not influence the killing efficacy, sub-lethal Cu exposure before dry surface test led to prolonged survival of pco strains	Santo et al. (2008)
Copper alloy (99.9% Cu)	<i>E. hirae</i> and related copper mutants	Up to 7 log <sub>10</sub>	Wet	Not investigated, but authors showed different reduction for the wild type and the mutant cells	Molteni et al. (2010)
Copper alloy (50–99.9% Cu)	<i>C. albicans</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , <i>MRSA</i>	Up to 8 log <sub>10</sub>	Dry	Not investigated	Mehtar et al. (2008)
Copper alloy (70% Cu)	<i>E. coli</i> , <i>S. enterica ssp.</i> , <i>S. aureus</i>	Up to 6 log <sub>10</sub>	Dry	Not investigated, but authors assume that the technology helps to prevent transfer of antibiotic resistance due to extensive DNA degradation	Warnes et al. (2012)
Copper alloy (99.9% Cu)	different bacteria and viruses	Up to 6 log <sub>10</sub>	Dry	Not investigated, but resistance development is assumed unlikely due to an assumed complete cell killing	Bleichert et al. (2014)
Copper electrolytic purity	<i>S. enterica</i> , <i>C. jejuni</i>	Up to 6 log <sub>10</sub>	Wet	Not investigated	Faundez et al. (2004)
Copper nanoparticles on polyamide membrane	<i>P. aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i>	~1 log <sub>10</sub>	Wet	Not investigated	Ben-Sasson et al. (2014)
Copper spray-based coating	<i>MRSA</i> , <i>Influenza A</i>	Up to 5 log <sub>10</sub>	Not defined	Not investigated	Champagne et al. (2019)
Copper spray-based coating	<i>MRSA</i>	Up to 5 log <sub>10</sub>	Not defined	Not investigated	Ben-Sasson et al. (2014)
Silver nanoparticles with chitosan	<i>E. coli</i> , <i>S. aureus</i>	Up to 8 mm inhibition halo	Wet	Not investigated	Su et al. (2021)
Silver nanoparticles	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , <i>S. enterica</i>	Up to 8 mm inhibition halo	Disc diffusion test	Not investigated	Kumavat and Mishra (2021)
Silver nano-composite	<i>P. mirabilis</i> , <i>M. luteus</i> , <i>A. baumannii</i> , <i>P. aeruginosa</i> , <i>L. lactis</i> , <i>S. epidermidis</i> , <i>S. cerevisiae</i> , <i>L. casei</i>	Up to 31 mm inhibition halo	Well diffusion test	Not investigated, but also disc diffusion tests with kanamycin and ampicillin, no mechanistic investigations	Viorica et al. (2021)
Silver silica coating	<i>S. aureus</i> , <i>E. coli</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>MRSA</i> , <i>A. baumannii</i> , <i>K. pneumoniae</i>	>5 log <sub>10</sub>	Wet	Not investigated	Varghese et al. (2013)
Silver in polymers	<i>S. aureus</i> , <i>E. coli</i> , <i>C. albicans</i>	Up to 8 log <sub>10</sub>	Wet	Not investigated	Scuri et al. (2019)
Silver nanoparticles in paint	<i>S. aureus</i> , <i>E. coli</i>	“Killing of almost all bacteria”	Wet	Not investigated	Kumar et al. (2008)
Silver sol-gel coating		~2 log <sub>10</sub>	Wet	Not investigated	Stobie et al. (2010)

(continued)

Table 6. Continued.

