



LEADER

Bacterial adaptation and resistance to antiseptics, disinfectants and preservatives is not a new phenomenon

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*Study the past if you would divine the future
(Confucius, Analects)
And he that will not apply New Remedies must
expect New Evils; for Time is the Greatest
Innovator (Francis Bacon, Essays: Of Innovation)*

Introduction

There has been considerable recent interest in bacterial adaptation and resistance to antiseptics and disinfectants (which, with preservatives, comprise the modern term, 'biocide').¹⁻⁵ It might thus be surmised that this insusceptibility is a new and worrying phenomenon, especially if it is associated with an increase in antibiotic resistance in the clinical, domiciliary and other environments.¹⁻⁹

It is the purpose of this short report to examine whether insusceptibility to biocides (1) is, indeed, an entirely new phenomenon, (2) is increasing, and (3) if so, is likely to pose a significant clinical and other problem.

Bacterial adaptation and resistance: historical perspective

Early studies

It is important to appreciate that some types of biocides have been used for a century or more, whereas others are of more recent vintage. The introduction of biocides as antiseptics or disinfectants into clinical practice³ or for the purposes of pharmaceutical, cosmetic or other types of preservation¹⁰ have been described. Alcohol, for example, was employed over 2000 years ago as an antimicrobial agent, although its usage as such was not then appreciated. In the 19th and early 20th centuries, phenolics and hypochlorites were used. Later came the introduction of quaternary ammonium compounds (quats, QACs) and more recently chlorhexidine (CHX) salts. Organic mercurials, silver salts, peroxygens (hydrogen peroxide, peracetic acid, ozone) and glutaraldehyde have also been described. The latest, very useful, biocide to be used in the clinical setting is *ortho*-phthalaldehyde (OPA).

From time to time, there have been reports of reduced bacterial susceptibility to some of these agents. Many of the investigations have been laboratory-based with little attempt to relate such findings to the clinical or other environment. It is

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now known that laboratory findings do not necessarily apply in such environments.⁶

As far back as 1887, Kossiakoff¹¹ found that bacteria acquired the faculty of developing resistance to gradually increasing doses of some chemical agents (including boric acid and mercuric chloride). Later, Masson¹² described the adaptation of *Bacillus pyocyaneus* (*Pseudomonas aeruginosa*), *Bacillus subtilis* and *Bacillus anthracis* to resorcinol, salicylic acid and mercuric chloride, although the involvement of bacterial spores was unclear, and Regenstein¹³ studied the adaptation of bacteria to disinfectants including phenol. Other early papers worthy of consideration described combinations of disinfectants¹⁴ and the theory of disinfection,¹⁵ both pertinent to bacterial resistance.

A particularly interesting set of ideas was propounded by Meader and Feirer,¹⁶ who examined what they termed 'drug fastness' in one strain of *Bacillus typhosus*, three strains of *Bacterium* (*Escherichia*) *coli* and one strain of *Bacterium lactis aerogenes* to 'familiar germicides' (silver nitrate, mercurochrome, formaldehyde, acriflavine, hexylresorcinol and phenol). These authors determined whether drug fastness (1) developed in vitro, (2) was specific to any type of organism, (3) was related to reduced susceptibility to other agents, and (4) persisted when the organisms were cultivated in a germicide-free environment. All of these aspects remain highly relevant.

A year later, Fleming and Allison¹⁷ described the development of stable lysozyme-resistant cultures of *Micrococcus lysodeikticus*, using a method (individual colony development within inhibition zones) often employed today. Stepwise training of *M. lysodeikticus* and *Streptococcus* (*Enterococcus*) *faecalis* also resulted in lysozyme resistance. Furthermore, there was acquired resistance to the bactericidal power of blood and to intracellular digestion by leucocytes.

In 1943, McIntosh and Selbie¹⁸ produced drug- and biocide-resistant cultures in vitro of *Streptococcus pyogenes* group A and *Staphylococcus pyogenes* by stepwise training. In the latter organism, cross-resistance was observed between the acridine, proflavine and the diamidine, propamidine.

Bacterial adaptation and resistance to different types of biocides

Acridines and other dyes

In the 1940s and 1950s, a series of papers on the

adaptation of '*B. lactis aerogenes*' to acridines appeared from the Oxford group, led by Sir Cyril Hinshelwood, who further considered some of their findings in a classical book.¹⁹ In these papers,²⁰⁻³² several aspects were examined, notably the effect of pH on the antibacterial activity of proflavine (PF), 'training' to PF adaptation, the number of subcultures needed to confer adaptation, stability of adaptation, influence of PF on the lag phase of growth of control (unadapted) and adapted cultures, similarity in the actions of PF and methylene blue and cross-adaptation between the two agents (this only partially occurred to crystal violet) and the induction of filamentous forms on agar containing PF. Baskett³³ found that the ability of cells to grow in the presence of PF was a function of their previous treatment with the aminoacridine, and that adaptation was greatly accelerated if PF was added gradually to actively growing cultures rather than on a single occasion. However, this effect of gradual PF addition might be more apparent than real because lowering of pH of the medium as a consequence of metabolic activity would be expected to lead to decreased activity of PF, as pointed out below also.

