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Prevention of the Dramatic Increase of Multi Resistant Microorganisms. *In Situ* Generated Biocides Provided by Catalysts Show Fast and Strong Antimicrobial Activity on Surfaces

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ABSTRACT

A worldwide increase of multi-resistant microorganisms, responsible for millions of deaths per year requires immediate, innovative and ambitious action. Pathogens are distributed by the hands of the personnel. *In situ* generated biocides by catalysts (molybdenum oxide, tungsten blue oxide, Zinc molybdate and polyoxometallates) show fast antimicrobial activity against a very broad spectrum of bacterial pathogens including microorganisms embedded in a biofilm, fungi, moulds and several viral pathogens including COVID 19 on surfaces. Transition metal oxides can be incorporated into various coatings and polymers, they are water insoluble and have a documented duration of activity of at least 10 years and 10 000 cleanings. Transition metal oxides are not toxic, there is no induction of resistance. The activity and marketability have been documented by external laboratories and the BPR of the EU.

Keywords: Transition Metal Oxides; *In Situ* Generated Biocides; Non-Toxic; Permanent Activity >10 Years; Activity and Marketability Approved by the BPR of EU

Introduction

For people in the 21st century, it is hard to imagine the world before antibiotics. At the beginning of the 20th century, as many as nine women out of every 1,000 who gave birth died, 40 percent from sepsis. In some cities as many as 30 percent of children died before their first birthday. One of every nine people who developed a serious skin infection died, even from something as simple as a scrape or an insect bite. Pneumonia killed 30 percent of those who contracted it; bacterial meningitis was almost universally fatal. Ear infections caused deafness; sore throats were not infrequently followed by rheumatic fever and heart failure. Surgical procedures were associated with high morbidity and mortality due to infection [1,2]. This picture changed dramatically with three major developments:

1. Improvements in public health,
2. Vaccines, and
3. Antibiotics.

Over the course of the 20th century, deaths from infectious diseases declined markedly and contributed to a substantial increase in life expectancy. Antibiotics have saved millions of lives. But the world is now at dire risk of losing this progress. Bacteria and other microbes evolve in response to their environment and inevitably develop

mechanisms to resist being killed by antibiotics but also disinfectants. For many decades, the problem was manageable as the growth of resistance was slow and the pharmaceutical industry continued to create new antibiotics. Over the past decade, however, this brewing problem has become a crisis [3]. The evolution of antibiotic resistance is now occurring at an alarming rate and is outpacing the development of new countermeasures capable of thwarting infections in humans. The last decade has shown that the dramatic increase of antibiotic resistance which is one of the most pressing issues in the healthcare system, causing a financial burden on hospitals and societies due to the prolongation of illness and subsequent treatment. An international group of experts is trying to quantify the extent of the problem in the journal «The Lancet». It has been recently broadcast on NTV [4] 4.95 million deaths according to the recent study were linked to an antibiotic-resistant bacterial infection, even though the direct cause of death may have been different. 1.27 million people died directly from infection with a resistant bacterium - so without resistance, these deaths would have been preventable [5]. In the "Report on Antibiotic resistance" this was seen as one of the most common causes of death worldwide [6-8]. By comparison, an estimated 680,000 people died from HIV/AIDS in 2020, and 627,000 from malaria [9].

If bacteria are resistant to treatment with antibiotics, infections that are harmless in themselves can be fatal. According to the study, problems with resistance occurred particularly frequent with infections of the lower respiratory tract, such as pneumonia [10,11]. These alone have been responsible for 400,000 deaths. A particularly large number of people also died because of blood poisoning and appendicitis because the infection could not be controlled with antibiotics due to resistant pathogens. In international medical Journals 229,464 The germs that most frequently caused problems with resistance included Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Streptococcus pneumoniae. According to the study, the dreaded hospital germ MRSA - methicillin-resistant Staphylococcus aureus - alone caused 100,000 deaths. There have been previous studies on individual regions, specific pathogens, or individual antibiotics. The researchers looked at 204 countries and regions, 23 disease-causing bacteria, and 88 combinations of bacteria and antibiotics. Countries in western sub-Saharan Africa were most severely affected. There, nearly 2 440 deaths each year per 100,000 people were directly attributable to infection with a resistant pathogen per year. Children under the age of five were most at risk. In rich countries, the rate was 13 deaths per 100,000 population [12]. One signal emanating from the data: there is an urgent need for action. Antimicrobial resistance poses a growing threat to public health and the provision of health care. Affected individuals also face significant health and economic consequences. This burden costs 1.3 to 2.7 billion estimated in USD in the USA and 1.5 billion in the EU per year [13].

There is still open questions regarding the reasons for this development. John E. Walker, a Nobel prize laureate, reports on behalf of the WHO reasons on this development in 2015: [14]

1. Early discontinuation of antibiotics.
2. Absence of new antibiotics.

However, there is no evidence that these arguments have a sizeable impact on the critical development of resistant microorganisms in the ICU.

1. Unnecessary prescription of antibiotics for virus infections.
2. Administration of antibiotics in animal husbandry.

contribute more to this phenomenon. New resistant strains of superbugs are discovered at an alarming rate due to human negligence i.e. misuse or overuse of antibiotics. The inappropriate use of antibiotics for viral infections that do not respond to antibiotics must be reassessed. Viral infections of the upper respiratory tract are frequently observed in children and predispose patients for bacterial superinfections e.g. sinusitis, otitis media or bronchitis, rarely pneumonia or a bloodstream infections where the administration of antibiotics is mandatory [15,16]. Antibiotics have to be administered only once a bacterial superinfection is documented. This requires frequent clinical control of the patient. Prophylactic antibiotics are not helpful for the prevention of these bacterial superinfections; in essence they also complicate the treatment of these infections as already resistant microorganisms may have been selected. Prophylaxis of bacterial superinfections is feasible with an approach other than antibiotics. Effective alternatives are e.g., anti-inflammatory properties based on herbal extracts (thyme, gentian, primula) which open clogged paranasal sinus openings as well as the Eustachian tube and improve the mucociliary clearance [17]. These herbal extracts decrease the arachidonic acid metabolism, the precursor for the formation of proinflammatory cytokines [18].

The diagnosis and treatment of nosocomial bloodstream infections is challenging. Patients generally don't have fever. The symptoms consist of a breakdown of the peripheral circulation with requirement of increased oxygen concentration and ventilation pressure in patients on artificial ventilation, decreased urinary output. A subtle but reliable symptom is a delayed capillary recirculation which must be frequently checked on fingernails. Immediate antibiotic therapy must be instituted if a bacterial superinfection for instance a bloodstream infection with breakdown of the circulation develops as death is imminent within a few hours [19]. The choice of the appropriate antimicrobial agent can frequently not be based on the results of a microbiologicstigations. Experience of antimicrobial susceptibility of microorganisms isolated in previous patients is helpful, however carbapenem, frequently in combination with an aminoglycoside must be immediately initiated to avoid any

risk [20,21]. The antibiotic therapy can be adapted once the pathogen is identified, and the results of the antimicrobial susceptibility test are available. There is obviously a lack of understanding of the true origin of multi resistant microorganisms. Nature by law indicates that all antimicrobial active substances which have to be incorporated into the metabolism of microorganisms induce resistance. Subinhibitory concentrations of antimicrobial agents increase the probability of induction of resistance. This is known for antibiotics but also disinfectants [22,23]. In addition, disinfectants as well as antibiotics are ineffective against microorganisms in a biofilm which form on composite surfaces [24,25]. The reason: microorganisms in a biofilm are hibernating and don't take up anything from the outside; a broad resistance of microorganisms against virtually all antibiotics and most disinfectants is observed. Identification and culture of microorganisms in a biofilm requires special culture methods as these microorganisms are "VBNC" viable but not culturable [26].

The use of antibiotics in animal health seems to be an important reason for the development of resistant microorganisms. Antibiotic-resistant bacteria associated with animals may be pathogenic to humans, easily transmitted to humans via food chains, and widely disseminated in the environment via animal wastes [27]. There are however additional facets to be considered. Antibiotics are generally not found in meat except in broiler production as non-absorbable antibiotics e.g., colistin are used [28-30]. The problem is the selection of resistant microorganisms in the excretions which are usually distributed into the environment as fertilizers. Vegetables harvested on these fields can be contaminated. The majority of remaining microorganisms in vegetables is eradicated by the hydrochloric acid in the stomach. Some microorganisms escape and are found in the flora of the large intestine in low concentrations. There these microorganisms are under control of a stabile fecal flora. However, if a broad-spectrum antibiotic is administered to the patient, the sensitive flora is eliminated, and the resistant microorganisms prevail. There is also a solution for prophylactic antibiotics as growth enhancers [31]. The pathogenic property of microorganisms responsible for nosocomial infections is the formation of toxins of the pathogen but also - equally important - adherence on epithelial cells. The blockage of adherence of microorganisms on epithelial cells by receptor analogue carbohydrates i.e., acid galacturonides is an important treatment option and can prevent the use of antibiotics [32]. Animal studies demonstrate superior outcomes of this option compared to antibiotics without induction of resistance (Table 1).

Table 1: Frequency of diarrhea in weaning piglets.

	Diarrhea		No Diarrhea		Total number	
Galacturo-nides	10	14.70%	58	85.30%	68	100%
Control	31	50.00%	31	50.00%	62	100%
Antibiotic Tylosine phosphate	17	24.60%	52	75.40%	69	100%
Total Number	58	29.10%	141	70.90%	199	100%

Prevention of adnexitis and death from sepsis in a laying hen battery with acid galacturonides. Inadequate hygiene in hospitals has a proven impact on the development of resistant microorganisms (Figure 1). However strong emphasis, based on alcoholic hand hygiene, has been reported responsible for a massive increase of vancomycin resistant and alcohol insensitive enterococci [33]. Inadequate sanitation in developing countries is also of high relevance. Global distribution of resistant microorganisms is a real problem, threatening people in developed countries as well by public transportation. The UN Interagency Coordination Group on Antimicrobial Resistance demands immediate, coordinated, and ambitious measures [34]. This situation threatens patient care, economic growth, public health, agriculture, economic security, and national security. Resistance of pathogens has been referred as "Ineffective antibiotics are a slow-motion pandemic more dangerous than Covid-19». «These new data reveal the true extent of the problem of antimicrobial resistance worldwide and send a clear signal that we need to act now,» said Chris Murray of the University of Washington, according to a news release from the journal. «We must use these data to correct course and drive innovation if we are to stay ahead in the race against antibiotic resistance» [35].

