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Replication capacity as a basis for assessing the sensitivity of micro-organisms to disinfectant agents

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SUMMARY

Background: Interpretation of microbial tolerance and resistance to disinfectants has long been inconsistent, with heterogeneous definitions and no clinically meaningful threshold. We propose the concept of replication capacity after use (RCAU) as a practical endpoint to assess whether microbial survival after disinfectant exposure constitutes a clinically relevant phenomenon under recommended use conditions. RCAU is defined as the ability of micro-organisms to replicate after exposure at recommended application concentration and exposure time. A critical RCAU corresponds to failure of a standardized quantitative suspension test.

Methods: We reassessed published evidence across the most common disinfectant substances listed by the German Association for Applied Hygiene. Reported findings on survival, tolerance and resistance were re-evaluated against the RCAU definition, with particular attention to whether testing was performed using quantitative suspension methods at application concentration.

Results: No disinfectant group has demonstrated a critical RCAU under application conditions in standardized suspension testing. Reports of reduced susceptibility or microbial survival exist, but many were not based on suspension tests at use concentrations, making interpretation with respect to RCAU uncertain. Transient or reversible adaptations have been described, yet without evidence of a critical RCAU. Only triclosan and silver compounds show established resistance mechanisms, though even here no critical RCAU has been confirmed under standardized testing.

Conclusion: RCAU provides a transparent, use-condition-anchored framework to differentiate non-critical survival from clinically relevant resistance development. Applied across disinfectant classes, it shows that no critical failures have occurred at use concentrations, although many reported findings were not assessed by standardized suspension tests.

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1. Background

The pandemic spread of antibiotic resistance with its consequences for treatment failure is undisputed [1]. The question is therefore regularly raised as to whether tolerance or resistance to disinfectants and antiseptics is also possible. This would lead to a further restriction in the control of pathogens with serious consequences for infection prevention.

The terms tolerance and resistance are clearly defined in the evaluation of the clinical efficacy of usually very specifically effective antibiotics. Even though some research groups have recently attempted to develop new definitions for reduced susceptibility to biocides [2—4], it is recommended that the terms tolerance and resistance are not used when referring to the context of the efficacy of broadly multi-target disinfectants and antiseptics. In this paper, the Association for Applied Hygiene (VAH) presents a proposal for a new terminology that is useful for evaluating effectiveness and assesses the risk of reduced sensitivity to disinfectants and antiseptics based on the current state of knowledge.

Disinfectant surface cleaning or surface disinfection is intended to render surfaces that are inevitably contaminated with pathogens during patient care safe by killing or inactivating the pathogens as a pathogen reservoir, and it is also intended to limit or prevent the spread of pathogens during patient care and treatment [5]. The same applies to hand antisepsis [6]. Disinfection is an essential step in the reprocessing of medical devices in order to avoid putting patients at risk when the medical devices are subsequently used [7]. The aim of prophylactic antisepsis is to decontaminate skin, mucous membranes, eyes and wounds contaminated or colonized with pathogens, if necessary, also by local application in body cavities, to protect the affected person, to prevent the transfer of pathogens from contaminated or colonized to non-microbially colonized areas of the body or to implants, to normalize dysbiosis or to kill facultative pathogens after accidental contamination. The objective of therapeutic antisepsis is to kill micro-organisms or inactivate viruses on or in living tissue for the local treatment of infections [8]. The declared efficacy of the disinfectants or antiseptics, based on in-vitro and in-vivo tests carried out according to defined standards, is a prerequisite for the success of disinfection and antisepsis.

As antiseptic active ingredients are also used as combination partners in some disinfectants, e.g. polyhexanide in surface disinfectants and disinfectant cleaners, chlorhexidine digluconate (CHG), triclosan (TCS) and quaternary ammonium compounds (QACs) in hand antiseptics [9], and the latter three also in cosmetics for preservation [10], they are also taken into account in the following risk assessment in addition to disinfectants.

2. Adaptation possibilities of micro-organisms to changed living conditions

Micro-organisms are highly adapted to their respective natural habitats and in some cases have a high natural resistance to harmful environmental influences (tenacity). In addition, micro-organisms have different ways of defending themselves against newly occurring stress conditions such as those caused by disinfectants and antiseptics. They can also evade their antimicrobial effectiveness. Knowledge of these defence options is crucial to be able to counter them. The various options are explained in the following.

Due to different evolutionary adaptation strategies to their natural habitats, micro-organisms exhibit different intrinsic resistance to environmental stress factors, which is usually rooted in the chromosomal nuclear genome of the organisms (genetically manifested). Even for genetically unmodified wild-types of different species, different concentrations of biocides are needed to achieve the required inactivation of the organisms. In addition, a reduced sensitivity can occur in wild-type organisms due to a transition to an altered physiological status, usually induced by environmental factors (Figure 1). This has been demonstrated in particular for the formation of endospores or multicellular biofilms adhering to inanimate surfaces [11—15]. The persistence of micro-organisms in a dormant, viable but not culturable (VBNC) status is also associated with reduced susceptibility to the inactivating effect of disinfectants and antiseptics [16,17]. The evolutionary adaptation to stressful environmental conditions and the change in physiological status generally lead to increased robustness to various biocides and are therefore independent of the active substance.

Non-wild-types are organisms with newly acquired genetic changes (Figure 1), which lead to a phenotypic change in sensitivity to disinfectants and antiseptics and are usually selected by contact with these substances. In non-wild-type organisms, the genetic changes can result from (point) mutations in existing gene loci with changes to existing cellular structures or from the inclusion of new gene loci and expression of new structures [18—20]. The development of mutations in existing gene loci is induced and subsequently selected in particular by long-term or repeated exposure to subinhibitory concentrations of biocides [18,21,22]. The sensitivity can be gradually increased by multiple mutations [23]. Bacteria in particular, but also yeasts, have an ultra-short generation time compared with humans, which can be 10—60 min under optimal conditions [24], disproportionately higher possibilities for adapting to changing life circumstances. The probability of a spontaneous mutation as the cause of chromosomally induced reduced sensitivity is 10^{-9} to 10^{-11}/h [25]. As the entire population of organisms often does not exhibit mutations, wild-type or non-wild-type organisms with a lower sensitivity threshold can often reassert themselves in the population when the selection pressure is removed so that phenotypic reversion can occur [26,27].

In addition to the modification of existing structures, the genetic information for additional structures or metabolic pathways can be incorporated into micro-organisms via horizontal gene transfer and lead to reduced sensitivity. Horizontal gene transfer often takes place via mobile genetic elements (MGEs) such as plasmids, transposon insertion elements, etc. [28,29]. The uptake of MGEs usually leads to an irreversible

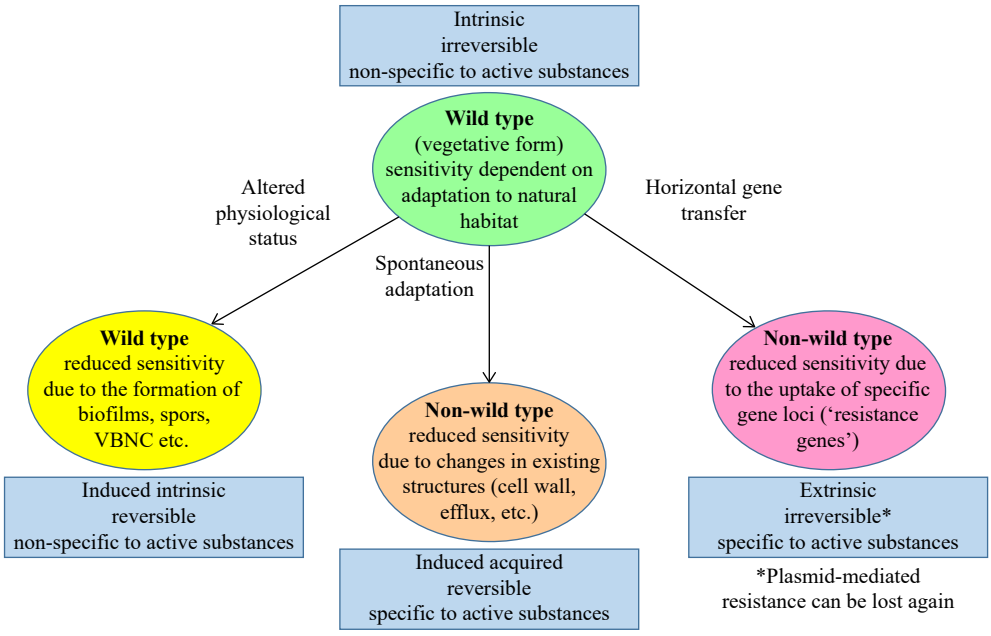

Figure 1. Distinction between genetic and non-genetic mechanisms in the development of bacterial strains with reduced sensitivity. VBNC, viable but not culturable.

phenotype as transposons and insertion elements are often anchored in the existing chromosome and the stability of plasmids is ensured by accompanying gene loci (e.g. toxin–antitoxin systems).

