ISSN: 2469-4096

Research Article

Integrative Biomedical Sciences

Dramatic Increase of Multiresistant Microorganisms We are approaching the Postantibiotic Era?

Guggenbichler S[1], Fey T[2] and Guggenbichler JP[3*]

[1]Division für Gefäßchirurgie und endovaskuläre Chirurgie der Universitätsklinik für Herzchirurgie, Gefäßchirurgie und endovaskuläre Chirurgie, LKH, Müllner Hauptstraße 48, A-5020 Salzburg

[2]Department of Material Science and Engineering, Institute of Glass and Ceramics, Martensstr. 5, 91058 Erlangen, Germany

[3]Department or Pediatrics, University of Erlangen, Germany, AmiSTec GMbH und Co KG, Kössen, Austria

Rec date: Nov 25, 2019 Acc date: Jan 21, 2020 Pub date: Feb 1, 2020

***Correspondance**: J Peter Guggenbichler, Erlangen, Department or Pediatrics, AmiSTecGMbH und Co KG, Kössen, Austria, E-mail: Prof.guggenbichler@amistec.at

Abstract

Clinical environments provide an ideal reservoir for the growth, proliferation, and transmission of pathogenic organisms. Surfaces in hospitals e.g. hospital furniture, ECG lead wires and other cables, push buttons of infusion pumps, control knobs of ventilation machines, textiles as well as implantable biomaterials like central venous catheters, urologic catheters, endotracheal tubes are contaminated increasingly frequent with multiresistant microorganisms. These microorganisms are distributed by the hands of the nursing personnel throughout the hospital with serious, life threatening consequences. 1.8 million patients suffer from a nosociomial infection per year in Europe; approximately 180,000 deaths are attributed to these infections. The Centers for Disease Control (CDC) estimates that 2 million U.S. patients per year acquire a hospital-related infection. These infections cause 90,000 deaths each year and cost an average of $47,000 per patient to treat. The added cost to hospitals is $4.8 billion annually for extended care treatment. Microorganisms show an increasing rate of resistance against the majority of antibiotics including carbapenems as last available antibiotic. 700,000 deaths have been reported worldwide, 30 000 deaths alone in Europe during the last year due to infections where no effective antimicrobial substance was available. The use of disinfectants is ostensibly intended to remove/kill pathogens on surfaces. However studies have shown that more than one-half the time, surfaces are not adequately cleaned or are recontaminated within minutes. Much emphasis has been put therefore on hand disinfection. However there are also reports of the emergence of alcohol tolerant/insensitive microorganisms e.g., vancomycin resistant enterococci. This phenomenon has the potential to undermine the effectiveness of alcohol-based disinfectant standard precautions. The reason for this dramatic development of resistant microorganisms is still in debate. The indiscriminate use of antibiotics for viral infections is frequently incriminated, however this seems to have little impacton the occurrence of multiresistant hospital pathogens. In contrast there is increasing evidence that the widespread use of disinfectants is responsible: disinfectants - analogous to antibiotics - must be incorporated into the metabolism of microorganisms. This is inevitably associated with induction of resistance by transfer of resistance plasmids e.g. induction of efflux pumps. 7880 publications (Aug 2019) are available in the international literature which document the induction of resistance by disinfectants and 649 papers describe the cross resistance with antibiotics.

Introduction

Key facts documented by the World Health Organization

- Health care-associated infections, or infections acquired in health-care settings are the most frequent adverse event in health-care delivery

worldwide.

- Hundreds of millions of patients are affected by health care-associated infections worldwide each year, leading to significant mortality and financial losses for health systems.
- Of every 100 hospitalized patients at any given time, 7 in developed and 10 in developing countries will acquire at least one health care-associated infection. The endemic burden of health care-associated infection is also significantly higher in low- and middle-income than in high-income countries, in particular in patients admitted to intensive care units and in neonates.
- While urinary tract infection is the most frequent health care-associated infection in high-income countries, surgical site infection is the leading infection in settings with limited resources, affecting up to one-third of operated patients; this is up to nine times higher than in developed countries.
- In high-income countries, approximately 30% of patients in intensive care units (ICU) are affected by at least one health care-associated infection. In low- and middle-income countries the frequency of ICU-acquired infection is at least 2-3 fold higher than in high-income countries; device-associated infection densities are up to 13 times higher than in the USA.
- Newborns are at higher risk of acquiring health care-associated infection in developing countries, with infection rates three to 20 times higher than in high-income countries [1].

Healthcare-associated infections (HAI) and antimicrobial resistance (AMR) are among the most serious public health problems globally [2]. 1.8 million patients suffer from a nosocomial infection per year in Europe; approximately 180,000 deaths are attributed to these infections [3]. The Centers for Disease Control (CDC) estimates that 2 million patients per year in the U.S. acquire a hospital-related infection. These infections caused 90,000 deaths each year and cost an average of $47,000 per patient to treat. The added cost to hospitals is $ 4.8 billion annually for extended care treatment [4,5].

