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Editorial

Contents

Our JHS team is thankful to all our readers for their ever increasing appreciation that has served as a reward & motivation for us. Here's another issue of JHS coming your way……..

Our Mini Review section talks about “Response of Microorganisms to Biocides”. Biocides are multi-targeted antimicrobial agents, unlike antibiotics. Bacteria themselves (Gram-positive and Gram-negative vegetative organisms, mycobacteria and spores) respond differently to biocides and this disparity is widened when yeasts, moulds, protozoa and algae are considered. The underlying reasons for these varied responses are poorly understood at present, but the chemical composition of outer cellular layers is likely to be a factor of prime importance.

This time's Current Trends section focuses on “Biochemical Tests to indentify bacteria”. Bacterial colonies can differ greatly in their morphologies. These differences can help us in identifying different species of bacteria. Likewise, bacterial species differ in their cellular morphologies and staining properties. Again, these differences can be used to aid in identifying different species. We can use selective and/or differential media to aid in identifying bacterial species. Generally, selective and differential media rely on some structural or metabolic property of the species that is preferentially selected.

Our In Profile scientist for the month is **Dr. Ananda Mohan Chakrabarty.** He is an Indian American microbiologist, scientist, and researcher, most notable for his work in directed evolution and his role in developing a genetically engineered a new species of *Pseudomonas* bacteria ("the oil-eating bacteria") in 1971.

Did you ever felt that you're irresistible to pesky mosquitoes? it probably isn't just paranoia, Numerous studies have revealed that mosquitoes really do prefer to bite some people over others when given the choice.

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. 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.

Ease your mind with light humour in our Relaxed Mood section…….

So go on, enjoy reading & don't forget to give us your valuable inputs & feedback.

Similarities and differences in the responses of microorganisms to biocides

Unlike antibiotics, biocides are multi-targeted antimicrobial agents. Several of the damaging effects reported to occur in the most widely studied organisms, bacteria, may also take place to varying degrees in other organisms. Nevertheless, there is considerable variation in the response of different microorganisms to biocides. Bacteria themselves (Gram-positive and Gram-negative vegetative organisms, mycobacteria and spores) respond differently to biocides and this disparity is widened when yeasts, moulds, protozoa and algae are considered. The underlying reasons for these varied responses are poorly understood at present, but the chemical composition of outer cellular layers is likely to be a factor of prime importance. Other possible contributory factors may be differences in stress responses, the presence of efflux pumps and cells occurring within biofilms or algal mats.

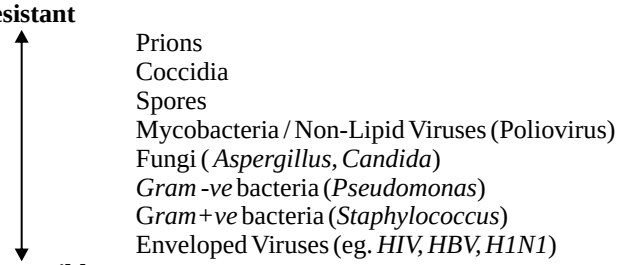
Introduction

Detailed information (Figure 1) is available about the activity spectra of biocides (antiseptics, disinfectants and preservatives). This provides an important basis for the never-ending attempt to control harmful microorganisms. Comparatively few biocides are bactericidal (including mycobactericidal), sporicidal, virucidal and fungicidal, whereas most are bactericidal (with or without being mycobactericidal), virucidal and fungicidal but do not inactivate spores. Some biocides show activity against protozoa and algae. Those factors that affect antimicrobial activity, namely period of contact, concentration, temperature, pH, presence of organic soiling matter, and type of organism, are well documented and influence the manner in which biocides are used for whatever purpose and against a variety of microorganisms. A particular biocide may thus inactivate (or sometimes inhibit) more than one type of microorganism. With our current levels of understanding of the mechanisms of biocidal action and of microbial resistance, it is pertinent to consider whether it is possible to explain why both similarities and differences in response to biocides occur in microbes that differ widely in their structure and physiology. In some ways, this presents a more formidable task than for antibiotics where a clear knowledge of mechanisms of inactivation and of resistance enables logical conclusions to be reached about specificity and selectivity of action. Thus, as pointed out by Ghannoum & Rice, a comparison between, for example, antibacterial and antifungal resistance to antibiotics is limited. Antibiotics are considered to have one major target site, usually inhibition of a particular biosynthetic process, although other effects may also be known. However, their actions tend to be much clearer-cut than those of biocides, whose effects are highly concentration-dependent. Consequently, delineating the reasons for activity against a range of organisms becomes more difficult with biocidal agents.