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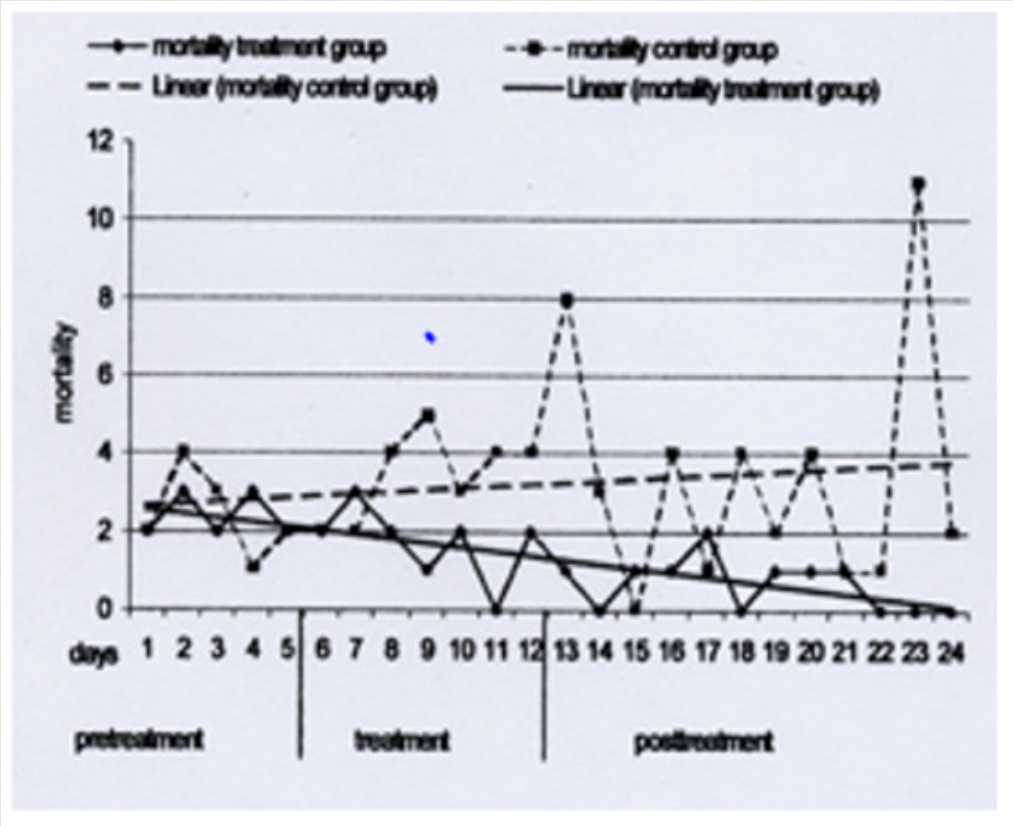

Figure 1.

The acquisition of new mutations is leading to rapidly evolving resistant bacterial strains and fungi. Interestingly, the data on the global spread of gene mcr-1, responsible for the resistance, were published in 2018. Later, the gene was found in several bacteria such as Escherichia, Enterobacter, Klebsiella, Kluyvera, Citrobacter, Salmonella and Cronobacter [36]. Colistin used to be the drug of last resort when all other antibiotics fail to treat an infection. With spreading colistin resistance, we have entered the post-antibiotic era [37]. The lack of attention of pharmacodynamic and pharmacokinetic properties of new antibiotics by regulatory authorities is a severe shortcoming. Broad spectrum antibiotics with incomplete bioavailability (Cefixim) or prodrugs with partly biliary elimination (Cefuroxime proxetil) select multi resistant microorganisms in the fecal flora just as Ceftriaxone with a 75% biliary elimination [38]. Azithromycin has an elimination half-life of 68 hours. Subinhibitory concentration in the oral cavity over 3 weeks select invariably high numbers of macrolide resistant microorganisms in contrast with Clarithromycin with a half-life of 2 hours. Macrolide insensitive microorganisms after administration of Azithromycin are also distributed to classmates in Kindergarten [39].

The most important issue however is the use of disinfectants. Disinfectants are widely used for elimination of microorganisms from a surface but proved to be a not reliable method any more due to a substantial increase of multi resistant microorganisms against disinfectants and cross resistance with antibiotics. Multi-drug resistant (MDR) bacteria have been reported as contaminating microorganisms of surfaces, commonly used medical equipment and high-contact communal surfaces (e.g., telephones, keyboard, medical charts) in a hospital. Contamination of inanimate surfaces in ICU has been identified in outbreaks and cross-transmission of pathogens among critically ill patients. Inanimate surfaces and equipment (e.g., bedrails, stethoscopes, medical charts, ultrasound machine) are frequently contaminated by bacteria, including MDR isolates. Contamination may occur either by transfer of microorganisms contaminating healthcare workers' hands or direct patient shedding of microorganisms in the immediate environment of a patient's bed [40]. It has been reported that both Gram-positive and Gram-negative bacteria are able to survive up to months on dry inanimate surfaces, with even longer persistence under humid and lower temperature conditions. Environmental contamination by fungi and

viral pathogens including coronavirus has been also described on surfaces in frequented customer areas viable for weeks [41]. Factors that may affect the transfer of microorganisms from one surface to another and cross-contamination rates are type of organisms, source and destination of surfaces, humidity level, and size of inoculum. Much emphasis is therefore based on the prevention of nosocomial infections with multi resistant microorganisms. The crucial initial step however is the investigation of the reasons for MDR development. The development of self-sanitizing surfaces with a broad spectrum of activity, fast eradication of microorganisms, long lasting to permanent antimicrobial activity without induction of resistance is the only promising solution for this problem if all requirements for the prevention of hospital acquired infections are met [42]. The requirements of self-sanitizing surfaces for the prevention of hospital acquired infections in hospitals, public transportation, the food industry is extraordinary high.

1. Intensive, fast and broad antimicrobial activity, against Gram-positive, Gram-negative microorganisms, irrespective of their antibiotic susceptibility, fungi, legionella, moulds, virus documented by the RODAC plate method.
2. Fast eradication of microorganisms i.e., minimum 5 log 10 reduction within 30 minutes.
3. Activity against a high inoculum size of 109 CFU on an area of 3 cm^2.
4. No induction of resistance.
5. Nontoxic, skin and soft tissue compatibility, no allergenicity, sbD (safe by design).
6. Long lasting/permanent antimicrobial activity water-, acid-, alkaline-, alcohol insoluble, UV Light stabile.
7. Cleanable with detergents.
8. Uncomplicated technical processability, heat stabile up to 400°C, non-corrosive.
9. Physical stability, activity irrespective of sweat, grease, blood, pus.
10. Not flammable, smoke reduction.
11. BP authorisation by the European commission on biocidal products.
12. Favourable cost/benefit analysis.

To combat multi-drug resistant (MDR) superbugs, a plethora of novel methods are under investigation, while old and momentarily forgotten strategies (nano-compounds, bacteriophages, physical factors) are being revised. There are however several shortcomings of these propagated technologies. Physical factors, such as UV light, high steam temperature is used for disinfection, especially in industries with a high risk of microbial contamination. UV light could kill a wide array of microorganisms including both vegetative and spore forming pathogens by induction of oxygen radicals. However, the activity is often not sufficient for bacterial eradication and has a number of adverse events like skin and eye problems and carcinogenicity [43]. Considerable variabilities in duration and the distance of the light emitting source have been determined. Steam shows an immediate germfree surface – however it does not exhibit a lasting antimicrobial activity. If someone touches the surface after 10 minutes, the surface is contaminated again. The steam technology would have to be reapplied in frequent i.e., in 30 minutes intervals. There however various technologies which have the potential to curb the dramatic increase of multi resistant microorganisms [44]. Since the early 1900s, bacteriophages are recommended for medical purposes. Several companies and research laboratories pursue a treatment strategy involving phages in infections caused by Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli [45].

In some countries (e.g., Russia, Georgia, Poland, USA), bacteria that do not respond to conventional antibiotics are treated by phages. The problem with phages is their specific activity against a certain bacterium. This requires the identification of the microorganisms prior to the application and selection of the suitable phage among thousands of phages [46]. A possible solution might be the use of a phage cocktail containing a multitude of different phages. Phages are not heat resistant and cannot be incorporated into polymers. There are high expectations of the efficient killing of bacteria using nanostructures and their non-chemical mechanisms such as contact killing, mechanical puncturing, and changes in the local microenvironment via nanoions [47]. Combination with metal oxides, silver, chitosan, gallic acid nanoparticles etc. have also been recommended. In addition, nanoparticles combined with antibacterial agents are also being studied [48]. Antibiotics however are not suitable for providing a permanent endowment of surfaces with antimicrobial properties. The induction of resistance is a great threat. The administration of antibiotics in animal husbandry may contribute to the increase of multi-resistant microorganisms [49]. The blockage of adherence of microorganisms on epithelial cells by acid galacturonides as receptor analogue carbohydrates is a successful treatment option and can prevent the administration of antibiotics except for documented bacterial infections.

Microorganisms are eliminated by natural paths [50,51]. Several animal studies using acid galacturonides also demonstrate superior outcomes compared to antibiotics and can completely replace the use of antibiotics for growth enhancers. The goal must be to avoid contamination of surfaces with multi resistant microorganisms as far as possible through improved hygiene or a new innovative ambitious approach i.e., self-sanitizing surfaces. [52]. Effective alternatives are available to prevent Infections with multi resistant microorganisms

mainly in hospitals but also beyond. In addition, the inappropriate use of antibiotics - for example, for viral infections that do not respond to antibiotics - must be properly reassessed [53]. New antibiotics would have to be developed and brought to market. The researchers cite limited data availability in some parts of the world and disparate sources for the data, which can lead to a bias, as weaknesses in their study [54]. An expert committee in 2015 comes to the conclusion: We are already in the postantibiotic era and recommend support of the pharmaceutical industry with billions of $$ [55,56]. This might not be the best approach. It has been shown that newly developed antibiotics like Linezolide developed resistance to the majority of microorganisms within 3 months [57]. R. Laxminarayan of the Center for Disease Dynamics describes the problem of antibacterial resistance as an «overlooked pandemic» in a commentary to the study [58].

We would be thrown back into the Dark Ages of medicine.» Although many more people die from such infections than from HIV, for example, far more money is donated to fight HIV and AIDS [59]. That needs to change, he said. «From an unrecognized and hidden problem, a clearer picture of the burden of antimicrobial resistance is finally emerging.» Paul deBarro writes in «The Guardian,» adding, «Covid doesn't even come close to the potential impact of AMR. We would be thrown back into the Middle Ages of medicine.» The reasons of antibiotic resistance are complex and include human behaviour at many levels of society; the consequences affect everybody in the world. It must be emphasized that the majority of these resistance mechanisms are self-inflicted. Many efforts have been made to describe the many different facets of antibiotic resistance and the interventions needed to meet the challenge [60]. However, coordinated action is largely absent, especially at the political level, both nationally and internationally. Antibiotics paved the way for unprecedented medical and societal developments and are today indispensable in all health care systems. Achievements in modern medicine, such as major surgery, organ transplantation, treatment of preterm babies, and cancer chemotherapy, which we today take for granted, would not be possible without access to effective treatments for bacterial infections.