Microorganisms show an increasing rate of resistance against the majority of antibiotics including carbapenems as last available antibiotic which adds additional problems for therapy [6]. 700,000 deaths have been reported worldwide, 30 000 deaths alone in Europe during the last year due to infections where no effective antimicrobial substance was available [7,8].

A potentially catastrophic crisis regarding the uncontrolled and dramatic increase of multiresistant microorganisms has been prognosticated by the UN Interagency Coordination Group on Antimicrobial Resistance. The group warns: If nothing is done, infections due to microorganisms resistant to all available antibiotics can be responsible until 2050 for ten million deaths annually, mostly people in Africa and Asia [9].

A large proportion of these deaths are due to the most common multidrug-resistant bacteria, i.e., *Staphylococcus aureus*, *Enterobacteriaceae, Pseudomonas aeruginosa* for which the number of directly attributable deaths is currently estimated. In 2000, the US Centers for Disease Control and Prevention estimated the total costs of NIs to be in excess of 5 billion US $. In Germany, it is estimated that approximately 2.4 billion € are spent annually for treatment of these infections [10]. Inversely, antibiotic-resistant bacteria (AMR), including multidrug resistant strains, are not only responsible for HAI but are also responsible for infections in outpatients and found as part of the flora of healthy individuals, in pet animals and in the environment. They are also isolated from food-producing animals and from food products we well as in public transportation.

Contamination of hospital surfaces with resistant bacteria and fungi is of great concern. Microorganisms grow and reproduce on almost every surface, impacting the general hygiene, causing odors, discoloration and lowered mechanical properties [11]. Surfaces containing composite materials even support the growth of microorganisms and biofilm formation. Especially in healthcare and medical facilities serious consequences are observed by the widespread contamination of surfaces [12,13]. Contamination includes implantable biomaterials with external and intraluminal contamination of short and long term central venous catheters. The contamination occurs during exchange of infusions at the luer lock [14]. Also transurethral catheters and endotracheal tubes, but also hospital furniture, ECG lead wires, wash basins, bed pans, hospital beds, trays, chairs, door handles, computer keyboards, telephones and a plethora of other plastic items e.g. push buttons of infusion pumps, turn knobs of mechanical ventilators are frequently touched by the personnel spreading the microorganisms throughout the hospital [15-17]. Contaminated surfaces are also of concern in public transportation, trains and trolleys, buses, cruise ships, airplanes and airports and

also in food production and distribution. Car sharing and rental cars are considered areas of considerable concern for acquisition of resistant and potentially pathogenic microorganisms - germfree cockpits are therefore in the focus of car makers for car sharing [18-20].

The vast majority of deaths due to infectious pathogens, resistant against all available antibiotics, affect hospitalized patients followed by patients in nursing homes.This situation threatens not only patient care, but also public health, agriculture, economic and national security and economic growth. These germs will however be distributed worldwide. Damage to the economy could cause problems comparable to the global financial crisis in 2008 and 2009. In addition resistant microorganisms could throw 24 millionen people until 2030into extreme poverty, reports Prof. Dr. J. Walker, a former Nobel Prize laureate [7,21]. A recent report released by the World Health Organization (WHO) indicates that the decreasing effectiveness of antibiotics and disinfectants signals the postantibiotic era. This situation threatens our presently practiced medicine. Resistant microorganisms are now recognized as a global health problem and an upcoming major crisis in clinical medicine. This issue has been escalated by major world health organizations to one of the top health challenges facing the 21st century [22]. Immediate coordinated and ambitions countermeasures are immediately required.

Resistance

There is overwhelming evidence of microorganisms developing resistance to chemical disinfectants commonly used in the clinic. They also show cross resistance with antibiotics especially since the mechanisms-of-action between antibiotic resistance and resistance to disinfectants is similar [23].

Overuse and misuse of antibiotics are considered generally as one reason for the rise in antibiotic resistance. The lack of attention to pharmacokinetic andpharmacodynamic properties of antimicrobial substances as well as the use of antibiotics with incomplete bioavailability, biliary elimination and long halflifes with subinhibitory concentrations over a prolonged period of time contribute more to survival conditions of microorganisms in community medicine. However, this has virtually no impact on the emergence of multiresistant hospital pathogens.

Biocides are critical components of intervention strategies used in clinical medicine for preventing the dissemination of nosocomial infections. The widespread use of antiseptic and disinfectant products has raised great concern on the development of microbial resistance, in particular cross-resistance with antibiotics [24]. Biocides are also used in community environments for personal hygiene and to prevent cross-contamination with foodborne pathogens. In vitro studies show that the ostensible use of disinfectants results in reduced susceptibility to biocides and antibiotics by intrinsic or acquired mechanisms of resistance [25]. Disinfectants - similar to antibiotics - must be incorporated into the metabolism of microorganisms, the induction of resistance by disinfectants is potentially inevitable.