Microbial cells

Two types of organisms, prokaryotes (bacteria) and eukaryotes, are considered here. Of the latter, moulds (filamentous fungi) and yeasts (unicellular fungi), protozoa and algae will be discussed. The fungi and algae (except euglenoids) possess rigid cell walls, whereas protozoa lack a 'true' cell wall. Many different types of microorganism (bacteria, fungi and protozoa) have been associated with serious human infections. Certain filamentous algae may produce thick carpet-like mats in freshwaters. Algal growth causes problems in swimming pools and cooling towers, and their control by algicides is often necessary. Food poisoning by micro-algae has been described. Thus, it is important to appreciate not only appropriate chemotherapeutic measures but also suitable procedures, often involving biocides, for controlling or preventing the spread of infections or other hazards caused by a variety of prokaryotic and eukaryotic microbes. There are obviously considerable differences in the structure and composition of microbial cells and **Table 1** summarizes the chemical nature of the outer layers of some of these organisms. There is no consistent theme. There are variations between similar types of organisms, e.g. cocci, Gram-negative bacteria and mycobacteria, whilst spores have coats that are also entirely different chemically from the cell walls of non-sporulating bacteria and indeed from the walls of germinating, outgrowing and vegetative forms produced from spores themselves. Further, variations in chemical composition may occur on growth under different conditions producing altered responses to antimicrobial agents. It is not surprising, therefore, that microbial susceptibilities to biocides and especially to antibiotics differ greatly. It should be noted, however, that even with antibiotics, some degree of 'cross-activity' occurs. For example, many antibiotics that specifically interfere with the 70S ribosome function in bacteria will also inhibit protein synthesis in mitochondria and chloroplasts. Fusidic acid has some activity against a range of protozoa, including *Giardia lamblia*. Mupirocin is active at low concentrations against staphylococci but has some clinical effect at much higher concentrations against fungi, and chloramphenicol shows a broad spectrum of activity against actinomycetes, mycoplasmas, *Leptospira* species and *Treponema pallidum*. Metronidazole, a 5-nitroimidazole derivative, has activity against anaerobic bacteria and protozoa and interferes with DNA synthesis via a metabolite in which the NO2 group of metronidazole has been reduced. For their part, biocides usually show a low degree of selectivity in their action against different types of microorganism. Chlorhexidine salts (CHX), quaternary ammonium compounds.

Resistant



Susceptible

Figure 1. Relative susceptibility of entities (prions, viruses) and microorganisms to biocides. Algae not shown, but likely to be susceptible to at least some biocides.

Biocide adsorption and uptake into cells

Interaction of a biocide with the whole microbial cell is conventionally measured by determining its adsorption. As a result, five different classes of adsorption are known. These may be summarized as follows: (i) S-shaped pattern, in which the solute molecule is mono functional, is orientated vertically and meets strong competition from the solvent molecules or by another adsorbed species; (ii) L (Langmuir) pattern in which, as more sites are filled, it becomes increasingly difficult for a solute to find a vacant site; (iii) H (high affinity) pattern, obtained when the solute is almost completely adsorbed; (iv) C (constant partition) pattern,

obtained when the solute penetrates more readily into the adsorbate than the solvent; and (v) Z pattern, in which there is a sharp break in the pattern followed by an increased uptake, which is interpreted as being caused by a breakdown of the structure of the adsorbing species with the generation of new adsorbing sites.

Table 1. Composition of outer cell layers of different microorganisms

Organism	Outer cell layers	Example(s)
Gram-positive cocci	CW: predominantly PTG	staphylococci
Gram-negative bacteria	OM: PL, LPS	*P. aeruginosa*, *E. coli*
Mycobacteria	CW: mycolate of AG, lipid	*Mycobacterium tuberculosis*
Bacterial spores	OSC: alkali-resistant (S-S bonds) ISC: alkali-soluble (acidic polypeptides) cortex: PTG, including spore-specific	*Bacillus* spp.
Yeasts and moulds	CW: chitin + chitosan CW: chitin + glucan *A. niger* CW: glucan + mannan	*Mucor rouxii* *S. cerevisiae*, *C. albicans*
Intestinal protozoa	cysts have thick outer coverings	*C. parvum*
Other protozoa	double-walled cysts containing cellulose (during encystation) *A. castellanii*	 green (Chlorophyta)
Algae	CW: cellulose + other polysaccharides + other constituents,	brown (Phaeophyta) red (Rhodophyta) algae

*a*CW, cell wall; PTG, peptidoglycan; OM, outer membrane; PL, phospholipid; LPS, lipopolysaccharide; AG, arabinogalactan; OSC, outer spore coat; ISC, inner spore coat.

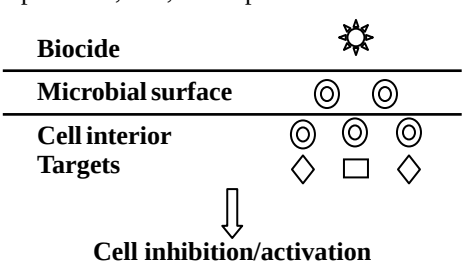


Figure 2. General pattern of biocide entry into different types of microorganisms (for simplicity, no barrier function is envisaged). 1, Adsorption of biocide to cell surface; 2, interaction with outer cell layers; 3, uptake into cell; 4, interaction with target site(s).

Similar adsorption patterns for a test biocide may be shown by different types of microorganisms. For example, the Z adsorption pattern is shown by phenoxyethanol and *Escherichia coli* (but not *Pseudomonas aeruginosa*) and *Candida lipolyica*. Hugo & Newton showed that uptake (adsorption?) of iodine differed between bacteria and yeast. The initial stages followed the H (high affinity) pattern, indicative of a high affinity of iodine for substrates. Thereafter, the shape varied, depending upon the iodine system (solution or iodophor) and the substrate (type of cells).

Biocides and antibiotics must traverse the outer cell layer(s) to reach their target sites, usually present within microbial cells (Figure 1). The information as to how this uptake is achieved is somewhat limited.