In the late 1950s, a series of papers was published by Yudkin and colleagues,³⁴⁻³⁸ who investigated the resistance of the Gram-negative organisms, *Aerobacter aerogenes* and especially *E. coli*, to PF. The distribution of resistance of *E. coli* was measured by the number of organisms able to multiply in the presence of PF and then in its absence. Their studies with *E. coli* demonstrated that training to PF resistance occurred, that the organism formed spontaneous mutants resistant to PF with cross-resistance to chloramphenicol in five out of six mutants,³⁵ that there were cycles of resistance at different stages in the growth cycle without cross-resistance to chloramphenicol or other antibiotics³⁷ and that PF resistance was lost on subculture in PF-free medium.³⁷ They also pointed out³⁶ that the apparent rapid development of resistance when PF was added gradually to actively metabolizing cells³³ was, in fact, the result of pH changes in the culture that rendered the acridine much less active.

Sugino³⁹ produced mutants of *E. coli* that were sensitive to methylene blue and acridines. Nakamura^{40,41} found that the *acrA*⁺ gene controlled resistance not only to basic dyes but also to phenethyl alcohol. Acquisition of the resistance gene by an acriflavine-sensitive *E. coli* resulted in a reduction in the cellular accumulation of the acridine.⁴² Wody-Karner and Greenberg⁴³ also described PF resistance in *E. coli*. Lowick and James⁴⁴ trained cells of *A. aerogenes* to crystal

violet resistance and observed alterations in electrophoretic mobilities, indicating that changes in the bacterial cell surface had taken place.

Phenolics and salicylanilides

Hinshelwood¹⁹ stated that *B. lactis aerogenes* could be subcultured as many as 100 times in a sub-inhibitory concentration of phenol without showing any recovery towards a normal growth rate. In contrast, the organism rapidly acquired resistance to sulphonamides, acridines, propamidine and triphenylmethane and other dyes. Nevertheless, bacterial resistance to phenols has been observed, but has been less extensively studied than the acridines.

For example, in very early studies, Regenstein¹³ described the adaptation of bacteria to disinfectants including phenol, and Masson¹² to the phenolic, resorcinol. Fogg and Lodge⁴⁵ and Berger and Wyss⁴⁶ also examined bacterial resistance to phenols. The latter authors used *Micrococcus pyogenes* var. *aureus* (*S. aureus*) as their test organism and showed that if it was grown just previously in the presence of phenol, the phenol-resistant strain appeared to be considerably more resistant to the lethal effects than the wild-type strain. Furthermore, the resistant strain maintained its resistance through 40 transfers in broth. Mrozek⁴⁷ considered that resistance to disinfectants was unlikely, although by serial passage a certain increase in insusceptibility was apparent, more so with a QAC than with phenol.

Bennett⁴⁸ reviewed the factors influencing phenol action and discussed the possible resistance of bacteria to phenols. Bean and Walters⁴⁹ showed that the intracellular material leaked from cells inactivated by phenols could serve as a possible source of nutrients for survivors, thereby accounting for the increase in colony-forming units during the latter stages of a disinfection treatment. Hugo and Franklin⁵⁰ examined the effects of lipid enhancement on the resistance of *S. aureus* to a series of 4-*n*-alkylphenols (phenol to hexylphenol). They found that not until the pentylphenol was reached did increased cell wall lipid content protect cells from the inhibitory action; protection was even greater against the hexyl derivative. However, Hamilton^{51,52} observed no protection of wall lipid against the phenolic, hexachlorophane, or salicylanilides.⁵³

QACs

The QACs have found a useful place in reducing microbial infection. First synthesized nearly 90

years ago, they have been used since the mid-1930s for a variety of medical, pharmaceutical and other purposes.^{3,10}

Over 50 years ago, tolerance and adaptation to the QACs were noted by several workers. Chaplin^{54,55} and Crocker⁵⁶ studied the resistance by training of bacteria to QACs. It was shown that *S. marcescens* and *E. coli* could develop resistance to QACs, although some of the procedures involved the use of very high QAC concentrations in nutrient liquid media such that precipitation or cloudiness of the media could be a limiting factor. It was not possible to increase the resistance of *S. aureus*, but this could have been due to the high concentrations of QAC employed.^{54,55} Physical characteristics of *E. coli* colonies were altered.⁵⁶

The role played by pH in the adaptation of *S. marcescens* to Roccal^R (alkyldimethyl benzylammonium chloride) was evaluated by Fischer and Larose⁵⁷ and Fischer.⁵⁸ QACs are more active at alkaline than at acid pH, and it was found that at pH 6.8 *S. marcescens* increased its resistance from growth in 1/100 000 [0.001% (w/v)] to 1/5000 [0.02% (w/v)] in three transfers, whereas at pH 7.7 there was an increase only to 1/45 000 [0.0022% (w/v)].