The broad application of chemical disinfectants used as a standard infection control strategy requires an understanding of the development of antimicrobial resistance in bacteria following a biocide exposure. Resistance of microorganisms to disinfectants has now been documented in more than 7900 publications in the international literature. The evidence of the occurrence of bacterial resistance and its mechanisms of action as well as a debate how to measure bacterial resistance to biocides is of crucial importance. If disinfection resistance occurs but remains unrecognised by the hospital staff, inadequate disinfection of surfaces may lead to ineffective eradication of infectious microorganisms, resulting in serious consequences such as uncontrolled spread of multidrug resistant microorganisms with outbreaks.

The current sterilisation and disinfection regimens do not meet the standard anymore and select multiresistant strains including the most formidable carbapenem resistant Klebsiella pneumoniae (CRKP) present in the hospital environment: This is an urgent problem that needs to be addressed in each individual hospital. As we cannot rely on adequate eradication of microorganisms by disinfectants highest emphasis is now put on adequate hand hygiene measures [26]. Over recent years, researchers have noted a steady rise in the number of serious infections caused by one particular drug-resistant bacterium - *Enterococcus faecium*. Despite the wide use of alcohol-based disinfectants, *E. faecium* is now a leading cause of hospital-acquired infections. The ethanol tolerance of this strain appears to result in large part from adaptive and evolutionary changes in cell membrane composition. The development of alcohol-tolerant strains of *E. faecium* has the potential to undermine the effectiveness of alcohol-based disinfectant standard precautions [27-29]. This is confirmed by a large outbreak involving 230 patients in a Swiss hospital in 2018 [30].

Eliminating bacteria from the hospital environment is especially pertinent for *K. pneumoniae* or Vancomycin resistant *Enterococci*, as these bacteria are easily spread via contact with surfaces on which they have been shown to survive for a prolonged period of time, sometimes >30 months [31]. Carbapenem-resistant *Klebsiella pneumoniae* infections have become an independent risk factor for in-hospital death and strategies to prevent initial infection by eliminating or at least reducing the presence of these bacteria in the clinical environment is of critical importance and should be given high priority [32]. The finding that admission to a room previously occupied by a patient infected with a hospital pathogen increases the risk of acquiring that pathogen and the combination with intervention studies provides the most powerful evidence that contaminated surfaces contribute to transmission [15]. Much emphasis has therefore to be payed to improve surface decontamination.

Microorganisms have adapted to biocide exposure by acquiring plasmids and transposons that confer biocide resistance, the same survival strategies to disseminate acquired mechanisms of resistance to biocides as they have for resistance to antibiotics [33]. *qacE* and *qacΔE* were isolated from a class I integron in the R751 plasmid, which was first isolated from *K. pneumoniae*. Both genes mediate resistance by a proton pump, and both confer bacterial resistance to quaternary ammonium disinfectants (e.g. benzalkonium bromide, benzalkonium chloride and domiphen bromide), biguanide compounds (such as chlorhexidine) and hydrazones [34]. This efflux pumps have a wide range of substrates and can discharge acridine orange, crystal violet, ethidium bromide and many other agents including virtually all antibiotics from the bacterial cell.

There has been previous precedence to support that drug-resistant bacterial strains with co-resistance to a particular disinfectant could have a survival advantage in hospital settings, potentially leading to an outbreak [35]. Pan-resistant pathogenic CRKP strains contained various drug-resistance genes and exhibited relatively high resistance to ethyl alcohol, chlorhexidine acetate and iodophor [25]. Monitoring the drug-resistance rates of CRKP strains displaying disinfectant resistance may facilitate appropriate and effective sterilisation and thus preventing the spread of these pan-resistant strains [36]. Energy-driven drug efflux systems are increasingly recognized as mechanisms of antibiotic resistance [37]. Chromosomally located or acquired by bacteria, they can either be activated by environmental signals or by a mutation in a regulatory gene. Two major categories exist: those systems energized by proton motive force and those dependent on ATP. The pumps may have limited or broad substrates, the so-called multiple drug resistance pumps, which themselves form a number of related families. The multiple antibiotic resistance (mar) locus and mar regulon in Escherichia coli and other members of the Enterobacteriaceae is a paradigm for a generalized response locus leading to increased expression of efflux pumps [37]. One such pump, the AcrAB pump extrudes biocides such as triclosan, chlorhexidine and quaternary ammonium compounds as well as multiple antibiotics. In Pseudomonas aeruginosa, a number of multidrug efflux pumps export a broad range of substrates [38].

Indeed, one study showed that MRSA carrying this genotype persisted in patients after chlorhexidine treatment, and another showed that the MBC of the *qacA/B*-containing MRSA for chlorhexidine was three times higher compared to other MRSA strains. Interestingly, it has been observed that a CRKP (Carbapenem resistant Klebsiellapnumoniae) population had the highest resistance rates against chlorhexidine (78%), which is consistent with previous reports showing high MIC values for chlorhexidine against MRSA. In addition the results of various studies showed that clinically isolated CRKP were highly resistant not only to anti-infective drugs but also to many of the disinfectants commonly used in the clinic [39]. Indeed, the resistance rate of the CRKP strains to the common disinfectants chlorhexidine acetate, iodophor, iodine tincture, benzalkonium bromide, glutaraldehyde and ethyl alcohol was 78, 74, 67, 63, 59 and 52%, respectively.