Figure 2 shows the general pattern of entry of a biocide into microbial cells. It is believed that antibiotics and biocides generally pass through the staphylococcal cell wall by passive diffusion. Little is known about the manner in which biocides enter other Gram-positive bacteria. Enterococci, for example, are generally less susceptible than staphylococci, and the cell wall could act as a barrier to limit intracellular uptake. In Gram-negative bacteria, passage across the outer membrane (OM) depends upon the chemical nature of the inhibitor, hydrophilic antibiotics utilizing the porin channels (hydrophilic route) and hydrophobic antibiotics entering via the hydrophobic route. Generally, large molecular weight hydrophilic molecules (e.g. the polypeptide antibiotic vancomycin) enter Gram negative bacteria poorly as do relatively hydrophobic antibiotics such as fusidic acid, erythromycin, novobiocin and rifampicin. Self promoted entry occurs as a result of OM damage induced by cationic agents that include CHX, QACs and the polymyxin antibiotics. Studies with smooth, rough and deep rough strains of *E. coli* and *Salmonella typhimurium* have demonstrated that deep rough strains are more susceptible to QACs than wild-type (smooth LPS), but generally of equal susceptibility to CHX. Interesting relationships were also found with a homologous series of esters of *para*(4)- hydroxybenzoic acid. Few data are available about the uptake of antibiotics and biocides by mycobacteria, fungi or other types of microorganisms. The varied composition of the outer cell layers of different types of microorganisms means that only very general conclusions can be reached about uptake into such cells. Virtually all members of the domain Bacteria have cell walls containing peptidoglycan; the *Chlamydia– Mycoplasma* groups lack a cell wall. Eukaryotes, of the domain Eukarya, do not contain peptidoglycan, and cell walls (if present) contain cellulose or chitin (Table 1). Different cell wall types exist in members of the domain Archaea, with the peptidoglycan analogue, pseudopeptidoglycan, or polysaccharide, protein or glycoprotein being present. Thus, it is not surprising that uptake of biocides might differ greatly in such a wide range of organisms in which the composition of the outer cell layers might have a limiting role, albeit for different reasons. The possible role of yeast cell walls in modifying cellular response to CHX has been studied. The relative porosity (RP) and thickness of cell walls of *Saccharomyces cerevisiae* and their glucan, but not mannan, composition influence susceptibility to CHX. Decreases in RP and increased wall thickness would be expected to reduce CHX uptake into the cells. The pores in fungal cell walls have been suggested as being too small for the entry of very large molecules, with compounds of molecular weight not greater than about 700 capable of diffusing freely.

A comparison of the effects of some biocides on different types of microorganisms produces some interesting results. For example, Dychdala considered the biocidal effect of free available chlorine on some algae, bacteria, fungi, protozoa, viruses and bacteriophages. Generally, algal growth was inhibited at low concentrations, whereas considerable variation was observed with bacteria. The two fungal test organisms (*Aspergillus niger* and *Rhodotorula flava*) needed high concentrations for a lethal effect to be achieved, whilst the only protozoon studied (*Entamoeba histolytica* cysts) required a low concentration albeit for a long contact period. It is difficult to come to meaningful conclusions about biocide uptake from these comparisons. Of greater significance, perhaps, is the comparison of inhibitory concentrations of a range of QACs against bacteria, fungi and

algae. Gram-positive bacteria were considerably more susceptible than Gram negative organisms or fungi, with test algae usually being inhibited at still lower concentrations. This suggests, but does not prove, that these algae presented no barrier to the uptake of the QACs. Low molecular weight substances are believed to diffuse freely across the algal cell wall, which is impermeable to larger molecules and to macromolecules. Iodine may not control algae, particularly black algae. Effective algal control in pool water can be achieved by CRAs, QACs and modified copper compounds. Interestingly, relative algicidal concentrations of various compounds may equate to their bactericidal properties. Bacterial spores present a different type of cell surface to biocides (Table 1). Adsorption (uptake?) of alkaline or acid GTA is greatest to vegetative cell forms of *Bacillus subtilis*, followed by germinating and then by resting spores of this organism. However, *E. coli* cells take up more, and *Staphylococcus aureus* cells less, GTA than *B. Subtilis* vegetative cells. *B. subtilis* spores take up considerably more chlorine (from sodium dichloroisocyanurate, NaDCC) and at a much more rapid rate than iodine (from Lugol's iodine). Chlorine is also a much more effective sporicide. In both cases, uptake is increased when coat-deficient spores are used. It is likely that the coats act as an efficient barrier especially to the entry of iodine. Uptake of both chlorine and iodine is greater with outgrowing and germinating cells than with spores.

In mycobacteria, it has long been known that the cell wall acts as an efficient barrier to the uptake of many biocides and antibiotics, as considered later. The microbial cell surface can thus act as a barrier to the uptake of some, but not necessarily all, types of antimicrobial agents. Impermeability or decreased uptake is a common mechanism for reduced susceptibility to antibiotics and biocides in a variety of microorganisms, notably mycobacteria, Gram-negative bacteria and bacterial spores, but can occur in some types of staphylococci also.

Microbial differentiation and changes in biocide response

The bacterial spore cycle and encystation and excystation in the simpler forms of protozoa provide excellent tools for associating morphological and biochemical changes in cells with susceptibility to antimicrobial agents, both antibiotics and biocides (Table 2). Members of the genera *Bacillus* and *Clostridium* have complex life cycles with the two extremes of dormant (spore) and metabolically active (vegetative cell) forms. Seven stages have been identified in the sporulation of *B. subtilis*, with stages IV (cortex development), V and VI (coat development) being the most relevant in relation to reduced susceptibility to biocides. Small acid-soluble proteins (SASPs) exist in the spore core as two types. These are (i) α,β-types associated with DNA and synthesized at about the third hour of sporulation, (ii) γ-types, also synthesized at around t3 but not associated with any macromolecules. Susceptibility to biocides decreases as the synthesis of the cortex and inner and outer spore coats proceeds, with late development of comparative insusceptibility to lysozyme and especially to GTA59 In addition, it has been shown that spores (α– β–) of *B. subtilis* deficient in α,β-type SASPs are much more susceptible to hydrogen peroxide but not to iodine, GTA or OPA than wild-type spores.