MacGregor and Elliker⁵⁹ stated that *P. aeruginosa* could acquire tolerance during continuous exposure to QACs, whilst Cousins⁶⁰ noted that residual QAC could remain on equipment with the possibility therefore of acting as a selective process for resistant organisms.

Soprey and Maxcy⁶¹ described the adaptation of *E. coli* and *P. fluorescens* to QACs. They observed a gradual build up in the numbers of individual cells that tolerated the essential plateau of maximum tolerance. A reverse process was true during the loss of tolerance resulting from growth in the absence of QACs. These authors also made the important point that there was no appreciable difference between adapted and non-adapted cultures when exposed to lethal, standard in-use concentrations of the QAC.

Geftic *et al.*⁶² described the long-term survival of *P. cepacia* in salt solution preserved with benzalkonium.

CHX salts

CHX salts appeared on the scene some considerable time later. Consequently, references describing CHX resistance are more recent in origin. Nakahara and Kozukue⁶³ found that most clinical isolates of *E. coli* showed minimum inhibitory concentrations (MICs) of CHX within the range of 0.39-56 mg/L. A few isolates had MICs above 5 mg/L and these were

also multidrug- and multiple-metal-resistant. Stickler *et al.*⁶⁴ made the important observation that cationic biocides, including CHX, could select for strains of Gram-negative with multiple antibiotic resistance. These and other aspects of CHX action and resistance have been discussed by Russell and Day.⁶⁵

Other antibacterial agents

The diamidines, propamidine and dibromopropamide isethionates, find a useful place as biocidal-type agents that are employed for therapeutic purposes.⁶⁶ Acquired resistance of *S. aureus* to propamidine and of *S. pyogenes* to dibromopropamidine have been demonstrated.^{67,68} In each case, there was cross-resistance to other diamidines but not to penicillin or PF.

Wille⁶⁹ examined the development of bacterial resistance to some commonly used disinfectants, and concluded that such an event occurred with formaldehyde and chloramine-80. Nolte⁷⁰ also found a low increase in resistance to the former.

Hospital disinfectants and bacterial contamination

Most of the findings presented above have been based on laboratory studies only. It is thus instructive to evaluate whether bacterial resistance to hospital disinfectants was considered to be a clinical or other problem.

Several papers have appeared that demonstrate the, at times, inadequacy of disinfectants. Lowbury⁷¹ described the contamination of cetrimide with *P. pyocyanea* (*P. aeruginosa*). Keown *et al.*⁷² observed that septicaemia from this organism resulted when another QAC, benzalkonium chloride, was used for the 'cold sterilization' of an oxygenator. Von Dold and Gest⁷³ cultivated *P. fluorescens* from QACs but not from phenols or an amphoteric surfactant. Plotkin and Austrian⁷⁴ reported 40 instances of bacteraemia from use of needles and catheters stored in QAC solutions contaminated with *P. aeruginosa* and benzalkonium chloride was considered a source of hospital infection with Gram-negative bacteria.^{75,76}

Alcaligenes faecalis was found to contaminate a phenolic disinfectant⁷⁷ and Parker⁷⁸ stated that places where disinfectants were inactive were often those in which Gram-negative survivors could multiply. Bassett⁷⁹ isolated *P. multivorans* from infected wounds and traced the source to

containers of a 1 in 30 dilution of Savlon^R [0.05% (w/v) chlorhexidine salt + 0.5% (w/v) cetrimide].

Dixon *et al.*⁸⁰ have emphasized the role played by QACs in clinical practice and have also discussed the disadvantages of these biocidal agents. Finzi *et al.*⁸¹ considered benzalkonium chloride, CHX and an iodophor to be problematic solutions. More recently, intrinsic microbial contamination of iodophors has again been observed,^{82,83} with biofilm formation being responsible in at least one instance.⁸²

Several short papers have appeared on CHX. Dulake and Kidd⁸⁴ studied postoperative urinary infections and found an organism, most nearly identified as *A. faecalis*, in the urine of 30 gynaecological patients during bladder drainage by indwelling catheter. Spigots used for the closure of catheters and a jar of 0.1% (w/v) solution in which the spigots were stored after heat disinfection were also heavily contaminated with this organism. Beeuwkes⁸⁵ noted that *Proteus* spp. were less sensitive than other bacteria to CHX, but Lubsen *et al.*⁸⁶ stated that there was no evidence that resistance of *P. rettgeri* had developed, a conclusion that was confirmed by Davies *et al.*⁸⁷ and Gillespie *et al.*⁸⁸ Beeuwkes and de Vries⁸⁹ supported the use of CHX in urology.

At first sight, therefore, there seems to be an occasional insurmountable problem, insofar as bacterial resistance to disinfectants in actual practice was known some 50 years ago, with many reports of the same within the following 10-20 years. However, as pointed out by Russell,³ it would be incorrect to state that bacterial insusceptibility was always found. In many instances, inactivation of a QAC by cotton, inadequate quality of water as diluent, the use of cork liners for containers, poor storage, and 'topping up', might all have contributed, at least in part, to the apparent bacterial resistance found in practice.

