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Editorial

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Mini Review section – Antibiotics have successfully been used to treat bacterial infections for decades. Their use however, is becoming threatened as bacteria evolve mechanisms to become resistant to these drugs. The resistance of microorganisms to antibiotics has been developing for more than 2 billion years and is widely distributed among various representatives of the microbiological world. Bacterial enzymes play a key role in the emergence of resistance.

Current Trends section – The current technology and market for pressurized household aerosol cans is described, with reference to the different products that are sprayed and the requirements for each type of product. The probable future legislative restrictions on volatile organic compounds in many countries are outlined with reference to the repercussions in the current aerosol products industry.

In Profile Scientist – Doudna first made her name uncovering the basic structure and function of the first ribozyme, a type of catalytic ribonucleic acid (RNA) that helps catalyse chemical reactions. In addition to her scientific contributions to CRISPR, Doudna is known for spearheading the public debate to consider the ethical implications of using CRISPR-Cas9 to edit human embryos.

Bug of the month – *Candida* species re one of the most common fungal pathogens, causing invasive infections worldwide and accounting for 600,000 infections each year, and are isolated from approximately 25% of all patients in an intensive care unit with central line–associated bloodstream infections in the United States. *C auris* has become a fungal pathogen of great clinical concern and is now considered a pathogen of urgent threat level by the US Centers for Disease Control and Prevention (CDC).

Did You Know? It is well known that a mother's environment plays a key role in child health. However, recent research, including more than 24,000 offspring, suggests that this may also be true for fathers.
"Offspring with a father who smoked only prior to conception had over three times more early-onset asthma than those whose father had never smoked," says Professor Cecilie Svanes at the Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen (UiB).

Best Practices – Problems associated with the development and spread of antibiotic resistance in clinics have been increasing since the early 1960s and are currently viewed as a major threat to clinical practice. It is generally accepted that the main cause of this problem has been and still is widespread inappropriate use and overprescribing of antibiotics in clinical medicine, animal husbandry, and veterinary practice. Concern about bacterial resistance has led to calls for increased education of both the public and professionals on the correct use of antibiotics and more stringent infection control measures to reduce the transmission of infection.

Unwind your mind with some light humour in our **Relaxed Mood section**. Feedback & suggestions are always welcomed.

Bacterial Enzymes and Antimicrobial Resistance

Antibiotics have successfully been used to treat bacterial infections for decades. Their use however, is becoming threatened as bacteria evolve mechanisms to become resistant to these drugs. The resistance of microorganisms to antibiotics has been developing for more than 2 billion years and is widely distributed among various representatives of the microbiological world. Bacterial enzymes play a key role in the emergence of resistance.

Classification of these enzymes is based on their participation in various biochemical mechanisms: modification of the enzymes that act as antibiotic targets, enzymatic modification of intracellular targets, enzymatic transformation of antibiotics, and the implementation of cellular metabolism reactions. The main mechanisms of resistance development are associated with the evolution of super-families of bacterial enzymes due to the variability of the genes encoding them. The collection of all antibiotic resistance genes is known as the resistome. Tens of thousands of enzymes and their mutants that implement various mechanisms of resistance form a new community that is called "the enzystome." Analysis of the structure and functional characteristics of enzymes, which are the targets for different classes of antibiotics, will allow in the development of new strategies for overcoming the resistance.

INTRODUCTION

Following the discovery of penicillin we enjoyed a 'golden age' of medicine whereby nearly all bacterial diseases could be treated with relative ease. This is now hampered by the evolution of bacteria that can resist antibiotics, and the propagation of these bacteria through improper use of the drugs.

Selman Waksman, a prominent researcher in the field of actinomycetes in the early part of the twentieth century, described the term antibiotic as a chemical compound generated from microorganisms that inhibits or destroys other microbes. Most antibiotics in use today originated from the phylum Actinobacteria with nearly80% of actinobacterial-derived antibiotics produced by soild welling bacteria of the genus *Streptomyces*. Before the discovery of natural antibiotics, synthetic compounds, including salvarsan, sulfa drugs and quinolones, were in use as chemotherapeutic agents. Penicillin was the first natural antibiotic to be discovered accidentally by Alexander Fleming in 1928 when the *Penicillium* fungus contaminated a culture plate in his laboratory however, penicillin was not developed for use until the late 1930s.Penicillin inhibits cell wall synthesis and was found to be very effective against Gram-positive but not against Gram-negative bacteria (due to the presence of the outer membrane)or the *tubercle bacillus* (because of the extra thick cell wall).

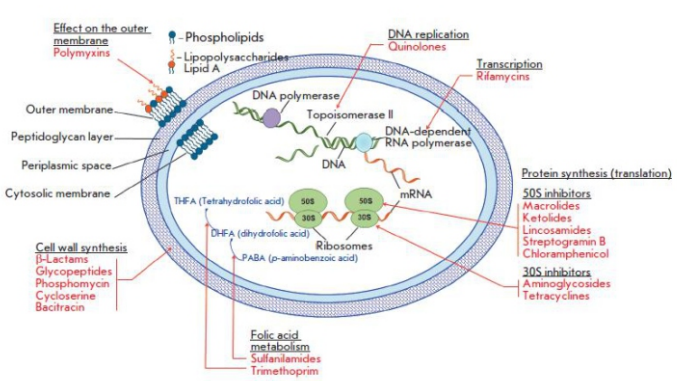


Fig. 1. The main classes of antimicrobial drugs, their targets, and their effect on the main processes of vital activity of a bacterial cell

Antibiotic resistance of the causative agents of infectious diseases is a global problem in biology and medicine. Modern Antimicrobial Drugs (AMDs) represent the largest group of pharmaceutical drugs, including 16 classes of natural and synthetic compounds (Fig. 1).

Synthesis of antibiotics has existed in nature for more than 2 billion years. During all this time, bacteria have been developing mechanisms of resistance to their toxic action. Resistance may occur as an adaptive process unrelated to the structure of an antibiotic or develop as a result of the selection of resistant strains of microorganisms under the influence of antibiotics. The anthropogenic factors associated with the application of antibiotics in medicine and, especially, in agriculture since the mid-20thcentury have led to a significant evolution of resistance mechanisms; the time it takes to develop resistance to new drug has significantly reduced.

The role of bacterial enzymes in resistance development is rather versatile and involves several key mechanisms (Fig. 2). The enzymes involved in cell wall biosynthesis, as well as the synthesis of nucleic acids and metabolites, serve as a direct target for antibiotics. The resistance mechanism is associated with structural changes in these enzymes. Another mechanism is associated with the enzymatic modification of the structural elements affected by antibiotics: for example, modification of ribosomes by methyltransferases. A large group of enzymes modify or destroy the structure of antibiotics by inactivating them. Enzymes catalyzing metabolic processes and modifying AMDs in the form of prodrugs are also involved in resistance development.

The bacterial enzymes that determine resistance usually belong to large superfamilies; many of them originated from enzymes that originally had other functions. The genes responsible for the synthesis of these enzymes and their mutational variability are of ten localized on mobile genetic elements, thus ensuring the rapid spread of resistance between microorganisms.

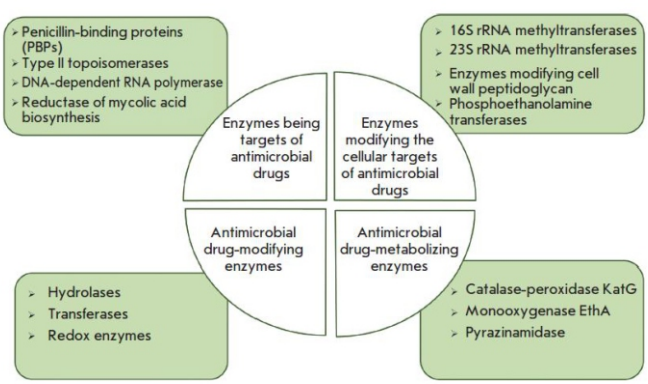


Fig 2: Classes of enzymes involved in various mechanisms of resistance to antimicrobial drugs

This review presents data on the functional features of the main classes and groups of the bacterial enzymes involved in the implementation of the mechanisms of bacterial resistance to AMDs.

I. BACTERIAL ENZYMES AS THE TARGETS OF AMDs

1. Penicillin-binding proteins

Penicillin-binding proteins (PBPs) play a key role in the synthesis of peptidoglycan, the main component of bacterial cell walls. PBPs are the targets of β-lactam antibiotics. Peptidoglycan is a polymer consisting of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) residues (Fig. 3). Peptides containing L-Ala, D-Glu, meso-diaminopimelic acid or L-Lys, and two D-Ala residues are attached to all NAM residues. PBPs are bound to the inner cell membrane or found in free form in the cytosol. PBPs are divided into high-molecular-weight (> 50 kDa) proteins consisting of two domains and low-molecular-weight proteins (< 50 kDa).

The N-terminal domain of high-molecular-weight PBP catalyzes transglycosylation reactions (sequential elongation of glycan chains by the addition of NAG-NAM-pentapeptide to the glycan backbone, 1 in Fig. 3). The C-terminal domain catalyzes transpeptidase reactions (cross-linking of peptide residues in two glycan chains, 2 in Fig. 3). Low-molecular-weight PBPs prevent cross-linking in peptidoglycan; they catalyze endopeptidase (hydrolysis of the peptide bond connecting two glycan chains, 3 in Fig. 3) and carboxypeptidase (hydrolysis of the bond in D-Ala-D-Ala dipeptide, 4 in Fig. 3) reactions.

The C-terminal domains of all PBPs are the targets of β-lactam antibiotics, which constitute more than half of all currently, used AMDs. These antibiotics contain a β-lactam ring, a structural analogue of D-Ala-D-Ala dipeptide, and, therefore, act as competitive inhibitors of PBPs. The interaction between the carbonyl group in the β-lactam ring and the hydroxyl group of serine in the active center of a PBP gives rise to an inactive acylated form of the enzyme. Irreversible inhibition disrupts the synthesis of the bacterial cell wall.

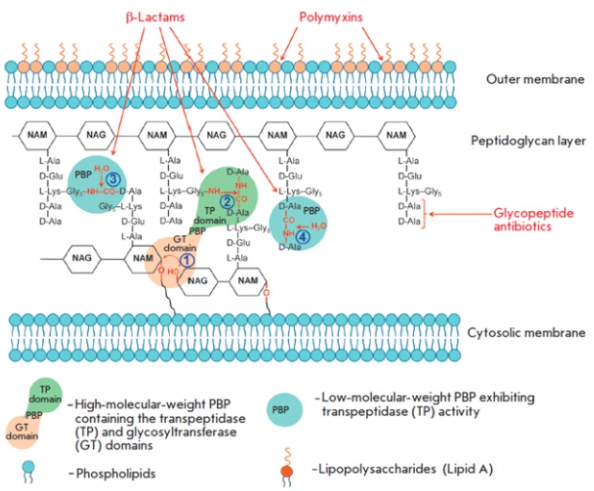


FIG 3: The structure of bacterial cell wall peptidoglycan and involvement of penicillin-binding proteins in different reactions of its synthesis: 1 – transglycosylation reaction, 2 – transpeptidation reaction, 3 – endopeptidation reaction, and 4 – carboxypeptidation reaction.