Within just a few years, we might be faced with dire setbacks, medically, socially, and economically, unless real and unprecedented global coordinated actions are immediately taken. Disinfection of surfaces with disinfectants is not constructive any more as ample evidence exists for tolerance of microorganisms to sublethal levels of various disinfectants e.g quaternary ammonium compounds (QAC) [61,62]. Numerous antimicrobial agents e.g., benzalkonium chloride as well as chlorhexidine, hexadecylpyridinium, polybiguanides, halogenated phenols and polyethylene imines have been investigated [63-65]. The majority of these technologies proved to be too toxic for application into frequent touched surfaces. Quaternary ammonium compounds (QAC) are cationic membrane active antibacterial agents widely used in disinfectants in health care, agriculture, home and the food industry. Benzalkonium chloride (BC) is a commonly used type of QAC, which typical contains a mixture of molecules with alkyl chain lengths of C12-C16. These compounds promoted the conjugation transfer of the RP4 plasmid; the optimal concentration of QACs was about 10-1-10-2 mg/L and their transfer efficiencies were between 1.33×10^{-6} and 8.87 × 10-5. QACs enhanced membrane permeability of bacterial cells and stimulated bacteria to produce ROS, which promotes the transfer of plasmids between bacteria. In conclusion, this study demonstrated that QACs may facilitate the evolution and gene transfer of antibiotic resistance gene among microbiomes.

QACs increase the resistance of bacteria to multiple antibiotics [63,64]. The resistance of QAC based disinfectants to antibiotics is conferred by the resistance determinants qac H and bcr ABC [65]. These genes induce the formation of efflux pumps which renders a microorganism resistant to virtually all antimicrobial agents. The presence and distribution of these genes have been anticipated to assume a role in the survival and growth of various even resistant microorganisms. Today more than 8000 publications in the international literature describe the resistance of microorganisms against disinfectants and in addition 678 publications document cross resistance with antibiotics. It has been described those disinfectants (e.g., quaternary ammonium compounds and benzalkonium chloride) even enhance the growth of Listeria monocytogenes in the food industry [66]. The use of disinfectants - ostensibly intended to remove/kill pathogens on surfaces which are ubiquitously contaminated with microorganisms - is not reliable anymore. Studies have shown that more than one-half the time, microorganisms on these surfaces are not adequately eradicated on surfaces. A 99% eradication rate of microorganisms of various disinfectants is by no means sufficient as the remaining microorganism proliferate every 20 minutes and end up with a contamination in millions of resistant microorganisms in 4 -6 hours. One additional major drawback however is the lack of sustainability of disinfection of surfaces.

In case the surface is decontaminated it can be re-contaminated within minutes if the surfaces are touched by a contaminated hand. These frequently emerging multi resistant microorganisms are distributed within the hospital by the hands of the personnel to patients. A microbial burden of >8000 CFU on a 100 cm^2 surface is associated with an incidence of 21% of a hospital acquired infection. In reality we see a 100–1000-time higher inoculum size. Improved surface cleaning and disinfection has been anticipated to reduce transmission of these pathogens and the risk of healthcare-associated infections [67]. Contact with the contaminated environment by healthcare personnel is equally likely as direct contact with a patient and can lead to contamination of the healthcare provider's hands or gloves that may result in patient-to-patient transmission responsible for nosocomial infections. Both detergent- and disinfectant-based cleaning have been used to control these pathogens, although

difficulties arose with the soaring resistance of pathogens to disinfectants. The vast majority of disinfectants are not resulting in a reliable and lasting decontamination of surfaces anymore [68]. This is also documented in the international literature in 9549 peer reviewed publications. 764 publications which describe cross resistance of disinfectants and antibiotics. There are in addition 10 664 publications in PUBMED which describe the toxicity of disinfectants on various levels: Much emphasis has been put therefore on hand disinfection [69].

However, there are also reports of the emergence of alcohol tolerant/insensitive vancomycin resistant enterococci after extensive hand disinfection with alcohol [70-72]. This phenomenon has the potential to undermine the effectiveness of alcohol-based disinfectant standard precautions. This prompted the WHO to publish a warning of an imminent crisis due to the lack of effective antibiotics and requests immediate, coordinate and ambitious measure. A completely new approach must be instituted and is demanded by the WHO. Traditional cleaning methods are notoriously inefficient for decontamination and new approaches have been proposed e.g., antimicrobial surfaces the correct terminus is self-sanitizing surfaces [73,74]. Considering the above information, it was mandatory to reassess preventive measures for hospital acquired infections and to curb the dramatic increasing rate of resistant microorganisms. Antimicrobial coatings hold promise based, in essence, on the application of materials and chemicals with persistent bactericidal or -static properties onto surfaces or in textiles used in the healthcare environments [75]. This belief is based on some preliminary studies involving, for example copper and silver ions, titanium or organosilane, albeit under laboratory conditions and with serious shortcomings for clinical applicability [76-81]. Many different chemical strategies and technologies for antibacterial coatings are described in the literature. Selection of the optimal additive as an *in situ* generated biocide where the additives don't need to be eluted from the surface is of crucial importance.

Several technologies are reported.

1. Nanocoatings have been propagated for antimicrobial technologies, however they are difficult to implement on surfaces: [82,83]. Antimicrobial technologies using nanomaterials e.g., chitosan, cellulose etc. must be incorporated into nanorods or nano mats on surfaces. There is a profound difficulty to fix these stabiles on the surface [84-86].

   a) Nanotechnologies are subject to approval by the Biocidal product regulation (BPR) of the European Union. The requirements of biocidal product regulation for nanotechnologies are time consuming and expensive. None of the nanoproducts passed the biocidal regulation up to this point in time.

   b) Nanocoating are generally not heat resistant, difficult to manufacture.

   c) Expensive

2. Antibacterial coatings may contain active eluting agents (e.g., ions or submicron particles of silver, copper, zinc, antibiotics, chloride, iodine, quaternary ammonium compounds, antimicrobial peptides, or light-activated molecules e.g., TiO2. or photosensitizers [87]. Active eluting agents show several disadvantages:

The active substances must be eluted from the polymer or the surface and incorporated into the metabolism of microorganisms. This means that the activity of these substances is limited to a short period of time. For antibiotics this means a duration of activity of a few hours, definitely less than 2 days, for silver 1 week. The antimicrobial activity of copper is too low to be considered as a valuable compound. Besides copper surfaces oxidize fast and must be constantly cleaned to show an acceptable optical appearance of the surface. The activity of a technology on the basis of Titanium oxide can only be documented with the JIS 25923 method. The activity is determined in the capillary space between the surface and a foil which prevents the evaporation of oxygen radicals. This method lacks completely clinical relevance. The determination of antimicrobial activity by the RODAC plate methods shows no efficacy!

3. Quaternary ammonium compounds must be excluded from further considerations as these products induce cross resistance with antibiotics by induction of efflux pumps and may even enhance the growth of microorganisms on surfaces [88].

4. Various drug eluting substances may show toxic side effects with human tissues. It is also difficult to get approval by the biocidal regulation of the EU. Last not least: There are other technologies available with substantial better antimicrobial activity and lack of toxicity.

5. In addition to chemical modifications, the topography of a surface can by itself significantly affect its hygienic status [89]. As such, modifications of surfaces to enhance antimicrobial properties should always consider the effect of surface wear on subsequent fouling and cleaning. Therefore, efforts should be undertaken to characterize typical wear, assess interactions with the most likely microorganisms in that environment, and define the most appropriate and least damaging cleaning and sanitizer regimes. Antimicrobial agents adjacent to surfaces have the risk of abrasion with cleaning. The agents must be insoluble in water-, alcohol-, detergents, acid, alkaline, in addition they must show UV light stability which is not guaranteed with several of the above-mentioned compounds. In essence the elution of the antimicrobial compounds necessary for incorporation into the metabolism of microorganisms requires however at least a moderate water solubility. The best way to achieve such outcomes is to ensure that multidisciplinary expertise is integrated into a developmental process, and that testing methods are appropriately robust. Therefore, the necessary requirements are

frequently not clearly defined and investigated! It seems obvious to engage in research in antimicrobial agents with a limited toxicological profile in the light of passing the requirements of the biocidal product regulation. The authorities supporting these research activities however did not favour research with compounds with promising activity but used a broad watering can like approach.

This was initially not necessarily wrong but after a certain period of investigation funding could have streamlined to more promising compounds. Through its Cooperation of Science and Technology Programme (COST), the European Commission has recently funded a four-year initiative to establish a network of stakeholders involved in development, regulation, and use of novel antimicrobial coatings for prevention of HCAI [90]. The network AMiCI (AntiMicrobial Coating Innovations) currently comprise participants of more than 60 universities, research institutes and companies across 30 European countries (www.amici-consortium.eu) and, to date, represents the most comprehensive grouping to target the use of these emergent technologies in healthcare settings. Within AMiCI, one of the working groups is collecting information on commercially available antimicrobial coatings with actual or potential application in healthcare, and the development of new coatings that are SbD. This review article is the result of extensive discussion within the working group and the AMiCI consortium as a whole, following the 'world cafe´ approach.

6. Chemical modifications of a surface are anticipated to achieve functional antimicrobial coatings. Strategies to achieve antimicrobial coatings can be classified according to their functional principle as:

(i) Antiadhesive,

(ii) Contact active, and

(iii) Biocide release [91].

Whereas the first two principles may be considered as SbD, biocide release incorporates the release of a toxic substance and can therefore be considered as toxic by design. Sometimes two functional principles are combined to achieve synergistic effects, e.g., by embedding biocidal substances into anti-adhesive surfaces. Today, the majority of chemical modifications include hydrogels or poly (ethylene glycol) (PEG) to repel approaching microbes, [92] metals (in particular, silver and copper), antimicrobial peptides (AMPs), quaternary ammonium compounds (QACs), and nanoparticles. Beyond those established approaches, state-of-the-art or potentially new strategies towards antimicrobial coatings were identified at the AMiCI meetings and were sorted and classified according to their functional principle. For many of the latest antimicrobial strategies, the mechanism of antimicrobial activity is still under investigation and there is not enough information available on whether antimicrobial activity happens preferably direct at the surface or whether small amounts of active compounds are released into the test media where they will exert their antimicrobial activity or whether both mechanisms are acting in parallel.

Besides engagement with known antimicrobial agents' emphasis should have been exerted in evaluating

a) Documentation of the mechanism of action of presently available compounds

b) Investigation of new principles with new and promising antimicrobial activity.

7. Anti-adhesive surfaces: Anti-adhesive surfaces can reduce the adhesion force between bacteria and a solid surface to enable the easy removal of bacteria before a biofilm layer is formed on the surface [88]. Such surfaces may suppress HCAI by blocking transmission paths involving surfaces, but they will not reduce the number of germs on the contacting media by killing them. Attachment of bacteria or cells starts with an initial adsorption of proteins on to the material surface [93]. The most important requirement of "self-sanitizing" surfaces is the ability of the surface to actively eradicate pathogens within a reasonable short period of time preferably less than 1 hour. This has been investigated by the laser scanning technology. Eradication of 10 million CFU on an ECG lead wire has been found in less than 15 minutes. It has to be emphasized that microorganisms are deposited by the hand of the personnel containing grease, sweat with considerable force. For prevention of recontamination of a second person, rapid eradication of microorganisms is mandatory. Reduction of adherence, blockage of proliferation and biofilm formation – although also important – are in no way sufficient as "self-sterilising" surfaces for clinical use to prevent recontamination of persons [94].