The evolution of resistance against antibiotics and desinfactants during the last decade is now occurring at an alarming rate.These data serve to alert clinicians that the use of any one of the disinfectants listed above may not effectively and reliably eliminate all CRKP strains from the environment, which may threaten patient prognosis and safety, and even promote the spread of such pathogens in endemic areas and within hospitals. These findings underscore the importance of consistently being aware of the types of strains present in an individual clinic over time as well as monitoring the antimicrobial activity of disinfectants that are most effective against the specific strains. Neglecting this type of monitoring may lead to more serious consequences as bacteria acquire more drug-resistant genes or become tolerant to disinfectants used in hospital settings.

Therefore, disinfectants to which bacteria have good sensitivity should be selected specifically for the pan-resistant strains found within a particular environment, which requires clinicians to identify which strains are present in their own hospital and which disinfectants are effective against these specific strains. Moreover, an appropriate extension of disinfection time and even the combined use of a variety of disinfectants may improve the effectiveness of disinfection, and comprehensive measures should be adopted, especially in strengthening hand hygiene before and after contact with infected patients or contaminated materials.

Disinfection of surfaces is therefore not constructive as ample evidence exists for tolerance of microorganisms to sublethal levels of various disinfectants e.g. quaternary ammonium compounds (QAC) i.e., benzalkonium chloride as well as chlorhexidine, hexadecylpyridinium and cetrimide. The resistance of QAC based disinfectants to antibiotics is conferred by the resistance determinants qacH and bcrABC. The presence and distribution of these genes have been anticipated to assume a role in the survival and growth of various microorganisms. It has been described that disinfectants (e.g. benzalkonium chloride) even enhance the growth of Listeria monocytogenes in the food industry [40].

Biofilm Formation

A particularly critical aspect of microbial growth on surfaces is biofilm formation. Microorganisms universally attach to surfaces and produce extracellular polysaccharides, resulting in the formation of a biofilm. A biofilm is a dense accumulation of bacteria and can be up to 150 µm thick. The bacteria in a biofilm protect themselves against external influences by various mechanisms. For instance, they expel sticky polysaccharides that help them to attach to surfaces and retain ambient conditions. Biofilms can form on various surfaces such as metals, ceramics and glass. Microorganisms on polymer surfaces are particularly prone to biofilm formation, as several polymers themselves or contained additives provide direct nutrition for bacteria. About 80% of human infections are caused by biofilm-associated microorganisms which are insensitive to antimicrobial agents i.e., antibiotics as well as disinfectants and the potential for these organisms to cause infections in patients e.g. with implantable biomaterials still prevails [41]. The lack of nutrients in a biofilm results in most bacteria results in so called "hibernation" with exceedingly low external metabolic activities.

Microorganisms in biofilms are difficult to eradicate with antibiotics and disinfectants and may even survive harsh cleaning and disinfection procedures. The mechanisms responsible in biofilm resistance to disinfectants are multifactorial. Until the last decade, it was assumed that biofilms conferred antibacterial resistance due to the density of their polysaccharide chains inhibiting diffusion of antibiotics and disinfectants [42]. However, it was demonstrated that antibiotics do in fact diffuse through biofilms well. Although the precise origin of such resistance remains still in debate, different studies have shown that it is a multifactorial process involving the spatial organization of microorganisms in the biofilm [43]. A relevant issue for resistant microorganisms in a biofilm is hibernation i.e., microorganisms don't take up foreign materials e.g. antibiotics and disinfectants from the outside. It has been demonstrated that in thick *P. aeruginosa* biofilms, cells are physiologically distinct spatially, with cells deep in the biofilm in a viable but antibiotic-tolerant slow-growth state. Disinfectants as well as antibiotics are of limited success against microorganisms embedded in biofilms and currently pose a difficult and complicated challenge for microbiologists and clinicians [44]. Antibiotic treatment alone is inadequate to overcome biofilm infections.

Distribution

For many decades, antibiotics and disinfectants were considered an effective weapon against infectious microorganisms. The evolution of resistance against antibiotics but also against disinfectants during the last decade is now occurring at an alarming rate and is outpacing the development of new countermeasures capable of thwarting infections in humans. Microorganisms on these surfaces contaminate the hands of the personnel and transfer these frequently multi-resistant microorganisms from inanimate surfaces to patients and vice versa.There is compelling evidence from modeling of transmission routes, microbiologic studies, observational epidemiology studies, intervention studies, and outbreak reports that contaminated surfaces contribute to the transmission of hospital pathogens [15,16]. A microbial burden of > 8000 CFU on a 100 cm^2 surface is associated with an incidence of 21% of a hospital acquired infection [45]. In reality we see 10 - 1000 time higher inoculum sizes.