The main reasons why Gram-positive bacteria develop resistance to β-lactam antibiotics include mutations in native PBPs, their hyperproduction, and the synthesis of new PBPs that are insensitive to inhibition by β-lactams. Today, the spread of *Staphylococcus aureus* strains resistant to methicillin and other semisynthetic penicillins and cephalosporins poses a threat. Resistance is determined by expression of the fifth enzyme, PBP2a (in addition to the four native PBPs), which has low affinity for β-lactam antibiotics and exhibits transpeptidase activity only. Figure 4 shows the resistance mechanism: without an antibiotic, both domains of a high-molecular-weight PBP are involved in peptidoglycan biosynthesis (A); only the glycosyltransferase domain remains active in a high-molecular-weight PBP in the presence of an antibiotic, while the transpeptidase domain is acylated and does not form crosslinks. It is the acquired low-molecular-weight PBP2a (B) that exhibits transpeptidase activity in the resistant strain. As a result, cell viability is restored.

PBP2a enzymes are encoded by the genes mecA or mecC. The mecA and mecC genes, together with the genes regulating their expression (mecI, mecR1 and mecR2), are the components of the mobile genetic element of the staphylococcal cassette chromosome mec.

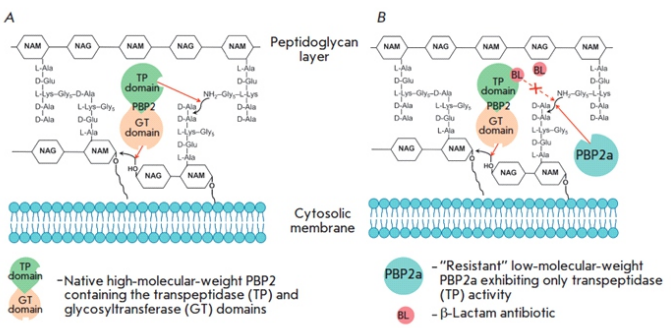


FIG 4: The role of penicillin-binding proteins in the resistance of Gram-positive bacteria to β-lactam antibiotics. A – sensitive strain, B – resistant strain

Proteins belonging to the PBP family play a crucial role in the formation of the bacterial cell wall and are precursors of the resistance caused by β-lactamase production (see Section "β-Lactamases").

2. Type II topoisomerases: DNA gyrase and topoisomerase IV

Type II topoisomerases include DNA gyrase and topoisomerase IV, which catalyze changes in the spatial configuration of the DNA molecule during replication, transcription, and cell division. DNA gyrase and topoisomerase IV are heterotetrameric enzymes: DNA gyrase consists of two GyrA subunits (97 kDa) and two GyrB subunits (90 kDa); topoisomerase IV consists of two ParC subunits (84 kDa) and two ParE subunits (70 kDa). The GyrA and ParC subunits form the catalytic domains involved in the formation of complexes with the DNA molecule for its break/ligation; the GyrB and ParE subunits exhibit ATPase activity to supply energy to the process.

DNA gyrase and topoisomerase IV serve as targets for quinolones and their derivatives, fluoroquinolones. Formation of the DNA–type II topoisomerase complex is a necessary condition for inhibition (Fig. 5). The site of antibiotic binding to the enzyme in the ternary complex is known as the quinolone-binding pocket.

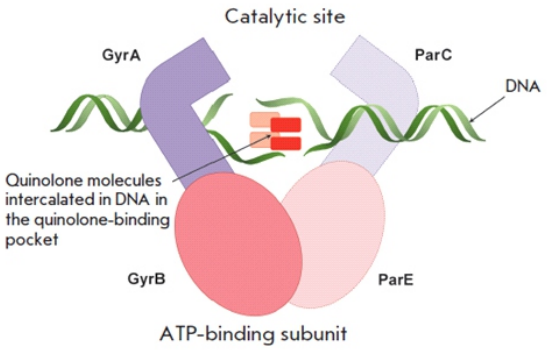


Fig. 5. The schematic structure of a ternary complex between type II topoisomerases, DNA, and quinolones. (Gyr A, Gyr B – gyrase subunits, Par C, Par E – topoisomerase IV subunits)

The antibiotic binds non-covalently to the active site of the enzyme, so the motion of the enzyme and the replication fork along the DNA molecule is stopped. The formation of the tertiary quinolone–topoisomerase type II–DNA complex stops not only replication, but also transcription, since the motion of RNA polymerase along the DNA template is inhibited. Therein, breaks are formed in the double-stranded DNA molecule, which also determines the bactericidal action of quinolones. Quinolones do not affect mammaliantype II topoisomerases, because they differ significantly from bacterial topoisomerases.

The development of quinolone resistance is mainly associated with a reduction in the efficiency of their interaction with the DNA-type II topoisomerase complex due to mutations in the genes, leading to amino acid substitutions in the quinolone-binding pocket. The region of the genes where mutations occur is called QRDR (the quinolone resistance-determining region). These mutations mainly localize to the N-terminal part of the GyrA subunit (the region between residues 67– 106 according to the E. coli numbering system) and/ or ParC subunit (amino acid residues 63–102) (Fig. 6) but can also affect the GyrB and ParE subunits.

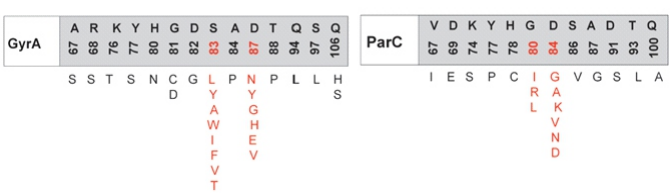


FIG 6: Amino acid mutations in the QRDR region of the GyrA and ParC subunits of type II topoisomerases from *E. coli*, which are responsible for the resistance to quinolones. The color indicates the positions of the mutations whose combination causes a synergistic effect.

The degree of reduction in sensitivity to an antibiotic depends on the mutation type and develops gradually. First, mutations occur in one enzyme and, only later, in another one. A single amino acid substitution at position 67 of the GyrA subunit in *E. coli* increases the MIC of all fluoroquinolones fourfold; at position 81 of the same subunit, eightfold; at position 87, 16-fold; and at position 83, 32-fold. The genes of both subunits carry several mutations, and a synergistic effect is often observed in microorganism strains with a high level of quinolone resistance. Thus, a combination of mutations at GyrA positions 83 and 87 and at ParC position 80 increases the MIC of fluoroquinolones over 4,000-fold.

3. DNA-dependent RNA polymerase

The bactericidal effect of rifamycins (rifampin, rifabutin) consists in inhibiting DNA-dependent RNA polymerase. This enzyme consists of five subunits: two α- (molecular weight of each subunit is 35 kDa), β- (155 kDa), β'- (165 kDa), and σ-subunits (70 kDa). The four subunits ββ'αα form the so-called apoenzyme, which exhibits catalytic activity and performs all the main stages of transcription. Transcription initiation and recognition of bacterial gene promoters require the formation of a holoenzyme, which occurs when the regulatory σ-subunit binds to the apoenzyme.

Rifamycins selectively bind to the β-subunit of the enzyme near the main channel and inhibit elongation of the originating RNA strand. The emergence of resistance to rifamycins in most cases is associated with mutations in a relatively small fragment of the rpoB gene (codons 507–533) encoding the β-subunit of RNA polymerase. Mutations in amino acid residues at positions 513, 516, 526, and 531 (Fig. 7) are characterized by the highest degree of polymorphism.

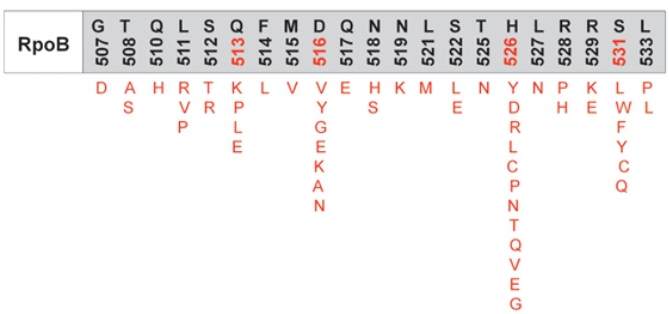


FIG 7: Amino acid mutations in the RpoB fragment of the β-subunit of RNA polymerase, which are responsible for the resistance to Rifamycins

4. Enzymes catalyzing the biosynthesis of mycolic acids

The term "mycolic acids" is a generic name for a group of long-chain branched fatty acids, components of the mycobacterial cell wall. Some antituberculosis drugs, derivatives of isonicotinic acid (isoniazid, ethionamide and prothionamide), suppress the synthesis of mycolic acids. These drugs are targeted at enoyl-acyl carrier protein reductase (known as InhA), which is a component of FAS-II fatty acid synthase. It catalyzes the reduction of D2-unsaturated fatty acids to saturated ones using the NADPH cofactor as a hydrogen donor. Disrupted synthesis of mycolic acids suppresses the synthesis of the mycobacterial cell wall.
Resistance to these drugs is caused by mutations in the *inhA* gene, which affect either both the promoter region of the *mabA–inhA* operon and cause hyperproduction of the enzyme, or the sequence encoding the enzyme, thus reducing its affinity for the complex between the isonicotinic acid radical and NAD+.

Pressure Can Technology

The current technology and market for pressurized household aerosol cans is described, with reference to the different products that are sprayed and the requirements for each type of product. The probable future legislative restrictions on volatile organic compounds in many countries are outlined with reference to the repercussions in the current aerosol products industry. It is described how these restrictions will generate new research interest in the more efficient production of sprays, and particularly in the reduction or replacement of hydrocarbon flashing propellants. The problems associated with these aims and their possible solutions are then described.

Introduction

The pressurized household aerosol can is found universally in the developed world and in most homes in the developing world. We here use the expression "household aerosol" to describe insecticide sprays introduced during WW2. Figure 1 shows a typical aerosol can which is pressurized, typically to 4 to 5 bar, using a liquefied gas. That is, the propellant is a low boiling point fluid (b.p. typically between 220K and 270K). Up to 15 years ago this fluid was a refrigerant such as Dichlorodifluoromethane (Refrigerant 12), which is of course a CFC and is now banned because it is a so-called greenhouse gas. The reasons for use of liquefied gas propellant and the replacements of the first CFC propellants are described in the next section which provides an overview of the current technology.