Despite being metabolically dormant, the spore contains a number of enzymes that act on the corresponding substrates in a very short period of time during germination. For example, 3-phosphoglycerate (3-PGA) is catabolized during the first few minutes to generate ATP, and germination endoprotease (GPR) is responsible for the degradation of SASPs to amino acids during the first 30 min. Profound degradative changes occur during germination whereas biosynthetic processes take place during outgrowth. During germination phase I, cation and DPA (dipicolinic acid) release, partial core hydration and SASP degradation occur, with cortex hydrolysis and further core hydration during germination phase II, followed by biosynthetic processes, escape from spore coats and eventual cell division. During periods of stress, trophozoites of *Acanthamoeba* spp. undergo a cell differentiation process (encystment), resulting in the formation of dormant cysts. Extensive changes occur during encystations in *A. castellanii*, with the development of acid-insoluble proteincontaining ectocyst wall and an alkali-insoluble cellulose endocyst wall. Resistance to several biocides commences with the synthesis of the cellulose-containing wall, implying that a physical barrier is responsible for this decreased susceptibility rather than it being a consequence of a metabolically dormant cyst. Suci & Tyler investigated the effect of CHX on yeast and filamentous forms in an early stage *C. albicans* biofilm and found that a portion of the yeast cells germinated to produce filamentous forms. The rates of propidium iodide penetration were substantially higher in filamentous forms when exposed to CHX.

Table 2. Microbial differentiation and changes in biocide responses

Organism	Form	Biocide response
B. subtilis	vegetative cell	usually susceptible
	spore	much less susceptible
	germinating cell	susceptibility usually increases
	outgrowing cell	susceptibility usually increases
C. albicans	yeast form	susceptible
	mycelial form	less susceptible
A. castellanii	trophozoites	usually susceptible
	cysts	less susceptible

Stress adaptation

Applied stress is (i) any deviation from the optimum growth condition that produces a reduced growth rate, (ii) exposure to an environmental situation that produces damage to cellular components in the absence of a cellular response, or (iii) a situation that stimulates the expression of genes known to respond to a specific environmental condition. Stress adaptation refers to the ability of bacteria or other microorganisms to adapt to a chemical or other applied stress. Gould pointed out that vegetative bacterial cells react homeostatically to stress in a variety of ways; these include the activation and expression of latent groups of genes following exposure to oxidative stress. Oxidative stress and the SOS response in *E. coli* and *Salmonella* are intrinsic defence mechanisms conferring tolerance to stress by hydrogen peroxide and involve the production of an array of neutralizing enzymes to prevent cell damage and of exonucleases to repair lesions in DNA. Peroxide-induced stress proteins overlap with heat shock proteins (HSPs). A regulated adapted response in growing *E. coli* cells exposed to hydrogen peroxide results in the cells becoming resistant to normally lethal doses of peroxide and the synthesis of around 40 new proteins. When *E. coli* is subjected to nutrient limitation or to antimicrobial agents, the growth rate is depressed and gene expression is markedly altered. This is essential for long-term survival of the cell and is partly mediated by alternative sigma factors. Programmed cell death (PCD) is a programmed suicide mechanism, with persisters being defective in PCD and using the exudate from lysed cells as a source of nutrient. It has been postulated that the metabolic imbalance following biocidal or other type of stress leads to free radical production and self-destruction. Highly metabolic cells, which are more susceptible to biocides, can be readily differentiated from stationary phase cells by this phenomenon. The adaptational network of *B. subtilis* involves the induction of stress proteins and the production of SASPs. Stress response proteins are induced when sporulating cells are heat-shocked. Nutritionally-limited cells expressing

starvation phenotypes are more resistant to biocides than 'normal' cells. Stress adaptation responses are also known in yeasts. Yeast cells have evolved a wide range of responses to many different types of stresses, both physical (e.g. heat, starvation) and chemical (such as oxidative stress, ethanol). These responses are (i) intrinsic (constitutive) and depend on growth phase and the stage of an organism in its life cycle, or (ii) inducible. The main toxic effects of the superoxide anion (O2–) and hydrogen peroxide results from their ready conversion into the highly reactive hydroxyl radicals (·OH). These can damage cellular nucleic acids, proteins and lipids. The main defence systems in *S. cerevisiae* involve (i) degradation or detoxification of the reactive oxygen species, (ii) maintenance of metal ion homeostasis to prevent free metal ions generating ·OH, and (iii) repair of damage. As with vegetative bacterial cells, yeast cells can adapt to a subsequent dose of hydrogen peroxide. During this adaptation, several polypeptides are induced, some of which are unique to peroxide treatment with others also being produced following heat shock. Catalase and possibly glutathione play a role in this adaptive response. A 'heat shock response' is also produced in *C. albicans* following exposure to ethanol. Stresses such as heat, oxidative stress and pH shock on *Acanthamoeba* trophozoites have been studied. Unstimulated pathogenic *Acanthamoeba* (*castellanii*, *cilbertsoni*) had relatively high levels of HSPs 60 and 70, whereas unstimulated trophozoites of freeliving *A. rhysodes* had the lowest background levels of these HSPs and were the most affected by the stresses.