Bacterial adaptation and resistance to preservatives

Preservative systems in pharmaceutical/medical and other types of products are of less interest to clinical and environmental microbiologists than are antiseptics and disinfectants. Preservatives are, nevertheless, worthy of brief consideration for three reasons. First, preservative concentrations are normally well below those used as antiseptics and especially as disinfectants, so that bacterial resistance could be a more potent threat than to antiseptics or disinfectants. Second, some

preservatives, e.g. QACs and CHX, may also, at higher concentrations, be used as antiseptics and disinfectants. Third, many of these preserved products, including cleaning solutions, are themselves widely used in hospitals, nursing homes and domiciliary environments.

Over the years, several authors have described the ability of bacteria to adapt to preservatives used in pharmaceutical and cosmetic formulations⁹⁰⁻¹⁰⁰ or to chemical agents used as sanitizers.⁹³ In some cases, this has meant reformulation using a new preservative system. Borovian⁹⁶ isolated a strain of *P. (Burkholderia) cepacia* that was not only able to grow in a product at low pH, but was also able to adapt to two chemically unrelated preservatives, benzoic acid and formaldehyde.

However, it must be remembered that several factors can influence activity of preservatives within a formulated product. These include possible incompatibility with the active and other ingredients, phase partitioning and pH.¹⁰⁰ Thus, caution and experience are needed to ensure that false conclusions about development of bacterial resistance to preservatives are not reached.

The present: lessons from the past

Biocidal agents have been used in one form or another for very many years.^{53,101-105} It is clear from the foregoing that bacterial adaptation and resistance to biocides is by no means a new phenomenon. Laboratory studies conducted over a century ago, together with many studies until the 1960s have demonstrated that this was appreciated and that attempts were made by some workers to determine its significance and, in some cases, to achieve a better understanding of the mechanisms involved. More recent studies, not considered here, but reviewed elsewhere in some detail,¹⁻⁹ have in many cases confirmed and extended these findings, although the level of resistance is often not of a high order.

Some of the chemical agents investigated in the earlier work, e.g. acridines, crystal violet, methylene blue, are little used nowadays and so their current relevance is minimal. In another context, however, the awareness that adaptation and resistance could arise, that it might be stable or unstable and that cross-resistance to other, chemically unrelated agents (sometimes to both antibiotics and non-antibiotics) could occur are all of considerable relevance. Unfortunately, much of this earlier work cannot be related to clinical or environmental situations.⁶ Furthermore, many of

the papers quoted relied solely on MIC determinations as an indicator of adaptation or resistance. It is now known that MICs provide a useful starting point in investigations of the antibacterial activity of biocides, but cannot be relied upon to show that reduced susceptibility has occurred to in-use bactericidal concentrations.⁶

Contamination of disinfectant solutions has been noted by several authors, although this is not necessarily associated with reduced susceptibility. Nevertheless, this did lead to a more rational approach in the correct procedures for both preparing and storing disinfectant solutions. The possible need for the introduction of biocide rotation in hospitals has also been appreciated.¹⁰⁶ Residual concentrations of QACs were considered by Cousins.⁶⁰ The possible effects of residual levels of biocides as a selective process for resistant bacteria have, much more recently, been re-examined.^{107,108}

Preservatives are an acceptable part of the formulation of many pharmaceutical and cosmetic products. In some instances, they were used as an aid to a thermal sterilization process. They have been employed for many years in both non-sterile and sterile pharmaceutical products, including various types of immunological ones.¹⁰⁹ It is of interest to note that concerns expressed some 20-30 years ago in relation to the possible development of resistance to preservatives⁹⁰⁻⁹⁷ are still being voiced today.¹⁰⁰ Balsams and phenols were used as preservatives in mummification,⁵³ and it would be thought that there would have been ample time for resistance to have developed in the intervening period.

There are, then, lessons that can be learned and conclusions that can be reached. As reduced susceptibility to biocides has been known for a long time, it might be expected by now to have resulted in the development of highly biocide-resistant strains. This does not appear to be the case.^{3,8} It might thus be argued that resistance to biocides is unlikely to occur in the future. This conclusion is also unwarranted, because in recent years there has been an explosion in the use of biocides, particularly in many household products. The nature of many such products leaves much to be desired. The inclusion of antibacterial agents is often unnecessary¹¹⁰ and has unfortunately increased the possibility of bacterial resistance arising. It is of interest to note that at least one testing method now requires information about the development of resistant bacteria.¹¹¹ There are current concerns about the usage of QACs, CHX and triclosan and possible bacterial resistance to them and to antibiotics. These aspects have been

considered elsewhere¹⁻⁹ and will not be re-examined here.