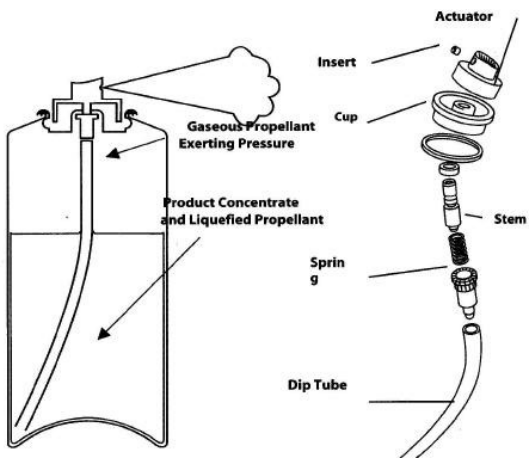


Fig. 1 Generic aerosol can

Fig. 2 Typical household sprays, from left to right: starch, air freshener with vertical spray, cleaning fluid using trigger pump, and body spray using finger pump.

Pressurized cans used for household purposes such as cleaning, polishing etc., and we include the so called "personal care" area, i.e. deodorants, hair sprays etc. Furthermore, the use of these devices extends into other areas, such as for lubrication, paint application and de-icing of vehicles. Pressurized aerosol cans are relatively recent in their mass use, with the first examples being

1. The present technology and markets

1.1 Present technology

Figure 2 illustrates some typical household spray products. The starch and air freshener cans on the left both utilized flashing propellants. The air freshener has a vertical nozzle, in line with the can axis, for ease of use. It also uses a more modern injection moulded actuator that forms part of the cap of the aerosol can. The two *pump sprays* on the right in Fig. 2 are shown to illustrate this technology, which tends to complement rather than compete with the pressurised aerosol can format. This pump technology is sometimes used either when droplet size is not ofgreat importance (for example for the window cleaning spray, second from the right in Fig. 2) or when a fine spray can be produced because the liquid has a low viscosity and surface tension and a low flow rate is needed (for example for the body spray on the right in Fig. 2). In these examples the *trigger pump* pumps the liquid to pressures up to around 5 bar. The *finger pump* achieves rather lower pressures. These devices have a restriction that the liquid to be sprayed must not be sensitive to contamination due to its prolonged contact with the air in the bottle. Although the pumps are relatively expensive to manufacture, they do not require the expensive high-pressure filling equipment needed for pressurised aerosol cans, and also refilling is straightforward. All the devices in Fig. 2 use a miniature swirl atomizer insert.

For pressurised aerosol cans, the principal replacement for CFC propellants has been liquefied hydrocarbon (HC), principally butane but with "blends" with, mainly, propane to "fine tune" the boiling point. These hydrocarbons are not powerful greenhouse gases and their thermodynamic properties are sufficiently close to CFC's for the changeover to be relatively straightforward for the industry with little noticeable deterioration in spray quality for different applications. There is an appreciation of the potential danger of hydrocarbons due to flammability and, in certain industrial applications in Europe, a Voluntary Code of Practice permits the use of non – flammable Hydrofluorocarbon (HFC) propellant instead of HC's. For all propellants, and as seen in Fig. 1, a simple spring loaded valve is attached to the can and, on depression by the injection moulded polymer attachment on top of the valve (referred to as an *actuator*) the can pressure forces the liquid phase propellant-product solution, or mixture, up through the *dip tube* and around the valve stem, into a central vertical channel in the actuator. For most cans the channel inside the actuator turns through approximately 90 degrees in order to produce a horizontal spray. Generally, it is not possible to injection mould both the actuator and the final nozzle and orifice as one unit, however, see the descriptions of novel technologies later in this paper. Thus, the final nozzle is a separate injection moulded item, which is referred to as an *insert.* As shown

schematically in Fig. 3, with very few exceptions these inserts are either of the *simple orifice* type, or they incorporate a pressure swirl atomizer which are referred to as a *Mechanical Break Up Unit* or "*MBU*".

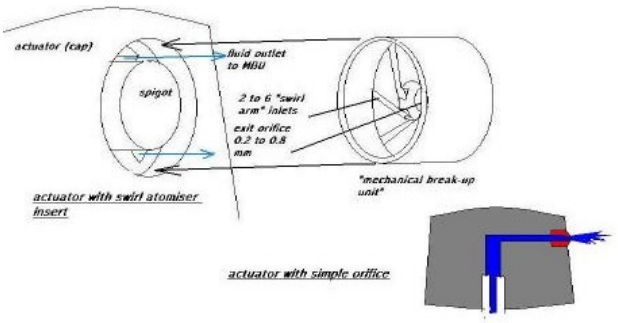


Fig. 3 MBU and simple orifice designs

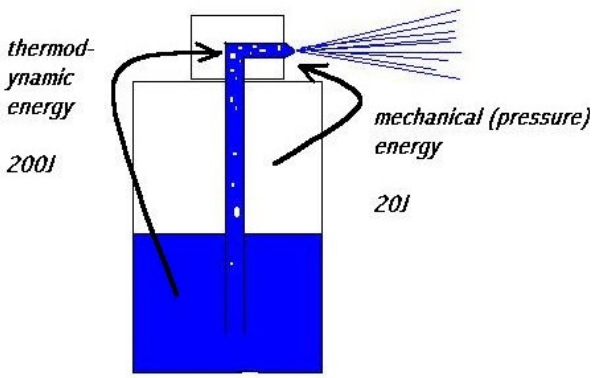


Fig. 4 Energy sources for flashing propellant

It is useful at this point to recognize the great advantages of using a flashing (liquefied) propellant. An aerosol can is typically safety accredited to around 12 bar internal pressure and allowing for use at high ambient temperature and for storage in the hold of an aircraft at low ambient pressure, safety margins limit the internal can pressure to typically 4.5 to 5 bar at STP for the surrounding environment. For "inert" compressed gas propellants such as Nitrogen, a can pressure around 8 bar is possible. All of the energy for atomization must be contained in a small (typically less than 0.5 litre) can and it is desirable that at least half the can contents, when new, should be liquid. As illustrated in Fig. 4 we can consider the mechanical energy available for atomizing where this is simply the work done by the gas pressure pushing the liquid out of the can: this may be typically 20J for a flashing propellant, for which the can pressure does not decrease greatly during evacuation of the liquid due to continual release of new vapour. However, if, say, Nitrogen were used to pressurize the can, the can pressure would halve during use giving only 15J approximately of mechanical pumping energy. The flashing propellant is also a source of energy from thermodynamic effects due to its phase change during flow through the device and particularly at the exit. This phase change results in high gas phase exit velocity so that the exit nozzle is effectively a two-fluid atomizer where the gas/liquid mass ratio may be at least 5:1. As shown in Fig. 4 this thermodynamic energy is at least two orders of magnitude greater than the mechanical pumping energy. Thus the following statement can be made:
"*Aerosol cans with flashing propellants have enough available energy to achieve any mean drop size that is realistically required and for most types of liquid formulation, even for difficult high viscosity liquids or suspensions: Take away the energy available due to flashing and there are very few applications, which have combinations of large drop size and "easy" liquid properties, such that current technology can be applied to give satisfactory sprays".*

Figure 4 indicates bubbles in the dip tube and the actuator. This is inevitable because the pressure drops with the flow along the tube, through the valve and around the bend in the actuator: every drop in pressure changes more of the propellant into the gas phase. An inevitable result of this pressure drop and phase change is that heat is removed to provide the latent heat and this heat comes from the liquid and also the actuator. Thus aerosol cans andtheir sprays are always cool to the touch when spraying. This cooling action may be important when the sprayed formulation contains oils, such as silicone oil found in antiperspirants: these oils will increase in viscosity and may thus result in larger droplets. Many aerosol cans use a *vapour phase tap* (VPT) to deliberately introduce vapour into the flow in the actuator. The VPT is simply one or two holes in the fitting between the dip tube and the valve, which allows vapour from the top of the can to enter the liquid flowing into the valve. The mass flow of this vapour is relatively very small compared with the flow of liquid phase up the dip tube. This is because the pressure drop across the VPT orifices, which controls the vapour flow, relies on there being a pressure drop in the dip tube from its inlet up to the VPT position: this drop in pressure is very much less than the can pressure. The VPT reduces drop sizes and also, due to the gas blockage effect, reduces the spray flow rate.

1.2 The markets

The size of the pressurised aerosol can market needs to be appreciated in order to understand the economic importance of the industry to many countries. In 2002, the world-wide production exceeded *11 billion units* and the approximate breakdown of this production by regions is shown in Fig. 5. Most but not all of these units are spraying devices, other devices being used in non-spraying formats to dispense mainly gels or foams.

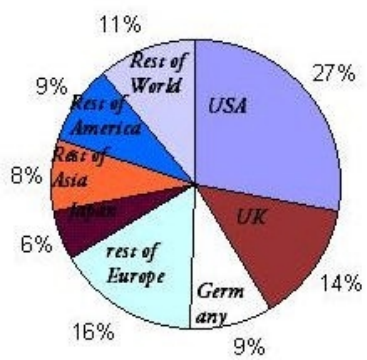


Fig. 5 Division of total world production of 11billion units in 2002.

Of course each unit is of relatively low value, however even allowing for this the value of the total market is comparable with, if not higher than other spray technology areas, including gas turbines, spray drying equipment, agricultural spraying, and medical spraying devices. However the published research relevant to the pressurised aerosol industry is completely negligible compared with these other fields. This is due entirely to the use of flashing propellant which makes "good sprays" so easy to produce: a luxury which, as described below, is now coming to an end.

Figures 6 and 7 provide data on production in Europe for 2002 for the main types of application, divided into "personal products", in Fig. 6, and "household products" in Fig. 7,

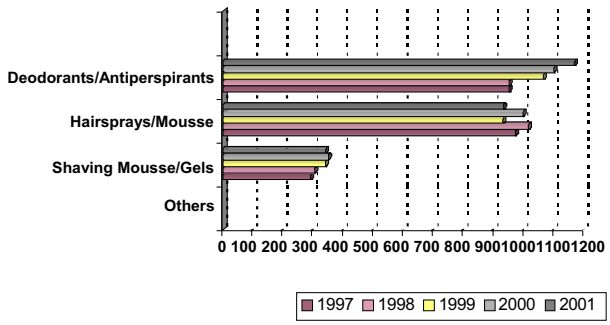


Fig. 6 Personal aerosol production in Europe, thousands of units (European Aerosol Federation)

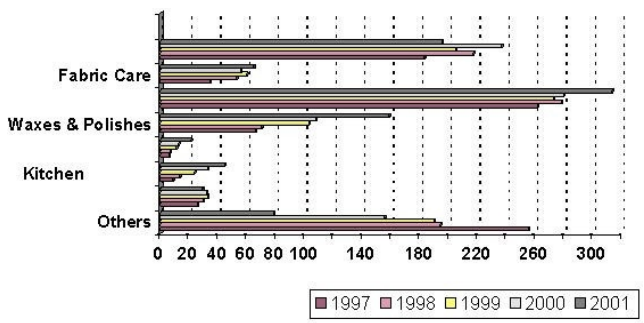


Fig. 7 Household aerosol production in Europe, thousands of units (European Aerosol Federation)

using the terminology of the industry. It can be seen that there are no signs of reductions in production in Europe, however legislation that may affect production is not yet implemented. The largest volumes of production are for deodorants/antiperspirants, hairsprays, air fresheners and insecticides. Also "other products", not shown here include cans for paints, lubricants and other industrial uses.