3. Phenotypic significance in relation to the application concentration

Disinfectants are applied at a defined concentration (application concentration) over a defined period (exposure time). Furthermore, the aim of disinfection is the inactivation of micro-organisms to a non-critical amount through a single application in the effective concentration and exposure time [30]. In contrast, treatment with antibiotics usually involves several doses over longer periods of time with variable concentrations of active substances at the site of infection [31,32]. In many cases, inhibiting the bacterial replication is sufficient for the therapeutic success. Therefore, the definitions of resistance and tolerance to antibiotics, which are based on the correlation of minimal inhibitory concentrations with clinical efficacy at the site of infection, are unsuitable in the context of disinfection.

Since disinfection measures are intended to reduce the number of remaining micro-organisms capable of replication to such an extent that there is no longer any significant risk of transmission from the surface, replication capacity after correct disinfection is a suitable correlate for assessing the effectiveness of disinfectants. Based on the concept presented by Kramer *et al.* [33], we therefore propose the new term 'replication capacity after use concentration (RCAU)' as an assessment criterion suitable for extended determinations of the RCAU.

RCAU can be determined *in vitro* using existing methods for testing disinfectants. The recently described microscale quantitative suspension test is particularly suitable for extended RCAU determination. It should be noted that the RCAU is determined according to the exposure time specified by the manufacturer of a product [34].

Definition

RCAU thus describes—in relation to a specific strain—the survivability of planktonic micro-organisms after exposure to a disinfectant under the recommended conditions of use (concentration and exposure time). Importantly, RCAU is distinct from a general postexposure replication capacity as the latter refers to an undefined exposure without specification of concentration or contact time. RCAU is a phenotypic and operational relevance criterion under recommended use conditions and is independent of predefined mechanistic or resistance-based classifications. In this context, RCAU reflects the ability of micro-organisms to survive the initial exposure under defined laboratory test conditions and to subsequently recover and resume replication after temporary stress. The concept of achieving a safe status of surfaces after disinfection resulted in the definition of a minimum reduction of the micro-organisms used in the test procedure, depending on the organism. Therefore, passing the test procedure does not require complete elimination of the test organisms used but allows the detection of replication-capable subpopulations of the organisms (persisters) if the overall reduction performance is sufficient. A retest of the persisters regularly results in sufficient reduction performance so that the occurrence of persisters is not associated with an adaptation of the tested organisms to the disinfectants. Therefore, a non-critical RCAU of individual persisters, which show sufficient overall reduction in a retest in standard test procedures, must be distinguished from a critical RCAU in which the overall reduction performance is no longer achieved in a retest.

For example, despite relatively low initial concentrations on surfaces, low amounts of replicable organisms can still be found in medical facilities even after adequate surface

disinfection [35,36]. For these organisms, a critical RCAU should only be assumed if they have subsequently been tested in a quantitative suspension test (micro or macro method) and show an insufficient lg-level reduction.

Due to the varying intrinsic resistance to environmental stress factors and the possibilities of altered physiological status in wild-type organisms, as well as the altered sensitivity to disinfectants due to mutation or horizontal gene transfer, different assessments of RCAU can be assumed for populations of micro-organisms (examples shown in Figure 2). For the care of patients, only those subpopulations that retain their ability to replicate after disinfection are relevant when disinfectants are used correctly. In the case of micro-organisms with very low intrinsic resistance (Figure 2A), a population with slightly

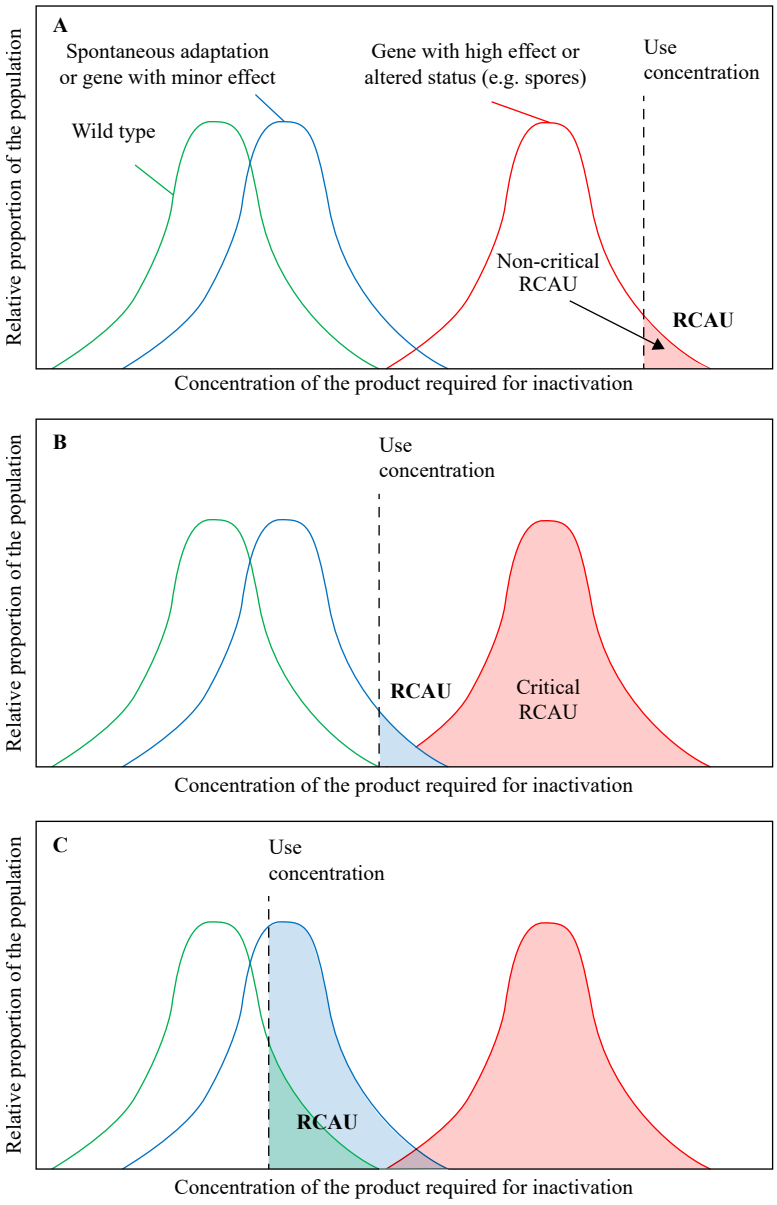

Figure 2. Schematic representation of the effects of genetic and non-genetic mechanisms in the development of bacterial strains with reduced sensitivity to biocides in relation to the application concentration and the RCAU. A: The application concentration is well above the sensitivity of the wild-type; neither spontaneous adaptations nor genetic changes in sensitivity lead to the survival of a relevant number of bacterial cells in the application concentration; there is no critical RCAU for any of the strains. B: The application concentration kills the entire wild-type population. Mechanisms with a minor effect cause reduced sensitivity in the blue strain, but the majority of the population is killed more reliably (5 lg reduction). Genetic changes with a strong effect mean that the majority of the population in the red strain is no longer killed. A critical RCAU exists for this strain only. C: The application concentration is already so close to the sensitivity of the wild-type that small parts of the population can survive, but sufficient killing kinetics with 5 lg levels are still achieved. In this case, both spontaneous adaptations and genetic changes can reduce sensitivity to such an extent that relevant parts of the population survive. There is a critical RCAU for both strains (blue and red). RCAU, replication capacity after use.