Much emphasis has been paid therefore to implement proper hand hygiene although this poses substantial problems. The personnel would have to wash/disinfect their hands approximately 60 - 80 times in an 8 hour shift with an alcoholic disinfectant [12].

Adequate hand hygiene takes at least 2 hours (60 x 2 minutes) in an 8 hours shift per person which is lost for patient care. All or nothing - If one person does not comply the entire construct is invalid.

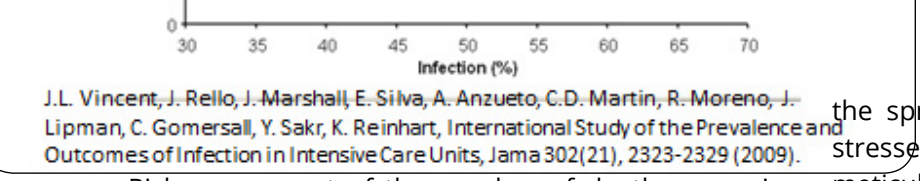
Clinical Infections

Healthcare associated infections due to antibiotic and disinfectant - resistant bacteria fail to respond to conventional therapy, resulting in greater risk of death and higher costs. Carbapenem-resistant Enterobacteriaceae (CRE) may be the most serious contemporary antibiotic resistance threat because of the number of different resistance mechanisms [32]. As a consequence nosocomial infections (NIs) or Hospital acquired infections (HAI) constitute an important world-wide health problem with high morbidity and mortality rates as well as economic consequences. NIs has become especially prominent in intensive care units (ICUs), where the incidence is two to five times greater than in the general in patient population. The causes of the increased risk for NIs in ICUs include the growing complexity of ICUs, the impaired host defense mechanisms of patients, invasive monitoring and procedures, exposure to multiple antibiotics and colonization by resistant microorganisms.

According to the European Center for Disease Prevention and Control, more than 3.2 million patients are estimated to acquire a healthcare-associated infection every year in European acute care hospitals [46]. Based on a conservative estimate, 10% of the European population is hospitalized each year. Thereof, it is assumed that 5% (3.8% on a general ward, 15.3% in intensive care units) acquire at least one nosocomial infection (NI). Based on these figures, it can be estimated that a NI in Europe affects some 1.75 million hospitalized patients annually. Assuming a conservative 10% attributable mortality rate, this equals to a minimum of 175,000 deaths every year [3]. These figures do not include the disabilities caused by NIs, the decrease of healthy life expectancy, the impact on the loss of productivity due to early death or chronic illness. In addition there are - besides unacceptably high numbers of affected patients and deaths - annual costs reaching into several billions of dollars. An excess length of stay (mean, 10 d; median, 5 d; p = 0.007) and increased direct costs (mean difference, $34,508; p = 0.008) have been described. In 2000, the US Centers for Disease Control and Prevention estimated the total costs of NIs to be in excess of 5 billion US $. In Germany, it is estimated that approximately 2.4 billion € are spent annually for treatment of these infections [47]. The costs of treatment of nosocomial infections as a complication of the underlying illness - in accordance with the DRG - not compensated by insurance.

Publications on the incidence and mortality of health care associated infections are scarce. An extended "Prevalence of Infection in Intensive Care (EPIC II) study" was performed about the global epidemiology of such infections. Demographic, physiological, bacteriological, therapeutic and outcome data were collected from 14,414 patients in 1265 participating ICUs from 75 countries on the study day. Analyses focused on the data from the 13,796 adult (>18 years) patients. On the day of the study, 7087 of 13,796 patients (51%) were considered infected; 9084 (71%) were receiving antibiotics. The ICU mortality rate of infected patients was more than twice that of non infected patients (25% [1688/6659] vs 11% [682/6352], respectively; P < .001), as was the hospital mortality rate

(33% [2201/6659] vs 15% [942/6352], respectively [48].



J.L. Vincent, J. Rello, J. Marshall, E. Silva, A. Anzueto, C.D. Martin, R. Moreno, J. Lipman, C. Gomersall, Y. Sakr, K. Reinhart, International Study of the Prevalence and Outcomes of Infection in Intensive Care Units, Jama 302(21), 2323-2329 (2009).

Risk assessment of the number of deaths occurring as a direct consequence of these infections has been estimated by the German society of Hospital hygiene: Taking the risk of death from a hospital acquired infection for the whole population in account a number of 1:2700 persons has been estimated. In contrast the risk of death from a car accident has been estimated with 1: 5000 which is substantially lower [49].