2. The challenges

The HFC propellants, e.g. HFC 134a, used for some products, particularly in the USA, remain relatively potent greenhouse gases and they are now subject to increasing legislation in countries abiding to the "Kyoto protocol". The EU has agreed an overall target to reduce emissions of a "basket" of greenhouse gases by 8% by 2010. Domestically DME is another propellant that is used where flammability is a particular issue, however it is a Volatile Organic Compound (VOC) as are, of course, hydrocarbon propellants (HC). VOC's are being subjected to increasing legislative control, with EU controls in force on major sources (IPC, IPPC and Solvents Directive) including Traffic (Auto Oil Programme). In Europe VOC emissions from aerosols are only around 3% of the problem: however they are very "visible" and are considered wasteful or particularly unnecessary because they are not resulting from some reaction in an essential transport or production process. What is classified as a VOC tends to vary from region to region, however in Europe ethanol (alcohol) *is* considered to be a VOC. This means that a deodorant using HC propellant to spray ethanol, with perfume traces, is 100% VOC. In the USA there are severe restrictions on the % of VOC in consumer products, including aerosol can products. The California Air Resources Board (CARB) has led the way with legislation, however it appears likely that the rest of the USA and also Europe will closely follow. However the main "solution" to VOC reduction in the USA has been the use of HFC propellant, and this is not an option for countries, including the EU, which abide with the Kyoto protocol. The problem approaching for the pressurised aerosol industry in these latter countries can be expressed quite starkly:

"*It is very likely that in the near future there will be restrictions in both VOC and greenhouse gas content in cans and there is no permitted flashing propellant available to replace the removed HC or HFC. Thus it will be necessary to make cans spray more efficiently, using lower HC content, or using inert gas propellant or other technologies, whilst at the same time replacing any ethanol in the can, by aqueous liquids*"

Table 1 provides a summary of the present main formats of pressurised aerosols, with the main emphasis on the EU marketplace. Some guidance on CARB legislation is also provided together with a description of some of the problems involved in meeting this type of legislation.

3. Possible solutions

3.1 Overview

It is envisaged that legislation will, in the foreseeable future, be mainly directed at severe reductions in the VOC content of aerosols. Eventually it is quite possible that the complete banning of VOC content may be applied, at least to a certain range of uses. The reduction of VOC leads to two major problems, plus a range of ancillary difficulties related to these problems. These major problems are:

(1) Reduction of VOC inevitably means using less flashing propellant: this leads to less thermodynamic energy for atomising and probably a lower can pressure, so less mechanical energy. An ancillary problem is that for most sprayed formulations, there is significant non- vaporised liquid propellant in the emerging spray so that these "carrier droplets" contain significant (usually HC) propellant. In practice it is likely that removed liquid phase propellant from the can must be replaced, and the only acceptable replacement is water. With its high surface tension, and high viscosity, compared with liquid HC, this gives greater demands on achieving good atomisation efficiency. There is also a marketing reason for such replacement: the customer may resist purchasing products that do not appear to have much liquid in the can.

(2) The can contents of ethanol and certain solvents will need removal or reduction. Ethanol is the main carrier fluid for many important applications. The problems caused by replacing it with water, which appears to be inevitable as there are no other replacements that are not targeted by legislation, have been described already.

In the next subsections some of the approaches to solving these problems are briefly described.

3.2 Reduced VOC cans with improved actuator fluid mechanics

With the exceptions of work by the groups of the present author, see Sharief et al (*ibid*), and also Sher (*ibid*), there is very little published research that is aimed at improving the designs of actuators and inserts when using flashing propellants. Clearly the large number of patents in the field of actuator and insert design indicates that manufacturers have had an interest in this subject: however examination of current products shows no significant fluid mechanical differences from those of 25 years ago. Sher's

work has shown that a chamber prior to the exit orifice causes extra vaporisation to give essentially a two-fluid atomizer with higher gas/liquid gas ratio and thus smaller droplets. That such improvements have not been generally implemented is due to the lack of incentives for the industry, either economic or legislative. Also, incorporating more complex flow control devices inside actuators would add to the number of component parts and thus the cost. However new methods of injection moulding of actuators and inserts as single units now permit more complex internal flow systems to be incorporated. In the present author's group quite intensive work is in progress to derive suitable devices, for different sprayed formulations, that permit acceptable sprays to be achieved with reduced VOC filling of the can.

3.3 Compressed gas aerosols

The use of an inert compressed gas in the can (usually Nitrogen, but Carbon Dioxide has some advantages due to its solubility in water) was the subject of considerable interest in the early 90's. This was driven by worries about the flammability of HC propellants as VOC legislation was then not decided. In particular ethanol-based deodorants with compressed gas around 6 bar were marketed with very small MBU's some with exit orifices of 0.15mm diameter. These products failed, possibly due to consumer non-acceptance of the spray quality. One difficulty with compressed gas propellants is that the can pressure drops during use, to perhaps 3 bar. Manufacturers incorporated miniature pressure regulators to maintain constant flow rate during can life, however these could only operate by maintaining the supply pressure to the MBU at the pressure of the can when it is empty, thus wasting pressure energy. In recent years some compressed gas aerosol cans have been introduced successfully to the market, polish sprays in particular. However the relatively large volume median diameter achieved, around 150 microns, does not appear create major difficulties in this application. Special devices have been introduced or patented, but without major market shares, which maintain a high can pressure during can lifetime. One device is a small high pressure storage reservoir inside the can, similar to a soda siphon refill, that gradually bleeds gas into the can. If (inert) compressed gas is to replace flashing propellants for a wide range of applications, then dramatic improvements are required in the efficiencies of the atomizer inserts that are used. There have been proposals for bleeding off some of the gas used to pressurise the can and adding it to the liquid flowing through the actuator. This would create two-fluid atomisation. However this is technically very challenging and simple calculations show that, assuming the new can is 50:50 liquid and gas with 8 bar gas pressure, the available gas/liquid mass ratio for atomization is no more than 0.5%.

3.4 Pumps

Pump technology for household sprays is well established but it has not necessarily been the subject of intensive research and development, reduction of manufacturing costs being an overriding factor. Improved, more easily used pumps, that provide higher pressures than at present are required and these need combining with improved atomizer inserts. Once again some interest has focused on combined liquid-air pumps, which could be used with two-fluid atomizer inserts. In practice there would be a resistance to changeover from pressurized aerosols to pump technology by customers, unless methods can be found for making the pump devices as easy and convenient to use as the pressurized cans.

3.5 "Exotic" devices

More than one person in the field has proposed that electrical power may be utilised with aerosol cans in some way in order to produce or enhance atomization. This power may be from a battery or supplied by applying pressure to piezoelectric transducers. This may be thought to be out of the question due to cost considerations, however it must be recalled that production is in billions so that the volume production savings are immense. Metered dose inhalers for asthma treatment are suffering similar needs for new formats without flashing propellant, although to a lesser extent than for pressurized aerosol cans. Successful portable inhaler products are now widely used, which are battery powered and use ultrasonic atomization. However, the values of these devices are two orders of magnitude greater than those of aerosol cans so that there would be considerable development work needed in order to introduce them to the supermarket shelves.

3.6 Liquefied gas

Liquefied propellants are gases that exist as liquids under pressure. As the aerosol is under pressure, the propellant exists mainly as a liquid, but it will also be in the headspace as a gas. As the product is used up as the valve is opened, some of the liquid propellant turns to gas and keeps the headspace full of gas. In this way, the pressure in the can remains essentially constant and the spray performance is maintained throughout the life of the aerosol. The propellant is an essential element in the formulation.

The main industry where LPG in its natural odourless form is required is, in fact, the aerosol industry, where it is considered an ideal 'propellant'.

Aerosol propellant grade LPG consists of high purity hydrocarbons derived directly from oil wells, and as a by-product from the petroleum industry.

It consists of a high purity mixture of propane, isobutane and n-butane. By the combination of these substances with regard to their different physical-chemical parameters, the producers of aerosol preparations achieve the required pressures inside aerosol cans, which ensure their problem-free use for the end customer. They are used in most aerosols today and have been used for many years in household aerosol products (especially for water-free environment or for products with small water content).

Aerosol propellant is used by manufacturers of Pesticides, Air-fresheners, Perfumes, Cosmetics, Spray Paint and Food grade packaging material.

Features

- LPG has a significantly lower cost when compared to other propellants such as dimethyl ether (DME) and Chlorofluorocarbons like 134a and 152a.
- LPG is a stable and pure propellant compound.
- LPG is an odourless, non-corrosive and non-toxic gas.
- The propellant is made up of natural compounds.
- LPG offers a wide range of applicable vapour pressures and boiling points.
- LPG is a versatile and efficient propellant.

References:

British Aerosol Manufacturers Association (BAMA) of London.
The European Aerosol Federation (FEA), of Brussels.