Table I
Assessment of the probability of critical RCAU and the development of resistance of frequently used active ingredients in disinfectants and antiseptics

Active ingredient group	Reproduction or survival in working concentration[a]	Derivation of a critical RCAU from results of a suspension test[b] possible	Reference	Probability of resistance
Aldehydes/aldehyde releasers	Possible (e.g. *M. massiliense)*	Yes, for application concentrations that are now obsolete	[44,45,47]	No evidence of genetically coded resistance to date
Alcohols	Not described to date	No	[49–51]	Unlikely
Halogens	Not described to date	No	[52]	Unlikely
Iodine-releasing compounds	Not described to date	No		Unlikely
Alkylamines/alkyl derivatives	Not described to date	No		No evidence of genetically coded resistance to date
Amphoteric surfactants	Not described to date	No	[55]	No evidence of genetically coded resistance to date
Glucoprotamines	Not described to date	No	[56]	No evidence of genetically coded resistance to date
Guanidines/guanidine derivatives	Possible (e. g. *A. xylosoxidans*, *S. marcescens*, *B. cepacian*)	No	[60–62,68]	No evidence of genetically coded resistance to date
Pyridine derivatives	Not described to date	No	[65]	No evidence of genetically coded resistance to date
QAC	Possible (e.g. *S. marcescens*, *Achromobacter species*)	No	[90]	No evidence of genetically coded resistance to date
Peracetic acid	Decreased sensitivity in biofilm for *K. pneumoniae*	No	[70–73]	Unlikely
Triclosan	Not described to date	No	[75–82]	Resistance mechanisms described; risk at sublethal dosage
Hexachlorophene	Not described to date	No	[83]	No evidence of genetically coded resistance to date
Acids (inorganic/organic) and bases	Not described to date	No	[84,88]	Unlikely
Heavy metal compounds/silver ions	Possible (e.g. *K. pneumoniae*, *Enterobacter cloacae*, *P. aeruginosa*	No	[89]	Possible due to plasmid resistance

QAC, quaternary ammonium compound; RCAU, replication capacity after use.
[a] With nosocomial infection as a possible consequence.
[b] e.g. EN 13727, VAH Method 9, or VAH Method 20.

reduced sensitivity will still be completely eliminated by a correct disinfection measure, and even in the case of changes with a high effect, only a very small proportion of the population will survive after the exposure time at the application concentration (non-critical RCAU). In the case of micro-organisms with medium intrinsic resistance (Figure 2B), a population with slightly reduced sensitivity will be largely eliminated and only a very small proportion of the population will be capable of replication after a disinfection process (non-critical RCAU). In micro-organisms with moderate intrinsic resistance (Figure 2B), a population with slightly reduced sensitivity is largely captured and only a very small proportion of the population will be capable of replication after a disinfection process (non-critical RCAU). However, in the case of changes with a high effect, the entire population may survive after the exposure time at the application concentration (critical RCAU). In micro-organisms with already high intrinsic resistance (Figure 2C), even a subpopulation of the wild-type population can survive a correct disinfection measure (critical RCAU).

4. Importance of the antimicrobial mode of action for altered phenotypes against disinfectants and antiseptics

With the exception of QAC, disinfectant agents destroy the cell as a whole non-specifically. In contrast, most antiseptic agents have targets in the bacterial cell due to their specific

mode of action comparable with antibiotics, e.g. damage to the cell membrane, inhibition of membrane transport processes or inactivation of metabolic steps. Reversible events include, for example, the initial release of intracellular potassium, which leads to a depletion of the membrane potential, to a reduction in ATP biosynthesis and subsequently to an interruption of transport and metabolic processes, inhibition of membrane transport processes up to the inhibition of replication. Continued exposure to the antiseptic eventually leads to irreversible damage including changes in cytosolic pH with disruption of enzymatic function and coagulation of intracellular material. If the cytoplasmic membrane is significantly damaged, cytoplasmic components of the cytoplasm including proteins, nucleotides, pentoses and other ions may be lost from the cell [37]. For antiseptic agents with a specific mechanism of action, the possibility of reduced sensitivity has been demonstrated in laboratory studies. However, the concentration increases demonstrated *in vitro* were far below the application concentration so that clinical efficacy is assured. However, in-vitro studies with bacteria adapted to the antiseptic agent have also shown reduced sensitivity to antibiotics, which could be due to common resistance mechanisms [38].

In contrast to antimicrobials with a specific mode of action, antimicrobials with a non-specific mode of action completely destroy the structure and thus the life processes of the cell. This is the case with alcohols, aldehydes, oxidants, peracetic acid (PAA), povidone-iodine (PVP-iodine) [39] and polyhexanide [40]. Thus polyhexanide forms nano-objects through the self-organization of biguanide groups, which can interact with the phospholipid membrane in a similar way to positively charged nanoparticles [41] or cell-penetrating peptides [42]. As a result, the bacterial cell cannot compensate for the drug attack metabolically or by changing the cell membrane. This means that no development of resistance is to be expected. QACs are an exception due to their specific mode of action [43].

5. Evaluation of antimicrobial agents regarding the risk of clinically relevant resistance development based on RCAU

Many publications mention the terms tolerance and resistance to disinfectants with different definitions. The following explanations describe the terms used in the original publications and evaluate the results with regard to the new term RCAU. A concise overview of all disinfectant classes, their reported mechanisms and the corresponding assessment according to RCAU is provided in Table I.

Aldehydes/aldehyde releasers

Glutaraldehyde (GDA) continues to be used for instrument disinfection (e.g. flexible endoscopes) and, in some cases, in surface disinfectants in combination with other active ingredients. *Mycobacterium massiliense* isolates isolated from surgical-site infections have been described which could not be completely inactivated in suspension tests even at concentrations of up to 7% GDA, whereas reference strains were killed at concentrations of 1.5—3% GDA [44]. Isolates of *Mycobacterium chelonae* did not show sufficient reduction in suspension tests at 2% in 60 min with 0.6 lg (4 lg would be required for *Mycobacterium avium*) [45]. It is therefore possible that critical RCAUs may occur in mycobacteria after the use of GDA.

M. massiliense was isolated from 2% GDA [46]. However, the species *Mycobacterium abscessus*, *Mycobacterium bovis*, *Mycobacterium chelonae*, *Mycobacterium neoaurum* and *Mycobacterium smegmatis*, which were tested for comparison, were killed within 30 min. Regarding the *M. massiliense* isolates, a critical RCAU may have occurred, but this was not sufficiently counter tested. Whether this is due to intrinsic resistance or tolerance after adaptation remains unclear.

During the inspection of the mechanical reprocessing of duodenoscopes, a *Pseudomonas aeruginosa* strain was isolated against which the GDA-based disinfectant used was ineffective at the concentration and exposure time declared by the manufacturer (1%, 5—10 min, temperature: 50—60 °C) (at 1%, 10 min, 55 °C, reduction 4.42 lg instead of 5 lg) [47]. Since no genomic analysis was performed, the cause remains unknown. However, it is likely to be a tolerance associated with biofilm formation as no R-plasmids against GDA are known to date. The results, which were confirmed by a suspension test, suggested a critical RCAU for the application concentration of 1% so that this was subsequently increased to 2%.

Passaging *Escherichia coli* in sublethal concentrations of GDA resulted in an 11—26% increase in sensitivity caused by mutations in the transcription activator yqhC, which led to an 8- to more than 30-fold overexpression of the aldehyde reductase gene. The protective effect was limited exclusively to *yqh*D as other aldehyde reductase genes of *E. coli* such as *yah*K, *ybb*O, *ygh*A and *ahr* did not offer protection against the biocide [48]. In the absence of standardized testing, it remains unclear whether a critical RCAU was achieved.

M. chelonae was isolated from cleaning and disinfection devices for endoscopes when using 2% GDA. Against this background, the sensitivity of three strains of *M. chelonae* var. *chelonae* (type strain NCTC 946) was compared with two machine isolates in a suspension test. The result was that the type strain, which was not exposed to selection pressure, was significantly more sensitive [45]. However, it remains unclear whether a critical RCAU was reached. Since no genetic resistance was detected, it can be assumed that this was a case of tolerance development.

Alcohols

Alcohols such as ethanol, propan-1-ol and propan-2-ol are mainly used in hand and skin antiseptics as well as in surface disinfectants. To date, there have been no reports of clinically relevant micro-organisms surviving or multiplying in application concentrations of these active ingredients or of isolates being insufficiently inactivated by these active ingredients in application concentrations [49].

Pidot *et al.* [50] described infections with multi-drug-resistant *Staphylococcus aureus* and *Enterococcus faecium* with a higher alcohol tolerance to 70% propan-2-ol determined in a mouse intestinal model. However, Gebel *et al.* [51] were able to demonstrate with a relevant *E. faecium* isolate (ATCC 6057, ST 796 [2-propanol-tolerant strain]) and the corresponding reference strain of EN 13727 (*Enterococcus hirae* ATCC 10541) with propan-2-ol at 60% and 70% sufficient reductions $>$ 5 lg levels within 15 s.