8. Superhydrophobic surfaces are characterized by a water contact angle >150° and they are inspired by the lotus leaf in nature. The Lotus effect requires a constant flow of water to remove the non-adhering particles. [94] This is not feasible on surfaces constantly in use e.g., in a hospital. It was further revealed that the lotus leaf has a hierarchical micro/nanostructure. Reducing bacterial adhesion via super hydrophobicity is a new topic and has yet to be studied thoroughly and systematically. Analysis of super hydrophobic siloxane and fluorosiloxane surfaces showed also minimal protein adsorption, both before and after protein adsorption trials [95,96]. Nanostructures are important, since effective air entrapment in the three-dimensional nanomorphology (nanopillars) renders them superhydrophobic and slippery [95,96]. On inherently nanostructured hydrophilic aluminium, adhesion forces of bacteria were reduced by a factor of 4 down to 2e4 nN compared to the electropolished flat surface, resulting in an 88% reduction of colony-

forming units for Staphylococcus aureus. This effect was even more pronounced after applying a hydrophobic Teflon coating, yielding a 99.9% reduction under flow conditions [97]. It has to be emphasized that microorganisms, deposited by the hand of the personnel with sweat, grease, blood are contaminating superhydrophobic surfaces. Therefore, a bactericidal surface property is mandatory.

Easier cleaning is not the answer – it must be bactericidal. Nanostructured surfaces were prepared using electrospun polystyrene nanofibres. When oxygen plasma-treated, a superhydrophilic surface was generated, which exhibited limited Escherichia coli attachment due to negative zeta potential of e40 mV. [98]. This technology however does not provide a lasting antimicrobial surface (less than 2 weeks). Addition of silver is a feasible technology for antimicrobial effectiveness, but only free silver ions show antimicrobial activity – in other words silver has to be released from the hydrophilic bottom layer and incorporated into the bacterial metabolism. The disadvantage is a limited activity to a few hours or days! The addition of hydrophilising agents is necessary for the release of free silver ions. An interesting anti-adhesive and killing approach is found in nature [98]. The nano-patterned cicada wing surface uses an adsorption and stretching mechanism with eventual rupture. As the bacterial cells adsorb on to the nanopillared structures present on the wing surfaces, the bacterial cell membrane stretches in the regions suspended between the pillars. If the degree of stretching is sufficient, cell rupture will occur. The cicadia wings technology – as well as the gecko foot technology which relies on the same principle - is not physically stabile – the cicadia wings surface is destroyed by mechanical wiping/cleaning, rubbing [99-101]. Strategies to prevent protein attachment include superhydrophobic surfaces, often augmented by a hierarchical nanostructure as well as zwitterionic polymers.

9. Zwitterionic polymer brushes may also delay or even prevent microbial attachment to a surface, since the hydration layer surrounding the ionic surface prevents non-specific protein adsorption. Using barnacle cement, a biological adhesive from barnacles, and 'click' chemistry, poly(2-(methacryloyloxy)ethyl trimethylammonium chloride) polymer brushes were successfully attached to stainless steel and antimicrobial properties were demonstrated. Zwitterionic polymer brushes cannot inactivate bacterial cells. Therefore, synergistic antiadhesion and bacterial inactivation was achieved by grafting zwitterionic poly (sulfobetaine methacrylate) brushes with embedded biocidal silver nanoparticles [102,103].

10. Addition of silver is a feasible approach but - as already described - only free silver ions are active – in other words silver has to be released from the hydrophilic bottom layer and incorporated into the bacterial metabolism. The importance of anti-adhesive properties for biofilm formation was also demonstrated by measuring the adhesive forces on brush-coated silicone rubber and uncoated silicon rubber. On the brush-coated rubber, adhesion was so weak that the bacteria were no longer able to sense the surface and therefore remained in their planktonic state, susceptible to antibiotics rather than forming a protected biofilm [104].

11. Contact-active surfaces: Contact-active surfaces exhibit antimicrobial activity without releasing biocidal substances. Several mechanisms are believed to take place in contact-active surfaces. These are:

1. A so-called spacer effect, where the biocidal group is attached to the surface through a polymer chain, allowing the biocide to reach the cytoplasmic membrane of the bacteria and to perforate them.

2. Alternatively, positively charged QACs, e.g., 3-aminopropyl trimethoxysilane grafted to cellulose nanofibers, can detach phospholipids from the cell membrane and thereby kill the microorganism [105]. This approach is also referred to as biomimetic with respect to the activity of chitosan i. e. a polysaccharide derived from the exoskeleton of crustaceans or cell walls of fungi. Hydrophobic parts of a surface can act similarly to QACs by deforming the membrane through adhesion. The activity of the spacer effect is obliterated by grease, proteins, sweat, pus and blood. The activity of chitosan has been investigated and a poor antimicrobial activity has been found [106].

12. Polymer brushes have been widely used in preparing contact-active antimicrobial surfaces without biocidal release. The rationale behind polymer brushes is the observation that antimicrobial molecules lose much of their activity, once attached to a surface. When providing an anchor for the active molecule through a flexible covalently bound polymeric chain, the active molecule should still be able to reach the site of action at or within the bacterium, e.g., by penetrating its cell wall, but leaching is still suppressed. Important parameters for polymer brush anchors are chain length and chain density. Polymer brushes have been shown to be effective for anchoring QACs or AMPs. Poly(amidoamine) dendrimer-immobilized CDs and Ag2S quantum dots conjugated chitosan nanospheres toward light-triggered nitric oxide release and near-infrared fluorescence [107]. The idea behind is attractive – unfortunately it is not feasible in a hospital environment. Again, it is definitely not recommended to use QACs (strong induction of resistance by induction of efflux pumps) or AMP (antimicrobial peptides.) AMPs belong to the bodys defense mechanisms., They are formed by epithelial cells upon contact with a pathogen and destroy the surface layer of microorganisms. Accumulating evidence shows that in addition to acting at the cell membrane, AMPs may act on the cell wall, inhibit protein folding or enzyme activity, or act intracellularly [108-110]. However, three problems arise with AMPs.

1. AMPs must be eluted from the surface and must be incorporated into the metabolism of microorganisms – activity by disruption of cell wall. Therefore, the activity is limited to a few days.

2. AMPs are difficult to obtain. They could be obtained as Magainins from frog skin with very limited availability [111].

3. Synthesis of AMPs is not solved as AMPs are lethal factors for microorganisms if produced by microorganisms. Investigations over 2 years have been performed by AmiSTec. Synthetic AMPs induce fast resistance against microorganisms – these microorganisms are in turn also insensitive to natural AMPs produced by the body. Definitely to be avoided! [112,113].

13. Using surface-initiated atom transfer radical polymerization, QACs with charge densities of >1.5e1015 accessible quaternary amine units/cm^2 were anchored through poly-2-(dimethylamino) ethyl methacrylate chains. Interestingly, these surfaces were bioactive even though the polymer chains were too short to penetrate the cells with envelope thicknesses of 46 nm for Gram-negative E. coli and 45.55 nm for Gram-positive Bacillus subtilis [114]. The spectrum of activity has to be very broad. Surfaces are contaminated with a variety of microorganisms which affect each other limiting the spread and growth. If one species is eradicated the remaining microorganisms have the possibility to proliferate and spread uninhibited. Limited spectrum of activity is therefore entirely unacceptable. This demonstrates that surface charge density can be more important than chain length. On the other hand, it was clearly shown that N-alkyl-pyridinium exhibited high antimicrobial activity when anchored through a 750 or 25 kDa N-alkyl-pyridinium exhibited high antimicrobial activity exhibited antimicrobial activity (PEI) but showed no activity when using the 2 kDa analogue [115]. Therefore, only long-chained, moderately hydrophobic immobilized polycations exhibit microbicidal activity. Interestingly, polycationic polymer brushes are not subject to existing mechanisms of resistance such as multidrug- resistance pumps or multidrug tolerance protein-expressing cells, presumably since there are no analogue structures in nature [116].

14. Polycations on a surface are not heat resistant and cannot be extrusion moulded, in addition the activity is obscured by any compounds e.g., grease, sweat etc. which are abundantly coating hospital surfaces close to patients. Anchoring on AMPs is again not helpful. In addition, the technology is complicated and expensive. Besides: These compounds cannot be added to various coatings [117]. Polymer brushes have also successfully been used for anchoring AMPs. AMPs are a logical alternative to conventional antibiotics due to their broad-spectrum antimicrobial activities. Surface concentrations of AMPs up to 5.9 mg/cm^2 were achieved by conjugating the peptides to surface-immobilized primary amine functionalized polymer chains obtained by aqueous surface-initiated atom transfer radical polymerization of N,N- dimethylacrylamide and aminopropyl methacrylamide hydrochloride The efficacy of AMPs attached to catheter material surface using polymer brushes was verified in vivo by using a catheter-associated urinary tract infection mouse model. By adding arginine glycine aspartate peptides to promote host-tissue cell adhesion to AMPs anchored through the block copolymer Pluronic F-127 two effects were achieved, namely thwarting bacteria from approaching and attaching to the surface and, simultaneously, enhancing tissue integration [118].

15. Another group of naturally occurring antimicrobials are claimed as alternatives to antibiotics are bacterial cell wall hydrolases (BCWHs) [119]. Antimicrobial peptides have a broad-spectrum against bacteria and fungi and are already investigated in my institution. No induction of resistance, no toxicity even at high doses but highly costly to produce has been described. BCWHs have limitations towards Gram-negative bacteria, due to the presence of the outer membrane, and some important Gram-positive pathogens such as S. aureus are already resistant to lysozymes.

16. Biocide-releasing surfaces:Biocide-releasing surfaces may have some conceptual disadvantages since they are toxic by design in terms of releasing biocidal substances. In addition, they will gradually become inactive, and they may induce the formation of resistance. Any substance eluting from the surface is also emanating into the environment. Toxic substances may affect various cell lines in the body e.g., epithelia cells, osteoblasts, fibroblasts if incorporated into the body. The chance that these biocides meet the requirements of the European Commission is improbable and requires extensive testing.