AMC technology	MoV	Reduction efficacy	Test condition	Investigations concerning resistance development	Reference
Silver silicate coating	<i>S. epidermidis</i> , <i>A. baumannii</i> <i>A. niger</i> , <i>S. aureus</i> , MRSA, VRE, <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>A. baumannii</i>	2–6 log <sub>10</sub>	Wet	Not investigated	Monte-Serrano et al. (2015)
Silver zeolite coating	MRSA	<0.3 log <sub>10</sub>	Dry	Not investigated	Michels et al. (2009)
Silver nanoparticle, nitrocellulose coating	<i>P. aeruginosa</i> , <i>S. aureus</i> , <i>C. albicans</i>	Up to 100% cfu/mL	Wet	Not investigated	Kumarasinghe et al. (2021)
Silver nanoparticle, coating	<i>E. coli</i>	~0.8 log <sub>10</sub>	Wet	Not investigated	Niyonshuti et al. (2020)
Isothiazolinone coated on PU	<i>S. aureus</i> , <i>E. coli</i>	Up to 25 mm inhibition halo	Wet	Not investigated	Peng et al. (2018)
Iodocarb coated on natural mineral	<i>A. niger</i> , <i>P. citrinum</i> , <i>T. viride</i> , <i>B. theobromae</i>	Up to 8 mm inhibition halo	Wet	Not investigated	Zhang et al. (2020)
Bronopol coated on PE	<i>S. aureus</i> , <i>E. coli</i>	79–82%	Wet	Not investigated	Zhang et al. (2006)
Zinc-pyrithione in thermoplastic	<i>S. aureus</i> , <i>E. coli</i>	up to 4 log <sub>10</sub>	Wet	Not investigated	Pittol et al. (2017)
TiO <sub>2</sub> -ZnO nano-composite	<i>S. aureus</i> , MRSA, <i>E. coli</i> , <i>K. pneumoniae</i>	Up to 13 mm inhibition halo	Disc diffusion test	Not investigated, nano-composites are effective against MDR bacteria	Harun et al. (2020)
ZnO and silver nanoparticles	<i>E. coli</i> , <i>S. aureus</i> , <i>C. albicans</i>	0–3 log <sub>10</sub> steps	Wet	Not investigated	Rosenberg et al. (2020)
TiO <sub>2</sub> coating	different bacteria and viruses	Up to 5 log <sub>10</sub>	Wet	Not investigated	Nakano et al. (2013)
TiO <sub>2</sub> doped with F, Cu	<i>S. aureus</i>	3.5 log <sub>10</sub>	Wet	Not investigated	Leyland et al. (2016)
TiO <sub>2</sub> carbon solid coating	<i>E. coli</i>	4.2 log <sub>10</sub>	Wet	Not investigated	Krumdieck et al. (2019)
QAC surface linked	<i>S. aureus</i> , <i>E. coli</i>	Up to 1 log <sub>10</sub>	Wet	Not Investigated	Ganewatta et al. (2015)
QAC surface linked	<i>S. aureus</i> , <i>E. coli</i>	~1.5 log <sub>10</sub>	Dry	Not Investigated	Gao et al. (2016)
QAC linked with PVC	<i>P. aeruginosa</i> , <i>B. cereus</i> , <i>E. coli</i> , <i>A. acidoterrestris</i>	~2 log <sub>10</sub>	Wet	Not investigated	Poverenov et al. (2013)
QAC immobilised on silicone rubber	<i>S. aureus</i> , <i>E. coli</i> , <i>S. epidermidis</i> , <i>P. aeruginosa</i>	Up to 3 log <sub>10</sub>	Wet	Not investigated	Gottenbos et al. (2002)
QAC covalent bound to plastic	<i>E. coli</i> , <i>S. enterica</i> , <i>B. subtilis</i>	Up to 6.6 log <sub>10</sub>	Wet	Not investigated	Fadida et al. (2014)
QAC immobilised on stainless steel	<i>E. coli</i>	Up to 3 log <sub>10</sub>	Wet	Not investigated, QAC resistance discussed only	Khaskin et al. (2015)
QAC added to copolymer	<i>E. coli</i> , <i>S. epidermidis</i>	Up to 5 log <sub>10</sub>	Wet	Not investigated, authors investigated leaching in order to prevent faster development of antibiotic co-resistance	Zhao et al. (2016)
ROS photodynamic coating with methylene blue	<i>S. aureus</i>	Up to 2 log <sub>10</sub>	Dry	Not investigated	Yao et al. (2019)
ROS photodynamic coating with porphyrin or phenothiazines	MRSA	Up to 3.6 log <sub>10</sub>	Dry	Not investigated	McCoy et al. (2014)
Singlet oxygen photodynamic coating with phenalenon	<i>S. aureus</i> , <i>E. faecalis</i> , <i>S. epidermidis</i> , <i>P. aeruginosa</i>	Up to 4 log <sub>10</sub>	Dry	Not investigated, the biocidal substance singlet oxygen is considered to provoke no resistance (Wainwright et al. 2017)	Eichner et al. (2020)

(Table 5). The MoV accordingly changes the habitat coming from, for example, a liquid inoculum *in vitro* or from skin surface during a touch under real-life conditions (Krasowska and Sigler 2014). Thus, MoV can be accompanied by constituents of cell culture media *in vitro* or by any soiling (e.g. fatty acids from skin) under real-life conditions.

### 5.3.1. Bacteria

As compared to solutions, solid surfaces offer an increased ionic strength, different nutrients, altered pH, and different osmolarity (Goodman and Marshall 1995; Berne et al. 2018). For instance, most Enterobacteriaceae can sense these changes mostly via CpxAR or EnvZ/OmpR, responsible for the expression of

adherence-related genes (Hall and Silhavy 1981; Danese and Silhavy 1998; Sato et al. 2000; Francez-Charlot et al. 2005; Clarke and Voigt 2011).

In the case of a hydrophobic surface, cell appendages of bacteria will adhere to the surface. That adherence process could be assisted by hydrophobic flagella. After an attachment via pili or flagella, or even both like for *P. aeruginosa*, the whole cell body may adhere to the surface and the attachment becomes gradually stronger within seconds or minutes (Tran et al. 2011; Berne et al. 2018). To assist that process, bacteria may change the conformation of proteins and may remove the obstructive water between the cell surface and the inanimate surface. For instance, *S. aureus* changes the shape and thickness of the cell wall during attachment (Chen et al. 2014; Gu et al. 2017). Initial attachment mechanisms occur within minutes while regulation on the genetic level might take significantly longer. Cellular activity is not always a prerequisite for sufficient attachment of a small body to a surface that was shown for passive adhesion of polystyrene particles to glass surfaces (Meinders and Busscher 1993).

Production of adhesins may even increase the attachment and this process surprisingly needs a few minutes only, however being different for different bacterial species (Kimkes and Heinemann 2020). Such described cell-surface interactions occur during the drying process of bacteria on AMC test samples in a laboratory and likewise in reality.

### 5.3.2. Attachment of fungi

In contrast to bacteria, the attachment of fungi or viruses on inanimate surfaces is less explored. In the case of fungal adherence, also hydrophobicity plays a pivotal role. In addition, adherence of fungi to inanimate surfaces is mediated by components of the extracellular matrix (Colling et al. 2005). Among the microorganisms, *C. albicans* is a major human pathogen. *Candida albicans* cells may alter the features of the cell wall to attach efficiently to surfaces with different physical or chemical properties (Varghese et al. 2013). This strain can switch its surface feature from hydrophobic to hydrophilic nature, depending on the prevailing temperature (Blanco et al. 1997). Also, agglutinin-like sequences could be involved in the attachment to abiotic surfaces (Hoyer and Cota 2016). This should have an impact when testing the antifungal activity of AMC with test protocols like ISO 22196 that requires wet conditions at 37 °C (see Table 4).

