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Review

Disinfection: A View from the Early 21st Century

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Introduction
The origins of today's disinfection practices go back to the 19th century. Although we have learned much since then, there are still gaps in our knowledge; gaps both in theory and in the practical application of disinfection. This collection of three short papers is taken from a session at the 6th Congress of the International Federation of Infection Control held in Istanbul, Turkey in October 2005. It provides an overview of the current status of disinfection, as well as an assessment of possible problems yet to come.

Is the overuse and abuse of biocides linked to increasing Antibiotic resistance?

Çiğdem Bal

Biocides (disinfectants, antiseptics, or preservatives) are antimicrobial agents widely used in hospitals, in industry, and in domestic settings in the community. There is an increasing trend towards a general and poorly directed use of biocides in the home environment for reducing microbial loads with the belief that this will reduce the risk of acquiring an infectious disease. It is now common to find biocides in floor cleaners, dishwashing detergents, plastics, ceramics, chopping boards, knife handles, toilet seats, wall paints, soaps, toothpaste, mouth rinse, cosmetics, socks, underwear, constructional materials and many other household products. A more carefully directed use of these agents in healthcare is needed.

From left to right: Çiğdem Bal, Manfred Rotter, Ulrika Ransjo, Ossama Rasslan Peter Hoffman

The questions we will try to answer are:

- Is there a risk of resistance against biocides due to their widespread and intensive use?
- Does abuse of biocides help emergence of coresistance against antibiotics due to the fact that biocides and antibiotics have common targets in bacteria?
- Is there a risk of resistance against biocides due to their widespread and intensive use?

The central problem here is defining "resistance". With antibiotics, there is a naturally attainable concentration in body tissues that can be used to define a level that separates sensitivity from resistance. With biocides however, this is not valid. Many claims of biocide "resistance" only show slightly decreased sensitivity instead of showing that a microbe is capable of withstanding the concentrations normally used. Biocides are normally used at a concentration many times that of the minimum inhibitory concentration (MIC), so what is the relevance of an increase in a MIC if it is still well within the biocide concentration used in practice? The relevance of a MIC may also be challenged. Biocide users are usually more interested in levels of kill within certain time limits than of mere inhibition of growth.

It should be remembered that biocides are crudely-targeted agents and will attack microbes at many different sites, making it impossible for single-point mutations to

confer resistance to biocides as they do for antibiotics. Biocides act concurrently on multiple sites or targets within a microorganism; change in one target brings a limited susceptibility change. Multiple resistance mechanisms have to work together (cell wall changes ± gene acquisition ± efflux pumps) to maintain true resistance in a microorganism against a biocide. This is quite unlikely, with a few exceptions, such as triclosan resistance.[1]

Does abuse of biocides help emergence of co-resistance to antibiotics?

The case for co-resistance

If biocide resistance is to occur, it may occur as a result of combined or concurrent action of several mechanisms. These could affect antibiotic sensitivity as well.

Cell wall changes ending up with reduced permeability to one or more biocides, similar to that for antibiotic resistance. Glutaraldehyde resistant *Mycobacterium chelonae* strains were found also resistant to ethambutol.[2] Chlorhexidine resistant laboratory strains of *Pseudomonas stutzeri* showed increased resistance to quaternary ammonium compounds (QAC), triclosan, polymyxin B, gentamicin, nalidixic acid, erythromycin and ampicillin, as a result of outer membrane permeability changes.[3] Cationic biocides enter bacteria by self-promoted uptake with a mechanism similar to that used by aminoglycosides. There have been suggestions that, if a bacterial cell adapts to being less accessible, then it becomes less susceptible also to aminoglycosides.[1]

The acquisition of resistance genes. Microorganisms adapt themselves to biocide exposure by acquiring plasmids and transposons with the possibility of also conferring biocide resistance. It was shown that gentamicin-resistant methicillin-resistant *Staph.aureus* (MRSA) contained a transferable multi-drug resistance (MDR) plasmid encoding for resistance to aminoglycosides, ethidium bromide, benzalkonium chloride, and chlorhexidine.[4] Benzalkonium chloride resistant mutants of MRSA had oxacillin MICs of ≥512 mg/L compared with 16 mg/L for the parent strain.[5]

Chromosomal changes. Isothiazolones are used as preservatives in cosmetics; bacterial filamentation occurs after low-level exposure to them. One hypothesis is that filamentation may be due to alterations in the topoisomerase enzymes that play a part in DNA replication or cellular septation, which could then result in cross-resistance to quinolones.[1]