17. There is a law by nature that all substances with antimicrobial activity which require the incorporation of the agent into the metabolism of microorganisms induce resistance. This is well known for antibiotics but also disinfectants. Therefore, it is required that a technology is developed which is not incorporated to the bacterial metabolism but attacks microorganisms from the outside i.e. acid water molecules, free radicals and most important a positive zeta potential i.e. a positive electrostatic charge at the surface which ruptures the phosphoplipid bilayer of microorganisms upon contact within minutes (documented by laser scanning microscopy). The use of a catalyst is considered a suitable technology as it meets all the basic requirements for the prevention of hospital acquired infections [120,121]. Therefore, it is required that a technology is developed where disinfectants are not incorporated to the bacterial metabolism but attack microorganisms from the outside i.e. acid water molecules, free radicals and most important a positive zeta potential i.e. a positive electrostatic charge at the surface which ruptures the phospholipid bilayer of electro negatively charged microorganisms upon contact within minutes [122,123]. The development of self-sanitizing surfaces with a broad spectrum of activity, fast eradication of microorganisms, long lasting to permanent antimicrobial activity

without induction of resistance is the only promising solution for this problem if all requirements for the prevention of hospital acquired infections are met. The technology is based on *in situ* generated biocides derived from transition metal oxides. The fast antimicrobial activity has been documented by laser scanning microscopy [124]. Transition metal oxides as *in situ* generated biocides are neither water nor alcohol/tensides soluble. Catalysts e.g. MoOxide, Tungsten oxides, Zinc Molybdate, Polyoxometallates, which generate reactive oxygen species provide the only technology which meets all the requirements for prevention of hospital acquired infections. The determination of an antimicrobial surfaces investigation is performed by the push plate method (RODAC Plate method) This technology is also effective against microorganisms embedded in a biofilm where antibiotics and disinfectants fail. Transition metal oxides are not toxic, in essence they are essential trace elements as stabilising molecules of enzyme systems involved in the detoxification of sulfur [125-127].

a) Sulfite oxidase catalyzes the oxidation of sulfite to sulfate, which is necessary for the metabolism of sulfur-containing amino acids. Sulfite oxidase deficiency or absence leads to neurological symptoms and early death [128].

b) Xanthine oxidase catalyzes the oxidative hydroxylation of purines and pyridines including the conversion of hypoxanthine to xanthine and xanthine to uric acid.

c) Aldehyde oxidase oxidizes purines, pyrimidines, pteridines and is involved in nicotinic acid metabolism.

d) Low dietary molybdenum content results in low uric acid concentrations in urine and serum and excessive xanthine excretion.

18. Another approach is triggered release depending on certain threshold concentrations of quorum-sensing molecules which are found in biofilms [129]. The antimicrobial activity of a quorum sensing technology has never been documented beyond reasonable doubt. It is considered an interesting idea but no one has ever been investigated the activity in comparison with other technologies.

19. Surface coating with carbon nanotubes (CNTs), graphene or diamond-like carbons (DLCs) promised interesting antimicrobial activity, since these materials show relatively low cytotoxicity towards mammalian cells [130]. Whether these materials are active on the surface or whether they achieve antimicrobial activity through releasing traces into the aqueous phase is not yet resolved, but their activity in microbial suspensions is clearly demonstrated, e.g., higher toxicity is found for surfactant dispersed CNTs [131]. The most frequently proposed mechanisms of action fall under four categories:

(i) Oxidative stress induction,

(ii) Protein dysfunction,

(iii) Membrane damage, and

(iv) Transcriptional arrest.

Recently, it was also demonstrated that the mechanism of action depends on the concentration of the bacteriocide in this case graphene oxide (GO): low GO concentrations cut membranes of the micro-organisms S. aureus and E. coli whereas high concentrations induce the formation of GO aggregates shielding their edges. When cluster size increases, bacterial deactivation through wrapping is observed. Graphene-based materials differ in their morphology (mono and multilayers) as well as in their surface chemistry (graphene, GO, reduced graphene oxide (rGO)). Lateral size for instance is important to enhance bacterial adhesion whereas the sharp edges may act as nanoknifes [132]. GOs can enhance the antimicrobial activity through oxidative stress with or without the production of reactive oxygen species. When comparing the antibacterial activity of graphite, graphite oxide, GO, and rGO towards E. coli under similar conditions, GO showed the highest antibacterial activity, followed by rGO, graphite, and graphite oxide [133]. The addition of silver for enhancement of antimicrobial activity has been propagated. Synergistic effects are reported for graphene-based silver nanocomposites and composites with other antibacterial nanoparticles, as well as with polymeric or enzymatic bactericides [134].

20. Graphene by itself show a very limited antimicrobial activity. However, in combination with silver the antimicrobial activity is enhanced. However again only silver ions show antimicrobial activity and must be incorporated into the metabolism of microorganisms with known consequences: limited duration of activity whereas graphene is enhancing the release of silver ions which is not favourable. Carbon nanotubes have also been widely studied as antimicrobial material since they can be easily embedded into polymers. Again, a variety of morphologies has been studied such as single wall or multi-wall, but it seems that GO-based materials show higher antimicrobial activity. Synergistic effects were obtained by making composites of CNTs and chitosan within a hydrogel, or by decorating CNTs with poly(amidoamine)dendrimer-immobilized CDs and Ag2S quantum dots which enhanced the antimicrobial activity in solution [135,136]. CNTs can also be used to prepare antimicrobial coatings either by electrodeposition of a polyvinyl-N-carba-zolee CNT film or by preparing spin-coated films. In the same work, the antimicrobial activity of dispersed CNTs was studied and it was found that such antimicrobial activity depended on the degree of dispersions. Antimicrobial activity of CNTs depends also on the length of CNTs, as was shown for poly (lactic-co-glycolic acid)-embedded CNTs, where the shorter ones were more active Diamond-like carbons represent a further morphology of carbon materials [137,138].

They can be prepared by chemical vapour deposition, e.g., on stainless steel surfaces, and they can be doped with known antimicrobial metals such as copper, silver, or platinum. When comparing the antimicrobial activity of pure DLCs and germanium doped DLCs, significant reduction in Pseudomonas aeruginosa biofilm formation was observed whereas these surface films showed no effect against Gram-positive S. aureus biofilms. This technology is highly experimental at this stage, In comparison with available technologies much more expensive with comparable activity (Figure 2). Carbon quantum dots (CDs) are a relatively new class of carbon materials which can be used for bacterial identification due to their tunable photoluminescence properties. CDs exhibit low toxicity and appreciable biocompatibility [139]. When decorating the surface of CDs with QACs or Ag NPs, it was possible to selectively attach C-dots to Gram-positive bacteria and to induce antimicrobial activity through the membrane-disrupting mechanism. Graphene oxide-silver nanocomposites modulate biofilm formation and extracellular polymeric substance (EPS) production [140,141]. Photocatalytic oxidation is a possible alternative strategy for antimicrobial coatings in the hospital environment Due to the self-regenerating biocidal effect of the catalytically released reactive oxygen species, such surfaces remain active throughout their lifetime [142].

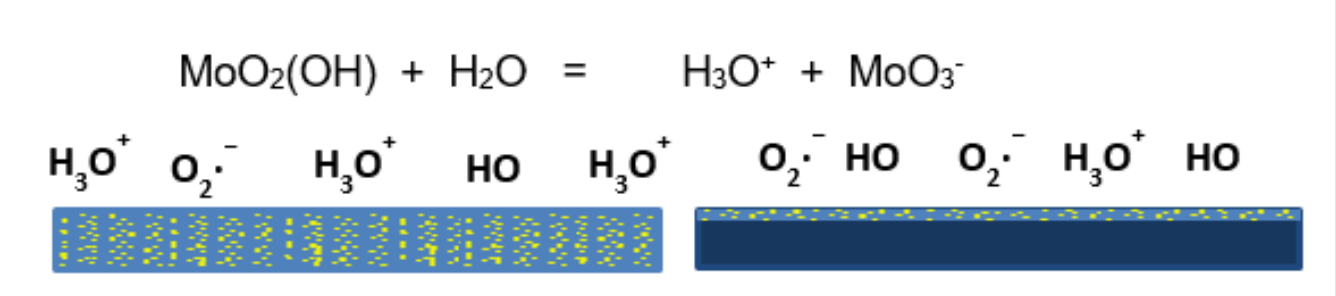

Figure 2.

Earlier published technologies fo antimicrobial surfaces contain the photocatalyst TiO_2, which generates active oxygen- and hydroxyl-radicals in the presence of water, oxygen, and UV-A light [143]. These highly reactive oxygen radicals can destroy bacteria. The antimicrobial activity can only be documented by the JIS 25923 method which means that coating the surface with a foil which prevents the radicals from emanating into the environment is mandatory. The antimicrobial activity is determined between the surface and the foil where unrealistic high concentrations of oxygen radicals are present. This method is not compatible with clinical use. The antimicrobial activity investigated with the RODAC-push plate method does not show any activity. Current research is focusing on shifting the photocatalytic activity of such coatings towards the visible light range, e.g., by adding silver nanoparticles which can act through their surface plasmon resonance effects, or molybdenum. When incorporating a combination of photosensitive dyes such as Crystal Violet with the inherently antimicrobial ZnO_2 nanoparticles into polymer surfaces, synergistic photocatalytic antimicrobial activity was reported [144]. The polymers exhibited significant bacterial kills using typical white light sources of hospital environments within 1 h against Gram-positive bacteria and within 6h against Gram-negative bacteria.

By combining a dye with Ag nanoparticles, bactericidal activity of the Ag nanoparticles could be enhanced under white light illumination. It is believed that the enhancement effect is due to an increase in bactericidal activity through the triplet state of the dye by biomolecular reaction rather than by enhancement of the concentration of reactive oxygen species. Photocatalytic oxidation is a mechanism with a potential as it eradicates bacteria without incorporation into the bacterial metabolism. A substantial disadvantage however is the requirement for an external light source. Prohibitive is that the technology can only be documented by the JIS method investigating the activity underneath a foil. Last not least free radicals don't penetrate the thick layer of a biofilm.

21. *In situ* generated Surfaces by transition metal oxides. Surfaces decorated with metal oxides e.g., Lewis acids such as MoO_3, oxygen deficient tungsten blue oxide WO3 and Zinc molybdate have also shown a broad-band and strong antimicrobial activity resulting in a reduction of the number of colony forming units by 6 – 7 log 10 within 1-3 hours [145,146]. Their mechanism of action is based on the in-situ generation of 4 mechanisms which work in a synergistic mode.

a) H_3O+ ions through the reaction with moisture from the air inspired by the bodys own defense mechanism imitating e.g., the acid coating of the skin [147]. The resulting acidified surfaces have a pH of 4.5 and the H_3O+ ions can diffuse through the cell membranes where they can distort the pH-equilibrium and transport systems of the pathogen.

b) In addition to this mechanism also free radicals e.g., oxygen radicals and hydroxyl radicals are formed which result in a synergistic mode of action [148]. Transition metal oxides embedded in polymers Transition oxides embedded in coatings (Figure 3).

c) A positive zeta potential has also been determined limited to a µm distance at the surface [149]. This is reflected by an extraordinarily fast eradication of microorganisms i.e., a reduction of 5 log 10 within 15 minutes documented by laser scanning microscopy [150].

d) Last but not least paramagnetic Ions also contribute to the antimicrobial activity [151]. This technology is the only one which meets all the requirements described initially for prevention of Hospital acquired infections. The additives are water, detergent and alcohol insoluble and are fixed in a polymer or a coating where they are not eluted [152]. There is no induction of resistance, no allergenicity, the additives are nontoxic and are essential trace elements in the body! Permanent (>10 years) activity – including activity against microorganisms in biofilms has been documented.