Appropriate use of antibiotics is important. There is increasing evidence to suggest that the use of appropriate and early antibiotics improves morbidity and mortality. Antibiotics should be administered at the right dose and for the appropriate duration. Daily ICU ward rounds with the microbiologist can lead to rational use of antibiotics tailored to benefit individual patients. Antibiotic-resistant bacteria prolong hospitalization, increase the risk of death, and require treatment with toxic and expensive antibiotics. Empirical use of broad spectrum antibiotics are often necessary as laboratory results are often not available for 48 h after the samples are sent to the laboratory for culture. Appropriate specimens include blood, urine, sputum, bronchoalveolar lavage, pus and wound swabs. Blood cultures are only positive for pathogen in one third of cases.

De-escalation involves early initiation of broad-spectrum antibiotic therapy in patients with suspected sepsis without the availability of microbiology results. The increase in antibiotic resistant pathogens such as MRSA has led some investigators to suggest broader antibiotic coverage by adding a glycopeptide to carbapenem as the initial empirical therapy. This aggressive empirical regimen is continued for 24-48 h by which time laboratory tests have confirmed the causative organisms and sensitivities. If the microorganisms have been identified and the susceptibility of antibiotics is available de-escalation i.e., the switch to a targeted small spectrum antibiotics can be implemented.

Health care-associated infections, which are widely seen as preventable and often caused by unsatisfactory hospital conditions or human error, are costing health care facilities more than $30 billion annually and are, more importantly, claiming at least 100,000 lives per year that's 271 lives each day. To combat the alarming 5% of all hospital admissions that result in readmission due to conditions acquired in health care facilities, all staff and contractors should be well educated on how to minimize the spread of fatal infections [50,51]. The issue most stressed for prevention of hospital acquired infections is meticulous hand hygiene. This however would require 50 - 80 times hand washing and disinfection. One problem remains the definition of "resistance" and how to measure resistance to a biocide. This has yet to be addressed globally, although the measurement of resistance is becoming more relevant, with regulators both in Europe and in the United States demanding that manufacturers provide evidence that their biocidal products will not impact on bacterial resistance. Alongside *in vitro* evidence of potential antimicrobial cross-resistance following biocide exposure, our understanding of the mechanisms of bacterial resistance and, more recently, our understanding of the effect of biocides to induce a mechanism(s) of resistance in bacteria has improved [52,53].

In order to block the spread of multiresistant microorganisms in the hospital, cutting off the transmission route is a particularly important strategy along with actively treating the infective source based on the sensitivity of the particular strain, which is determined by characterising the strain for drug resistance.

References

1. Key facts documented by the world health organization. https://www.who.int/gpsc/country_work/gpsc_ccisc_fact_sheet_en.pdf
2. National nosocomial infections surveillance (Nnis) system report, data summary from january 1992 to june 2002, issued august 2002. *American Journal of Infection Control*. 2002; 30(8):458-475. DOI: https://dx.doi.org/10.1067/mic.2002.130032
3. ESCMID (Europe) 2005: 1.75 million patients affected, 175 000 deaths/year. Consensus conference ESCMID, Arlanda, 2015.
4. Klevens RM, Edwards JR, Richards CL, et al. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. *Public Health Rep*. 2007; 122(2):160-166. DOI: https://dx.doi.org/10.1177/003335490712200205
5. Scott RD. The direct medical costs of healthcare-associated infections in US hospitals and the benefits of prevention.CDC. 2008. http://www.cdc.gov/ncidod/dhqp/pdf/Scott_CostPaper.pdf
6. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. *Perspect Medicin Chem*. 2014; 6:25-64. DOI: https://dx.doi.org/10.4137/PMC.S14459