General aspects of resistance mechanisms to biocide action

Resistance to biocidal agents has been widely studied in bacteria and to some extent in fungi, with some useful information beginning to emerge with some types of protozoa. In nonsporulating bacteria, the major mechanisms of resistance are reduced uptake (impermeability and/or efflux), with possible mutation, transferable resistance and biocide degradation worthy of consideration. In addition, biofilm formation is a major reason for the refractory response of many organisms to biocides. There could also be different target site affinities for biocides in different types of microorganisms, although this aspect has been less widely studied. *Cellular impermeability as a resistance mechanism* Gram-negative bacteria, and especially *P. aeruginosa*, *Proteus* spp., *Providencia* spp. and *Serratia marcescens*, generally show reduced susceptibility to biocides compared with the Gram-positive cocci. A major reason for this reduced susceptibility resides in the OM acting as a permeability barrier, so that uptake into the cell is reduced, as described earlier. This aspect has been considered in greater detail elsewhere. VRSA possess thickened cell walls as well as altered peptidoglycan and thus might limit the uptake of biocides. This could prove to be a worthwhile investigation. In terms of their biocide susceptibility, mycobacteria occupy an intermediate position between bacterial spores and other bacteria. There is no evidence that efflux plays a role in this. The major reason for their recalcitrance to biocide activity is the lipid-rich, waxy cell wall which limits intracellular uptake of many biocides. Bacterial spores tend to be much less susceptible to biocidal agents than non-sporulating bacteria. An obvious reason is to be found with the nature and composition of the spore coats and possibly cortex (Table 1) which present an effective permeability barrier to the entry of many biocides. Antibiotic resistance in yeasts is known to occur via target site mutations and reduced uptake (impermeability and efflux). Examples occur with fluconazole (modification in the quantity and quality of 14α-demethylase, leading to reduced azole affinity and uptake) and polyenes (membranes with modified sterols that have lower affinity for nystatin binding). The antibacterial antibiotic, rifampicin, is ineffective versus fungi. However, when used in combination with the polyenic antifungal drug, amphotericin B (which combines with fungal membrane sterol), it shows activity against several fungal species. This has led to the suggestion that increased uptake of rifampicin occurs as a consequence of amphotericin action and that membrane sterols pose a barrier to its entry. CHX and QACs cause damage to the yeast plasma membrane;, it is not, however, known whether this interaction is reduced by the presence of membrane sterols which could effectively limit further uptake into the cell interior. The outer layers of protozoal cysts are likely to act as a barrier to some biocides. The outer shell of *Cryptosporidium* oocysts renders them more resistant to biocides. In the protozoon, *A. castellanii*, resistance to biocides is likely to result from the cellulose content of the outer layers.

Target site: Biocides are considered to be multitargeted chemical agents. However, as pointed out earlier, the growth-inhibitory properties of triclosan involve inhibition of enoyl reductase. Mutation in the target enzyme or its overproduction can lead to considerable increases in MICs. With alcohols, the lipid composition and plasma fluidity play a role in the susceptibility of yeasts.

Efflux as a resistance mechanism: Efflux is a major mechanism for the resistance shown by bacteria to antibiotics. Efflux of biocides is known and has been the subject of several authoritative reviews. In bacteria, several classes of efflux pumps have been described. The silver resistance determinant from a hospital burn ward *Salmonella* plasmid encodes a periplasmic silver-specific binding protein (SilE) plus two parallel efflux pumps. One of these is a P-type ATPase (SilP) and the other a membrane potential-dependent, three component cation/proton antiporter (Sil CPA). Efflux of antifungal antibiotics has also been described. Efflux has been shown to play a role in the response of some strains of yeasts to organic acids as an inducible preservative elimination system, but there is no evidence to date that low-level resistance to cationic biocides occurs by active efflux pumps in yeasts.

Biofilms: The mechanisms of reduced susceptibility to biocides and antibiotics of bacterial cells present within biofilms have been the subject of considerable experimentation and debate. These mechanisms include (i) reduced access of biocide molecules to bacterial cells, (ii) chemical interactions between biofilm and biocide, (iii) modulation of the micro-environment, producing nutrient- and oxygen-limited and starved cells, (iv) production of degradative enzymes that might be effective at lower biocide concentrations within the biofilm, (v) genetic exchange between cells, (vi) quorum sensing, (vii) presence of persisters and of pockets of surviving organisms, (viii) adaptation and mutation within the biofilm, and (ix) biocide efflux. In nature, it is likely that biofilms will consist of mixed populations of different types of microorganisms. Many types of bacteria and yeasts interact with protozoa, e.g. the co-evolution between *Legionella* and *Acanthamoeba* and other protozoa, and between *Cryptococcus neoformans* and *Acanthamoeba*.157 This co-evolution of bacteria and lower order eukaryotes has equipped the organisms for environmental survival as well as virulence towards higher order eukaryotes. *Legionella pneumophila* within *Acanthamoeba* cysts are protected from the action of chlorine. With algae, the presence of mats equates to biofilms and constant dosing with biocides may be needed to prevent algal recontamination.

Other factors: An additional factor in bacterial spores, but not in other microorganisms, is the presence of α,β-type SASPs (referred to earlier). These can coat spore DNA, thereby protecting it from damage by enzymes and antibacterial agents. They thus play an important role in determining spore susceptibility to antibacterial agents. Other factors also need to be considered, namely the reduced water content in the core and the ability or otherwise to repair DNA damage during germination.

Biochemical Tests to identify Bacteria

Bacterial colonies can differ greatly in their morphologies. These differences can help us in identifying different species of bacteria. Likewise, bacterial species differ in their cellular morphologies and staining properties. Again, these differences can be used to aid in identifying different species. We can use selective and/or differential media to aid in identifying bacterial species. Generally, selective and differential media rely on some structural or metabolic property of the species that is preferentially selected.