Efflux, the active pumping out of a cell of certain molecules, is a resistance mechanism the importance of which has been reunderstood recently. Such efflux pumps include those for biocides and antibiotics and one efflux pump may be effective with multiple biocides and antibiotics. One example of this are the qac genes that are responsible for an efflux pump working on quaternary ammonium compounds, but are also associated with resistance to trimethoprim, sulphonamides, oxacillin and aminoglycosides.[6] This may end up in cross-resistance.[7]

Efflux pumps may be switched on by exposure to low-levels of biocides and antibiotics however mutant bacterial cells may be selected where this efflux is permanently switched-on. Multiply antibiotic resistant mutants (mar mutants/ efflux mutants) show biocide and antibiotic co-resistance. This may be most notable in biofilms where, due to the low levels of metabolism in cells deep in a biofilm and the failure of biocides and antibiotics to penetrate to these layers, sublethal doses can facilitate resistance. These are most easily seen in mutants of the mar regulon controlling one efflux mechanism, which confers resistance against both antibiotics and biocides;[8] in Pseudomonas aeruginosa, where the mexAB-OprM system, induced by beta lactams, pumps out beta lactams as well as fluoroquinolones, tetracyclines, trimethoprim, chloramphenicol, meropenem, aminoglycosides and triclosan;[9] and the MexCD-OprJ system, induced by benzalkonium chloride, which pumps out chlorhexidine, fluoroquinolones, macrolides, tetracyclines and beta lactams.[10]

Triclosan – the best explored example of possible co-resistance

The biocide that has been most cited as a possible risk for co-resistance to antibiotics is triclosan (also sometimes known as irgasan). This biocide works by inhibition of lipid synthesis in bacteria, by inhibiting enoyl reductase enzyme, and by membrane damage. Efflux is the major resistance mechanism against it used by bacteria[11] with marA/AcrAB overexpression in laboratory and clinical strains of *E. coli* also pumping out of triclosan, ampicillin, tetracyclines and fluoroquinolones.[12] Triclosan exposure can experimentally result in efflux resistance in *P. aeruginosa* to ciprofloxacin.[13]

Triclosan inhibits growth of *E. coli* by inhibiting enoyl reductase (fab1) associated with lipid biosynthesis. Mutation in enoyl reductase gene (fab1) at gly93 in *E. coli* results in triclosan resistance. Inh1 is an analogue of fab1 in mycobacteria and is a common target for both triclosan and isoniazid in *M. tuberculosis*. Therefore, overuse of triclosan may select for antibiotic resistant mycobacteria.[14, 15]

The case against co-resistance between biocides and antibiotics

Despite the isolated, mostly laboratory-based, examples provided earlier, the issue of whether biocide use can generate clinically-significant antibiotic resistance is still very much under debate. There is reportedly far wider use of biocides than previously,[1,11] but does this present a threat to the effective use of antibiotics? McBain *et al.*[16] explain that selection of resistant mutants by triclosan occurs with *E. coli*; however this phenomenon is not universal. Gilbert *et al.*[17] propose that *M. tuberculosis* is intrinsically resistant to triclosan but generally susceptible to isoniazid and conclude that this not clinically important. They also suggest that data on MRSA are not evidencebased. Lambert *et al.*[18] state that there is no relation between long-term triclosan use and antibiotic resistance in MRSA and *P. aeruginosa*. Cole *et*

al.[19] found no significant differences in concentrations and antibiotic susceptibilities of bacteria at homes of users and non-users of biocides. Murtough *et al*.[20] claim that studies to prove co-resistance are laboratory-based and use pure cultures, that resistance developed is genetically stable in the laboratory but not in the environment, and that clinical strains behave differently, so the risk of co-resistance is negligible.

The importance of the efflux mechanism, which seems to have a highlighted role for any co-resistance threat, has been questioned by Gilbert *et al*.,[17] who observe that many naturally occurring substances (pine oil, spices, garlic, chilli, mustard) also induce bacterial pumps. Since nutrients and metabolic intermediates are pumped out together with biocides and antibiotics, this will render bacteria with active efflux less able to compete with other bacteria and so it is rarely in a bacterium's favour to use efflux. This mechanism is also criticised as a laboratory phenomenon whose significance in the clinical setting is unknown. In addition, Gillespie[21] states that there is an inverse relation between virulence and resistance; resistance mutations generate bacteria that are less able to adapt and compete. In summary, it seems that there is insufficient evidence to arrive at any firm conclusion on whether biocide overuse generates clinically-significant antibiotic resistance. Indeed, after many decades of biocide use, the situation is still not proven, potentially evidence that any threat is not major. As it is still not possible to say conclusively that there is no such threat, it could be recommended that biocide use be restricted to areas of proven benefit within the framework of health care. However, given the multiplicity of biocide uses and commercial benefits of such use, this is unlikely to be possible without very good evidence.