### 5.3.3. Attachment of virus

In contrast to living organisms like bacteria or fungi, viruses may not actively control the attachment process. The major mechanism of virus adherence to surfaces is based on electrostatic forces, especially when the hydrophobic nature of inanimate surfaces encounters similar virus surface conditions (Vasickova et al. 2010; Armanious et al. 2016). Contributions to these electrostatic interactions may originate from ionizable special amino acids but also from negative charges inside the capsid (Nguyen et al. 2011; Dika et al. 2015). Some viruses like bacteriophages have negatively charged surfaces showing different extents of polarity (Armanious et al. 2016). The persistence of dried but intact viruses on surfaces depends on different physical and chemical factors like temperature, moisture, pH value, and type of surface (Firquet et al. 2015). All these factors may play a role when testing AMC activity against viruses, in particular when comparing dry and wet conditions.

### 5.4. Recovery

As explained above, MoV may firmly attach to dry, inanimate surfaces, and the strength of attachment depends on the type of MoV and the humidity of the surface. Thus, the detachment of MoV and hence the recovery should be determined prior to any other measurement by the respective standard methods to guarantee a correct quantification of their inactivation by AMC action. The detachment of MoV from inanimate surfaces is performed by using various technologies including different swabs, extraction media, vortexing, sonification, and the assistance of small-sized materials (Rawlinson et al. 2019). For example, the recovery of *E. coli* from polyester-rayon ranged from 40.5% to 60.6% and for *B. cereus* from 65.9% to 81.3% for different extraction solvents (Downey et al. 2012).

In case MoV are not quantitatively detached from AMC surface after testing, the subsequent quantification would count the MoV, which still adhere to the surface, as inactivated MoV leading to false-positive results. Thus, the quantification of recovery of MoV from inanimate surfaces plays a very important role in the quantification of AMC efficacy.

The drying of MoV to surfaces may lead to a “dry biofilm.” This expression describes the condition of MoV like living microorganisms, which firmly attach to surfaces and their recovery requires a substantially higher effort. In that case, usual contact plates might be not appropriate for a sufficiently high recovery (Vickery et al. 2012; Ledwoch et al. 2021).



### 5.5. Neutralization of biocidal action and exposure time

Once biocidal substances are taken up by or permanently attached to MoV (e.g. metal ions, QACs), the biocidal action may not stop after removal of MoV from the AMC surface. Some AMC releases certain amounts of the biocidal substance, which likely can be found in the solvent that is used to recover MoV. Such biocidal substances are rather carried together with MoV to the next procedure steps in the test and continue to act during the following procedure of bacterial cultivation or measuring virus infectivity for many hours. According to international standards, the testing must include neutralization of biocidal agents to prevent a test bias (EN 13727 2015; EN 14476 2019).

In case, neutralization of biocidal action is not possible, the exposure time to the biocidal substance is longer than the time MoV is placed on AMC. Consequently, one must consider that the exposure time of MoV to the biocidal action of such an AMC is then definitely longer than documented in the test protocol, in particular when comparing to other AMC technologies.

### 5.6. Review of laboratory studies that tested antimicrobial efficacy

When reviewing the literature, numerous studies in laboratories were performed to investigate AMC on solid, porous, or non-porous surfaces. The studies used coated material ranging from small nanoparticles to large, solid surfaces. The antimicrobial technologies were mainly based on metal ions and more recently on QACs (Rosenberg et al. 2019). In light of the high number of studies published, only a selection of such studies is presented in Table 6 including the biocidal material used, the reduction achieved, the test conditions applied (dry or wet surface), and whether resistance development was investigated. The present selection neglected the use of coatings designated for medical implants.

The microbial reduction on coated surfaces, as shown in Table 6, ranged from about 1 to 8 log<sub>10</sub> steps, whereat these diverse results may have several reasons. Among others, the AMC efficacy should depend on the different organisms, the respective test, and the quantification method. Methods such as image-based live-dead staining evaluation can hardly achieve experimental accuracy compared to culture-based methods that include dilution series as for example propidium iodide staining may have limitations and bias (Shi et al. 2007; Rosenberg et al. 2019). In summary, each of the

methods discussed here has its weaknesses but also its strengths. Depending on the method used, it is important to take possible bias into account. It, therefore, requires an interplay of several methods and the necessary expertise to select the appropriate approach regarding the analysis of the antimicrobial activity of surfaces.

On the other hand, when using culture-based methods, bacteria in VBNC (viable but not cultivable) state should also be considered (Colwell et al. 1985; Oliver 2010). Lastly, the maximum obtainable values depend on the number of applied cells to the surface. Consequently, a common and standardized test procedure is highly recommended to quantify the antimicrobial efficacy of AMC against defined MoV (Wiegand et al. 2018).

In the case of copper, the results of laboratory studies reveal that the greater the copper content of a copper AMC, the better is the reported antimicrobial efficacy (Dauvergne and Mullie 2021). Tests were performed on wet surfaces but also on dry surfaces claiming the contact killing of copper surfaces (Table 6). It should be mentioned that in case of dry conditions, the copper surfaces (e.g. different alloys) were thoroughly cleaned before bacteria were applied on the AMC to perform antimicrobial testing. Such a perfectly clean surface condition is difficult to maintain under real-life conditions, for example, in health care settings. It was already stated that some factors could potentially inhibit the action of copper: antioxidants, organic soil, or the repeated use of cleaning products (Chyderiotis et al. 2018). The efficacy of silver or QACs containing AMC is mainly tested under wet conditions, which is rather unrealistic for surfaces under real-life conditions. In the case of a dry surface condition, silver failed to inactivate bacteria and QACs showed a rather low efficacy (Table 6) (Michels et al. 2009; Gao et al. 2016).

It becomes obvious that most of the laboratory studies did not investigate any resistance development, which might be provoked by repeated exposure of MoV to different biocidal substances used in AMC. To the best of our knowledge, the only true resistance study was published by Bleichert et al., who investigated the resistance development of bacteria on antimicrobial surfaces (Bleichert et al. 2021). The authors used artificial laboratory evolution to produce mutant strains of *E. coli* and MRSA, which were able to survive on antimicrobial metallic copper surfaces being 12- and 60-fold less susceptible to the copper-mediated contact killing process than their respective parent strains.