7. Report: Prof.Dr. John Walker at the yearly nobellaureate meeting Lindau Germany August 2018. Spiegel online 2018
8. Multiresistente Mikroorganismen, Süddeutsche Zeitung 23./24. Februar 2018
9. Antibiotikaresistenzen: Expertengruppe der Vereinten Nationen schlägt Alarm. Deutsches Ärzteblatt. 2019; Available from: https://www.aerzteblatt.de/nachrichten/102758/Antibiotikaresistenzen-Expertengruppe-der-Vereinten-Nationen-schlaegt-Alarm
10. SepNet Critical Care Trials Group. Incidence of severe sepsis and septic shock in German intensive care units: the prospective, multicentre INSEP study. *Intensive Care Med*. 2016; 42(12):1980-1989. DOI: https://dx.doi.org/10.1007/s00134-016-4504-3
11. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health*. 2015; 109(7):309-318. DOI: https://dx.doi.org/10.1179/2047773215Y.0000000030
12. FDA 2000. FDA Task Force on Antimicrobial Resistance: key recommendations and report, Washington, DC. FDA, Washington, DC. 2000; Available from: https://www.researchgate.net/publication/239857970_FDA_Task_Force_on_Antimicrobial_Resistance_Key_Recommendations_and_Report
13. Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol Rev*. 2011; 35(5):736-755. DOI: https://dx.doi.org/10.1111/j.1574-6976.2011.00268.x
14. Guggenbichler JP, Assadian O, Boeswald M, et al. Incidence and clinical implication of nosocomial infections associated with implantable biomaterials - catheters, ventilator-associated pneumonia, urinary tract infections. *GMS Krankenhhyg Interdiszip*. 2011; 6(1):Doc18. DOI: https://dx.doi.org/10.3205/dgkh000175
15. Russotto V, Cortegiani A, Raineri SM, et al. Bacterial contamination of inanimate surfaces and equipment in the intensive care unit. *J Intensive Care*. 2015; 3:54. DOI: https://dx.doi.org/10.1186/s40560-015-0120-5
16. Oumokhtar B, Lalami AEO, Benaicha N, et al. Environmental surfaces in healthcare setting: a great potential risk of pathogens transmission. 2017. http://www.biomedres.info/abstract/environmental-surfaces-in-healthcare-setting-a-great-potential-risk-of-pathogens-transmission-6779.html
17. Smiseth OA, Steg PG, Sipido K, et al. Antibiotic resistant pathogens found on 77 % of ECG lead wires: News from the european society of cardiology congress in vienna, august 30 to september 3, 2003. *J Am Coll Cardiol*. 2004; 43(4):691-697. DOI: https://dx.doi.org/10.1016/j.jacc.2003.11.025
18. Boyce JM. Environmental contamination makes an important contribution to hospital infection. *J Hosp Infect*. 2007; 65 Suppl 2:50-54. DOI: https://dx.doi.org/10.1016/S0195-6701(07)60015-2
19. McManus CJ, Kelley ST. Molecular survey of aeroplane bacterial contamination. *J Appl Microbiol*. 2005; 99(3):502-508. DOI: https://dx.doi.org/10.1111/j.1365-2672.2005.02651.x
20. Stephenson RE, Gutierrez D, Peters C, et al. Elucidation of bacteria found in car interiors and strategies to reduce the presence of potential pathogens. *Biofouling*. 2014; 30(3):337-346. DOI: https://dx.doi.org/10.1080/08927014.2013.873418
21. Jarvis WR. Selected aspects of the socioeconomic impact of nosocomial infections: morbidity, mortality, cost, and prevention. *Infect Control Hosp Epidemiol*. 1996; 17(8):552-557. DOI: https://dx.doi.org/10.1086/647371
22. Dame Sally Davies: Warning over “post-antibiotic apocalypse”. YouTube October 2019.
23. Hoff JC, Akin EW. Microbial resistance to disinfectants: mechanisms and significance. *Environ Health Perspect*. 1986; 69: 7-13. PMID: https://www.ncbi.nlm.nih.gov/pubmed/3816738
24. CDC 2010. Get smart: know when antibiotics work. Centers for Disease Control, Atlanta, GA: www.cdc.gov/Features/GetSmart
25. Zhang Y, Gu AZ, He M, et al. Subinhibitory concentrations of disinfectants promote the horizontal transfer of multidrug resistance genes within and across genera. *Environ Sci Technol*. 2017; 51(1):570-580. DOI: https://dx.doi.org/10.1021/acs.est.6b03132
26. WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge *Clean Care Is Safer Care*. Available from: https://www.who.int/gpsc/5may/tools/who_guidelines-handhygiene_summary.pdf
27. Ingram LO. Ethanol tolerance in bacteria. *Crit Rev Biotechnol*. 1990; 9(4):305-319. DOI: https://dx.doi.org/10.3109/07388558909036741
28. Haft RJF, Keating DH, Schwaegler T, et al. Correcting direct effects of ethanol on translation

and transcription machinery confers ethanol tolerance in bacteria. *Proc Natl Acad Sci USA*. 2014; 111(25):E2576-2585. DOI: https://dx.doi.org/10.1073/pnas.1401853111