It is thus essential that antiseptics and disinfectants, together with preservatives incorporated into formulated products, should be employed only when necessary and then only with a full appreciation of the factors influencing their activity. Additionally, more detailed information is required about the actions and activity of biocidal agents on bacteria and other types of micro-organisms^{112,113} and of the mechanisms involved in bacterial insusceptibility.^{6,114}

References

- Levy SB. Antibacterial household products: cause for concern. *Emerg Infect Dis* 2001;**7**:512–515.
- Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. *J Appl Microbiol* 2002;**92**:725–775.
- Russell AD. Introduction of biocides into clinical practice and the impact on antibiotic resistance. *J Appl Microbiol* 2002;**92**:1215–1355.
- Chapman JS. Biocide resistance mechanisms. *Int Biodet Biodeg* 2003;**51**:133–138.
- Chapman JS. Disinfectant resistance mechanisms, cross-resistance and co-resistance. *Int Biodet Biodeg* 2003;**51**:271–276.
- Russell AD. Biocide usage and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. *Lancet Infect Dis* 2003;**3**:794–803.
- Aiello A, Larson E. Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community. *Lancet Infect Dis* 2003;**3**:501–506.
- Gilbert P, McBain A. Potential impact of increased use of biocides in consumer products on prevalence of antibiotic resistance. *Clin Microbiol Rev* 2003;**16**:189–208.
- Orth DS. The impact of antibiotic resistance on the development of cosmetics and drugs. *IFSCC Mag* 2000;**3**:27–34.
- Wallhäuser KH. Antimicrobial preservatives used by the cosmetic industry. In: Kabara JJ, editor. *Cosmetic and drug preservation. Principles and practice*. New York: Marcel Dekker; 1964. p. 605–745.
- Kossiakoff MG. De la propriété que possèdent les microbes de s'accommoder aux milieux antiseptiques. *Ann Inst Pasteur* 1887;**1**:465–476.
- Masson ML. Sur l'accoutumance des bactéries aux antiseptiques. *CR Acad Sci* 1910;**150**:189–191.
- Regenstein H. Studien über die Anpassung von Bakterien an Desinfektionsmittel. *Zbl Bakt Hyg, I Abt Orig* 1912;**63**:281–298.
- Frei W. Versuche über Kombination von Desinfektionsmitteln. *Z Hygiene Infektionskr* 1913;**75**:433–496.
- Frei W. Zur Theorie der Desinfektion. Über des Mechanismus der Elektrolytwirkung bei der Desinfektion durch Kreselseifenlösungen. *Z. Infektionskr* 1913;**15**:407–426.
- Meader PD, Feirer WA. Drug fastness in its relation to the resistance of certain organisms toward familiar germicides. *J Infect Dis* 1926;**39**:237–249.
- Fleming A, Allison VD. On the development of strains of bacteria resistant to lysozyme action and the relation of lysozyme action to intracellular digestion. *Br J Exp Pathol* 1927;**8**:214–218.
- McIntosh J, Selbie FR. The production of drug-resistant cultures of bacteria in vitro and a study of their inter-relationships. *Br J Exp Pathol* 1943;**24**:246–252.
- Hinshelwood CN. *The chemical kinetics of the bacterial cell*. Oxford: Clarendon Press; 1946. chapters V and VI, p. 95–128, and 129–59, respectively.
- Davies DS, Hinshelwood CN, Pryce JM. Studies in the mechanism of bacterial adaptation. II. Action of methylene blue. *Trans Faraday Soc* 1944;**40**:405–409.
- Davies DS, Hinshelwood CN, Pryce JM. Studies in the mechanism of bacterial adaptation. III. Degree of specificity of the adaptative processes. *Trans Faraday Soc* 1944;**40**:409–412.
- Davies DS, Hinshelwood CN, Pryce JM. Studies in the mechanism of bacterial adaptation. I. Action of proflavine. *Trans Faraday Soc* 1944;**40**:397–404.
- Davies DS, Hinshelwood CN, Pryce JM. Studies in the mechanisms of the adaptive process. II. Methylene blue. *Trans Faraday Soc* 1944;**40**:405–409.
- Davies DG, Hinshelwood CN, Pryce JM. Studies in the mechanism of the adaptive process. III. Degree of specificity of the adaptive process. *Trans Faraday Soc* 1944;**40**:409–412.
- Davies DS, Hinshelwood CN, Pryce JM. Studies in the mechanism of the adaptive process. IV. Theoretical discussion of adaptation. *Trans Faraday Soc* 1944;**40**:412–417.
- Davies DS, Hinshelwood CN, Pryce JM. Studies in the mechanism of the adaptive process. V. Conditions affecting the occurrence of rate of training. *Trans Faraday Soc* 1944;**40**:417–419.
- Davies DS, Hinshelwood CN, Pryce JM. Adaptation of *Bacterium lactis aerogenes* to varying concentrations of an antibacterial drug (proflavine). *Trans Faraday Soc* 1945;**41**:163–169.
- Davies DS, Hinshelwood CN, Pryce JM. Adaptation of *Bacterium lactis aerogenes* to high concentrations of proflavine. *Trans Faraday Soc* 1945;**41**:778–785.
- Pryce JMG, Davies DS, Hinshelwood CN. Quantitative relation between the adaptations of *Bacterium lactis aerogenes* to two antibacterial agents (methylene blue and proflavine). *Trans Faraday Soc* 1945;**41**:465–471.
- Peacocke AR, Hinshelwood C. The adaptation of bacteria to acridine derivatives. The influence of pH. *J Chem Soc* 1948;**1235**–1244.
- Peacocke AR, Hinshelwood CN. The absorption of antibacterial substances (2,8-diaminoacridine and methylene blue) by cells of *Bacillus lactis aerogenes*. *J Chem Soc* 1949;**2290**–2303.
- Dean ACR, Hinshelwood CN. Colony formation by *Bacterium lactis aerogenes* on solid medium containing antibacterial agents. *Proc Roy Soc* 1952;**B140**:339–352.
- Baskett AC. The resistance of *Bacillus lactis aerogenes* to proflavine (2:8-diaminoacridine). II. The direct induction of adaptation. *Proc Roy Soc B* 1952;**139**:251–262.
- Thornley MJ, Yudkin J. The origin of bacterial resistance to proflavine. I. Training and reversion in *Escherichia coli*. *J Gen Microbiol* 1959;**20**:355–364.
- Thornley MJ, Yudkin J. The origin of bacterial resistance to proflavine. II. Spontaneous mutation to proflavin resistance in *Escherichia coli*. *J Gen Microbiol* 1959;**20**:365–372.
- Sinai J, Yudkin J. The origin of bacterial resistance to proflavine. III. The alleged rapid adaptation to proflavine resistance in *Bacterium lactis aerogenes* (syn. *Aerobacter*