Table 1 Summary of current aerosol products

Product Category	CARB VOC limit	Current Products (Europe)	Typical Current Spray Characteristics	Problems
Hair spray	55%	European formulations 80% - 90% VOC (+), butane/propane, polymer in ethanol. European moves to restrict VOCs. Also DME or DME/butane at 30% propellant; 50% alcohol and 15% water. MBU (Swirl) inserts common.	Vol Med Dia. 50-80 microns spray angle 30 degrees (+), hollow cone. Flow rate 0.3 to 1.2g/sec. Would like to limit fraction of droplets less than 10micron e.g. less than 5- 7%	Require good atomisation, negatively affected by viscosity increase with water addition. Fast drying rate negatively affected by water and increased droplet size. Products must wet out hair effectively. Non - Newtonian with complex polymers in product, extensional viscosity?
Air fresheners	Progressively, 30% to 18% (liquid/pump sprays)	Typically around 30% butane 68% water, plus fragrance, and emulsifier. Simple orifices common.	Vol Med Dia 30 to 45 microns. Flow rate 0.8g/s. 30degree full cone. 1.5m minimum throw (penetration)	Finer sprays preferred, but must have adequate penetration (throw), difficult to get finer than 30-40 micron with aqueous formulation. Larger particle sizes rapidly settle, taking fragrance with them. Inhalation problems?
Anti-perspirants	HVOC 40% and MVOC 10%	Simple orifice with "bull-nose" & VPT. Typ. composition: silicones 13%, hydrocarbon propellant 75%, Aluminium chlorohydrate powder 10%.	Vol Med Dia 10-20 microns. Flowrate 0.75-1g/s. Spray angle 20-30degrees full cone.	Interest in reducing inhalable fraction (sub-7 microns say) currently 20%+. Particulates + silicone = high viscosity: Non flashing spray would be coarse. Cool feel attractive to customer.
Personal Deodorants	HVOC 0% and MVOC 10%	Powder-free often essentially 97% VOC: 50% ethanol plus propellant plus perfume etc. MBU (Swirl) insert common.	Vol Med Dia up to 40 microns. Flowrate 0.6g/s. Spray angle up to 40 degrees, full or hollow cone.	Replacing ethanol with water can give poor "feel" to spray.
Spray Paints	88% but reducing	Hydrocarbon and/or DME propellant. Typically acrylic resins plus solvent . Solids 10-15%, HC 25-30% solvent 55%. MBU (Swirl) insert, or fan jets.	Ideally Vol Med Dia 40-50 micron. Flowrate 1g/s (+) Spray angle wide rages used.	Water based products for low VOC have drop size challenges. Narrow size distribution ideal: less than 30 microns = drift, greater than 60 microns = runs.
Insecticide	crawling bug 15% (31/1/2/04) flying bug 25% (31/12/04)	Hydrocarbon propelled, aqueous or solvent formulation. MBU or simple orifice, with VPT.	For flying insects Vol Med Dia 30-40 microns. Flowrate 0.5-1.0g/s (+). For crawling insects larger droplets and narrow coverage, 1-2.5 g/s	Good penetration with no "fall out" of large drops plus low inhalable fraction: low VOC aqueous formulations have difficulty in achieving this.
Furniture Polish	25% (01/01/94) 17% 31/12/04	Butane propellant, water, butane and solvent, usually MBU insert. Compressed air-driven systems: wax, solvent, surfactant, water: MBU	Vol Med Dia 110 (HC propellant) to 150 microns (+) compressed gas. Flowrate 1.5 g/s (+) Spray angle 30 to 90 degrees hollow cone.	Wax-water solution is viscous and non-Newtonian, problems with reduced solvent and no flashing.

Jennifer Doudna

Doudna first made her name uncovering the basic structure and function of the first ribozyme, a type of catalytic ribonucleic acid (RNA) that helps catalyse chemical reactions. This work helped lay the foundation for her later helping to pioneer CRISPR-Cas 9, a tool that has provided the means to edit genes on an unprecedented scale and at minimal cost. In addition to her scientific contributions to CRISPR, Doudna is known for spearheading the public debate to consider the ethical implications of using CRISPR-Cas9 to edit human embryos.

Family

Jennifer Anne Doudna was born in Washington DC. When she was seven years old she moved with her parents to Hilo, a small non-touristy town on the largest island of Hawaii, where her father became a professor in English literature at the University of Hawaii and her mother taught history at a local community college. Landing up in a place where most of the children were of Polynesian and Asian descent and came from a blue-collar background, Doudna always felt slightly out of place with her fair hair, blue eyes and academic parents. She often retreated into books or exploring the rugged volcanic landscape, beaches and lush vegetation of the island.(Kahn, Pollack)

Career

Doudna's doctorate proved to be just the beginning of a long career trying to work out the chemistry underlying RNA's many biological functions. Like many around her, she was intrigued by the idea that RNA could provide some clues about the origins of life.

Following her doctorate, Doudna spent some time working with Szostak and then left to take up a postgraduate fellowship in the laboratory of Thomas Cech at the University of Colorado in Boulder. One of the attractions of going to work with Cech was that he had just won the Nobel Prize, in 1989, for discovering the catalytic properties of RNA.(Marino) He also had the equipment to carry out X-ray diffraction which would help her decipher the three-dimensional atomic structure of RNA. With no formal training in x-ray diffraction, Doudna spent many of her early days in Cech's laboratory acquiring the skill alongside crystallising RNA molecules in preparation for imaging them.

In 1994 Doudna left Cech's laboratory to take up a position as an assistant professor at Yale University. Six years later she was promoted to become the Henry Ford II Professor of Molecular Biophysics and Biochemistry. In 2002, Doudna moved to the University of California, Berkeley, where she was appointed Professor of Biochemistry and Molecular Biology. Berkeley was particularly appealing to Doudna because it allowed her to be closer to her mother in Hawaii and her extended family. It also gave her access to the facilities of the Lawrence Berkeley National Laboratory. She was particularly keen to use the Laboratory's synchroton, a huge machine that provides high intensity X-ray beams. This would provide her with the means her to delve deeper into the complex structure of proteins and other molecules. (Russell)

Achievements

One of Doudna's first breakthroughs occurred when she was still a doctoral student in Szostak's laboratory. She helped demonstrate that RNA not merely carries instructions from DNA for synthesising proteins but also helps catalyse the process. (Doudna, Szostak) Published in 1989, their work helped revolutionise RNA research. Seven years later Doudna announced, together with Cech, the three-dimensional structure of the P4-P6 domain of the Tetrahymena thermophila group I intron ribozyme, a particular type of RNA. It was a major achievement because prior to this only one other single RNA structure had been unravelled, transfer RNA (tRNA), and it was much smaller and simpler than the ribozyme. Working with Cech and others, including Cate - her future husband, Doudna helped demonstrate that the ribozyme had a defined shape and an organised structure similar to proteins.(Cate et al; Marino) By 1998, Doudna and her team had determined the crystal structure of their first viral RNA - the hepatitis delta virus (HDV), a human pathogen linked to hepatitis B. By working on the structure of HDV they hoped to determine how viral RNAs functioned so as to develop treatments to combat viral disease. (Marino)

Doudna is now closely linked to the invention of a new tool for gene editing that has radically reduced the time and work needed to edit the genome. Originally this began as just a side-project from her main research. As narrated in her book with Sternberg (2017), it all started in 2005 with a phone call from Jillian Banfield, a colleague at Berkeley, who wanted Doudna to help her understand some repetitive sequences she had spotted in the genomes of some bacterial communities she was studying from highly acidic wastewater from a mine in northern California. Banfield was curious to know whether the sequences, known as CRISPR (clustered regularly interspaced short palindromic repeats), could be some form of RNA mechanism the bacteria used to protect themselves from viral infection. Never having come across CRISPR before, Doudna quickly got swept up in trying to figure out how CRISPR worked. Such work she believed could provide some clues into how small RNA molecules in human cells regulated genes and the pathways of RNA interference, a topic that she and her group were then investigating. She was also intrigued by the notion that bacteria might have a human-style immune system that recorded previous diseases to curb a future attack. Up to this moment scientists had assumed bacteria only had a rudimentary immune system.(Kahn, Mukhopadyay; Witowski)

A few years later, in March 2011, Doudna went to an American Society for Microbiology conference in Puerto Rico where she met Emmanuelle Charpentier, a French microbiologist and geneticist then based at Umea University in Sweden. Charpentier had noticed a mysterious enzyme, Cas9, associated with CRISPR that appeared to help Streptococcus pyogenes, a type of flesh-eating bacteria that causes many important human diseases, fend off invading viruses. Immediately warming to Charpentier, Doudna agreed to partner with her to find out more and sent over Martin Jinek, her postdoctoral researcher from the Czech Republic, to work with her. Thereafter a number of other researchers came on board, including Michael Hauser, a master's student from Germany who worked in Doudna Laboratory, and Krysztof Chylinski a Polish doctorate student of Charpentier who was based in her old laboratory at the University of Vienna.(Doudna and Sternberg)

After many months the collaborators figured out that the CRISPR defense mechanism consisted of two separate RNA molecules (CRISPR RNA and tracRNA) which helped guide Cas9 to snip out a piece of DNA at a precise point on the genome. Bacteria used the mechanism as a means to slice up viral DNA whenever and wherever it invaded a cell. Soon after they had puzzled this out, it suddenly dawned on Doudna and Jinek that the same bacterial defense system could be re-engineered in the laboratory to provide a tool for editing genes in all kinds of cells from different organisms. Within a short time they had demonstrated in test tubes using a jellyfish gene, called green fluorescent protein, that this was possible. What surprised them was how straightforward and easy the system was to use. Indeed, it was much less laborious and much faster than previous methods for gene editing, such as Zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). In 2012 the whole team published their findings in Science, concluding 'our methodology based on RNA-programmed Cas9 …. could offer considerable potential for gene targeting and genome editing applications.'(Jinek) The paper quickly grabbed the attention of molecular biologists and geneticists who grasped the method's significance. Following this, both Doudna and other scientists proved the technique could be used in human cells.

Since 2012 Doudna has been swept up in the whirlwind of excitement that CRISPR-Cas9 has fueled for many different applications. CRISPR-Cas-9 has the key advantage that it is easy to engineer. It is also very inexpensive. For example, it is 150 times cheaper than ZFNs. In addition, it is more precise. (Wang) Just how revolutionary the technique is proving can be seen from the case of engineering genetically modified mice, an animal model widely used to study genetics and the pathways of disease. Prior to the arrival of CRISPR-Cas9 engineering a mouse with a single mutation could take nearly two years. Now this can be achieved in just one month. (Cohen, Doudna & Sternberg)

While a great achievement, CRISPR-Cas9 poses many ethical questions for Doudna. Her main concern is the use of the technology in human embryos before it has been adequately shown to be safe. She has been at the forefront of public debates on this issue and was behind the 2015 effort to get a temporary worldwide moratorium on the clinical use of the technique in human embryos before its safety had been proven and its consequences fully considered. Since then she has begun to rethink her position after hearing some of the heart-breaking stories of children suffering from genetic disorders. (Devlin)

Doudna has won numerous awards in her time and many now argue she should be nominated for the Nobel Prize based on her CRISPR work. When asked about her success, Doudna comments that much of it was down to her luck in having good mentors early on in her career and having had the freedom to build up her laboratory team with people with whom she shares a personal chemistry and the same scientific vision and drive. A key essence for her is to have laboratory with a supportive environment where people work together as a team and older members are prepared to mentor those who are younger. Much of her achievement she also attributes to Kaihong Zhou, her laboratory manager, who has worked with her for over thirty years, starting from when Doudna was at Yale University. The two of them confer on everything all the way from what projects to run through to what staff to hire and how to allocate funds. (Mukhopadyay)

Jokes

This Is Why Husbands Avoid Questions
Wife: “What Would You Do If I Died? Would You Get Married Again?”
Husband: “No.”
Wife: “Why Not? Don't You Like Being Married?”
Husband: “Of course, I Do.”
Wife: “Then Why Wouldn't You Remarry?”
Husband: “Ok Ok, I'd Get Married Again.”
Wife: “Would You Live In Our House”
Husband: “Yes, It's A Great House.”
Wife: “Would You Let Her Drive My Car?”
Husband: “Yes, It's Almost New.”
Wife: “Would You Give Her My Jewellery?”
Husband: “No, I'm Sure She Would Want Her Own.”
Wife: “Would She Wear My Shoes?”
Husband: “No, Her Size Is 6.”
Wife: “…….”
Husband: “Oh Shit!”