Disinfection and Human Failures

Peter N. Hoffman

Disinfection

Chemical disinfectants are reactive compounds. They kill microbes by altering systems that the microbe relies on for metabolic integrity. This can be accomplished by chemical interference with metabolic pathways, such as oxidising agents, or physical disruption of membranes, such as surface active disinfectants. Disinfectants will also react with organic material that is not part of their intended target and, in doing so, will become inactivated. Thus the same disinfectant acting against the same pathogen may have vastly different effectiveness depending on the medium in which it is expected to act. It is up to the user to assess the situations in which disinfectants will be expected to act and, if necessary, modify the situation, the disinfectant, or its concentration so as to help effective disinfection occur.

Disinfection and human failures

Disinfectants should be chosen that have passed appropriate tests that are, or should be, highly standardised and reproducible procedures. (The latest advances in disinfectant testing are explored later in this paper.) Tests may model one particular use-situation however they can never reproduce all the varying conditions that real-life use entails. Thus they can only be a guide to how disinfectants may behave and not a guarantee that they will behave similarly in real-life. The selection and application of a particular chemical are only a part of the process of chemical disinfection. Among the essential matters to consider and control are:

The microbicidal spectrum of the chosen disinfectant must include all the relevant pathogens likely to be present in a given situation.

Micro-organisms such as non-enveloped viruses and mycobacteria are amongst the more resistant likely to be encountered; bacterial spores are highly resistant. However, there is no correlation between pathogenicity and disinfectant resistance, so micro-organisms such as methicillin-resistant *Staph. aureus* (MRSA) and human immunodeficiency virus are easily inactivated. Many disinfectants are sold on the basis that they can kill pathogens of interest; this, however, is no guarantee that they are good disinfectants.

Examples: Using a disinfectant with an inappropriate microbicidal spectrum can often be observed when wards reprocess instruments and items of equipment using the surgical scrub that they also use for hand washing. This can either be thought of as a very expensive general purpose detergent or an inappropriate disinfectant for that purpose.

This type of disinfection is highly uncontrolled and could also fail for a number of other reasons such as: the disinfectant is inactivated due to over-dilution, poor pre-cleaning of an item (both disinfectant unable to reach target and inactivation by organic matter), or insufficient exposure time.

The disinfectant is inactivated.

This could occur during use, usually because there is too much organic matter present. It can also be caused by mixing with incompatible chemicals, for example, mixing a disinfectant with a cleaning compound. Inactivation could also happen if the disinfectant was incorrectly prepared at too low a concentration, if it were stored incorrectly (too long, too hot or too much light) or if there were materials present that could inactivate it (rubber, cork and cotton wool have been known to inactivate disinfectants). Another common cause of inactivated disinfectants is too long a time of storage, particularly of diluted solutions.

Examples: Amongst the many examples in this category, there is a recurring example involving quaternary ammonium compound (QAC) disinfectants inactivated by cotton wool. QACs are surface active compounds and will attach to surfaces; it is by such disruption of membranes and other hydrophobic areas that they kill microbes. It is intended that the QAC will be applied to the patient on cotton wool and, in preparation for this, cotton wool balls are kept in the disinfectant. The fibres in these cotton wool

balls have a very large surface area and will thus remove substantial amounts of the QAC from effective solution, sometimes enough to allow bacterial growth. Several examples of this can be found from 1958[22] to 1996.[23]

The disinfectant is used at the wrong concentration.
The concentration of a disinfectant can be critical. Unfortunately it is common for many users to dilute disinfectants without accuracy and rely on the appearance of a solution to guide them. This is an issue of staff training and supervision.

Examples: This is an area where there are many anecdotal examples. Most of us have seen people preparing disinfectants in a hospital or laboratory by estimation. I was involved in an incident where disinfectants were measured accurately; however an error of a factor of ten was made in the dilution calculation. There should also be caution against deliberately making in-use disinfectants too concentrated as a "safeguard"; this will often increase their corrosivity and toxicity, as well as increasing the cost unnecessarily.

The disinfectant must be able to reach its target.
This process can be impeded by lumps of organic matter, coagulated proteinaceous material, occluded or air-filled lumens, or items that should be immersed in disinfectant left floating on the surface.