Gram staining, for instance, can allow us to distinguish Gram positive from Gram negative organisms and rod-shaped organisms from coccii-shaped organisms, but does not allow us to make a more specific identification. Likewise, a selective and differential medium like MacConkey allows us to identify Gram negative, lactose fermenting organisms, but does not allow us to positively identify what specific Gram negative, lactose fermenting organism we are examining. Which sugars bacteria ferment, which antibiotics they haveresistance to and which enzymes they produce are all important identifying characteristics that can be reasonably easily tested.

To aid in the more definitive identification of bacteria, microbiologists have developed a series of **biochemical tests** that can be used to differentiate even closely related organisms. These various tests were designed to identify various metabolic properties of different bacterial species. More importantly, these tests, in conjunction with a **dichotomous tree**, can lead to the unambiguous identification of an organism.

Tests used to identify Gram Positive Bacteria

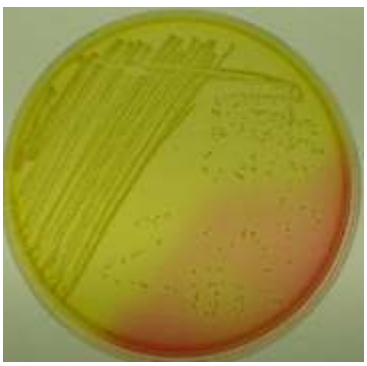
Catalase Test
Mannitol Salt Agar (MSA)
Blood Agar Plates (BAP)
Taxos P (optochin sensitivity testing)
Taxos A (bacitracin sensitivity testing)
Bile Esculin Agar
Nitrate Broth
Spirit Blue agar
Starch hydrolysis test
Motility Agar
Coagulase Test

Tests used to identify Gram Negative Bacteria

Oxidase Test
Sugar (eg glucose) broth with Durham tubes
Methyl Red / Voges-Proskauer (MR/VP)
Kliger's Iron Agar (KIA)
Nitrate Broth
Motility Agar
MacConkey agar
Simmon's Citrate Agar
Urease test
Sulfur Indole Motility Media (SIM)
Triple sugar iron medium

Mannitol Salt Agar (MSA)

This type of medium is both selective and differential. The MSA will select for organisms such as *Staphylococcus* species which can live in areas of high salt concentration. The differential ingredient in MSA is the sugar mannitol. Organisms capable of using mannitol as a food source will produce acidic byproducts of fermentation that will lower the pH of the media. The acidity of the media will cause the pH indicator, phenol red, to turn yellow. *Staphylococcus aureus* is capable of fermenting mannitol while *Staphylococcus epidermidis* is not.



Glucose broth with Durham tubes

This is a differential medium. It tests an organism's ability to ferment the sugar glucose as well as its ability to convert the end product of glycolysis, pyruvic acid into gaseous byproducts. This is a test commonly used when trying to identify Gram-negative enteric bacteria, all of which are glucose fermenters but only some of which produce gas.

Like MSA, this medium also contains the pH indicator, phenol red. If an organism is capable of fermenting the sugar glucose, then acidic byproducts are formed and the pH indicator turns yellow. *Escherichia coli* is capable of fermenting glucose as are *Proteus mirabilis* and *Shigella dysenteriae*. *Pseudomonas aeruginosa* is a nonfermenter.

The end product of glycolysis is pyruvate. Organisms that are capable of converting pyruvate to formic acid and formic acid to H_2 (g) and CO_2 (g), via the action of the enzyme formic hydrogen lyase, emit gas. This gas is trapped in the Durham tube and appears as a bubble at the top of the tube. *Escherichia coli* and *Proteus mirabilis* (right) are both gas producers. Notice that *Shigella dysenteriae* (left) ferments glucose but does not produce gas.

*Note - broth tubes can be made containing sugars other than glucose (e.g. lactose and mannitol). Because the same pH indicator (phenol red) is also used in these fermentation tubes, the same results are considered positive (e.g. a lactose broth tube that turns yellow after incubation has been inoculated with an organism that can ferment lactose).

Shigella dysenteriae | *Escherichia coli* | *Proteus mirabilis*

Blood Agar Plates (BAP)

This is a differential medium. It is a rich, complex medium that contains 5% sheep red blood cells. BAP tests the ability of an organism to produce hemolysins, enzymes that damage/lyse red blood cells (erythrocytes). The degree of hemolysis by these hemolysins is helpful in differentiating members of the genera *Staphylococcus*, *Streptococcus* and *Enterococcus*.

Beta-hemolysis is complete hemolysis. It is characterized by a clear (transparent) zone surrounding the colonies. *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus agalactiae* are b-hemolytic (the picture on the left below shows the beta-hemolysis of *S. pyogenes*).

Partial hemolysis is termed alpha-hemolysis. Colonies typically are surrounded by a green, opaque zone. *Streptococcus pneumoniae* and *Streptococcus mitis* are a-hemolytic (the picture on the right below shows the a-hemolysis of *S. mitis*).

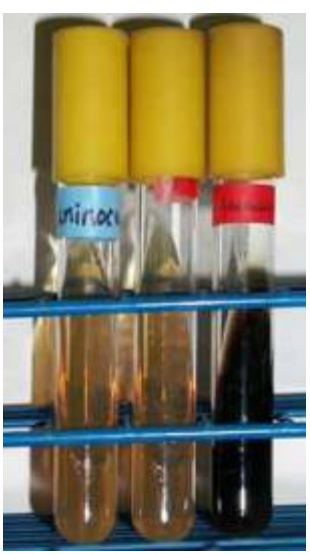
If no hemolysis occurs, this is termed gamma-hemolysis. There are no notable zones around the colonies. *Staphylococcus epidermidis* is gamma-hemolytic.