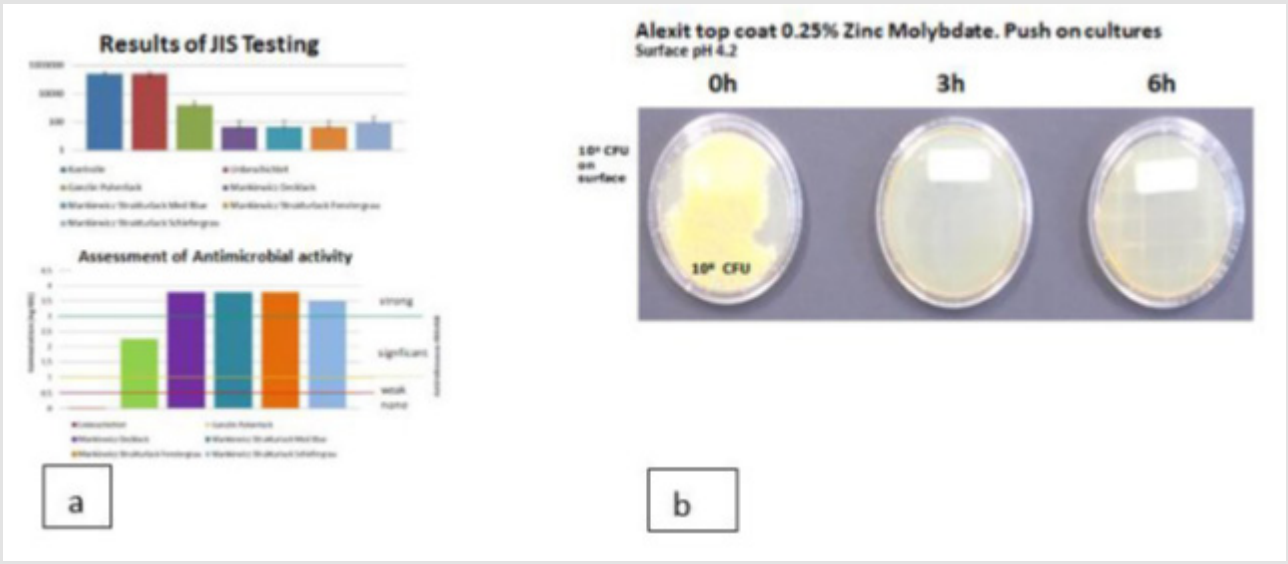

Figure 3.

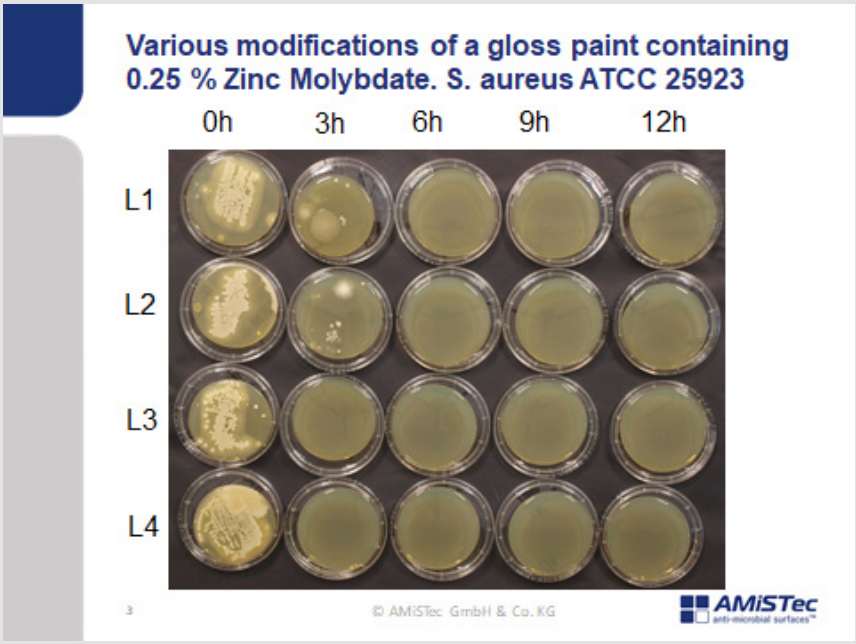

Figure 4.

Easy cleaning has been documented with water and detergents as microorganisms don't adhere on acid surfaces. 1000 cleanings with water and a detergent did not impair the antimicrobial activity of this technology (Figure 4). The technology is also active against microorganisms embedded in a biofilm! [153]. Microorganisms in a biofilm are hibernating and don't take up anything from the outside. Therefore, all technologies which are based on incorporation of the antimicrobial agent into the bacterial metabolism are ineffective. Again, technologies which attack microorganisms from the outside also eradicate microorganisms in a biofilm, an important asset. However also technologies based on oxygen radicals alone are also not sufficiently active as these free radicals are not able to penetrate the biofilm. The antimicrobial technology of this technology is approved by the BPR of the EU as *in situ* generated biocides and is legitimately on the market. As these additives are not eluted to the surface no toxicity is observed. Molybdenum is an essential trace element in the body as stabilizing molecule for several enzyme systems responsible e.g., for the elimination of sulfur in the body. The antimicrobial activity and marketability of these *in situ* generated biocides has been documented by the Austrian ministry of environmental protection as rapporteur of the BPR of the EU. In the future no easy approval for "*in situ* generated biocides" is possible by ECHA.

This opportunity expired September 1, 2018. This is also an additional favorable asset of the presently available technology. Reduced toxicity and prolonged durability of the antimicrobial effect may also be achieved by the triggered release of biocidal molecules. Additional technologies with limited usefulness or profound toxicity have been propagated: A completely unacceptable technology, based on *in situ* generated biocides has been propagated a well-known German company; AgXX = Silber + catalyst imitating the technology *in situ* generated biocides. Initially the catalyst has not been disclosed and after intense investigations Ruthenium has been detected as XX. All ruthenium compounds should be considered highly toxic as well as carcinogenic. Ruthenium compounds are highly discoloring to the skin. Ingested ruthenium is believed to be accumulating in bones. Ruthenium oxide (RuO4) is very volatile and highly toxic. Contact should be strictly avoided [154]. Finally: For health claims all ingredients must be disclosed. This was not the case! The technology is not approved by the BPR of the European union. Ruthenium 106 is one of the radionuclides used in nuclear weapons testing in the atmosphere, which began in the United States in 1945 and ended with Chinese testing in 1980. It is one of the long-lived radionuclides that pose an increased cancer risk to humans and will continue to do so for decades and centuries to come.

Another technology which shows the lack of a broad understanding of the requirement has been pursued by BASF: Nickelous hydroxide plating of surfaces is used as a catalyst. The problem: Nickel hydroxide is a poor electron donor and is highly allergenic [155]. The incorporation of antibiotics in particular results in the induction of resistance to a group of antibiotics indispensable in clinical practice. The duration of the activity is limited to a few days. This is in no way a practical approach and must be abandoned. Also, the incorporation of disinfectants must be banned due to the induction of cross resistance with antibiotics. Some plant extracts are well known for their antimicrobial properties and much research is devoted to their application to protect food from pathogens [156]. It has been shown that a tea-tree oil coating may induce zones of inhibition against MRSA after a two-day incubation. The problem: these technologies are not sustainable and do not withstand cleaning. However, limited research has been done on investigating their efficacy on surfaces of healthcare units or on medical devices. Some plant extracts may exhibit antimicrobial activity by fermentative properties and formation of free fatty acids. There are other less well documented mechanisms. Plant extract however are not heat resistant, difficult to work with and water soluble [157].

Impact of topography on surface effectiveness: It is generally acknowledged that defects or design features on any inert surface can retain soil and/or micro-organisms, and therefore affect cleanability, disinfection, and hygienic status of the surface. Implications in the clinical environment in terms of cross-infection control, the choice of surface material to be used, and the cleaning and sanitization protocols are significant issues. However, the assumption 'the rougher the surface, the worse the hygienic status' is somewhat simplistic, although many publications make this type of claim. Cells are easily removed from 'smooth' surfaces, but they may be retained within features approximating in size to that of the cells. In larger features, the cells may again be relatively easily removed.

Easy cleanability is in addition to an optical attractive surface one of the prerequisites for hospital furniture. If easy cleaning is also accompanied by a germfree surface this is the ideal approach as this can avoid the use of disinfectants which hence are responsible for the dramatic induction of resistant microorganisms. Technologies which combine easy cleaning and antimicrobial activity are preferable. It has been documented that the technology with surfaces decorated with metal oxide Lewis acids such as MoO_3, WO_3, Zinc molybdate and Polyoxometallates (incorporation of molybdenum oxide into the tungsten blue oxide crystal structure) have also shown a broad-band and strong antimicrobial activity resulting also in a reduction of the number of colony forming units by 6-7 log 10 within 3 hours [158-160]. Their mechanism of action is based on the in-situ generation of H_3O+ ions through the reaction with moisture from the air inspired by the body's own defense mechanism e.g., the acid coating of the skin. The resulting acidified surfaces have a pH of 4.5 and the H_3O+ ions can diffuse through the cell membranes where they can distort the pH-equilibrium and transport systems of the cell. In addition, by this mechanism free radicals e.g., oxygen radicals and hydroxyl radicals

are formed which result in a synergistic mode of action [161]. Also, a strong positive zeta potential is formed. This is a positive electric charge in µm distance at the surface which attracts electronegative charged microorganisms.

Upon contact the bacterial membrane is immediately disrupted. This is reflected by an extraordinarily fast eradication of microorganisms i.e., a reduction of 5 log 10 within 10 minutes. There is an additional favorable property of such a surface. Bacteria don't adhere to these surfaces, don't proliferate and don't form a biofilm. Microorganisms can also efficiently be removed by mechanical cleaning. Cleaning 2 hours after deposition of 109 colony forming units per $3cm^2$ on a surface endowed with this technology resulted in a complete eradication of microorganisms [162]. Self-disinfecting surfaces - the correct term is self-sanitizing surfaces - are highly cost effective as they save the daily application of disinfectants for years. At the same time the emergence of resistant microorganisms is prevented by stopping the application of disinfectants. Indeed, the previously mentioned 'lotus effect' reveals that a hierarchical micro/nanostructure can significantly reduce retention, enabling cells to 'roll off' the surface. The fabrication of surfaces with well-defined nano-topographies provides a new avenue for the design of anti-adhesive/easily cleanable (and therefore hygienic) surfaces depending on the intended environment of use. The Lotus effect was investigated in great detail a number of years ago; however, the efficacy was very limited. It has been documented that bacteria adhere less vigorously on a microrough surface. This requires however an active elimination of microorganisms by a constant flow of water or constant active wiping of the surface (Figure 5).