Besides the mentioned study, the literature, in general, lacks investigations on the true resistance

development of a bacterial culture over time. Future research could use bacterial cultures, expose them to AMC surfaces under sub-lethal conditions, re-cultivate the remaining organisms and repeat the process with a sufficient frequency. How fast such a microbial evolution can be observed in general was strikingly shown for antibiotics by Baym et al. (Baym et al. 2016) where they demonstrated visible evolutionary dynamics within the scope of 12 days, underlining the need for such investigations also for AMC.

### 5.7. Test procedures outside laboratories (field studies)

All laboratory tests of AMC are intrinsically artificial because it is impossible to mimic real-life conditions in laboratories, in particular when using wet conditions for a test procedure like ISO 22196 (2020). Thus, laboratory tests of AMC are appropriate to perform research and development but inappropriate to recommend its use under real-life conditions.

It is mandatory that laboratory tests of AMC efficacy are followed by tests on inanimate surfaces in the subsequent field of application (Dancer 2014; Muller et al. 2016; de Jong et al. 2018; Albarqouni et al. 2020; Eichner et al. 2020; Dauvergne and Mullie 2021). The quantification of AMC efficacy in field studies is rather different compared to laboratory experiments. In laboratory tests, a known number of MoV are placed on the AMC. After a defined time span, the AMC efficacy is expressed as  $\log_{10}$  reduction of viable or infectious MoV.

In contrast, the number of MoV on inanimate surfaces under real-life conditions (e.g. healthcare settings) is highly variable (Tables 1 and 2) (Eichner et al. 2020). The respective contamination on a specific surface site depends on many factors like varying contacts with hands or items, existing soiling, humidity, and particularly the survival time of different MoV species (Kramer et al. 2006; Kramer and Assadian 2014). Thus, the number of MoV may change from time to time for specific sites on different surfaces. Therefore, quantification of MoV on surfaces should yield different results concerning the moment of surface sampling.

These unpredictable numbers of MoV on surfaces require statistical procedures to quantify AMC efficacy under real-life conditions. Firstly, AMC efficacy cannot be correlated to a starting inoculum and must be always referenced to an inactive control coating on an inanimate surface. Such a control coating should carry the same coating material but without the biocidal agent. Ideally, the control coating should be located in

the same room with comparable touch frequencies to serve as an adequate reference. Both, AMC and inactive control coating should be sampled in parallel at the same time.

Contact plating and/or swabs are common sampling technologies to quantify the number of MoV on each surface on-site. In contrast to laboratory studies, evaluation of AMC efficacy yield no  $\log_{10}$  steps per mL reduction when comparing active AMC and inactive control coating (Khan et al. 2016). The sampling should be expressed as cfu or pfu per  $\text{cm}^2$  of sampled surface area. Noteworthy, the use of contact plates in field studies might underestimate the number of MoV present on surfaces, in particular in the case of firmly attached microorganisms (see also Section 5.3).

Using contact plates from different suppliers, the recovery was rather low for *S. epidermidis* (23–38%) and *S. aureus* (38–56%) (Pinto et al. 2009). Another experiment showed that swab culture with broth enrichment detected the target MDR bacteria more frequently than RODAC plates (37.5% vs 26.0%,  $p=0.06$ ) (Okamoto et al. 2018). Among many other factors, the recovery can also depend on the type of bacteria (Rawlinson et al. 2019). When comparing swab and RODAC, the sensitivity for Gram-positive bacteria was higher for RODAC (69.5%) as compared to swab (54%), but lower for Gram-negative bacteria (RODAC: 42.7%, swab: 74.2%) (Lemmen et al. 2001). A mechanical component in swab recovery (rubbing) might assist in removing Gram-negative bacteria from hard surfaces. But even moist swabbing can fail to detect bacteria from surfaces like keyboards, whereat the problem was overcome using enrichment culture (Ledwoch et al. 2021).

Due to the statistical nature of the sampling, it should be performed at regular time intervals for at least 3 months, far better 6 months (Reid et al. 2018; Eichner et al. 2020). A study protocol should contain the sampling frequency and the number of sampling sites, which should be sufficiently high, ideally based on the prospective calculations of a statistician. This guarantees an evaluation at the study end showing results of AMC efficacy with a statistically significant outcome.

Findings concerning dry biofilms may have an impact on field studies in hospitals, in which AMC are tested and it should be further investigated whether AMC enables the prevention of biofilm emergence on inanimate surfaces by the permanent antimicrobial action of the coating (Hu et al. 2015; Ledwoch et al. 2021). In addition to quantification, the analysis of the detected MoV species may provide an insight into their diversity on inanimate surfaces. When comparing such

results for AMC and uncoated surfaces, a possible influence of the respective AMC technology on the diversity should be investigated. It is known that the microbiome of humans may be influenced by the diversity of microorganisms present on environmental surfaces (Brooks et al. 2017).

Another important outcome of a field study would be the correlation of the presence of AMC and the transmission of pathogens, for example tracking the MoV by using whole-genome sequencing, which was shown for MRSA and *C. auris* (Eyre et al. 2018; Popovich et al. 2020). Another important step could be the correlation of AMC presence and the rate of nosocomial infections in health care settings. Each of both, the reduction of pathogen transmission and nosocomial infection rate would be a clear and positive vote for the application of AMC in hospitals (Dancer 2014; Albarqouni et al. 2020).

When summarising the differences between laboratory tests (including the respective norms) and the field studies, only the field study is able to provide clear evidence of whether an AMC technology reduces the burden of MoV on surfaces under real-life conditions.