The RCAUs are within the non-critical range across the entire population.

Halogens

Sodium hypochlorite/tosylchloramide sodium: Ten passages of Gram-negative bacteria, particularly *Klebsiella pneumoniae* and *P. aeruginosa*, with concentrations below the MIC of sodium hypochlorite (NaOCL) were sufficient to increase the MIC to >2500 μg/mL. After overnight incubation with a sublethal concentration of NaOCl, a significant increase in MIC was observed for imipenem. The investigation revealed that resistance to imipenem was increased due to the enhanced expression of resistance nodulation divisional (RND) efflux pumps such as AcrAB-TolC and MexAB/XY-OprM. It was therefore hypothesized that exposure to NaOCl may influence the expression of RND efflux pump genes, contributing to imipenem cross-resistance [52]. This is an example of the need for strictly indicated use of disinfectants at effective concentrations.

Again, no data from standardized tests are available. Since typical application concentrations are around 0.5% (5000 μg/mL), it is unlikely that a critical RCAU is reached.

Iodine-releasing compounds: No data indicating critical RCAUs have been published in the literature to date for iodine-releasing compounds.

Surface-active compounds

Cationic compounds: Similar to QACs, tertiary amines as surface-active agents lead to a significantly stronger depolarization of the membrane potential [53], resulting in increased membrane permeability [54]. Since the bacterial cell is not immediately and completely destroyed in a non-specific manner, it can be assumed that not only the development of tolerance is possible but also resistance genes could be selected, similar to QACs. In contrast to QACs, our research has not yet found any published studies on the development of tolerance and resistance.

Amphoteric surfactants: For amphoteric biocides, it has been shown in *P. aeruginosa* that repeated subcultivation in increasing sublethal concentrations leads to gradual, reversible adaptation. This was associated with cross-resistance to biguanides, increased hydrophobicity and ultrastructural changes in the outer membrane. In addition, changes in fatty acid composition were observed, indicating a modification of the outer membrane [55]. A relevant development of resistance, indicating the attainment of a critical RCAU, has not yet been described.

Glucoprotamine: In *Acinetobacter baumannii*, it has been shown that overexpression of RND-type efflux pumps (AdeABC, AdeIJK) increases the survival rate after exposure to glucoprotamine and other quaternary biocides. In standardized sensitivity tests, the effect was only slight (non-critical RCAU), but in time-kill assays, pump-overexpressing strains showed prolonged survival. Stable, genetically fixed resistance to glucoprotamine has not yet been described [56].

Guanidines

CHG is found in some alcohol-based skin antiseptics to achieve a sustained effect. The literature describes isolates of Gram-negative species that were able to survive in aqueous solutions at concentrations of 2.5% (*Achromobacter xylosoxidans*), 2% (*Serratia marcescens*) or 0.12% CHG (*Burkholderia cepacia*) [57—59]. In addition, isolates of *Enterococcus faecalis* and *E. faecium* have been described that were insufficiently killed by 4% CHG in suspension tests within 5 min (4%, 5 min, ≤ 2.4 lg) [60]. In general, the risk of cellular adaptation to low CHG concentrations is higher in Gram-negative than in Gram-positive species [61]. This is due to specific adaptation mechanisms that occur in the outer membrane or arise through the expression of efflux pumps [57]. Since the concentrations of CHG in aqueous solution tested to date do not indicate a critical RCAU and the behaviour in combination with alcohols in microbicidal concentrations has not been investigated, the relevance of the *in vitro* findings remains open.

The use of CHG and octenidine dihydrochloride (OCT) for the prevention of vascular catheter-associated infections resulted in a decreased sensitivity in coagulase-negative staphylococci. Critical RCAUs were not detected under the conditions tested. There was no evidence of the presence of resistance-coding genes [62]. After passage *in vitro*, bacteria with reduced sensitivity to CHG were also less sensitive to antibiotics. This could be due to common mechanisms. In the future, it will be important to investigate the replication capacity at the application concentration to understand the role of CHG in selection [63]. Due to the widespread use of CHG and QAC, the resistance genotypes (resistance genes and integrons) in *P. aeruginosa* and *A. baumannii* were associated with resistance patterns to antibiotics and antiseptics [64]. Although clinical studies to support the hypothesis of cross-resistance of CHG with antibiotics are currently lacking, its use should be limited to essential indications.

Pyridines

Planktonic cultures of *P. aeruginosa* survived at >50% of the application concentration of a common OCT formulation during the recommended exposure time. Seven strains of *P. aeruginosa* adapted after continuous exposure to increasing concentrations of OCT. Adaptation increased tolerance to OCT formulations and CHG by up to 32-fold. Continuous OCT exposure of a multi-species community in a simulated clinical environment resulted in up to an eightfold increase in tolerance to OCT and CHG in *P. aeruginosa*, which was lost again after OCT was removed [65]. To date, there is no evidence of critical RCAU.

Quaternary ammonium compounds

QACs such as benzalkonium chloride (BAC) and didecyldimethylammonium chloride are mainly used in surface disinfectants. Isolates of some bacterial species such as *Achromobacter* species or *S. marcescens* were able to multiply at room temperature in application solutions of QAC-based products [66]. In a patient with a brain cyst, the clonal identity between the towel dispenser isolate and the clinical isolate led to the assumption that the source of the infection could have been the disinfectant solution in the towel dispenser [67]. This implies that QACs are ineffective against individual strains at the application concentration, i.e. critical RCAUs may occur [49]. If we follow the definition that QAC-resistant microorganisms are able to survive in a ready-to-use concentration of a product, then examples of QAC-resistant bacteria also include those obtained from contaminated disinfectants or antiseptic solutions in clinical settings. Contaminated disinfectants and antiseptics have repeatedly caused nosocomial

outbreaks [43]. However, the evidence did not include any analyses of the genetic origin of the resistance, so it was probably a case of tolerance development. The risk of tolerance development to low BAC concentrations is higher in Gram-negative than in Gram-positive species [68,69]. However, the detection of bacterial strains in disinfectant working solutions is not evidence of critical RCAU as there may be a variety of causes for this, including incorrect concentrations or biofilms. Testing in a standardized suspension test is therefore essential for assessing the relevance of such isolates.

The finding that exposure to BAC in microbial soil ecosystems increased both the absolute and relative frequency of antimicrobial resistance genes is alarming. This enrichment was most pronounced at BAC concentrations such as those observed in sediments and sludge. Although the development of tolerance is certain, no clinically defined antibiotic resistance has been detected to date. However, the emergence of mobile genetic determinants carrying multiple genes encoding QAC or antibiotic tolerance raises the question of whether the widespread use of QAC could promote the development of antibiotic resistance [67]. Despite some evidence from laboratory-based studies, there is currently insufficient evidence to conclude that the frequent use of QAC-based disinfectants and antiseptics has promoted the development of antibiotic resistance [43]. However, the association between QAC and carbapenemase-producing Gram-negative clusters requires further observation.

Oxidizing agents/peroxide compounds

Peroxides such as PAA or hydrogen peroxide are found, for example, in surface, instrument and laundry disinfectants. The probability of a relevant reduction in sensitivity to substances in these groups of active ingredients is low due to the non-specific mode of action of these active ingredients. To date, there have been no reports of clinically relevant micro-organisms surviving or multiplying in the concentrations in which these active substances are used, and thus no critical RCAUs have been identified, i.e. isolates have not been sufficiently inactivated by these active substances in the concentrations in which they are used [49].

In an isolate of OXA-48 carbapenemase-producing *K. pneumoniae* from an endoscope-washer-disinfector in which PAA was used as an active ingredient, a slightly increased tolerance of the outbreak strain in planktonic form to PAA was found, but not to other disinfectants tested. The biofilm formed by the outbreak strain could not be eliminated with the standard PAA concentration used for the reprocessing of duodenoscopes at the time of the outbreak [70]. Although this was not a case of resistance but rather the development of tolerance, the example illustrates that even increased tolerance can jeopardize the success of disinfection. However, no critical RCAUs have been described; only increases in tolerance have been observed to date in combination with biofilms and long-term presence of the microbicidal active ingredient in solutions that closely resemble the conditions in a bioreactor [71—73].

Surprisingly, antibiotics-resistant *E. coli* were rendered viable but not culturable (VBNC) after exposure to PAA by destroying proteins. However, this does not indicate a risk of resistance development. As with any microbicidal agent, selection is possible at microbiostatic concentrations [74]. This once again underscores the need for standardized disinfectant testing (using RCAU determination) to assess relevance.