Examples: Alcohol is the most common disinfectant to be linked to this form of failure. Alcohols will coagulate the outer layer of any protein and form a barrier to further alcohol penetration. Another similar example is that of biofilms (explored earlier in this paper), where the outer layers of a biofilm block effective entry to the lower layers. Here the target is in proteinaceous matter that the disinfectant cannot penetrate.

The disinfectant is not brought into contact with all the surfaces
of the item to be disinfected when items are floating on the surface of a disinfectant tank, when air bubbles are not removed from tubes, or when lumens, such as those in endoscopes, are not irrigated by either a manual process or an automated washer-disinfector.

Microbes acquiring resistance to disinfectants. Whilst this does exist, it is nowhere near as common as bacterial resistance to antibiotics. Failures of disinfection are more usually the result of human failings than of microbial resistance. (There is a full exploration of resistance to disinfectants earlier in this paper).

Advances in Disinfection

Manfred L. Rotter

This section is an update on recent changes in disinfection practices in the medical area and starts with a roundup of new or reformulated disinfectants, either available or in development. Many of these products are intended for use on instruments, primarily flexible endoscopes, and are designed to replace glutaraldehyde-based products, which are being phased-out in some parts of the world due to concerns about staff health and safety. Most of these new agents are more expensive than glutaraldehyde.

Ortho-phthalaldehyde (example: Cidex OPATM):
A high molecular weight, and therefore non-volatile, aldehyde. In-use concentration 0.55%. Bactericidal, mycobactericidal, fungicidal, virucidal, but not sporicidal. Odourless, active in the presence of organic matter, non-corrosive, stains proteins (skin, cloths and any residual proteins not cleaned-off endoscopes), stable on storage, there is some irritant and allergenic potential.

Applications: instrument disinfection, especially endoscope disinfection. Possible use on surfaces, however less expensive agents are available for this purpose.

"New" hydrogen peroxide (H_2O_2) formulations:
In contrast to unformulated H_2O_2, which has weak and slow microbicidal activity, stabilized and "accelerated" formulations have very good bactericidal (including mycobacteria), fungicidal and virucidal activity, however they act only slowly against bacterial spores.

These preparations are slight irritants but are not allergenic and are stable on storage. They exist in various forms: Stabilized and "accelerated" by appropriate detergents and acids (pH 1.3, "Virox™") or alkalis (pH 12.5, "Hvèzda S.C.H™") or combined with peracetic acid (PA) (e.g. "Compliance™", 7.35% H_2O_2 + 0.23% PA or "Cidex PATM", 1% H_2O_2 + 0.08% PA). These combinations are corrosive. Applications: Instrument disinfection, especially endoscopes, surfaces and, in its "AHPTM" formulation, for hygienic hand wash.

Peracetic acid (peroxyacetic acid):
Examples include Nucidex, Perasafe, Perascope, Gigasept PA. They have excellent broadspectrum microbicidal activity, although in the presence of organic matter higher concentrations are necessary. An irritant (eye and skin damage) but not allergenic, pungent odour, corrosive, explosive and flammable in high concentrations, unstable when diluted. Often combined with hydrogen peroxide (see above).

Monopercitric acid:
Another new peroxygen compound that is virucidal within 0.5-1 minute at 0.5% against poliovirus 1 and at 0.1% against adenovirus 2. In qualitative suspension tests it has been shown to be sporicidal at 1%.

Super-oxidized water:
This product is a novel approach to disinfection where the disinfectant is produced in or near the location where it will be used. Users can buy or rent the production machine. The disinfectant is produced from a sodium chloride solution by electrolysis and contains a variety of oxidizing agents, mainly hypochlorous acid at low pH (2.3-6.5), and has high redox potential (>950 mV). There are various electrolysis systems: e.g. "Super Oxseed alpha 1000™" (Janix Inc., Japan) producing a pH of 2.3-2.7 or "Sterilox 2500™" (Sterilox Medical Ltd., USA) producing a pH of 5.0-6.5.

Antimicrobial activity: It is microbicidal against all forms of microorganisms with short application times (0.5-10 minutes); however, depending on the equipment used, the age since production of the super-oxidized solution is important. It should be used shortly after production.

Super-oxidized water is neither toxic nor harmful for tissue and skin but may damage certain instrument surface materials. It is inactivated by organic matter and not stable during storage. It can be used for instrument disinfection, particularly in endoscope washer-disinfectors.