## 6. Risks of antimicrobial resistance emergence

Various microorganisms already show reduced sensitivity or resistance to a variety of biocidal substances, among them many antibiotic drugs as well as disinfecting substances like chlorhexidine, triclosan, povidone-iodine, metals, and quaternary ammonium compounds (Williamson et al. 2017; Alquethamy et al. 2020). Horizontal gene transfer and *de-novo* mutation are two important pathways by which resistance may emerge (Pietsch et al. 2020). The spread of antimicrobial coatings in health care settings and beyond leads to increased, permanent exposure of the applied biocidal substances to MoV that may have the potential to contribute to the resistance emergence in MoV especially for those conditions present in health care settings. Comparably to antibiotics, antiseptic or biocidal stewardship should be recommended (Kampf 2016; Zamudio et al. 2019).

In 2019, Graves and co-authors stated that the spread of resistance to traditional antibiotics has spurred the search for new antimicrobial substances like ionic and nanoparticle metals (Graves et al. 2019). The authors also criticized the AMC testing is frequently performed by material scientists and engineers, who had little understanding of the evolutionary dynamics of populations exposed to biocidal substances (Graves et al. 2019).

Adaptation of microorganisms to its surrounding, that should include AMC surfaces, is a well-known escape mechanism. Biocidal substances can be released from coatings and enter the cells of microorganisms, potentially triggering the same mechanisms when directly applied as suspensions. Even the contact of biocidal substances to cell surfaces has the potential to provoke cellular reaction and defence as a normal evolutionary process. For instance, *S. aureus* may become resistant to membrane-damaging cationic antimicrobial molecules following exposure (Staubitz et al. 2004). Thus, special attention should be paid to the fraction of MoV, which survives the biocidal attacks on AMC and have the potential of adaptation giving way for even more resistant MoV on inanimate surfaces, in particular in health care settings. It should be additionally considered that MoV like bacteria are permanently exchanged between humans and inanimate surfaces. For instance, a genome-resolved metagenomic study compared microbial genotypes from the gastrointestinal tracts of infants and from the neonatal intensive care unit (NICU) room environment. The authors found that a component of premature infant gut colonization is the cycle of microbial exchange between the room environment and the occupant (Brooks et al. 2017). Nevertheless, a potential contribution of AMC to resistance emergence is not clearly proven yet but should be considered and checked for each biocidal technology used for AMC.

In 2021, the European Medical Association (EMA) stated that compounds like metals (e.g. copper, zinc, and silver) are also known to elicit co-selection for AMR genes and thus are attributed to play a role in the development and spread of AMR (Association EM 2021). This review emphasizes the possible antimicrobial resistance emergence induced by metals, which are mostly used in AMC (Rosenberg et al. 2019). Being present in many natural environments, such metals pose a permanent threat to microorganisms, which developed various defense mechanisms against metals.

Comparable to bacteria, the widespread fungi *C. albicans* produce biofilms to survive toxic metal concentrations (Harrison et al. 2006). *Candida tropicalis* showed significant tolerance against ions of metals like copper and zinc (Rehman and Anjum 2011). Nevertheless, bacteria are in the focus for an antimicrobial application of various metals and beyond. Bacteria are capable of performing various strategies to survive in a toxic metal environment. Bacteria use electrostatic repulsion of ions, may switch on the cellular ion efflux pumps, may form chelates, or may change the charge of metal ions (Table 7) (Nino-Martinez et al. 2019).

**Table 7.** Tolerance/resistance of microorganisms towards metals used in AMC.

Metal	Microorganism	Reference
Copper	Enterococci of several species and from human, animal, environment and food samples	Silveira et al. (2014)
Copper	Gram-positive staphylococci and micrococci	Santo et al. (2010)
Copper	<i>E. coli</i>	Balouiri et al. (2016)
Copper, silver, zinc	<i>E. coli</i> (human and chicken isolates)	Marazzato et al. (2020)
Copper	<i>Cupriavidus metallidurans</i>	Maertens et al. (2020)
Copper	<i>P. aeruginosa</i>	Hausrath et al. (2020)
Copper	<i>A. baumannii</i>	Williams et al. (2016)
Copper	MDR <i>S. enterica</i>	Branchu et al. (2019)
Silver	different <i>E. coli</i> , <i>P. aeruginosa</i>	Panáček et al. (2018)
Silver	<i>A. baumannii</i>	Deshpande and Chopade (1994)
Silver	<i>E. coli</i> , <i>E. cloacae</i> , <i>K. pneumoniae</i> , <i>P. mirabilis</i> , <i>C. freundii</i>	Hendry and Stewart (1979)
Silver	<i>E. faecalis</i>	Cui et al. (2020)
Silver	<i>Enterobacter</i> spp., <i>Klebsiella</i> spp.	Elkrewi et al. (2017)
Silver	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>Enterobacter cloacae</i> , <i>S. aureus</i>	Keđziora et al. (2020)
Silver	<i>S. aureus</i>	Valentin et al. (2020)
Silver	<i>E. coli</i>	Graves et al. (2019)
Silver	<i>K. pneumoniae</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>E. cloacae</i> , <i>P. aeruginosa</i> , <i>A. Baumannii</i>	Hosny et al. (2019)

### 6.1. Metal nanoparticles

Metal may appear as ions or may form large aggregates with various diameters in the nanometre range (nanoparticles). Such nanoparticles can physically interact with the cell wall or the cell membranes affecting cellular integrity (Nino-Martinez et al. 2019). Bacteria counteract that damage via the generation of extracellular matrix, which may embed nanoparticles (Nino-Martinez et al. 2019). Another shielding process is based on overexpressing a flagellin matrix (Nino-Martinez et al. 2019). For instance, *E. coli* and *P. aeruginosa* overexpress such a flagellin matrix, inducing an agglomeration of small silver nanoparticles and thereby avoid direct contact (Panáček et al. 2018).