Phenol/phenol derivatives/phenol ethers

Tolerance against triclosan (TSC) is caused by mutations in the target site, the gene for acyl carrier protein reductase and its overexpression, as well as increased efflux of the substance through upregulation of multi-drug efflux pumps [75—77]. Isolated cases of changes in the composition of the cell membrane and different morphologies in colony-forming units have been reported [78,79]. As with QACs, the broad substrate spectrum of multi-drug efflux pumps in triclosan-induced overexpression means that there is a possibility of a simultaneous decrease in sensitivity to antibiotics [80,81]. Initial results indicate that the unrestricted use of TCS-containing antiseptics enhances both chromosomal and horizontally acquired resistance mechanisms [82] as triclosan is used below microbicidal concentrations in both consumer products and oral antiseptics. It is therefore not possible to make a statement on RCAU in disinfectants.

Against hexachlorophene, two R-plasmids against sulfadiazine and gentamicin simultaneously reduced the MIC of *P. aeruginosa* fivefold. It is not possible to make a statement about RCAU because the application concentrations are unknown and no quantitative suspension tests were performed. The investigation of the same plasmids against 12 other phenol derivatives or chlorophenols showed no influence on the MIC [83].

Acids (inorganic/organic) and bases

Organic acids such as formic acid, tartaric acid or citric acid act via pH-dependent diffusion of uncharged molecules into the cell, followed by intracellular dissociation into protons and anions, which causes a decrease in intracellular pH and toxic anion accumulation. This leads to membrane damage, disruption of central metabolic processes and inhibition of proton-dependent transport mechanisms [84].

Previous studies show that micro-organisms can develop temporary adaptation under repeated exposure to sublethal concentrations, for example through altered membrane permeability or activation of stress responses [85,86]. However, classic, genetically fixed resistance mechanisms have not been detected. In a study with *Salmonella enterica*, exposure to subinhibitory acetic acid concentrations did not lead to a stable 'acid tolerance response' [87].

The clinical relevance of resistance development is therefore considered to be low. Even though moderate changes in sensitivity have been described under laboratory conditions, these remain well below the concentrations used in practice [84,88]. Critical RCAU is therefore not to be expected.

Heavy metal compounds

Plasmid resistance to silver ions has been known for more than 60 years and has been the cause of nosocomial outbreaks. The plasmids often also contain resistance determinants against other heavy metals such as cobalt, nickel, cadmium, lead, zinc and antibiotics. As a consequence, antibiotic-resistant pathogens can be spread by silver-containing antiseptics or preservatives [89].

In Table I an overview is provided of the risk of a critical RCAU or resistance occurring.

6. Surveillance of phenotypic changes to disinfectants and antiseptics

In contrast to antibiotics, there is no surveillance of bacterial sensitivity to disinfectants and antiseptics because there has been no need to date [91]. The reason for targeted investigations was the detection of bacteria in working solutions of an active agent or from disinfectant solutions from automated reprocessing procedures, whereby in these cases the (additional) release from biofilms cannot be ruled out.

In the event of an outbreak caused by a pathogen that is multi-resistant or pan-resistant to antibiotics, it may be advisable to check whether there is tolerance to a QAC. However, a test carried out in this regard did not reveal any evidence of tolerance development [92]. In such cases, it is easier to carry out surface disinfection with disinfectants based on oxidants or PAA, for example. This also reduces the risk of co-selection of QAC-tolerant and carbapenemase-producing strains, e.g. in the biofilm of sanitary areas.

7. Discussion

With long-term exposure to subinhibitory concentrations of active agents, a change in sensitivity to a single active substance is possible for each substance. However, this does not necessarily mean that the RCAU will be undershot. In contrast, the probability of the occurrence of chromosomally encoded dual resistance is only 10^{-18}—10^{-24}/h and should not be achievable in practice [93].

As the disinfection result can be called into question in the event of tolerance, the VAH offers to check whether the listed concentration-to-time ratios are effective in the event of an accumulation of infections caused by a specific bacterium. For this purpose, the VAH reference laboratory at the Institute for Hygiene and Public Health (IHPH) has established a microtitre plate procedure that enables simplified screening of isolates and comparison with reference strains [34]. At the same time, the isolates could be examined for the presence of resistance genes to clarify the genetic background.

To date, there is no evidence that resistance can develop to antimicrobials with a non-specific mode of action, i.e. a non-targeted destructive effect on the cell. Only in the case of disinfectants and antiseptics with specific mode of action, which contain the active ingredient below the microbicidal concentration, can the risk not be ruled out and requires further analysis.

None of the known mechanisms necessarily leads to a remaining replication capacity of the micro-organisms after exposure to the substances at the usual application concentration and exposure time. The term RCAU is best suited to describe relevant increases in resistance because it refers to the correct conditions of use. It therefore summarizes mechanisms that have previously been referred to as resistance, tolerance and adaptation and applies equally to wild-types and genetically modified micro-organisms. Testing is carried out using methods suitable for disinfectant testing in quantitative suspension tests under low stress conditions in accordance with the relevant European standards or VAH methods or the VAH micro method. If a critical RCAU is reached, the disinfection or antisepsis result is called into question. In this context, the identification of clinically relevant changes in susceptibility relies primarily on standardized disinfectant efficacy tests performed under recommended use conditions. Further experimental or mechanistic studies may provide valuable complementary insights into the underlying causes of an increased RCAU but are considered supportive rather than decisive for assessing practical relevance, which is determined by the reproducible demonstration of a critical RCAU. In infection prevention practice, RCAU provides a pragmatic framework for interpreting recurrent or epidemiologically linked microbial findings. It allows differentiation between a non-critical RCAU, reflecting occasional survivors without loss of efficacy upon retesting, and a critical RCAU, where the disinfectant or antiseptic fails to achieve the required reduction under recommended application concentration and exposure time. This distinction supports targeted follow-up testing and informed decision-making in outbreak situations.

If disinfectants are used on surfaces and not under conditions where biofilm formation is possible (e.g. in siphons, water-bearing systems, washer-disinfectors, medical devices with cavities, especially endoscopes), the VAH Disinfectant Commission assumes, based on current knowledge, that the products published in the VAH disinfectant list are also effective against antibiotic-resistant micro-organisms in the certified application concentrations. However, it should be noted whether the intrinsic resistance of a vegetative pathogen to be detected by disinfection is higher than that of the test organisms for determining bactericidal/levurocidal activity (see Figure 1C). This applies to *Candida auris*, for example. As QAC may be less effective against *C. auris*, the European Centre for Disease Prevention and Control (ECDC) [94] and the CDC [95] recommend the use of sporadically effective surface disinfectants and, after release from isolation, room fogging with hydrogen peroxide. However, more recent studies do not confirm this. Since QACs cannot be used for room disinfection by fogging for toxicological reasons, only hydrogen peroxide or NaOCl can be used for fogging if desired. Although studies confirm that QACs are sufficiently effective against *C. auris*, whereas gaps have been identified in the case of peri-compounds [96,97]. In these cases, too, in order to assess relevance, it should always be checked whether there is a critical RCAU for the strain concerned.

The colonization of carbapenemase-producing Gram-negatives in wastewater-carrying systems (sink drains, shower drains, sinks and toilets as well as the sewer system) in continuous exposure to the different groups of active ingredients of disinfectants and antiseptics in diluted concentrations, in biofilms in wastewater systems provide opportunities for micro-organisms to develop tolerance to disinfectants and antiseptics. Wastewater reservoirs are therefore one of the most important sources of infection for antibiotic-resistant and disinfectant-tolerant micro-organisms [98]. This is one of the major challenges in preventing and controlling nosocomial infections. This problem cannot be solved solely using disinfection procedures but requires structural—functional solutions that safely prevent patient exposure to the micro-organisms present.

If antiseptics are known to have plasmid-controlled resistance, they should only be used for medical indications and not for cosmetic purposes, e.g. in deodorants and antiperspirants,

in personal hygiene products, in mouthwashes or as conservans. Even in the case of antiseptic indications, it must be considered whether an active agent with the potential to select R-plasmids or an active substance with an unspecific mechanism of action should be used.

In conclusion, this conceptual framework introduces RCAU as a unifying concept for interpreting microbial survival and postexposure replication under recommended use conditions of disinfectants and antiseptics. RCAU provides a structured basis for distinguishing transient survival from reproducible postuse replication and for assessing when microbial findings have practical relevance. In infection prevention practice, failure of an antiseptic or disinfectant to achieve the expected effect or persistent outbreak situations despite intensified disinfection measures should prompt an assessment of RCAU in laboratory tests under recommended use conditions.