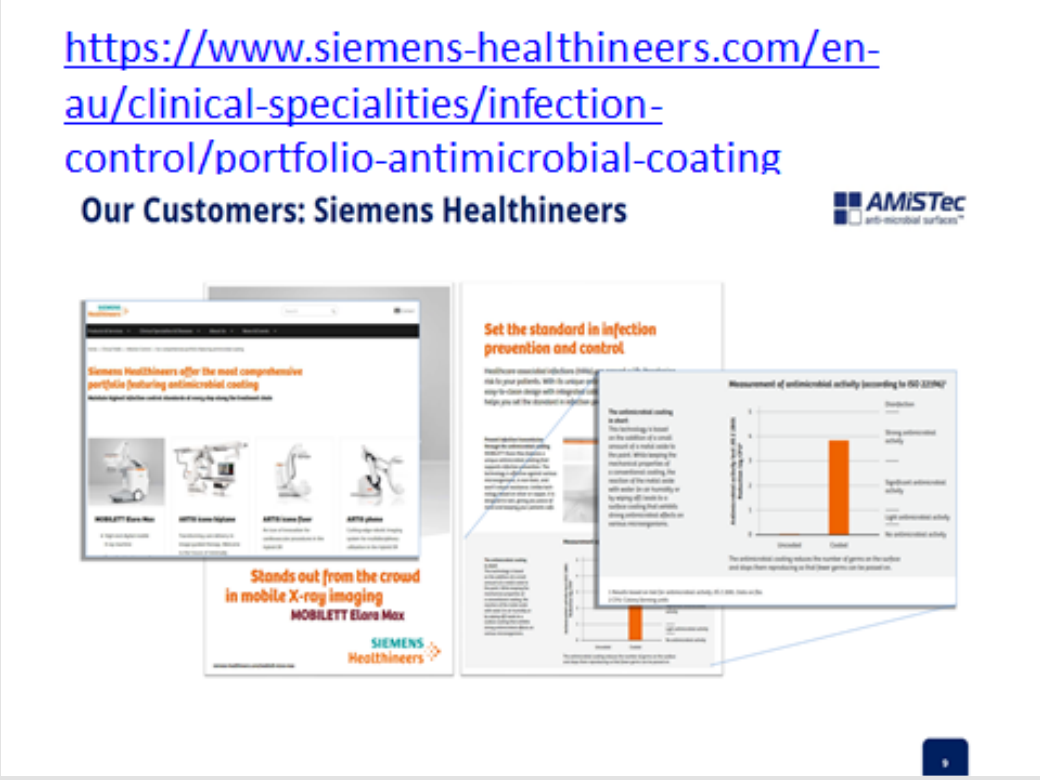

Figure 5.

The environment in which surfaces are placed will also affect their hygienic status. At a flowing solid- liquid interface, cells will move across the surface, and may be retained in features where they may replicate and form biofilms with accompanying 'streamers' which may detach and contaminate downstream. However, on open surfaces, at a solid air interface, the cells tend to be deposited on the surface through contact with vectors such as food, fingers, equipment, or splashing. Surfaces endowed with *in situ* generated biocides have been cleaned 1000 times and no elution of the active ingredients has been observed. Also, no loss of antimicrobial activity has been observed after 1000 cleaning cycles as the lack of e.g. water alcohol, tensid solubility of the additive in particular Zinc Molybdate, Tungsten blue oxide and Polyoxometallates has been documented [163]. Antimicrobial surfaces, and/or surfaces which are hard or

difficult to abrade, coupled with effective cleaning regimes, are strategies employed to counter this phenomenon. The continued cleaning/soiling cycle can itself affect the surface, causing abrasions that result in increased soiling and require increasing force in cleaning which in turn may increase abrasion. The nature of the surface itself can affect how it wears: steel and other metals tend to scratch; glass and ceramics tend to fracture; softer materials such as plastics will abrade more easily, however if the additives are incorporated into the composite material this again no problem.

The presence of retained organic material (blood, food, sputum) i.e., soiling has been investigated and no decrease in antimicrobial activity has been observed in numerous experiments with various substrates. It might be argued that the increase in surface area presented by surfaces with increased roughness is the driver for the increased retention, but this has not been convincingly proven. The features themselves, in terms of shape, profile, and size clearly provide an increased area of contact for cells, enhancing their ability to remain on surfaces [164]. All these issues should be considered when developing novel and effective antimicrobial surfaces, focusing on broad, strong and fast antimicrobial activity as well as minimizing wear to maintain cleanliness and cleanability. The atomic force microscope is one means of assessing the strength of attachment of cells on a surface [165]. The probe scans repeatedly across the surface, moving vertically in response to surface features. This movement is captured and imaged using lasers. By increasing the force of the scan, less strongly attached cells are removed. Thus, the strength of attachment as well as the amount of retention can be assessed. This work has revealed that the size of cells and their relationship with the feature size affect strength of attachment: as might be expected, comparable feature size and cell size is the least desirable combination, enabling maximum contact area between cell and surface.

In addition, cell shape will also affect this interaction, with rod-shaped cells having a larger area of contact available for interaction with the cell surface in comparison to cocci. Investigation of the strength of attachment of cells on linear features where the force is applied either across or along the feature has revealed different results: demonstrating easier removal along well-defined features on titanium-coated stainless steel, but easier removal by applying force across features on softer polymeric surfaces containing transition metal oxides as *in situ* generated biocides. This work has led to the fabrication of surfaces with designed topographies that are targeted at inhibiting attachment of particular cells, where surface features smaller than cells might reduce their ability to strongly attach to the surface, and therefore improve cleanability. The robustness of these surfaces is essential to ensuring a long- lasting effect, and the potentially interfering effect of organic material must also be considered [166]. In the clinical/medical environment, high-touch surfaces (worktops, walls, doorhandles, telephones, patient surrounds) are the prime focus for antimicrobial endowment and/or effective cleaning. Solide-liquid interfaces, where biofilms could form, would likely be encountered on hospital furniture as wells around taps, showers or drains.

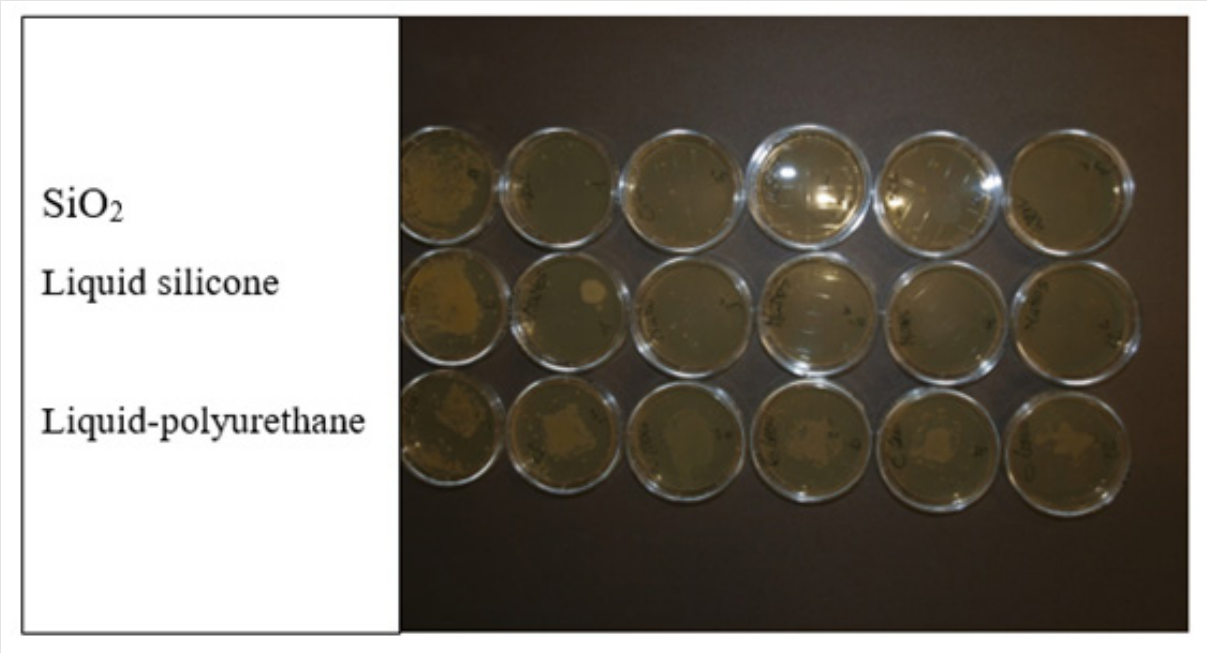

Figure 6: Leather. 2 % Zinc Moylbdate in SiO_2. S. aureus ATCC 25923.

 DOI: 10.26717/BJSTR.2023.50.007883

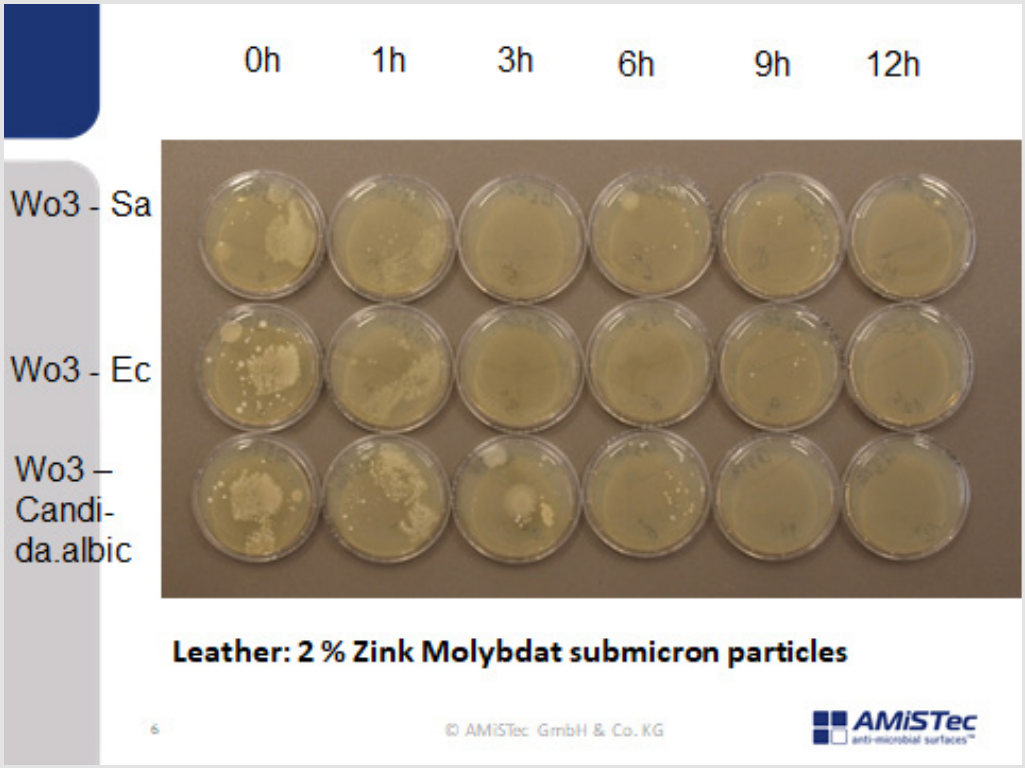

Figure 7: Textiles. 2 % Zinc Molybdate in SiO2. S. aureus ATCC 25923.