The surface charge of a nanoparticle in a suspension or fixed at AMC may be important to pull bacteria to nanoparticles or AMC surface enabling the antimicrobial effect. However, some bacteria counteract that electrical force by changing their own surface charge. Such an effect was exemplarily shown for cationic *E. faecalis* (Kumariya et al. 2015). Besides changes in the charge of the cell surface, researchers described an altered membrane composition ultimately leading to a higher membrane rigidity. The authors assigned this resistance mechanism to the *mprF* gene, which was initially described in *S. aureus* (Oku et al. 2004; Staubitz et al. 2004).

### 6.2. Silver

Efflux pumps are common bacterial constituents by which bacteria get rid of toxic metals (Pal et al. 2017; Squadrone 2020). In Gram-negative bacteria, trans-envelope protein assemblies such as tripartite efflux complexes enable the bacteria to extrude different antibiotics and other toxic chemicals. CusCBA is a tripartite copper and silver ion efflux complex in *E. coli* that is

relevant for multidrug resistance to Gram-negative bacteria (Genova et al. 2019; Fu et al. 2020).

Silver is already known to provoke resistance in *E. coli*, *E. cloacae*, *K. pneumoniae*, *P. mirabilis*, and *C. freundii* taken from patients' wounds (Hendry and Stewart 1979). A clinical study showed resistances for *E. coli* and *P. aeruginosa* after repeated exposure to silver when treating burn wounds with silver sulfadiazine. For instance, 92 % of isolated Enterobacteriaceae showed resistance to sulphonamides and 42 % to silver (Panáček et al. 2018). Another study investigated the resistance to silver in 444 clinical isolates of bacteria using a silver challenge test. The authors showed that 76 % of *Enterobacter* spp. and 58 % of *Klebsiella* spp. showed resistance to silver ions (Elkrewi et al. 2017). Even the worldwide major pathogen *Staphylococcus aureus* shows mutations, which indicate protection against silver and its nanoparticles (WHO 2017; Valentin et al. 2020). Increased silver resistance was also found in *Cupriavidus metallidurans* in which the AgrRS system was upregulated (Ali et al. 2020).

One of the striking examples of silver-mediated bacteria resistance is the international space station (ISS). Onboard, the drinking water is reprocessed using silver. A recent study found isolates of *Cupriavidus metallidurans* and *Ralstonia pickettii* in water samples from ISS (Mijnendonckx et al. 2013), of which several isolates were capable to form biofilms and were resistant to several antibiotics. Especially representatives from the genus *Ralstonia* may cause severe disease in patients with pre-existing conditions (Ryan and Adley 2014).

### 6.3. Copper

As shown for *Pseudomonas aeruginosa*, the protein CopG may assist to change the copper state that

enables the Cus RND transporter efflux system to remove toxic copper (Hausrath et al. 2020). *Salmonella enterica* has dramatically increased in prevalence worldwide causing many diseases in humans with clones being resistant against metals like copper and silver (Clark et al. 2020). That resistance may also constrain macrophages in bacteria-killing (Clark et al. 2020). Silveira et al. showed a correlation of copper tolerance and selection/maintenance of multidrug-resistant Enterococci, which is related to an environmental use of copper products (Silveira et al. 2014). The authors described the distribution of the multi-copper-oxidase cueO and the co-transfer of ampicillin resistance along with copper tolerance genes (Silveira et al. 2014). Recently, a novel community-acquired MRSA strain was identified in Japan showing a copper and mercury resistance mobile element (COMER) (Takadama et al. 2020). In addition, copper is able to destroy bacterial cells and the genomic DNA at the same time that would reduce the risk of AMR gene transfer (Warnes and Keevil 2016).

Also, copper AMC may provoke resistance mechanisms as shown by Santo et al for bacteria isolated from copper alloy coins (Santo et al. 2008; 2010). The most resistant of 294 isolates were Gram-positive staphylococci and micrococci (Santo et al. 2010). Maertens et al found that long-term bacterial survival on copper surfaces is possible upon induction of metal resistance mechanisms (Maertens et al. 2020). Another study showed that the preadaptation of *E. coli* to copper enhanced its survival rates on copper AMC (Santo et al. 2008). These findings should have an impact on future applications of copper as AMC technology.

#### 6.4. Metals and antibiotic resistance

Besides tolerance and resistance, also the influence of biocidal metal on antibiotic resistance mechanisms may play an important role, in particular heavy metal tolerance of hospital pathogens (Andrade et al. 2018). Such

an interplay of metal resistance and antibiotic resistance is a concern since 1974 (233). This is also called co-selection that can be found as resistant genes to heavy metals and antimicrobial agents (Seiler and Berendonk 2012; Alquethamy et al. 2020; Bazzi et al. 2020). The underlying mechanisms are usually attributed to co-resistance, co-regulation, or cross-resistance. In co-resistance, resistance genes are linked or located in adjacency on plasmids, transposons, or integrons (Seiler and Berendonk 2012; Bazzi et al. 2020). In co-regulation, resistance to metals and antibiotics is governed by a common regulator (Pal et al. 2017; Bazzi et al. 2020). Cross-resistance mainly appears when the cells use efflux pumps, by which the bacteria can efficiently remove metals and antibiotic substances at the same time (Table 8) (Pal et al. 2017; Bazzi et al. 2020).

A pivotal experiment showed that a long-lasting exposure of different multidrug-resistant Gram-negative strains (*E. coli*, *K. pneumoniae*, *Enterobacter cloacae*) and Gram-positive bacteria strains (*S. aureus*) to silver clearly affected their sensitivity to silver and/or antibiotic substances (Kędziora et al. 2020). Therefore, it is not surprising that 150 clinical isolates from wounds contained 19 isolates (*K. pneumoniae*, *S. aureus*, *E. coli*, *E. cloacae*, *P. aeruginosa*, *A. baumannii*) with silver resistance (Hosny et al. 2019). The authors added PCR investigations and found different *sil* genes on the extracted plasmids (Hosny et al. 2019).