Key points of the framework are as follows:

1. There are many mechanisms that can increase the resistance of micro-organisms to disinfectants or antiseptics. The term 'RCAU' summarizes mechanisms that have previously been referred to as resistance, tolerance and adaptation and applies equally to wild-types and genetically modified micro-organisms.
2. Increases in resistance below a critical RCAU are irrelevant in practice, except for mechanisms that are also linked to antibiotic resistance.
3. For mechanisms described as 'resistance', 'tolerance' and 'adaptation', the presence of a critical RCAU should always be checked to assess their relevance.
4. MIC determinations or the mere observation that micro-organisms are present after a disinfection measure are not sufficient to speak of a critical RCAU or a situation requiring intervention.
5. Critical RCAU can only be detected using methods suitable for disinfectant testing in quantitative suspension tests under low load conditions in accordance with the relevant European standards or VAH methods including the VAH micro method.

CRediT authorship contribution statement

A. Kramer: Writing — review & editing, Writing — original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **J.K. Knobloch:** Writing — review & editing, Writing — original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **J. Gebel:** Writing — review & editing, Writing — original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **K.-M. Roesch:** Writing — review & editing, Methodology, Investigation, Data curation. **C. Ilschner:** Writing — review & editing, Investigation, Formal analysis, Conceptualization. **N.T. Mutters:** Writing — review & editing, Validation, Resources, Formal analysis. **M. Exner:** Writing — review & editing, Validation, Formal analysis, Conceptualization. **B. Hornei:** Writing — review & editing, Writing — original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Rausch:** Writing — review & editing, Writing — original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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References

[1] Mendelson M, Sharland M, Mpundu M. Antibiotic resistance: calling time on the 'silent pandemic'. JAC Antimicrob Resist 2022;4:dlac016.
[2] Kampf G. Proposal for a definition to describe resistance to biocidal active substances and disinfectants. J Hosp Infect 2023;139:251—2.
[3] Krewing M, Mönch E, Bolten A, Niesalla H. Resistance or tolerance? Highlighting the need for precise terminology in the field of disinfection. J Hosp Infect 2024;150:51—60.
[4] Neuhaus S, Feßler AT, Dieckmann R, Thieme L, Pletz MW, Schwarz S, et al. Towards a harmonized terminology: a glossary for biocide susceptibility testing. Pathogens 2022;11:1460.
[5] Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO). Anforderungen an die hygiene bei der reinigung und desinfektion von flächen: empfehlung der kommission für krankenhaushygiene und infektionsprävention (KRINKO) beim Robert Koch-Institut. Bundesgesundheitsbl Gesundheitsforsch Gesundheitsschutz 2022;65:1074—115.
[6] Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO). Händehygiene in einrichtungen des gesundheitswesens: empfehlung der kommission für krankenhaushygiene und infektionsprävention (KRINKO) beim Robert Koch-Institut. Bundesgesundheitsbl Gesundheitsforsch Gesundheitsschutz 2016;59:1189—220.
[7] Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO). Bundesinstitut für arzneimittel und medizinprodukte (BfArM). Anforderungen an die hygiene bei der aufbereitung von medizinprodukten. Bundesgesundheitsbl Gesundheitsforsch Gesundheitsschutz 2012;55:1244—310.
[8] Kramer A, Assadian O, Kampf G. Antiseptische indikationen, konzepte und antiseptic stewardship. In: Kramer A, Assadian O, Exner M, Hübner NO, Scheithauer S, Simon A, editors. Krankenhaus- und Praxishygiene. München: Elsevier; 2022. p. 88—109.
[9] World Health Organization. WHO guidelines on hand hygiene in health care: first global patient safety challenge, clean care is safer care. Geneva: World Health Organization; 2009.
[10] Halla N, Fernandes IP, Heleno SA, Costa P, Boucherit-Otmani Z, Boucherit K, et al. Cosmetics preservation: a review on present strategies. Molecules 2018;23:1571.
[11] Richter AM, Konrat K, Osland AM, Brook E, Oastler C, Vestby LK, et al. Evaluation of biofilm cultivation models for efficacy testing