Chlorine dioxide (example Tristel):
An oxidising disinfectant with good bactericidal, fungicidal, virucidal and sporicidal activity. Stable on storage but unstable after activated for use. Can be an irritant to skin and mucous membranes. May damage some materials. Inactivated by organic matter.

The properties of the above mentioned agents are listed in the following table:

Disinfectants in Development

Glucoprotamin:
Antimicrobial activity at 1.5% in 60 min. Bactericidal, mycobactericidal, fungicidal, and virucidal. Only sporicidal in undiluted solution. Dissolves in water, active in the presence of organic matter, non-toxic, non-mutagenic, non-teratogenic, and non-corrosive.

Tea tree oil:
An essential oil from the leaves of *Melaleuca alternifolia* (an Australian plant) obtained by steam distillation. It has a long history of use as a topical antiseptic due to its antiflammatory and antimicrobial activity.

Antimicrobial activity: Bactericidal (minimum bactericidal concentration: 0.003 – 8%), Fungicidal (minimum fungicidal concentration for yeast: 0.12 – 1%; for filamentous fungi: 0.12 – 8%), Virucidal (HSV1& HSV2 - IC50: 0.0009 – 0.008%). The actual antimicrobial activity depends on the composition of the batch of oil.

Table 1: Properties of some new disinfectants

Agent	Microbial activity					*Inact by organic matter*	*Irritant allergenic*	*Corrosive damage*	*Stability of in-use sol*
	Bacteria		Viruses						
	Veg. Form	Spore Form	Myco-bact.	Env.	Non-env.				
Glutaraldehyde 2%	++	slow	+	++	++	NO	I/A	No	+
O-Phthalaldehyde 0.55%	++	-	++	++	++	NO	I/A	NO*	++
Hydrogen peroxide	++	slow	+	++	++	NO	I	slight	++
Peracetic acid 0.2%	++	++	++	++	++	slight	I	Yes	-
Mono-Percitric acid	?	++	?	++	+	YES	?	?	--
ClO_2, Cl_2-releasers	++	++	++	++	++	YES	I	YES	--
Superoxidized water	++	++	+	++	+	YES	--	YES	--

++ very good, + moderate, - poor, -- not, I irritant, A allergenic *stains protein

Table 2: New European standards for disinfectants used in the medical field

Standard number	Application	Target organisms	Phase/step*
EN 12791	Hands (surgical)	bacteria	2/2
EN 14561	Instruments	bacteria	2/2
EN 14562	Instruments	fungi/yeasts	2/2
EN 13624	Instruments	fungi	2/1
EN 13727	Instruments	bacteria	2/1
EN 14348	General	mycobacteria	2/1
EN 14476	General	viruses	2/1
prEN**14563	Instruments	mycobacteria/TB	2/2
prEN 12054	Hands	bacteria	2/1
prEN 13623	Water	legionella	2/1
prEN 13713	Surfaces	bacteria	2/1

* Phase 2 step 1 tests are performed in suspension; phase 2 step 2 tests are performed on surfaces (including skin). Surface tests simulate most practical use situations more accurately than suspension tests.
**prEN standards are standards that are still provisional

Octoxy hand rub:
Consists of ethanol (approx. 68% V/V), octoxyglycerine (emollient) and preservatives. In a pig skin model with artificial contamination, a 15 second exposure results in synergistic activity against *Staphylococcus aureus*, demonstrating an increased reduction compared with ethanol. Shows good reductions against *Staph. aureus* on volunteers' hands. This compound also shows a sustained action 15 min. after application to a challenge with *Staph. epidermidis, Staph. aureus* or *E. coli*.

N-Chlorotaurine (a derivative of the amino acid taurine):
A weak oxidant produced by stimulated human PMNs and monocytes, which destroys pathogens in oxidative bursts. It has low toxicity and is non-allergenic. It has been synthesized as a sodium salt.

It is bactericidal (*S. aureus, E. coli, P. aeruginosa*, etc.), fungicidal (yeast, filamentous fungi), and virucidal (HSV1 and 2, Adeno 5, HIV). It has enhanced microbicidal activity at low pH and in presence of N-H compounds such as glycine. NH_4Cl formation of a "chlorine cover" on microbial cell surface causes lethal effects on microorganisms by oxidation of proteins. This compound has possible therapeutic use as an antiseptic on mucous membranes.

Developments in European Disinfectant Standards
In addition to standards already published, CEN Technical Committee 216, Working Group 1 (medical application) has approved, as of the end of 2005, the following standards for publication in the near future. All apply to disinfectants for use in medical areas.

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