Bile Esculin Agar

This is a medium that is both selective and differential. It tests the ability of organisms to hydrolyze esculin in the presence of bile. It is commonly used to identify members of the genus *Enterococcus* (*E faecalis* and *E. faecium*).

The first selective ingredient in this agar is bile, which inhibits the growth of Gram-positives other than enterococci and some streptococci species. The second selective ingredient is sodium azide. This chemical inhibits the growth of Gram-negatives.

The differential ingredient is esculin. If an organism can hydrolyze esculin in the presence of bile, the product esculetin is formed. Esculetin reacts with ferric citrate (in the medium), forming a phenolic iron complex which turns the entire slant dark brown to black. The tube on the right was inoculated with *E. faecalis* (positive). The tube in the center was inoculated with a bilieesculin negative organism and the tube on the left was uninoculated.



uninoculated P.mirabilis E.faecalis

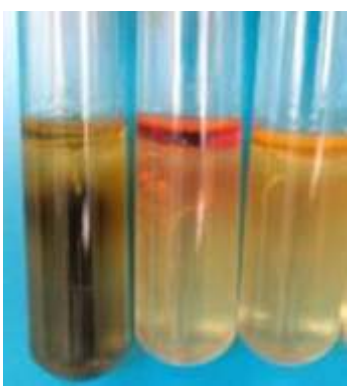
Sulfur Indole Motility Media (SIM)

This is a differential medium. It tests the ability of an organism to do several things: reduce sulfur, produce indole and swim through the agar (be motile). SIM is commonly used to differentiate members of *Enterobacteriaceae*.

Sulfur can be reduced to H_2S (hydrogen sulfide) either by catabolism of the amino acid cysteine by the enzyme cysteine desulfurase or by reduction of thiosulfate in anaerobic respiration. If hydrogen sulfide is produced, a black color forms in the medium. *Proteus mirabilis* is positive for H_2S production. The organism pictured on the left is positive for hydrogen sulfide production.

Bacteria that have the enzyme tryptophanase, can convert the amino acid, tryptophane to indole. Indole reacts with added Kovac's reagent to form rosindole dye which is red in color (indole +). *Escherichia coli* isindole positive. The organism pictured second from left is *E. coli* and is indole positive.

SIM tubes are inoculated with a single stab to the bottom of the tube. If an organism is motile than the growth will radiate from the stab mark and make the entire tube appear turbid. *Pseudomonas aeruginosa* and the strain of *Proteus mirabilis* that we work with are motile.



P. mirabilis E. coli P. aeruginosa

Kliger's Iron Agar (KIA)

This is a differential medium. It tests for organisms' abilities to ferment glucose and lactose to acid and acid plus gas end products. It also allows for identification of sulfur reducers. This media is commonly used to separate lactose fermenting members of the family *Enterobacteriaceae* (e.g. *Escherichia coli*) from members that do not ferment lactose, like *Shigella dysenteriae*. These lactose nonfermenting enterics generally tend to be the more serious pathogens of the gastrointestinal tract.

The first differential ingredient, glucose, is in very short supply. Organisms capable of fermenting this sugar will use it up within the first few hours of incubation. Glucose fermentation will create acidic byproducts that will turn the phenol red indicator in the media yellow. Thus, after the first few hours of incubation, the tube will be entirely yellow. At this point, when the glucose has been all used up, the organism must choose another food source. If the organism can ferment lactose, this is the sugar it will choose. Lactose fermentation will continue to produce acidic byproducts and the media will remain yellow If gas is produced as a result of glucose or lactose fermentation, then fissures will appear in the agar or the agar will be lifted off the bottom of the tube.

If an organism cannot use lactose as a food source it will be forced to use the amino acids / proteins in the media. The deamination of the amino acids creates NH_3, a weak base, which causes the medium to become alkaline. The alkaline pH causes the phenol red indicator to begin to turn red. Since the incubation time is

short (18-24 h), only the slant has a chance to turn red and not the entire tube. Thus an organism that can ferment glucose but not lactose will produce a red slant and a yellow butt in a KIA tube. These organisms are the more serious pathogens of the GIT (gastrointestinal tract)such as *Shigella dysenteriae*.

If an organism is capable of using neither glucose nor lactose, the organism will use solely amino acids / proteins. The slant of the tube will be red and the color of the butt will remain unchanged (picture on the far right below). *Pseudomonas aeruginosa* is an example of a nonfermenter.

KIA tubes are also capable of detecting the production of H_2S. It is seen as a black precipitate. Sometimes the black precipitate obscures the butt of the tube. In such cases, the organisms should be considered positive for glucose fermentation (yellow butt). *Proteus mirabilis* is a glucose positive, lactose negative, sulfur reducing enteric.

Uninoculated *S.dysenteriae* *P.mirabilis* *P.aeruginosa*

Nitrate Broth

This is a differential medium. It is used to determine if an organism is capable of reducing nitrate (NO_3) to nitrite (NO_2) or other nitrogenous compounds via the action of the enzyme nitratase (also called nitrate reductase). This test is important in the identification of both Gram-positive and Gram-negative species.