of disinfectants against *Salmonella Typhimurium* biofilms. Microorganisms 2023;11:761.
[12] Günther F, Scherrer M, Kaiser SJ, DeRosa A, Mutters NT. Comparative testing of disinfectant efficacy on planktonic bacteria and bacterial biofilms using a new assay based on kinetic analysis of metabolic activity. J Appl Microbiol 2017;122:625—33.
[13] Ahmed H, Joshi LT. *Clostridioides difficile* spores tolerate disinfection with sodium hypochlorite disinfectant and remain viable within surgical scrubs and gown fabrics. Microbiology (Reading) 2023;169:001418.
[14] Rashid T, Haghighi F, Hasan I, Bassères E, Alam MJ, Sharma SV, et al. Activity of hospital disinfectants against vegetative cells and spores of *Clostridioides difficile* embedded in biofilms. Antimicrob Agents Chemother 2020;64:e01031-19.
[15] McSharry S, Koolman L, Whyte P, Bolton D. Investigation of the effectiveness of disinfectants used in meat-processing facilities to control *Clostridium sporogenes* and Clostridioides difficile spores. Foods 2021;10:1436.
[16] Robben C, Witte AK, Schoder D, Stessl B, Rossmanith P, Mester P. A fast and easy ATP-based approach enables MIC testing for non-resuscitating VBNC pathogens. Front Microbiol 2019;10:1365.
[17] Fu Z, Zhang H, Chen T, Ning D, Xu H. Quantitative detection of VBNC state *Pseudomonas aeruginosa* contributing to accurate assessment of microbial inactivation in drinking water disinfection. Water 2024;16:236.
[18] Webber MA, Woodward MJ, Piddock LJ. Disinfectant resistance in bacteria. In: Aarestrup FM, editor. Antimicrobial resistance in bacteria of animal origin. Washington, DC: ASM Press; 2005. p. 311—25.
[19] Tong C, Hu H, Chen G, Li Z, Li A, Zhang J. Disinfectant resistance in bacteria: mechanisms, spread, and resolution strategies. Environ Res 2021;195:110897.
[20] Russell AD. Mechanisms of bacterial insusceptibility to biocides. Am J Infect Control 2001;29:259—61.
[21] Pereira AP, Antunes P, Bierge P, Willems RJL, Corander J, Coque TM, et al. Unraveling *Enterococcus* susceptibility to quaternary ammonium compounds: genes, phenotypes, and the impact of environmental conditions. Microbiol Spectr 2023;11:e0232423.
[22] Soumet C, Fourreau E, Legrandois P, Maris P. Resistance to phenicol compounds following adaptation to quaternary ammonium compounds in *Escherichia coli*. Vet Microbiol 2012;158:147—52.
[23] Schmidt SBI, Täschner T, Nordholt N, Schreiber F. Differential selection for survival and for growth in adaptive laboratory evolution experiments with benzalkonium chloride. Evol Appl 2024;17:e70017.
[24] Weller C, Wu M. A generation-time effect on the rate of molecular evolution in bacteria. Evolution 2015;69:643—52.
[25] Gillissen G, Gillissen IM. Die Resistenzbildung bei Mikroorganismen. In: Kikuth W, Miescher K, Nauck EG, Pappenheimer AM, Tomcsik J, editors. Ergebnisse der Mikrobiologie, Immunitätsforschung und Experimentellen Therapie. Berlin, Heidelberg: Springer; 1960. p. 128—241.
[26] Kastbjerg VG, Nielsen DS, Arneborg N, Gram L. Response of *Listeria monocytogenes* to disinfection stress at the single-cell and population levels as monitored by intracellular pH measurements and viable-cell counts. Appl Environ Microbiol 2009;75:4550—6.
[27] Nordholt N, Sobisch LY, Gödt A, Lewerenz D, Schreiber F. Heterogeneous survival upon disinfection underlies evolution of increased tolerance. Microbiol Spectr 2024;12:e0327622.
[28] Mc Carlie S, Boucher CE, Bragg RR. Molecular basis of bacterial disinfectant resistance. Drug Resist Updat 2020;48:100672.
[29] Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev 2018;31:e00088. 17.
[30] Rutala WA, Weber DJ. Surface disinfection: treatment time (wipes and sprays) versus contact time (liquids). Infect Control Hosp Epidemiol 2018;39:329—31.
[31] Koch BCP, Zhao Q, Oosterhoff M, van Oldenrijk J, Abdulla A, de Winter BCM, et al. The mysteries of target site concentrations of antibiotics in bone and joint infections: what is known? A narrative review. Expert Opin Drug Metab Toxicol 2022;18:587—600.
[32] Janattul-Ain J, Mohd-Hafiz AA, Lipman J, Roberts JA. Defining antibiotic dosing in lung infections. Clin Pulm Med 2013;20:121—8.
[33] Kramer A, Lexow F, Bludau A, Köster AM, Misailovski M, Seifert U, et al. How long do bacteria, fungi, protozoa, and viruses retain their replication capacity on inanimate surfaces? A systematic review examining environmental resilience versus healthcare-associated infection risk by "fomite-borne risk assessment". Clin Microbiol Rev 2024;37:e00186-23.
[34] Gebel J, Rausch M, Bienentreu K, Droop F, Eggers M, Gebel L, et al. Evaluation of a microscale quantitative suspension test to determine the bactericidal and yeasticidal activity of glutaral—one step to improve sustainability in disinfectant testing. GMS Hyg Infect Control 2024;19:9.
[35] Knobling B, Ulatowski A, Franke G, Belmar Campos C, Büttner H, Klupp EM, et al. Superiority of manual disinfection using presoaked wipes over automatic UV-C radiation without prior cleaning. J Hosp Infect 2023;140:72—8.
[36] Frickmann H, Bachert S, Warnke P, Podbielski A. Validated measurements of microbial loads on environmental surfaces in intensive care units before and after disinfecting cleaning. J Appl Microbiol 2018;124:874—80.
[37] Maillard JY, Pascoe M. Disinfectants and antiseptics: mechanisms of action and resistance. Nat Rev Microbiol 2024;22:4—17.
[38] Maillard JY. Impact of benzalkonium chloride, benzethonium chloride and chloroxylenol on bacterial antimicrobial resistance. J Appl Microbiol 2022;133:3322—46.
[39] Lepelletier D, Maillard JY, Pozzetto B, Simon A. Povidone iodine: properties, mechanisms of action, and role in infection control and *Staphylococcus aureus* decolonization. Antimicrob Agents Chemother 2020;64:e00682-20.
[40] Kramer A, Eberlein T, Müller G, Dissemond J, Assadian O. Re-evaluation of polihexanide use in wound antisepsis in order to clarify ambiguities of two animal studies. J Wound Care 2019;28:246—55.
[41] Lin J, Alexander-Katz A. Cell membranes open "doors" for cationic nanoparticles/biomolecules: insights into uptake kinetics. ACS Nano 2013;7:10799—808.
[42] Zorko M, Langel U. Cell-penetrating peptides: mechanism and kinetics of cargo delivery. Adv Drug Deliv Rev 2005;57:529—45.
[43] Boyce JM. Quaternary ammonium disinfectants and antiseptics: tolerance, resistance and potential impact on antibiotic resistance. Antimicrob Resist Infect Control 2023;12:32.
[44] Lorena NS, Pitombo MB, Côrtes PB, Maya MC, Silva MG, Carvalho AC, et al. *Mycobacterium massiliense* BRA100 strain recovered from postsurgical infections: resistance to high concentrations of glutaraldehyde and alternative solutions for high level disinfection. Acta Cir Bras 2010;25:455—9.
[45] Griffiths PA, Babb JR, Bradley CR, Fraise AP. Glutaraldehyde-resistant *Mycobacterium chelonae* from endoscope washer disinfectors. J Appl Microbiol 1997;82:519—26.
[46] Lorena NS, Duarte RS, Pitombo MB. Infecção por micobactérias de crescimento rápido após procedimentos videocirúrgicos — a hipótese do glutaraldeído. Rev Col Bras Cir 2009;36:266—7.
[47] Tschudin-Sutter S, Frei R, Kampf G, Tamm M, Pflimlin E, Battegay M, et al. Emergence of glutaraldehyde-resistant *Pseudomonas aeruginosa*. Infect Control Hosp Epidemiol 2011;32:1173—8.
[48] Merchel Piovesan Pereira B, Adil Salim M, Rai N, Tagkopoulos I. Tolerance to glutaraldehyde in *Escherichia coli* mediated by overexpression of the aldehyde reductase YqhD by YqhC. Front Microbiol 2021;12:680553.
[49] Kampf G. Biocide resistance and clinical implications. In: Antiseptic stewardship. Cham: Springer International Publishing; 2018.