An important example of this interplay is shown in complex isolates of MDR *Enterobacter cloacae*, which simultaneously show antibiotic and heavy metal tolerance genes (Andrade et al. 2018). Another typical example is ST664 XDR *P. aeruginosa* strains, which were isolated from burn wounds. On one hand, the authors found 11 AMR genes, including a *blaKPC-2* gene that confers resistance to carbapenem. On the other hand, the mega-plasmid pNK546a harbours also silver resistance modules (Li et al. 2020).

Moreover, when metal ions or their nanoparticles leave the AMC, either incorporated in microorganisms

**Table 8.** Co-, Coreg-, Cross-Resistance of metals used in AMC.

Metal	Antibiotics	Microorganism	Reference
Copper	Erythromycin, vancomycin	<i>E. faecium</i>	Silveira et al. (2014)
Copper silver	Chloramphenicol, kanamycin, tetracycline	<i>S. marcescens</i>	Gilmour et al. (2004)
Silver	Carbapenem	XDR <i>P. aeruginosa</i>	Li et al. (2020)
Silver	Piperacillin-tazobactam, norfloxacin, imipenem, meropenem, ertapenem, gentamicin, ciprofloxacin, tigecycline and others	21 clinical isolates of <i>E. cloacae</i>	Andrade et al. (2018)
Silver copper	Carbapenem	<i>K. pneumoniae</i>	Chen et al. (2020)
Zinc	Erythromycin, clindamycin	<i>L. monocytogenes</i>	Mata et al. (2000)
Zinc	$\beta$ -Lactams, erythromycin, novobiocin, ofloxacin	<i>B. cepacia</i>	Hayashi et al. (2000)
Copper	Ciprofloxacin, $\beta$ -lactams	<i>C. jejuni</i>	Lin et al. (2002)
Zinc	Carbapenem	<i>P. aeruginosa</i>	Perron et al. (2004)
Silver	Ampicillin, Pen-Strep	<i>E. coli</i> , <i>S. aureus</i>	Kaweeteerawat et al. (2017)

or when abrading the coating, the metals will encounter also different bacteria in wastewater and marine water. A recent review listed numerous microorganisms in freshwater and marine ecosystems, which show metal and antibiotic resistance at the same time as copper and zinc (Squadrone 2020).

### 6.5. QAC

AMC may sequester different QACs substances or have the QACs permanently bound to the surface (Jennings et al. 2015; Zhang et al. 2015). QACs attacks MoV with unspecific and broad mechanisms like membrane damages (Alkhalifa et al. 2020). Therefore, the use of QACs was thought to entail a low risk of resistance mechanisms. However, bacteria like *P. aeruginosa* can change their cellular morphology upon small concentrations of QACs (Voumard et al. 2020). Such modifications of the cell wall properties may affect the efficacy of QACs as the biocidal substance in AMC (Fox et al. 2011).

In addition, recent investigations clearly show the existence of various bacterial genes (*qac* genes) responsible for efflux pumps (Han et al. 2019). Comparable to metals and antibiotics, such efflux pumps remove many QACs from bacteria thereby reducing antimicrobial efficacy (Han et al. 2019). It has been also shown that *qac* genes can undergo horizontal gene transfer together with antibiotic-resistant genes (Jennings et al. 2015). Interestingly, QACs may provoke the generation of ROS, which promotes the transfer of plasmids between bacteria. Another study showed that QACs may thereby facilitate the evolution and gene transfer of antibiotic resistance genes among the microbiome (Han et al. 2019).

All these results appear to be rather similar to the resistance mechanisms induced by metals, see above. A major concern is the longevity and hence the accumulation of QACs in the environment. Unfortunately, persistent exposure of microorganisms to QACs may continuously spur the resistance mechanisms, including co- and cross-resistance of antibiotics (Jennings et al. 2015; Soumet et al. 2016).

### 6.6. ROS and singlet oxygen

Upon generation of ROS like superoxide anions inside living cells, microorganisms may react with anti-oxidative defence mechanisms like the use of superoxide dismutase, catalase, radical scavengers, and protective proteins (Lemire et al. 2017). Bacteria offer a complex regulation system for an antioxidant defence network including OxyR, PerR, and SoxR, which enables a quick

response to elevated intracellular levels of ROS (Imlay 2015). Inside cells, any additional source of ROS production like the presence of metals might strengthen that defence network.

In the case of the specific member of ROS (singlet oxygen, photodynamic AMC, Table 3), this gaseous molecule is generated in the coating and approaches the cells from outside causing unspecific, oxidative damages at cell surfaces. Due to a very short range of diffusion in cellular environments, singlet oxygen hardly penetrates into cells and thereby bypasses the cellular defence network (Hatz et al. 2008). That might prevent the onset of resistance, which was investigated in experiments via a repeated sub-lethal photodynamic action against bacteria. The results showed that the photodynamic process caused no resistance mechanisms and did not affect the sensitivity to antibiotics (Lauro et al. 2002). Thus, it is generally assumed that the photodynamic approach with using singlet oxygen as a biocidal substance should not provoke resistance (Wainwright et al. 2017).

## 7. Potential risks of AMC for humans and the environment

In 2020, the EU commission stated in a communication to the European Parliament that chemicals with hazardous properties can cause harm to human health and the environment. As a consequence, the EU requires safe chemicals while preventing harm to humans and the environment by avoiding substances of concern for non-essential use (Chemicals Strategy for Sustainability Towards a Toxic-Free Environment) (European Commission 2020). For example, the antimicrobial agent tributyltin was used as antifouling additives in paints to decrease the growth of this biological community (marine bacteria, algae, mollusks). That compound is very harmful to the environment and was therefore banned in the EU in 2008 (Gipperth 2009).

The increasing trend to coat inanimate surfaces with AMC technologies may have also an impact on the environment. To date, little is known about the Ecotoxicological impact of AMC (Rosenberg et al. 2019). In most of the AMC technologies, the respective biocidal product will leave the coating due to its mode of action (leaching of metal ions, biocides, etc.) or simply due to mechanical abrasion.