29. Lusta KA, Leonovitch OA, Tolstorukov II, et al. Constitutive biosynthesis and localization of alcohol oxidase in the ethanol-insensitive catabolite repression mutant ecr1 of the yeast Pichia methanolica. *Biochemistry Mosc*. 2000; 65(5):604-608. PMID: https://www.ncbi.nlm.nih.gov/pubmed/10851039
30. https://www.srf.ch/news/p/schweizweit-groesster-fall-eines-multiresistenten-spitalkeims.
31. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infectious Diseases*. 2006; 6(1):130. DOI: https://dx.doi.org/10.1186/1471-2334-6-130
32. Guo W, Shan K, Xu B, Li J. Determining the resistance of carbapenem-resistant Klebsiella pneumoniae to common disinfectants and elucidating the underlying resistance mechanisms. *Pathog Glob Health*. 2015; 109(4):184-92. PMID: https://www.ncbi.nlm.nih.gov/pubmed/26184804
33. Abuzaid A, Hamouda A, Amyes SG. Klebsiella pneumoniae susceptibility to biocides and its association with cepA, qacΔE and qacE efflux pump genes and antibiotic resistance. *J Hosp Infect*. 2012; 81(2):87-91. PMID: https://www.ncbi.nlm.nih.gov/pubmed/22498639
34. Duran N, Temiz M, Duran GG, Eryılmaz N, Jenedi K.Relationship between the resistance genes to quaternary ammonium compounds and antibiotic resistance in staphylococci isolated from surgical site infections. *Med Sci Monit*. 2014; 20:544-550. PMID: https://www.ncbi.nlm.nih.gov/pubmed/24691183
35. Longtin J, Seah C, Siebert K, et al. Distribution of antiseptic resistance genes qacA, qacB, and smr in methicillin-resistant Staphylococcus aureus isolated in Toronto, Canada, from 2005 to 2009. *Antimicrob Agents Chemother*. 2011; 55(6):2999-3001. DOI: https://dx.doi.org/10.1128/AAC.01707-10
36. Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. *J Appl Microbiol*. 2002; 92 Suppl:65S-71S. PMID: https://www.ncbi.nlm.nih.gov/pubmed/12000614
37. Soto SM. Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. *Virulence*. 2013; 4(3): 223–229. PMID: https://www.ncbi.nlm.nih.gov/pubmed/23380871
38. Nolivos S, Cayron J, Dedieu A, et al. Role of AcrAB-TolC multidrug efflux pump in drug-resistance acquisition by plasmid transfer. *Science*. 2019; 364(6442): 778-782. DOI: http://10.0.4.102/science.aav6390
39. McGann P, Kwak YI, Summers A, et al. Detection of qacA/B in clinical isolates of methicillin-resistant Staphylococcus aureus from a regional healthcare network in the eastern United States. *Infect Control Hosp Epidemiol*. 2011; 32(11):1116-1119. PMID: https://www.ncbi.nlm.nih.gov/pubmed/22011540
40. Møretrø T, Schirmer BCT, Heir E, et al. Tolerance to quaternary ammonium compound disinfectants may enhance growth of Listeria monocytogenes in the food industry. *Int J Food Microbiol.* 2017; 241:215-224. DOI: https://dx.doi.org/10.1016/j.ijfoodmicro.2016.10.025
41. Dubois-Brissonnet F, Trotier E, Briandet R. The biofilm lifestyle involves an increase in bacterial membrane saturated fatty acids. *Front Microbiol*. 2016; 7:1673. DOI: https://dx.doi.org/10.3389/fmicb.2016.01673
42. Bridier A, Briandet R, Thomas V, et al. Resistance of bacterial biofilms to disinfectants: a review. *Biofouling*. 2011; 27(9):1017-1032. DOI: https://dx.doi.org/10.1080/08927014.2011.626899
43. Williamson KS, Richards LA, Perez-Osorio AC, et al. Heterogeneity in Pseudomonas aeruginosa biofilms includes expression of ribosome hibernation factors in the antibiotic-tolerant subpopulation and hypoxia-induced stress response in the metabolically active population. *J Bacteriol*. 2012; 194(8):2062-2073. DOI: https://dx.doi.org/10.1128/JB.00022-12
44. Costerton JW, Montanaro L, Arciola CR. Biofilm in implant infections: its production and regulation. *Int J Artif Organs*. 2005; 28(11):1062-1068. DOI: https://dx.doi.org/10.1177/039139880502801103
45. http://www.copper.com.au/copper/wcms/en/home/Infection-Control.pdf
46. Martin M, Zingg W, Hansen S, et al. Public reporting of healthcare-associated infection data in Europe. What are the views of infection prevention opinion leaders? *J Hosp Infect*. 2013; 83(2):94-98. PMID: https://www.ncbi.nlm.nih.gov/pubmed/23273963
47. Safdar N, Crnich CJ, Maki DG. Nosocomial infections in the intensive care unit associated with invasive medical devices. *Curr Infect Dis Rep*. 2001; 3(6):487-495. DOI: https://dx.doi.org/10.1007/s11908-001-0085-5
48. Vincent JL, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA*. 2009; 302(21):2323-2329.

DOI: https://dx.doi.org/10.1001/jama.2009.1754

49. Weber DJ, Rutala WA. Understanding and preventing transmission of healthcare-associated pathogens due to the contaminated hospital environment. *Infection Control & Hospital Epidemiology*. 2013; 34(5):449-452. DOI: https://dx.doi.org/10.1086/670223

50. https://www.krankenhaushygiene.de/informationen/hygiene-tipp/hygienetipp2015/557

51. Locci R, Peters G, Pulverer G. Microbial colonization of prosthetic devices. IV. Scanning electron microscopy of intravenous catheters invaded by yeasts. *Zentralbl Bakteriol Mikrobiol Hyg B*. 1981; 173(6):419-424. PMID: https://www.ncbi.nlm.nih.gov/pubmed/7034404

52. Otter JA, Yezli S, Salkeld JAG, et al. Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. *Am J Infect Control*. 2013; 41(5 Suppl):S6-11. DOI: https://dx.doi.org/10.1016/j.ajic.2012.12.004

53. Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. *J Appl Microbiol*. 2002; 92(Suppl):65S-71S. PMID: https://www.ncbi.nlm.nih.gov/pubmed/12000614