Have You Ever Noticed That a Women's "I Will Be Ready in 5 Minutes”
and Man's, " I Will Be Home in 5 Minutes" Are Exactly the Same...!
A Man and a Women Are Propotional to Each Other...!

Santa Had A Leakage In The Roof Over His Dining Room.
Plumber Asked: "Sir When Did U Notice Leakage in Roof?”
Santa: "Last Night.... When It Took Me 3 Hours To Finish My Delicious Chicken Soup".

Santa bought split AC.
He installed outdoor unit in room and indoor unit on Roof because he thought-
outdoor unit has Big Fan to provide much air to room.

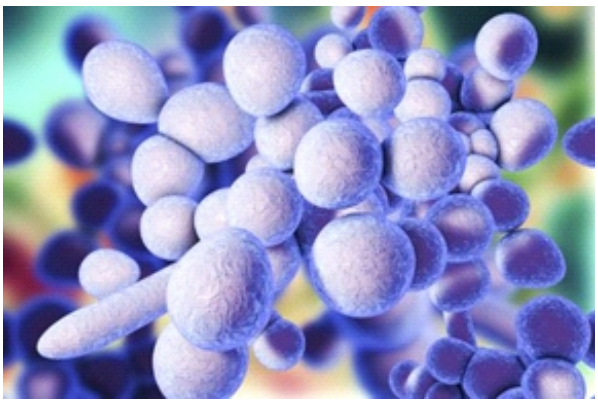
A man went to Renown lawyer and told him, "My neighbor owes me 50,000 Rupees and he won't pay up. What should I do?”
“Do you have any proof he owes you the money?" asked the lawyer. "No," replied the man.
"OK, then write him a letter asking him for the 500000 Rupees he owed you," said the lawyer.
"But it's only 50,000," replied the man. "Precisely That's what he will reply and then you'll have your proof!"

Diagram in book was not clear..
So, Madam drew diagram on blackboard and announced...
“Don't look at Book Figure, Look at my Figure!”
It is Bold and Clear - a student said.

An E.N.T. Professor retired from Renown college. In the Farewell college faculty gifted him a silver ear.
Thanking the faculty the professor said: "Thank god I am not a gynecologist."

Husband found his wife's old school report card FAINTED
The comment written.....
"most obedient and soft spoken student".

Candida auris is Changing the Paradigm of Antifungal-Resistant Candida



Candida species re one of the most common fungal pathogens, causing invasive infections worldwide and accounting for 600,000 infections each year, and are isolated from approximately 25% of all patients in an intensive care unit with central line–associated bloodstream infections in the United States.

Therapeutic options for the treatment of candidemia and other forms of invasive candidiasis are unfortunately limited, with only 3 classes of antifungal agents currently available to clinicians. Thus, antifungal resistance against clinical isolates, estimated to include 35,000 infections each year and 7% of all cases of candidemia in the United States, is a threat to public health. Unlike drug-resistant bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* and carbapenem-resistant Enterobacteriaceae, infections caused by antifungal drug-resistant *Candida* are most often reported as individual cases among at-risk patients following previous antifungal exposure, and they are generally not associated with transmission in health care settings. The discovery and emergence of *Candida auris*, however, have significantly changed the way clinicians need to consider antifungal-resistant *Candida,* and it represents new challenges not previously associated with this genus of fungi.

Shortly after the initial identification of *C auris* was described in 2009, clinical outbreaks of infections caused by this organism occurred in South Africa, Venezuela, India, and Pakistan. Less than a decade later, invasive infections caused by *C auris* have been reported in 40 countries, with approximately 1000 infections and more than 2000 patients colonized in the United States alone. Furthermore, nearly all clinical *C auris* isolates are found to be resistant to at least 1 antifungal agent, and a large proportion of isolates are multidrug resistant (defined as resistance to agents from more than 1 class of antifungal agents). As a result, *C auris* has become a fungal pathogen of great clinical concern and is now considered a pathogen of urgent threat level by the US Centers for Disease Control and Prevention (CDC). Although considerable research efforts continue to expand the epidemiology and pathogenesis of *C auris*, 3 factors define its threat to public health: the organism's ability to rapidly spread among patients and health care environments, challenges in the detection of *C auris* in the clinical microbiology laboratory, and the prevalence of antifungal resistance among clinical isolates.

RAPID SPREAD AMONG PATIENTS AND WITHIN HEALTH CARE

The first clinical specimens of *C auris* were isolated from patients with ear infections in Japan and South Korea, and this is how the species acquired its name (*auris* is Latin for "ear"). Within a few years, *C auris* was identified as the cause of unrelated outbreaks of invasive infections on 3 continents. In India and South Africa, where *C auris* appeared earliest and before widespread recognition, *C auris* quickly became a leading cause of candidemia in some institutions. In fact, the most recently available data from South Africa show that *C auris* was isolated from 14% of nearly 6000 cases of candidemia in private sector hospitals. In health care settings outside these regions, introduction of a single clinical isolate has been reported to cause prolonged outbreaks as a result of the unique ability of *C auris* to resist commonly employed disinfectants, persist on surfaces for weeks, and colonize patients for months. In the Chicago, Illinois, metropolitan area, where it was first clinically identified in 2016, *C auris* has now spread across health care facilities, most notably long-term post–acute care facilities with large populations of patients at high risk for infections due to multidrug-resistant organisms. A point prevalence study at one institution reported that 71% of screened patents were colonized with *C auris*, and 49% of patients were positive for both *C auris* and carbapenemase-producing bacterial pathogens. This high rate of colonization among high-risk patients is particularly concerning considering no established methods exist to effectively decolonize patients; an estimated 5% to 10% of colonized patients develop invasive *C auris* infections.

Thus, excellent infection control practices are essential to controlling the spread of *C auris* colonization and infection. As with other, similar bacterial multidrug-resistant threats, clinicians should report cases of *C auris* colonization or infections to local and state departments of public health, and they should be aware if *C auris* has been identified in their region. Clinicians must also evaluate patients for recent travel or direct personal contacts who may have exposed them to *C auris*, and those who are positive for *C auris* should be placed under contact precautions. Health care workers should be reminded to employ good hand hygiene, and facilities and equipment should be cleaned and sanitized with agents carrying Environmental Protection Agency claims for *C auris* or *Clostridioides difficile* (list K). Further detailed recommendations for the prevention and control of *C auris* in health care settings are available on the CDC's *C auris* website.

DIFFICULT IDENTIFICATION IN THE CLINICAL MICROBIOLOGY LABORATORY

Reliable and timely identification of *C auris* in the clinical setting is imperative to the prevention of its spread in health care environments. Unfortunately, many clinical microbiology laboratories are unable to provide definitive species-level identification of *Candida* isolates in-house. Among those that can, standard phenotypic methods often misidentify *C auris* (platform-specific examples are available on the CDC's *C auris*

website), and in some cases, misidentification occurs even after implementing updated testing panels. Taken together, the most reliable methods of identifying *C auris* from clinical specimens are matrix-assisted laser desorption ionization–time of flight platforms with *C auris* spectral libraries, the T2 magnetic resonance platform with the updated T2Cauris panel, and molecular techniques such as sequencing of ribosomal DNA markers. However, these methods require costly equipment or advanced technical skills that may not be feasible in every clinical setting. Thus, clinicians must be aware of resources available at their own institutions for the identification of *C auris*, and if limitations exist, clinicians should also carefully consider them when caring for patients in whom *C auris* is suspected. Clinical isolates exhibiting multidrug antifungal resistance should also be carefully identified to the species level by methods capable of identifying *C auris*. Clinicians can send clinical isolates in question to the CDC's Antibiotic Resistance Laboratory Network for confirmatory identification and susceptibility testing.

HIGH RESISTANCE TO ANTIFUNGALS

Further contributing to the clinical significance of this emerging fungal pathogen, *C auris* demonstrates higher-level resistance to most antifungal drug classes than do other *Candida* species. Although clinical experience and epidemiologic data relating to *C auris* are currently insufficient to support the establishment of true clinical breakpoints at this time, in an effort to provide provisional guidance for clinicians, the CDC has put forth tentative antifungal breakpoints for the treatment of *C auris* infections. These breakpoints are based on available in vitro susceptibility data, limited in vivo pharmacodynamics studies, and the distribution of mutations in genes associated with antifungal resistance in other species of *Candida*. The specific breakpoints and comments relating to their appropriate application are available on the CDC's *C auris* website.

Approximately 90%, 30%, and 5% of clinical *C auris* isolates from the United States are resistant to fluconazole, amphotericin B, and the echinocandins, respectively, when applying these tentative breakpoints. Moreover, one-third of isolates are resistant to agents from more than 1 class of antifungals, and isolates resistant to all available therapies have repeatedly been identified. The CDC currently recommends empiric therapy with echinocandins (using labeled dosing) for the treatment of infections in which *C auris* is suspected in patients at least 2 months of age. Furthermore, fungal cultures with species-level identification and antifungal susceptibility testing are recommended, as is the consultation of an infectious disease specialist. Clinicians should obtain repeat cultures and antifungal susceptibilities and monitor patients carefully for signs of clinical response, as cases of patients developing antifungal-resistant *C auris* infections on therapy have been reported. Most of these instances of acquired antifungal resistance have occurred following prolonged courses of treatment with echinocandins. However, the rate at which *C auris* may develop resistance to echinocandins or other classes of antifungals is unknown. In the event of insufficient patient response or the development of echinocandin-resistant disease, clinicians should consider switching to liposomal amphotericin B. However, clinicians need to consider specific patient-, pathogen-, and infection-related factors on a case-by-case basis in collaboration with infectious disease specialists.

CONCLUSIONS

Although much research is needed on *C auris* and its long-term impact on invasive candidiasis as a whole, ultimately the best strategies to overcome the challenges posed by *C auris* focus on not only therapeutics but also infection control and mitigation. Clinicians must be aware of any cases of *C auris* in their local area, monitor new patients for travel or contacts with risk of *C auris* exposure, and practice good antimicrobial stewardship and infection control when *C auris* is suspected or identified. Finally, clinicians should regularly check the CDC's *C auris* website for the latest information and recommendations.

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Smoking Fathers increase Asthma risk in future Offspring

A Norwegian study shows that asthma is three times more common in those who had a father who smoked in adolescence than offspring who didn't.

It is well known that a mother's environment plays a key role in child health. However, recent research, including more than 24,000 offspring, suggests that this may also be true for fathers.

"Offspring with a father who smoked only prior to conception had over three times more early-onset asthma than those whose father had never smoked," says Professor Cecilie Svanes at the Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen (UiB).

Early debut increases risk

The study shows that both a father's early smoking debut and a father's longer smoking duration before conception increased non-allergic early-onset asthma in offspring. This is equally true with mutual adjustment, and adjusting for the number of cigarettes smoked and years since quitting smoking.

"The greatest increased risk for their children having asthma was found for fathers having their smoking debut before age 15. Interestingly, time of quitting before conception was not independently associated with offspring asthma," Svanes says.