[50] Pidot SJ, Gao W, Buultjens AH, Monk IR, Guerillot R, Carter GP, et al. Increasing tolerance of hospital *Enterococcus faecium* to handwash alcohols. Sci Transl Med 2018;10:eaar6115.
[51] Gebel J, Gemein S, Kampf G, Pidot SJ, Buetti N, Exner M. Isopropanol at 60% and at 70% are effective against "isopropanol-tolerant" *Enterococcus faecium*. J Hosp Infect 2019;103:e4—6.
[52] Nam JH, Yoo JS. Sublethal sodium hypochlorite exposure: impact on resistance-nodulation-cell division efflux pump overexpression and cross-resistance to imipenem. Antibiotics (Basel) 2024;13:828.
[53] Burckhardt BC, Thelen P. Effect of primary, secondary and tertiary amines on membrane potential and intracellular pH in Xenopus laevis oocytes. Pflugers Arch 1995;429:306—12.
[54] Endo Y, Tani T, Kodama M. Antimicrobial activity of tertiary amine covalently bonded to a polystyrene fiber. Appl Environ Microbiol 1987;53:2050—5.
[55] Jones MV, Herd TM, Christie HJ. Resistance of Pseudomonas aeruginosa to amphoteric and quaternary ammonium biocides. Microbios 1989;58:49—61.
[56] Meyer C, Lucaßen K, Gerson S, Xanthopoulou K, Wille T, Seifert H, et al. Contribution of RND-type efflux pumps in reduced susceptibility to biocides in *Acinetobacter baumannii*. Antibiotics (Basel) 2022;11:1538.
[57] Tena D, Carranza R, Barberá JR, Valdezate S, Garrancho JM, Arranz M, et al. Outbreak of long-term intravascular catheter-related bacteremia due to *Achromobacter xylosoxidans* subspecies *xylosoxidans* in a hemodialysis unit. Eur J Clin Microbiol Infect Dis 2005;24:727—32.
[58] de Frutos M, López-Urrutia L, Domínguez-Gil M, Arias M, Muñoz-Bellido JL, Eiros JM, et al. *Serratia marcescens* outbreak due to contaminated 2% aqueous chlorhexidine. Enferm Infecc Microbiol Clin 2017;35:624—9.
[59] Zurita J, Mejia L, Zapata S, Trueba G, Vargas AC, Aguirre S, et al. Healthcare-associated respiratory tract infection and colonization in an intensive care unit caused by *Burkholderia cepacia* isolated in mouthwash. Int J Infect Dis 2014;29:96—9.
[60] Kampf G, Höfer M, Wendt C. Efficacy of hand disinfectants against vancomycin-resistant enterococci in vitro. J Hosp Infect 1999;42:143—50.
[61] Kampf G. Adaptive bacterial response to low level chlorhexidine exposure and its implications for hand hygiene. Microb Cell 2019;6:307—20.
[62] Sethi DK, Felgate H, Diaz M, Faust K, Kiy C, Clarke P, et al. Chlorhexidine gluconate usage is associated with antiseptic tolerance in *staphylococci* from the neonatal intensive care unit. JAC Antimicrob Resist 2021;3:dlab173.
[63] Abbood HM, Hijazi K, Gould IM. Chlorhexidine resistance or cross-resistance, that is the question. Antibiotics (Basel) 2023;12:798.
[64] Elkhatib WF, Khalil MAF, Ashour HM. Integrons and antiseptic resistance genes mediate resistance of *Acinetobacter baumannii* and Pseudomonas aeruginosa isolates from intensive care unit patients with wound infections. Curr Mol Med 2019;19:286—93.
[65] Shepherd MJ, Moore G, Wand ME, Sutton JM, Bock LJ. *Pseudomonas aeruginosa* adapts to octenidine in the laboratory and a simulated clinical setting, leading to increased tolerance to chlorhexidine and other biocides. J Hosp Infect 2018;100:e23—9.
[66] Boyce JM, Havill NL. In-use contamination of a hospital-grade disinfectant. Am J Infect Control 2022;50:1296—301.
[67] Kupfahl C, Walther M, Wendt C, von Baum H. Identical Achromobacter strain in reusable surface disinfection tissue dispensers and a clinical isolate. Infect Control Hosp Epidemiol 2015;36:1362—4.
[68] Cieplik F, Jakubovics NS, Buchalla W, Maisch T, Hellwig E, Al-Ahmad A. Resistance toward chlorhexidine in oral bacteria—is there cause for concern? Front Microbiol 2019;10:587.
[69] Kampf G. Adaptive microbial response to low-level benzalkonium chloride exposure. J Hosp Infect 2018;100:e1—22.
[70] Brunke MS, Konrat K, Schaudinn C, Piening B, Pfeifer Y, Becker L, et al. Tolerance of biofilm of a carbapenem-resistant *Klebsiella pneumoniae* involved in a duodenoscopy-associated outbreak to the disinfectant used in reprocessing. Antimicrob Resist Infect Control 2022;11:81.
[71] Voloshchuk O, Rolon ML, Bartlett KV, Mendez Acevedo M, LaBorde LF, Kovac J. *Pseudomonadaceae* increased the tolerance of *Listeria monocytogenes* to sanitizers in multi-species biofilms. Food Microbiol 2025;128:104687.
[72] Haak J, Klempien I, Hans JB, Schaefer S, Meyer-Bothling K, Gatermann S, et al. Endoscope-associated outbreak of OXA-181-carbapenemase-producing *Klebsiella pneumoniae* and its implications for hygiene management. J Hosp Infect 2025;158:19—28.
[73] Lima LS, Müller TN, Ansiliero R, Schuster MB, Silva BL, Jaskulski IB, et al. Biofilm formation by *Listeria monocytogenes* from the meat processing industry environment and the use of different combinations of detergents, sanitizers, and UV-A radiation to control this microorganism in planktonic and sessile forms. Braz J Microbiol 2024;55:2483—99.
[74] Turolla A, Sabatino R, Fontaneto D, Eckert EM, Colinas N, Corno G, et al. Defence strategies and antibiotic resistance gene abundance in enterococci under stress by exposure to low doses of peracetic acid. Chemosphere 2017;185:480—8.
[75] Schweizer HP. Triclosan: a widely used biocide and its link to antibiotics. FEMS Microbiol Lett 2001;202:1—7.
[76] Pycke BF, Crabbé A, Verstraete W, Leys N. Characterization of triclosan-resistant mutants reveals multiple antimicrobial resistance mechanisms in *Rhodospirillum rubrum* S1H. Appl Environ Microbiol 2010;76:3116—23.
[77] Saleh S, Haddadin RN, Baillie S, Collier PJ. Triclosan—an update. Lett Appl Microbiol 2011;52:87—95.
[78] Tkachenko O, Shepard J, Aris VM, Joy A, Bello A, Londono I, et al. A triclosan—ciprofloxacin cross-resistant mutant strain of *Staphylococcus aureus* displays an alteration in the expression of several cell membrane structural and functional genes. Res Microbiol 2007;158:651—8.
[79] Seaman PF, Ochs D, Day MJ. Comment on: Triclosan resistance in methicillin-resistant *Staphylococcus aureus* expressed as small colony variants: a novel mode of evasion of susceptibility to antiseptics. J Antimicrob Chemother 2007;60:175—6. author reply 176—176.
[80] Bailey AM, Constantinidou C, Ivens A, Garvey MI, Webber MA, Coldham N, et al. Exposure of *Escherichia coli* and *Salmonella enterica* serovar *Typhimurium* to triclosan induces a species-specific response, including drug detoxification. J Antimicrob Chemother 2009;64:973—85.
[81] Usui M, Nagai H, Hiki M, Tamura Y, Asai T. Effect of antimicrobial exposure on AcrAB expression in *Salmonella enterica* subsp. *enterica* serovar *Choleraesuis*. Front Microbiol 2013;4:53.
[82] Yang QE, Ma X, Li M, Zhao M, Zeng L, He M, et al. Evolution of triclosan resistance modulates bacterial permissiveness to multidrug resistance plasmids and phages. Nat Commun 2024;15:3654.
[83] Sutton L, Jacoby GA. Plasmid-determined resistance to hexachlorophene in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1978;13:634—6.
[84] Rebelo A, Almeida A, Peixe L, Antunes P, Novais C. Unraveling the role of metals and organic acids in bacterial antimicrobial resistance in the food chain. Antibiotics (Basel) 2023;12:1474.
[85] Zinn M-K, Dobariya B, Heidkamp H, Ade C, Flemming H-C, Bockmuehl D. How effective are cleaners with "effective microorganisms". J Surfactants Deterg 2025;28:e12883.
[86] Ji QY, Wang W, Yan H, Qu H, Liu Y, Qian Y, et al. The effect of different organic acids and their combination on the cell barrier and biofilm of *Escherichia coli*. Foods 2023;12:3161.
[87] Gavriil A, Thanasoulia A, Skandamis PN. Sublethal concentrations of undissociated acetic acid may not always stimulate acid resistance in *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* phage type 4: implications of challenge substrate-associated factors. PLoS One 2020;15:e0234999.

[88] Baik HS, Bearson S, Dunbar S, Foster JW. The acid tolerance response of *Salmonella typhimurium* provides protection against organic acids. Microbiology (Reading) 1996;142:3195—200.
[89] Lebek G. Genetische Grundlagen der Resistenzentwicklung von Mikroorganismen gegenüber Antiseptika bzw. Desinfektionsmitteln. In: Weuffen W, Kramer A, Gröschel D, Berencsi G, Bulka E, editors. Grundlagen der Antiseptik. Teil 2: Allgemeine Prinzipien der Antiseptik. Stuttgart, New York: Fischer; 1982. p. 170—86.
[90] Kampf G, Degenhardt S, Lackner S, Jesse K, von Baum H, Ostermeyer C. Poorly processed reusable surface disinfection tissue dispensers may be a source of infection. BMC Infect Dis 2014;14:37.
[91] Nădăş G, Manchon A, Bouari C, Fit N. Veterinary clinics as reservoirs for *Pseudomonas aeruginosa*: a neglected pathway in One Health surveillance. Antibiotics (Basel) 2025;14:720.
[92] Hornschuh M, Zwicker P, Kramer A, Schaufler K, Heiden SE, Bohnert JA, et al. Extensively drug-resistant *Klebsiella pneumoniae* ST307 outbreak strain from north-eastern Germany does not show increased tolerance to quaternary ammonium compounds and chlorhexidine. J Hosp Infect 2021;113:52—8.
[93] Kramer A, Kedzia W, Lebek G, Grün L, Weuffen W, Poczta A. In-vitro- und In-vivo-Befunde zur Resistenzsteigerung bei Bakterien gegen Antiseptika und Desinfektionsmittel. In: Krasilnikow AP, Kramer A, Gröschel D, Weuffen W, editors. Grundlagen der Antiseptik. Bd I, Teil 4: Faktoren der mikrobiellen Kolonisation. Stuttgart, New York: Fischer; 1984. p. 79—121.
[94] European Centre for Disease Prevention and Control (ECDC). Candida auris in healthcare settings - Europe. First update, 23 April 2018. Stockholm: ECDC; 2018 [last accessed 2023 Jun 7]. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/RRA-Candida-auris-European-Union-countries-first-update.pdf.
[95] Centers for Disease Control and Prevention (CDC). Infection control guidance: Candida auris. Atlanta, GA: CDC; 2024 [last accessed 2024 Jan 16]. Available at: https://www.cdc.gov/candida-auris/hcp/infection-control/.
[96] Brill FHH, Gabriel H, Ulatowski A, Steinmann J. Wirksamkeit von Desinfektionsverfahren gegen klinische Isolate der internationalen *Candida auris*-Ausbruchsstämme im Vergleich zum Referenzstamm *Candida auris*. Hyg Med 2024;49:59—60.
[97] Pitten FA, Meckel M, Gebel J, Hornei B. Candida auris, *Trichophyton tonsurans* und Co: Neues zur Testung der Wirksamkeit von Desinfektionsmitteln gegen Pilze. Hyg Med 2025;50.
[98] Exner M. Zur Bedeutung von Krankenhausabwässern als Reservoir und Emittent für antibiotikaresistente Bakterien — Konsequenzen für die Krankenhaushygiene und die Umwelt — mit besonderer Berücksichtigung der Ergebnisse des HyReKA-Projektes. Hyg Med 2024;49:D52—63.