- aerogenes*, *Klebsiella pneumoniae*). *J Gen Microbiol* 1959; **20**:373–383.
37. Sinai J, Yudkin J. The origin of bacterial resistance to proflavine. IV. Cycles of resistance in *Escherichia coli* and their bearings on variations in resistance in cultures. *J Gen Microbiol* 1959; **20**:384–399.
 38. Sinai J, Yudkin J. The origin of bacterial resistance to proflavine. V. Transformation of proflavine resistance in *Escherichia coli*. *J Gen Microbiol* 1959; **20**:400–413.
 39. Sugino Y. Mutants of *Escherichia coli* sensitive to methylene blue and acridines. *Genet Res* 1966; **7**:1–11.
 40. Nakamura H. Gene-controlled resistance to acriflavine and other basic dyes in *Escherichia coli*. *J Bacteriol* 1965; **90**: 8–14.
 41. Nakamura H. Genetic determination of resistance to acriflavine, phenethyl alcohol and sodium dodecyl sulfate in *Escherichia coli*. *J Bacteriol* 1968; **96**:987–996.
 42. Nakamura H. Acriflavine-binding capacity of *Escherichia coli* in relation to acriflavine sensitivity and metabolic activity. *J Bacteriol* 1966; **92**:1447–1452.
 43. Woody-Karrer P, Greenberg J. Resistance and cross-resistance of *Escherichia coli* S mutants to the radio-mimetic agent proflavine. *J Bacteriol* 1964; **87**:536–542.
 44. Lowick JHB, James AM. The electrokinetic properties of *Aerobacter aerogenes*. A comparison of the properties of normal and crystal-trained cells. *Biochem J* 1957; **65**: 431–438.
 45. Fogg AH, Lodge RM. Mode of antibacterial action of phenols in relation to drug-fastness. *Trans Faraday Soc* 1945; **41**: 359–365.
 46. Berger H, Wyss O. Bacterial resistance to inhibition and killing by phenols. *J Bacteriol* 1953; **65**:103–110.
 47. Mrozek Von H. Untersuchungen zum problem der resistenzentwicklung gegenüber desinfektionsmitteln. *Brauwissenschaft* 1967; **20**:229–234.
 48. Bennett EO. Factors affecting the antimicrobial activity of phenols. *Adv Appl Microbiol* 1959; **1**:123–140.
 49. Bean HS, Walters V. Studies on bacterial populations of solutions of phenols. Part II. The influence of cell exudate upon the shape of the survivor–time curve. *J Pharm Pharmacol* 1961; **13**:183T–194T.
 50. Hugo WB, Franklin I. Cellular lipid and the antistaphylococcal action of phenols. *J Gen Microbiol* 1966; **52**: 365–373.
 51. Hamilton WA. The mechanism of the bacteriostatic action of tetrachlorosalicylanilide. *J Gen Microbiol* 1968; **50**: 441–458.
 52. Hamilton WA. Membrane active antibacterial compounds. In: Hugo WB, editor. *Inhibition and destruction of the microbial cell*. London: Academic Press; 1971. p. 77–93.
 53. Onlooker, The art of the embalmer. *Pharm J* 2003; **271**:754.
 54. Chaplin CE. Observations on quaternary ammonium disinfectants. *Can J Bot* 1951; **29**:372–382.
 55. Chaplin CE. Bacterial resistance to quaternary ammonium disinfectants. *J Bacteriol* 1952; **63**:453–458.
 56. Crocker CK. Variations in characteristics of *E. coli* induced by quaternary ammonium compounds. *J Milk Food Technol* 1951; **14**:138–140.
 57. Fischer R, Larose P. Factors governing the adaptation of bacteria against quaternaries. *Nature* 1952; **170**:715–716.
 58. Fischer R. pH and the adaptation of bacteria versus quaternary ammonium disinfectants. *Mfg Chem* 1953; **24**: 195–196.
 59. MacGregor DR, Elliker PR. A comparison of some properties of strains of *Pseudomonas aeruginosa* sensitive and resistant to quaternary ammonium compounds. *Can J Microbiol* 1958; **4**:499–503.