Smoking fathers may influence gene control in children

Concerning mother's smoking, the research found more offspring asthma if the mother smoked around pregnancy, consistent with previous studies. However, no effect of maternal smoking only prior to conception was identified. The difference from father's smoking suggests effects through male sperm cells.

"Smoking is known to cause genetic and epigenetic damage to spermatozoa, which are transmissible to offspring and have the potential to induce developmental abnormalities," explains Svanes.

It is previously known that nutritional, hormonal and psychological environment provided by the mother permanently alters organ structure, cellular response and gene expression in her offspring. Father's lifestyle and age appear, however, to be reflected in molecules that control gene function.

"There is growing evidence from animal studies for so called epigenetic programming, a mechanism whereby the father's environment before conception could impact on the health of future generations," Svanes says.

Welding increases risk

Svanes and her team also investigated whether parental exposure to welding influenced asthma risk in offspring, with a particular focus on exposures in fathers prior to conception.

The study shows that paternal welding increased offspring asthma risk even if the welding stopped prior to conception. Smoking and welding independently increased offspring asthma risk, and mutual adjustment did not alter the estimates of either.

“For smoking and welding starting after puberty, exposure duration appeared to be the most important determinant for the asthma risk in offspring," says Cecilie Svanes.

Potential Impact of Increased Use of Biocides in Consumer Products on Prevalence of Antibiotic Resistance

Introduction

Problems associated with the development and spread of antibiotic resistance in the clinic have been increasing since the early 1960s and are currently viewed as a major threat to clinical practice. It is generally accepted that the main cause of this problem has been and still is widespread inappropriate use and overprescribing of antibiotics in clinical medicine, animal husbandry, and veterinary practice. Concern about bacterial resistance has led to calls for increased education of both the public and professionals on the correct use of antibiotics and more stringent infection control measures to reduce the transmission of infection.

In recent years, a number of scientists have expressed concern that the use of antimicrobial chemicals (biocides, preservatives) in general practice and in domestic and industrial settings may be a contributory factor to the development and selection of antibiotic-resistant strains. This has been particularly the case with regard to the recent trend towards inclusion of antibacterial agents within a multitude of otherwise traditional consumer products and apparent increases in the environmental impact of many active ingredients used in personal care and consumer products, together with pharmaceuticals. The general concerns are (i) that commonality of target site between biocide and antibiotic might lead to selection of mutants altered in such targets by either agent and the emergence of cross-resistance, (ii) that subtle differences in the biocide and antiseptic susceptibility of antibiotic-resistant strains might facilitate their selection and maintenance in the environment by low, sub-effective concentrations of biocides and antiseptics as well as the primary antibiotic, and (iii) that indiscriminate biocide application might cause the evolution and selection of multidrug-resistant strains through polygamous mechanisms such as efflux pumps.

The current indications are that if the concerns that the widespread deployment of biocidal molecules impacts antibiotic efficacy are genuine, then its contribution is likely to be relatively minor. Conversely, the tremendous contributions of disinfection and acceptance of hygienic measures towards advances in public health over the last century cannot be denied. Indeed, if reductions in the number of infections requiring antibiotic treatment can be achieved through effective hygiene, including the use of biocidal products, then this is likely to decrease rather than increase the incidence of antibiotic resistance. Accordingly, it is important to ensure that biocide use, as an integral part of good hygiene practice, is not discouraged when there is real benefit in terms of preventing infection transmission. This means that it is also necessary to assess the possibility that the indiscriminate use of biocides and antibacterial products might compromise the in-use effectiveness of such biocides in truly hygienic applications. Use of such products must be associated with appropriate analyses of added value to the consumer, particularly when there is no apparent gain in public health.

I has to consider the mechanisms by which bacteria may become less sensitive to biocide action and then to look at the potential links between antibiotic and biocide resistance and their implications for the inclusion of antibacterial agents within consumer products. The relevance of laboratory monoculture experiments in particular, where competitive selection pressures are absent, will be viewed in the context of field studies and complex ecologies. First, however, it is necessary to consider the precise meaning of some of the terms used and misused by various opinion-forming groups.

Possible associations between biocide use and resistance – Field studies

Association between chronic sub lethal exposure of bacterial monocultures to biocides and changes in susceptibility to both the biocides themselves and third-party antibiotics has been demonstrated unequivocally in the laboratory. Such phenomena have not yet been demonstrated to have any relevance to the real world. In such situations, individual species of bacteria are in fierce competition with other forms of bacteria, and their competitive fitness determines their survival. Arguably, the clinic represents an environment where biocide use has been and still is extreme. If the increasing use of antibacterial agents within consumer products is likely to impact antibiotic resistance within the home, similar effects should already be apparent in clinical and hospital settings. Accordingly, a large number of studies have been carried out to evaluate whether clinical and environmental isolates taken from such settings show any evidence of significant reductions in their susceptibility to biocides and whether this might be linked with antibiotic resistance.

The results of such studies have been largely ambiguous. Thus, no differences were found in the MICs of hospital and laboratory gram-negative isolates for cationic antiseptics and two organo mercurial compounds. Three separate studies by Stickler's group assessed the MICs of a range of antiseptics, disinfectants, and antibiotics for gram-negative bacteria isolated from a hospital environment and found that approximately 10% of the isolates (mainly*Pseudomonas*, *Proteus*, and *Providencia* spp.) exhibited some level of reduced susceptibility to chlorhexidine and cetrimide and were also generally more resistant to multiple antibiotics. More recently, Block and Furman isolated 251 strains of staphylococci, *Klebsiella*, *Pseudomonas*, *Acinetobacter*, and *Candida* spp. from a hospital environment and detected an inverse correlation between chlorhexidine use and susceptibility. It was noteworthy that when individual taxa were analyzed separately, no significant correlation was noted. This indicates a clonal expansion of existing less-susceptible strains rather than adaptation of individual species, as has been noted in other recent studies of hospital isolates.

Similar results showed that 12.8% of 148 clinical *E. coli* isolates selected for their elevated chlorhexidine MICs were no less susceptible to use concentrations. Such changes, in the case of the *Providencia* isolates, were thought to affect binding of the biguanides to the cell surface and therefore reflected envelope modification. Freney et al. found no evidence of decreased susceptibility within 169 novel *Enterobacteriaceae* isolated from the general environment relative to clinical isolates. Arguably, such studies support the view that antiseptic use in hospitals does

not contribute to the biocide susceptibilities of enterococcal isolates. Equally, Lear et al. examined over 100 factory isolates and compared the MICs of triclosan and chloroxylenol for these to those of the equivalent culture collection strains. They concluded that there was no evidence suggesting that the residual levels of biocides in the factory environment had led to changes in susceptibility. Equally, Braid and Wale showed that triclosanimpregnated storage boxes were effective at reducing the numbers of various challenge inocula and that the susceptibilityof the strains was unaffected after repeated exposure on these treated items.

By way of contrast, Reverdy et al. showed that antibioticsensitive *S. aureus*, and other staphylococci, for which the MICs of various antiseptics were elevated, were nevertheless less sensitive to a wide variety of antibiotics. Increased MICs for methicillin-resistant *S. aureus* strains have been reported for some biocides, including chlorhexidine, cetrimide, benzalkonium chloride , hypochlorite, triclosan, parahydroxybenzoates, and betadine. Thus, while the MIC of chlorhexidine was higher against methicillin-resistant S. aureus clinical isolates (4 to 8 µg/ml) than for susecptible ones (0.37 to 21 µg/ml), there was no significant difference in the efficacy of this agent when these strains were tested on the arms of volunteers with a bactericidal assay. No significant differences were noted in the chlorhexidine susceptibility of 33 clinical methicillin-resistant and -susceptible *S. aureus* isolates, and there was no loss of sensitivity to the bactericidal effects of triclosan when a clinical methicillin-resistant *S. aureus* isolate showing an elevated MIC (2 to 4 µg/ml) was challenged.

Bamber and Neal found that of 16 methicillin-resistant *S. aureus* that exhibited low-level mupirocin resistance, none had increased MICs of triclosan, but Suller and Russell found clinical methicillin-resistant *S. aureus* isolates to have slightly decreased susceptibility, relative to susceptible isolates, to a range of biocides that included chlorhexidine, cetylpyridinium chloride, benzalkonium chloride, and triclosan. Most of the strains described in the above studies remained equally susceptible to bactericidal concentrations of the biocidal agents, an observation that was repeated recently for vancomycin-resistant *Staphylococcus aureus* (L. M. Sehulster and R. L. Anderson, Abstr. 98th Annu. Meet. Am. Soc. Microbiol., 1998, abstr. Y3). Four antiseptic formulations (Savlon, Dettol, Dettol hospital concentrate, and Betadine) retained their bactericidal activity in a European suspension test against a variety of antibiotic-resistant strains, including methicillin-resistant *S. aureus* and vancomycin-resistant enterococci. These data bear testimony to the multiplicity of target sites implicated in the bactericidal action of biocides.

Many other studies failed to observe any change whatsoever in MIC. Thus, Stecchini et al. showed that, despite widespread antibiotic resistance in 100 strains of *Enterobacteriaceae* isolated from minced meat, these were not resistant to the bactericidal activity of an amphoteric Tego disinfectant. Similarly, among 330 psychrotrophic non-fermenting gram-negative strains isolated from vegetables, those antibiotic-resistant strains were demonstrated to be susceptible to the bactericidal action of quaternary ammonium compounds and hypochlorite disinfectants.

Baillie et al. evaluated the chlorhexidine sensitivity of 33 clinical isolates of *Enterococcus faecium* that were sensitive to both vancomycin and gentamicin with vancomycin-resistant and gentamicin-resistant strains. The results showed no increase in resistance to chlorhexidine as indicated by MIC. lnterestingly, a study of 67 ciprofloxacin-resistant isolates of *P. aeruginosa* yielded four which were hypersensitive to chlorhexidine (MIC, 5 mg/liter), while none were found among 179 ciprofloxacinsensitive isolates.

Marshall et al. (P. J. Marshall, P. Rumma, and E. Reiss-Levy, presentation at the 11th National Conference of the Australian Infection Control Association, 7-9 May 1997, Melbourne, Australia) reported that during an intensive policy of antiseptic handwashing involving a triclosan-based medicated soap, aimed at combating a methicillin-resistant *S. aureus* infection episode, not only did the incidence of methicillin-resistant *S. aureus* decrease significantly, but the percentage of ciprofloxacin-sensitive isolates increased from 8.1% to 22.5% within the trial. In a study of *Streptococcus mutants* isolated from the mouths of 114 school children and students from families in which about 70% used oral preparations containing chlorhexidine on a regular basis, there was no evidence of decreased susceptibility either to chlorhexidine or to a range of antibiotics, as tested with MICs.