Generally, silver and copper salts and the respective nanoparticles are highly toxic to aquatic organisms (Bondarenko et al. 2013). A recent investigation showed that L1 larval worms (*Caenorhabditis elegans*) were impaired in growth, fertility, and reproduction upon

exposure to silver nanoparticles (Ayeche et al. 2020). In another investigation freshly collected sandy loam soil was spiked with silver nanoparticles. Bacterial genes involved in heavy metal resistance (e.g. the CzcA efflux pump) and other toxicity response pathways were highly upregulated in the presence of silver (Meier et al. 2020). The authors stated that regulatory agencies should investigate and monitor such substances, in particular when the environment is their final destination (Meier et al. 2020). In addition, the introduction of silver nanoparticles and silver ions in natural brackish waters results in distinct bacterial community composition and structure as well as reduction of richness and diversity (Zou et al. 2020). Another study found potential ecological risks of silver as ions or nanoparticles for the spreading antibiotic resistance genes (Lu et al. 2020).

TiO<sub>2</sub> nanoparticles present in cosmetics have the potential to penetrate the skin and the extent of penetration depends on the size of the nanoparticles (Filon et al. 2015). After evaluation of the available data in 2021, the concern with respect to the genotoxicity of TiO<sub>2</sub> could not be ruled out (EFSA 2021).

Ecotoxicological factors should also concern QACs because these compounds can be tremendously toxic for aquatic organisms (Zhang et al. 2015). It is remarkable that many AMC components have already been found in the wastewater of hospitals (Orias and Perrodin 2013). Consequently, especially non-degradable biocides of AMC like metal ions or QACs may affect the work of microorganisms in wastewater treatment plants (Ochoa-Herrera et al. 2011; Zhang et al. 2018; Guo et al. 2019). Clinical data suggest that exposure to some QACs may cause multiple types of hypersensitivity reactions upon skin exposure (Shane et al. 2019).

Meanwhile, other chemical compounds were investigated concerning its use in AMC and also offered in products on the AMC market. These compounds are well-known biocides and partially exhibit concerns regarding the environment as discussed in the following.

The substance zinc-pyrithione was added to thermoplastic elastomers, which can be applied for control keyboards and telephones (Pittol et al. 2017). According to the European Chemical Agency (ECHA), Zinc-pyrithione is classified as fatal if inhaled, is toxic if swallowed, may damage the unborn child, causes damage to organs through prolonged or repeated exposure, is very toxic to aquatic life with long-lasting effects, and causes serious eye damage (ECHA 2021a).

Biocidal reagents such as bronopol can be coated on the surface of low-density polyethylene for its use in healthcare (Popelka et al. 2015). However, these

compounds are easily released due to poor bonding with the substrate yielding a short time of antibacterial effects only (Wu et al. 2011). According to ECHA classification, bronopol is very toxic to aquatic life, is harmful if swallowed, is harmful in contact with skin, causes serious eye damage, causes skin irritation, and may cause respiratory irritation (ECHA 2021b).

The textile industry and others may use different isothiazolinones for AMC (Aerts et al. 2017), as well as for coatings (Peng et al. 2018). Derivatives of isothiazolinone are widely used as preservatives or biocides in household and industrial products and show a high prevalence of contact dermatitis and allergic reactions (Herman et al. 2019). Another substance intended for antifouling is 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) was tested *in vitro*. The results show that doped polymers are able to release diuron for months (Fay et al. 2007). Diuron is very toxic to aquatic life with long-lasting effects, is harmful if swallowed, is suspected of causing cancer and may cause damage to organs through prolonged or repeated exposure (ECHA 2021c). 3-iodo-2-propynyl butylcarbamate (iodocarb) was coated on natural minerals and tested against mould and blue-stain fungi (Zhang et al. 2020) as well as biocidal agents in coatings against fungi in general (Vallieres et al. 2021). Iodocarb is toxic if inhaled, causes damage to organs through prolonged or repeated exposure, is very toxic to aquatic life with long-lasting effects, is harmful if swallowed, causes serious eye damage, and may cause an allergic skin reaction (ECHA 2021d).

A safe and positive example is the generation of singlet oxygen via photodynamic mechanisms (see Table 3). Singlet oxygen has been safely applied in medicine for many years to treat retina pathologies and tumours (Kent 2014; Hu et al. 2021). After excitation, singlet oxygen is present for a few microseconds only. It may escape the AMC to kill MoV but it will not reach the environment (Maisch et al. 2007; Felgentrager et al. 2014; Wang et al. 2020).

## 8. Conclusions

AMC technology may play an important role in hygiene measures of inanimate surfaces in the near future, especially inside health care settings and also in other environments, in which frequent and alternate hand touches of such surfaces occur.

AMC should not directly be compared with common surface disinfection technologies. AMC acts slowly but autonomously and persistently thereby reducing the mean number of MoV on the coated surface. Common

surface disinfection acts more rapid (within minutes) but only at the moment of its execution with no permanent effect. Any consecutive touch of the disinfected surface inevitably causes a re-contamination. Both technologies complement one another that may yield a higher safety level for patients and staff in health care settings.

AMC is a rather new technology that should be tested in laboratories using standardized methods that are as close as possible to real conditions, in particular using dry surfaces. Such laboratory testing is needed as a first step only towards the clinical application of AMC. It should be mandatory to perform the second step, testing outside the laboratory in the future fields of AMC application (field studies). In such field studies, the AMC must prove itself under real-life conditions when coated surfaces are cleaned and disinfected, soiled with substances of daily life, especially skin fat of hands. Field studies should be performed to investigate the effect of AMC on the incidence of nosocomial transmission and infections of MoV.

In light of a potential large-scale application of AMC, the used biocidal substances should not contribute to antibiotic or antiseptic resistance of microorganisms and should not harm the environment.

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