 60. Cousins CM. Methods for the detection of survivors on milk handling equipment with reference to the use of disinfectant inhibitors. *J Appl Bact* 1963; **26**:376–382.
 61. Soprey PR, Maxcy RB. Tolerance of bacteria for quaternary ammonium compounds. *J Food Sci* 1968; **33**:536–540.
 62. Gefitic SG, Heymann H, Adair FW. Fourteen-year survival of *Pseudomonas cepacia* in salt solution preserved with benzalkonium chloride. *Appl Environ Microbiol* 1979; **39**: 505–510.
 63. Nakahara H, Kozukue H. Chlorhexidine resistance in *Escherichia coli* isolated from clinical lesions. *Zbl Bakt Hyg, I. Abt Orig A* 1981; **251**:177–184.
 64. Stickler DJ, Thomas B, Clayton CL, Chawla JC. Studies on the genetic basis of chlorhexidine resistance. *Br J Clin Pract* 1983; **25**:23–28.
 65. Russell AD, Day MJ. Antibacterial activity of chlorhexidine. *J Hosp Infect* 1993; **25**:229–238.
 66. Hugo WB. Amidines. In: Hugo WB, editor. *Inhibition and destruction of the microbial cell*. London: Academic Press; 1971. p. 121–136.
 67. Wien R, Harrison J, Freeman WA. Diamidines as antibacterial compounds. *Br J Pharmacol* 1948; **3**:211–218.
 68. Wien R, Harrison J, Freeman WA. New antibacterial diamidines. *Lancet* 1948; **i**:711–712.
 69. Wille B. Möglichkeiten einer resistenzentwicklung von mikroorganismen gegen desinfektionsmittel. *Zbl Bakt Hyg, I. Abt Orig B* 1976; **162**:217–220.
 70. Nolte H. Zur Resistenzsteigerung von Bakterien bei der Anwendung chemischer Desinfektionsmittel—1. *Mitteilung Hosp-Hyg, Gesund und Desinfekt* 1977; **69**:22–27.
 71. Lowbury E.J.L. Contamination of cetrimide and other fluids with *Pseudomonas pyocyanea*. *Br J Ind Med* 1951; **8**:22–26.
 72. Keown KK, Gilman RA, Bailey CP. Open heart surgery: anaesthesia and surgical experiences. *J Am Med Assoc* 1957; **165**:781–787.
 73. Von Dold H, Gust R. Über das Vorkommen Lebender Bakterien in Desinfektionsmitteln. *Arch Hyg* 1957; **141**: 321–333.
 74. Plotkin SA, Austrian R. Bacteraemia caused by *Pseudomonas* sp. following the use of materials stored in solutions of cationic surface-active agents. *Am J Med Sci* 1958; **235**: 621–627.
 75. Lee JC, Fialkow PJ. Benzalkonium chloride—source of hospital infection with Gram-negative bacteria. *J Am Med Assoc* 1961; **177**:708–710.
 76. Anon. Failure of disinfectants to disinfectant. *Lancet* 1958; **ii**:306.
 77. Simmons NA, Gardner DA. Bacterial contamination of a phenolic disinfectant. *BMJ* 1969; **2**:668–669.
 78. Parker MT. Causes and prevention of sepsis due to Gram-negative bacteria. Ecology of the infecting organisms. *Proc Roy Soc Med* 1971; **64**:979–980.
 79. Bassett DJC. Causes and prevention of sepsis due to Gram-negative bacteria. Common source outbreaks. *Proc Roy Soc Med* 1971; **64**:980–986.
 80. Dixon RE, Kaslow RA, Mackel DC, Fulkerson CC, Mallison GF. Aqueous quaternary ammonium antiseptics and disinfectants. *J Am Med Assoc* 1976; **236**:2415–2417.
 81. Finzi GF, Domenicoli W, Porcari L. Studi inerenti la resistenza di ceppi batterici nei confronti delle sostanze disinfettanti. *Arch Sci Med* 1980; **137**:495–498.
 82. Anderson RL. Iodophor antiseptics: intrinsic microbial contamination with resistant bacteria. *Infect Control Hosp Epidemiol* 1989; **10**:443–446.
 83. O'Rourke EO, Runyon D, O'Leary J, Stern J. Contaminated iodophor in the operating room. *Am J Infect Control* 2003; **31**:255–256.