Anderson et al. determined the susceptibilities of vancomycin-resistant and vancomycin-sensitive enterococci to various concentrations of commonly used hospital disinfectants, including quaternary ammonium compounds, phenolics, and a iodophore, at recommended use dilutions and extended dilutions with suspension tests. They concluded that there was no relationship between levels of vancomycin resistance and their susceptibility to disinfectants at the use dilution. Such findings have been confirmed by showing that a series of vancomycin-resistant and vancomycin-resistant enterococcal clinical isolates had no significant differences in their growth-inhibitory or bactericidal sensitivities to chlorhexidine, cetylpyridinium chloride, or triclosan.

Published data for triclosan state that the expected MIC for staphylococci should be between 0.01 ppm and 0.1 ppm. Bamber and Neal determined the MIC for 186 isolates of methicillin-resistant and methicillin-sensitive *S. aureus* and found 14 isolates (7.5%) with MICs greater than 1.0 ppm. These were, however, equally distributed between the methicillin-resistant and methicillin-sensitive *S. aureus* strains.

A series of antibiotic-resistant clinical and environmental isolates that included *P. aeruginosa*, *Klebsiella* species, *E. coli, S. aureus,* and *S. epidermidis* were found to be no less susceptible to the bactericidal activity of phenolic and quaternary ammonium disinfectants, chloroxylenol, cetrimide, and povidone iodine. Similarly, some variation in the vancomycin susceptibility and biocide (chlorine, alcohol, aldehyde) susceptibility of enterococci has been noted, but the two did not correlate.

The food processing industry represents an environment other than the clinic where the use of biocidal products is high. In this respect, Heir et al. reported that 13% of staphylococcal isolates from a food manufacturing environment had MICs of benzalkonium chloride that were between 4 and 11 mg/liter, compared with 70% of remaining isolates, which had MICs of

less than 2 mg/liter. This resistance probably related to the presence of qac efflux mechanisms and encoded only small changes in susceptibility. Accordingly, suspension tests showed that recommended use concentrations of the agent produced the desired 5-log reduction in viable count in 5 min. In an examination of poultry carcasses, two strains of *Pseudomonas* were isolated that were deemed resistant to benzalkonium chloride by virtue of possessing a MIC greater than 200 µg/ml. Only one of these organisms failed the suspension test. A more recent study showed that *S. aureus* cells that expressed qacG efflux suffered reduced killing in environments that contained low concentrations of benzalkonium chloride but 5-log reductions in viable counts at higher concentrations. The latter were nevertheless still well below the recommended use concentrations.

Latterly, Heir et al. found a new member of the qac family of genes in Staphylococcus saprophyticus (qacH) isolated from a poultry processing plant. The same authors, however, conceded that quaternary ammonium compound use in the production facilities might have led to a selection for staphylococci bearing the qacAB genes. Bass et al. demonstrated that approximately one third of diseased poultry carried plasmids that encoded multiple antibiotic resistance; 63% of these contained markers for the class 1 integrons intI and qacEand were part of transposon Tn21. The selection pressure for Tn21, which also encodes mercury resistance, could not be determined.

The field studies discussed so far suggest strongly that the variable nature of the observable links between biocide and antibiotic susceptibility have no single underlying cause and that worries and concerns raised through laboratory monoculture experiments cannot be echoed in the environment. There are, however, a few published studies that indicate the contrary and show reductions in susceptibility to various oxidizing biocides that are sufficient to compromise their in-use effectiveness. In most instances, such studies make no distinction between phenotypic and hence reversible changes in susceptibility and that which may be acquired. In other instances, data were collected from large numbers of isolates taken from environments where biocide use is widespread but without reference to control habitats. The extent to which the data reflect adaptation to the biocides or the natural selection and clonal proliferation of existing strains is therefore often unknown. These studies are discussed below.

Several reports have described isolates, especially among gram-negative species, from various food processing environments that possess a reduced susceptibility to chlorine and quaternary biocides that relates to practical usage. Thus, an early report noted that after changing the sterilization practices from steam to chlorine-based disinfectant compounds, there was a higher occurrence of dairy isolates that were resistant to hypochlorite. Similarly, Mead and Adams and Bolton et al. found that chlorine concentrations of 1 mg/liter produced a 4-log reduction in viability of *S. aureus* strains isolated from turkeys and turkey products, but only a 2-log reduction when tested against endemic strains that had colonized the processing equipment. All three reports could be related to growth of the resistant isolates as coaggregates within extracellular slime. This was also the explanation for the apparent resistance of lactobacillus strains isolated from packed meat that could survive exposure to 200 mg of benzalkonium chloride per liter. The resistance in all of these instances was therefore phenotypic in nature.

Pseudomonads are not generally noted for their susceptibility to quaternary ammonium compounds, a property that is generally attributed to the unique properties of the *Pseudomonas* cell envelope. Approximately 30% of *Pseudomonas* isolates taken from poultry carcasses were able to grow at concentrations of 200 µg/ml. While it was recognized that clonal selection of existing resistant strains, through a constant usage regimen involving benzalkonium chloride as the disinfectant, might have been the cause, these workers later reported (S. Langsrud and G. Sundheim, 1997, *Pseudomonas* '97, p. 102) that the resistance was lost within 4 to 8 h of removal from the quaternary ammonium compound and was developed in batch culture only during the lag phase. These observations therefore more probably reflect a regulated process involving efflux genes, and the resistance shown for these cells could not be replicated in a bactericidal assay.

In a similar study, the susceptibility of 350 isolates collected from commercial chicken hatcheries to commercial preparations of quaternary ammonium compounds, phenolics, and glutaraldehyde was examined. Nineteen isolates (ca. 6%, including *Serratia marcescens*, *Bacillus* species, *Enterococcus* species, and *P. stutzeri*) from two of three hatcheries were resistant to disinfectant at and above the recommended use concentrations and exposure times. Some isolates were multi-resistant, but only three showed resistance to quaternary ammonium compounds compared with 7 to phenol and 15 to glutaraldehyde. The authors suggested that this might be correlated with the usage of glutaraldehyde in U.S. hatcheries over many years. No investigations were carried out to determine whether the resistance was reversible, although all isolates had been grown once through tryptone soy medium.

In a study of the effects of repeated antiseptic use on the bacterial flora of the urethral meatus in patients undergoing intermittent bladder catheterization, the bacterial flora was examined from the date of injury to the time at which urinary tract infection developed after daily washing with aqueous chlorhexidine (600 µg/ml). Prior to the regular application of chlorhexidine, the predominant flora comprised gram-positive, chlorhexidine-sensitive bacteria. These were superseded by a gram-negative flora that included some resistant strains (mainly *Proteus mirabilis*, *P. aeruginosa*, *Providencia stuartii*, and *Klebsiella* species) less sensitive to chlorhexidine, with MICs of 200 to 800 µg/ml. These were well above the levels of 10 to 50 µg/ml usually reported for gram-negative species.

In a subsequent study, the susceptibility to an array of antiseptics and disinfectants that included chlorhexidine, cetrimide, glutaraldehyde, and a phenolic formulation was assessed against a large collection of gram-negative isolates taken from a variety of clinical and hospital settings. The general conclusion drawn was that antiseptic and disinfectant resistance was not a widespread phenomenon in species responsible for urinary tract infections. They found that approximately 10% of the isolates (mainly *Pseudomonas*, *Proteus*, and *Providencia*) exhibited some resistance to chlorhexidine, but these came from situations where there was extensive use of chlorhexidine.

It would appear therefore that in the earlier study, the routine application of chlorhexidine had eliminated the natural colonization resistance provided by the sensitive autochthonous flora and had enabled innately resistant environmental strains to infect. The innate recalcitrance of environmental gram-negative bacteria to antiseptics has been demonstrated by Nagai and Ogas. They isolated strains of *Achromobacter xylosoxidans* from a 0.4% chlorhexidine solution handwashing reservoir for which minimum bactericidal concentrations were more than 10-fold higher than the chlorhexidine solution in the reservoir. Two separate investigations with *Providencia stuartii*, and an antibiotic-resistant clinical strain of *P. mirabilis* that was resistant to the growth-inhibitory action of chlorhexidine at 800 mg/liter failed to show any evidence of a plasmid link. Both sets of authors concluded that the resistance was most likely an intrinsic property induced by persistent exposure to the biocide.

More recently, strains of *P. stutzeri* and *P. aeruginosa* have been shown to become much less susceptible to chlorhexidine and cetylpyridinium chloride when passaged through gradually increasing concentrations of each. Such decreased susceptibility was stable for *P. stutzeri* but not for *P. aeruginosa* and could not be transferred by conjugation. The authors concluded that resistance resulted from a nonspecific decreases in cell permeability such as might arise from deletion or depression of a porin protein. In this context, passage with increasing concentrations of isothiazolone biocides has been shown to repress the synthesis of an outer membrane porin protein (OmpT) that appears to facilitate the entry of this group of thiol-interactive biocides into the cell.

Overall, there is good evidence to suggest that good standards of hygiene in the domestic setting, which includes not only day-today cleaning of the home but food hygiene, hand hygiene, and hygiene related to the protection of vulnerable groups, can have a significant impact in reducing the number of infections arising in the home. Indeed, a number of recent studies have reported increased incidence of critical pathogens such as methicillin-resistant *S. aureus* into the home environment, often associated with household pets like dogs and cats, and their transfer to humans. Such work highlights the need for targeted hygiene within the home. A variety of different procedures can be used to achieve hygiene in the home, and in some cases this may require the use of a disinfectant or antiseptic. This being the case, it can be seen that responsible use of biocides and antimicrobial cleaning products could contribute to reducing the impact of antibiotic resistance. Thus, if reducing the number of infections through effective hygiene is important, then it is also important to ensure that biocide use is not discouraged in situations where there is real benefit.

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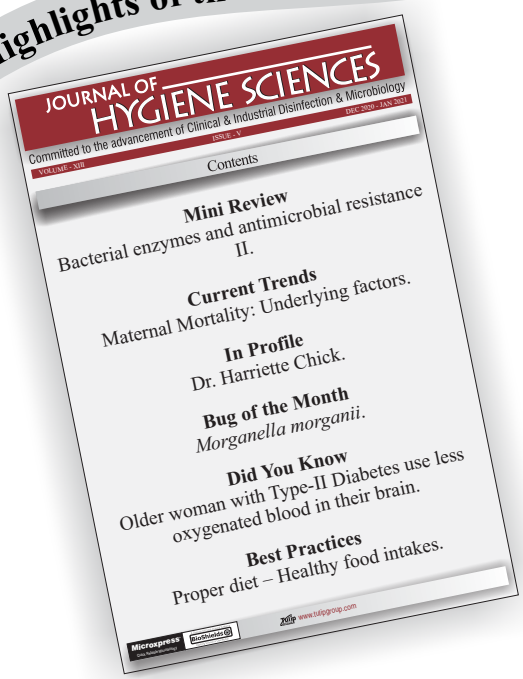
Highlights of the coming issue



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