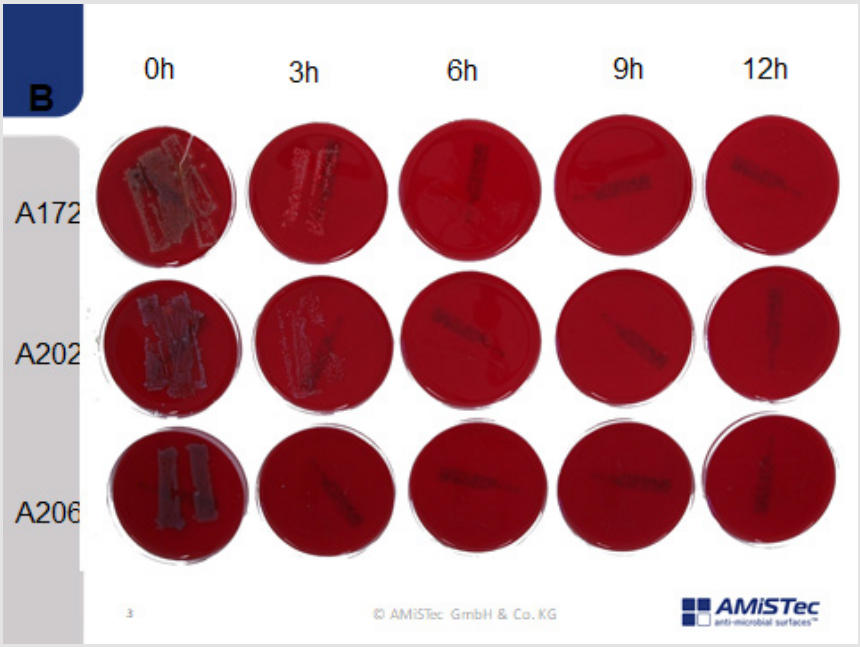

Figure 8.

DOI: 10.26717/BJSTR.2023.50.007883

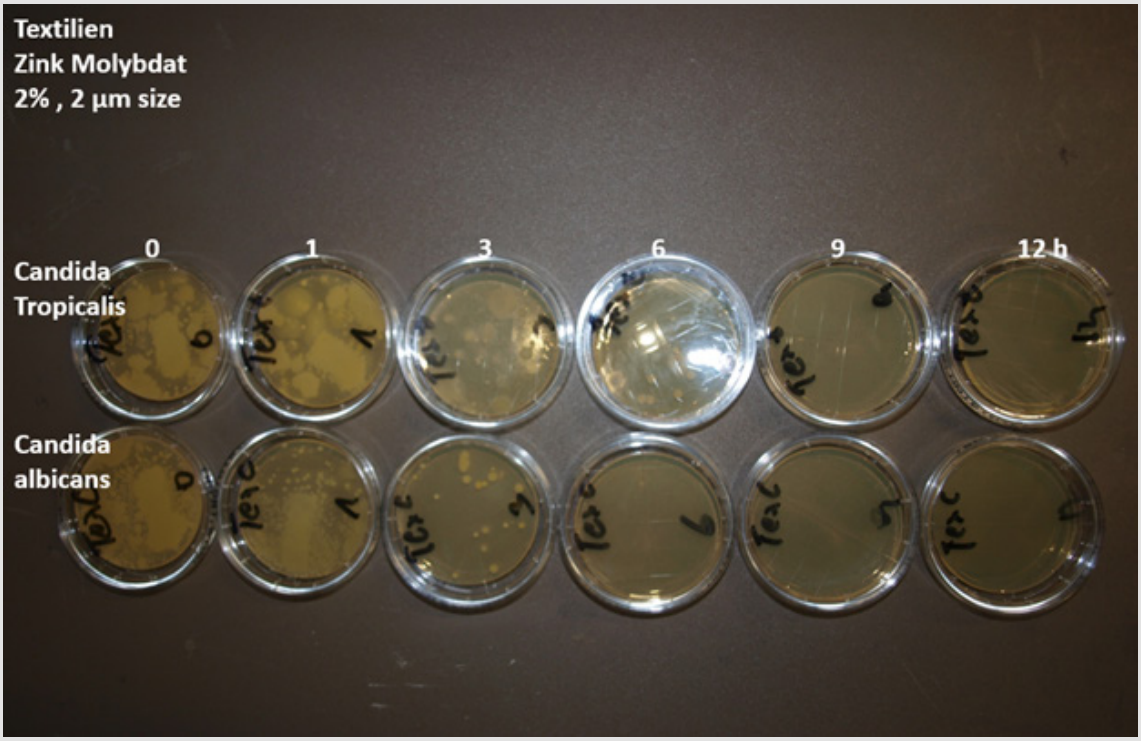

Figure 9.

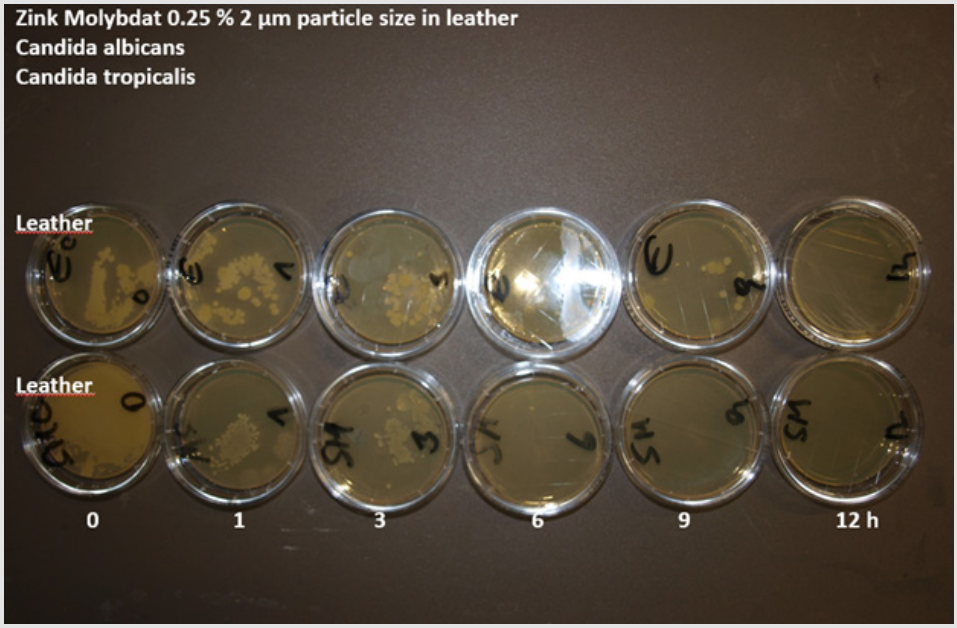

Figure 10.

The topography of the surface underlying the biofilm does not necessarily influence the quantity of the biofilm itself again depending on the scale of feature size, but after cleaning, the substratum will retain cells in features which can regrow and reduce the time taken for the biofilm to develop once more. The technology has been applied to a variety of surfaces either by incorporation of the additive into various polymers (Polyethylene, polypropylene, thermoplastic polyurethane, polystyrol etc.) or by application of the additives into various coatings like melamine resin, liquid polyurethane, liquid silicone or gloss paints. Important [167]. 0.2 µm particles sizes can be achieved by thermal fractioring with intact crystal structures which are mandatory for the antimicrobial activity [168]. Coating of Hospital furniture with various coatings containing 0.2 µm particle size. Addition of 3% zinc molybdate: S. aureus ATCC 25923 inoculum size 109 CFU/ml. to paints [169]. Gloss paints: 0.25% Zinc Molybdate, S. aureus ATCC 25923 (Figures 6-10). Results of various samples with different basic materials e.g., in gloss paints, coatings, leather, textiles with JIS 25923 testing. This technology has been submitted under the special application as *in situ* generated biocides in due time at the BPR of the EU. This technology meets the requirements of an *in situ* generated biocide and is legitimately on the market, approved by the BPR of the EU All other applications have to be submitted as single products.

This is time consuming /5 years +) and expensive (5 Mio € +). The BPR necessary for placement on the market is the pinhole for virtually all other technologies. Four additives are available as *in situ* generated biocides with important additional properties:

a) Molybdenum oxide. Molybdenum is incorporated in thermoplastic polyurethane in use for antimicrobial ECG lead wires. The antimicrobial activity has been documented: Immersion in 109 CFU/ml for 6 hours shows no growth at the surface after application on blood agar plates. The duration of the antimicrobial activity is more than 20 years. The results of numerous external investigations are available. Additional application in push buttons, artificial leather with a blue grayish appearance has been endowed with molybdenum oxide. The advantage Molybdenum oxide is an inexpensive additive, readily available in unlimited quantities. Transparent coatings are available with particle sizes of the additives e.g., Zinc Molybdate of 025 µm (Lambda half).

b) 5% Oxygen deficient Tungsten blue oxide is in use for surfaces in permanent contact with water e.g., pipes faucets in hospitals. This can prevent the growth of legionella in faucets. The endowment of water heaters e.g., incorporated into enamel has also been investigated providing a permanent coating. Oxygen saturate tungsten yellow oxide shows moderate antimicrobial activity [168].

c) The incorporation of Molybdenum into the zinc oxide crystal lattice results in a highly active white or - with submicron particles - transparent coatinging or paint. Zinc Molybdate has the broadest application for hospital furniture, for leather, textiles or artificial leather in numerous applications in public transportation, for office furniture in contact with different customers. Antimicrobial activity is very broad including several viral pathogens like bird flu, swine flu, influenza, Herpes, Epstein Barr virus.

d) The incorporation of Molybdenum oxide into the tungsten crystal lattice provides additional antimicrobial features due to a strong zeta potential: The antimicrobial activity against bacterial pathogens is fast and includes fungi and molds (Aspergillus spp), many viral pathogens like hepatitis B, C, COVID 19. Investigations of the antimicrobial activity against COVID 19 has been performed by MSL laboratory: The test product received has achieved a 99.22% reduction of feline coronavirus under the conditions stipulated. Documents MSL Laboratories, Gollinrod UK [170-172]. There is also a strong activity against algae providing antifouling surfaces for marine vessels. All additives can be used as transparent coating or paint if applied as submicron particle sizes (lambda half) However great caution has to be exhibited to leave the delicate orthorhombic and monocline crystal structure intact. These particles with a size of 0.2 µm can be achieved by thermal fracturing [168,173].

Summary

This technology based in in siu generated biocides is a new, innovative and ambitious approach to eradicate microorganisms in a surface preventing the spread of frequently observed multi resistant microorganisms from a surface. In almost five million deaths, such an infection was at least partly responsible for the death. Report on Antibiotic resistance was thus seen as one of the most common causes of death worldwide. This link to a TV report on NTV documents the dramatic increase of resistant microorganisms in hospital surfaces. By comparison, an estimated 680,000 people died from HIV/AIDS in 2020, and 627,000 from malaria [6].

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