84. Dulake C, Kidd E. Contaminated irrigating fluid. *Lancet* 1966;i:980.
85. Beeuwkes H. Chlorhexidine and *Proteus rettgeri* infection. *Lancet* 1961;ii:53.
86. Lubsen N, Boissevain W, Fass H. Chlorhexidine and *Proteus rettgeri* infection. *Lancet* 1961;ii:53–54.
87. Davies GE, Martin AR, Swain G, Rose FL, Stewart ASR. Chlorhexidine and *Proteus rettgeri* infection. *Lancet* 1961; i:1062.
88. Gillespie WA, Linton KB, Miller A, Mitchell JP, Slade N. Chlorhexidine and *Proteus rettgeri* infection. *Lancet* 1961; i:1287.
89. Beeuwkes H, de Vries HR. Chlorhexidine in urology. *Lancet* 1956;ii:913–914.
90. Smart R, Spooner DF. Microbiological spoilage of pharmaceuticals and cosmetics. *J Soc Cosmet Chem* 1972;23: 721–737.
91. Rosen WE, Berke PA. Modern concepts of cosmetic preservation. *J Soc Cosmet Chem* 1973;24:663–675.
92. Croshaw B. Preservatives for cosmetics and toiletries. *J Soc Cosmet Chem* 1976;28:3–16.
93. Yablonski JI. Microbiological aspects of sanitary cosmetic manufacturing. *Cosmet Toilet* 1978;93:37–50.
94. Lueck E. Acquired resistance to preservatives. *Antimicrobial food additives*, Berlin: Springer; 1980. p. 32–35.
95. Orth DS. Evaluation of preservatives in cosmetic products. In: Kabara JJ, editor. *Cosmetic and drug preservation: principles and practice*. New York: Marcel Dekker; 1984. p. 403–421.
96. Borovian GE. *Pseudomonas cepacia*: growth in and adaptability to increased preservative concentrations. *J Soc Cosmet Chem* 1983;34:197–203.
97. Orth DS, Lutes CM. Adaptation of bacteria to cosmetic preservatives. *Cosmet Toilet* 1985;100:57–64.
98. Lehmann RH. Synergisms in disinfectant formulations. *Crit Rep Appl Chem* 1988;23:68–90.
99. Hill G. Preservation of cosmetics and toiletries. In: Rossmore HW, editor. *Handbook of biocide and preservative use*. London: Blackie. 1995 p. 349–415.
100. Hodges NA. Microbiological contamination and preservation of pharmaceutical products. In: Aulton ME, editor. *Pharmaceutics. The science of dosage form design*, 2nd edn. Edinburgh: Churchill Livingstone; 2002. p. 658–667.
101. Hugo WB. Phenols: a review of their history and development as antimicrobial agents. *Microbios* 1978;23:83–85.
102. Selwyn S. Early experimental models of disinfection and sterilization. *J Antimicrob Chemother* 1979;5:229–238.
103. Hugo WB. A brief history of heat and chemical preservation and disinfection. *J Appl Bacteriol* 1991;71:9–18.
104. Block SS. Historical introduction. In: Block SS, editor. *Disinfection, sterilization and preservation*, 5th edn. Philadelphia: Lippincott Williams and Wilkins; 2001. p. 3–17.
105. Newsom SWB. Pioneers in infection control—Joseph Lister. *J Hosp Infect* 2003;55:246–253.
106. Murtough SM, Hiom SJ, Palmer M, Russell AD. Biocide rotation in the healthcare setting: is there a case for policy implementation? *J Hosp Infect* 2001;48:1–6.
107. Thomas L, Maillard J-Y, Lambert RJW, Russell AD. Development of resistance to chlorhexidine diacetate in *Pseudomonas aeruginosa* and the effect of a 'residual' concentration. *J Hosp Infect* 2000;46:297–303.
108. Russell AD. Biocides and pharmalogically active drugs as residues in the environment: is there a correlation with antibiotic resistance? *Am J Infect Control* 2002;30: 495–498.
109. Russell AD. History of pharmaceutical (drug) preservation. In: Orth DS, Kabara JJ, Denyer SP, editors. *Handbook of cosmetic microbiology*, 2nd edn. New York: Marcel Dekker, in press.
110. Tan L, Nielsen NH, Young DC, Trizna Z. Use of antimicrobial agents in consumer products. *Arch Dermatol* 2002;138: 1082–1086.
111. Hobson DW, Bolsen K. Methods of testing oral and topical antiseptics and antimicrobials. In: Block SS, editor. *Disinfection, sterilization and preservation*, 5th edn. Philadelphia: Lippincott Williams and Wilkins; 2001. p. 1328–1359.
112. Russell AD. Similarities and differences in the responses of micro-organisms to biocides. *J Antimicrob Chemother* 2003;52:750–763.
113. Russell AD. Mechanisms of antimicrobial action of antiseptics and disinfectants: an increasingly important area of investigation. *J Antimicrob Chemother* 2002;49:597–599.
114. Russell AD. Bacterial resistance to biocides: current knowledge and future problems. In: Lewis P, Moran AP, Mahony T, Stoodley P, O'Flaherty V, editors. *Biofilms in medicine, industry and environmental biotechnology*. London: IWA Publishing; 2003. p. 512–533.