After incubation, these tubes are first inspected for the presence of gas in the Durham tube. In the case of nonfermenters, this is indicative of reduction of nitrate to nitrogen gas. However, in many cases gas is produced by fermentation and further testing is necessary to determine if reduction of nitrate has occurred. This further testing includes the addition of sulfanilic acid (often called nitrate I) and dimethyl-alpha-napthalamine (nitrate II). If nitrite is present in the media, then it will react with nitrate I and nitrate II to form a red compound. This is considered a positive result. If no red color forms upon addition of nitrate I and II, this indicates that either the NO_3 has not been converted to NO_2 (a negative result), or that NO_3^- was converted to NO_2 and then immediately reduced to some other, undetectable form of nitrogen (also a positive result). In order to determine which of the preceding is the case, elemental zinc is added to the broth. Zinc will convert any remaining NO_3 to NO_2 thus allowing nitrate I and nitrate II to react with the NO_2 and form the red pigment (a verified negative result). If no color change occurs upon addition of zinc then this means that the NO_3 was converted to NO_2 and then was converted to some other undetectable form of nitrogen (a positive result).

If the nitrate broth turns red after nitrate I and nitrate II are added, this color indicates a positive result. If instead, the tube turns red after the addition of Zn, this indicates a negative result. If there is no color change in the tube after the addition of nitrate I and nitrate II, the result is uncertain. If the tube is colorless after the addition of Zn this indicates a positive test.

Catalase Test

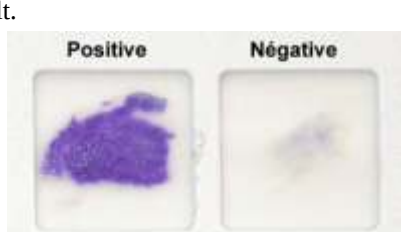
This test is used to identify organisms that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas.

The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result. The *Staphylococcus* spp. and the *Micrococcus* spp. are catalase positive. The *Streptococcus* and *Enterococcus spp. are* catalase negative.

Oxidase Test

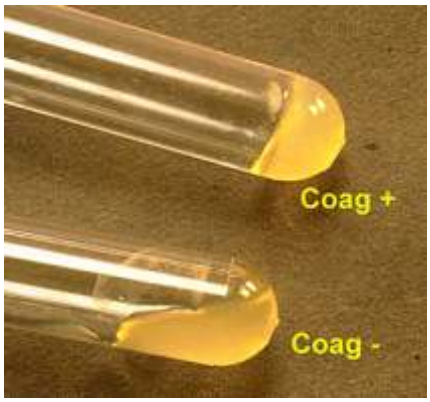
This test is used to identify microorganisms containing the enzyme cytochrome oxidase (important in the electron transport chain). It is commonly used to distinguish between oxidase negative *Enterobacteriaceae* and oxidase positive *Pseudomadaceae*.

Cytochrome oxidase transfers electrons from the electron transport chain to oxygen (the final electron acceptor) and reduces it to water. In the oxidase test, artificial electron donors and acceptors are provided. When the electron donor is oxidized by cytochrome oxidase it turns a dark purple. This is considered a positive result.



Coagulase test

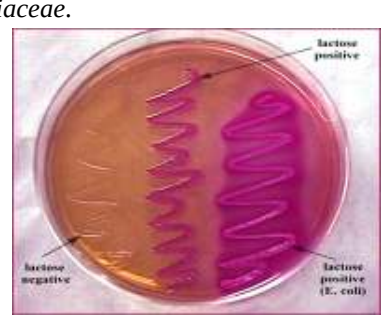
Coagulase is an enzyme that clots blood plasma. This test is performed on Gram-positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus.* Coagulase is a virulence factor of *S. aureus.* The formation of clot around an infection caused by this bacteria likely protects it from phagocytosis. This test differentiates *Staphylococcus aureus* from other coagulase negative *Staphylococcus* species.



Taxos A (bacitracin sensitivity testing)
This is a differential test used to distinguish between organisms sensitive to the antibiotic bacitracin and those not. Bacitracin is a peptide antibiotic produced by *Bacillus subtilis*. It inhibits cell wall synthesis and disrupts the cell membrane. This test is commonly used to distinguish between the b-hemolytic streptococci: *Streptococcus agalactiae* (bacitracin resistant) and *Streptococcus pyogenes* (bacitracin sensitive).

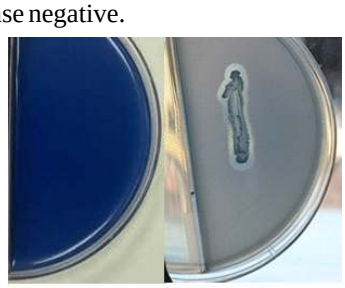
Taxos P (optochin sensitivity testing)
This is a differential test used to distinguish between organisms sensitive to the antibiotic optochin and those not. This test is used to distinguish *Streptococcus pneumoniae* (optochin sensitive (pictured on the right below)) from other a-hemolytic streptococci (optochin resistant (*Streptococcus mitis* is pictured on the left below)).

MacConkey agar
This medium is both selective and differential. The selective ingredients are the bile salts and the dye, crystal violet which inhibit the growth of Gram-positive bacteria. The differential ingredient is lactose. Fermentation of this sugar results in an acidic pH and causes the pH indicator, neutral red, to turn a bright pinky-red color. Thus organisms capable of lactose fermentation such as *Escherichia coli*, form bright pinky-red colonies. MacConkey agar is commonly used to differentiate between the *Enterobacteriaceae*.



Simmon's Citrate Agar
This is a defined medium used to determine if an organism can use citrate as its sole carbon source. It is often used to differentiate between members of *Enterobacteriaceae*. In organisms capable of utilizing citrate as a carbon source, the enzyme citrase hydrolyzes citrate into oxaloacetic acid and acetic acid. The oxaloacetic acid is then hydrolyzed into pyruvic acid and CO_2. If CO_2 is produced, it reacts with components of the medium to produce an alkaline compound (e.g. Na_2CO_3). The alkaline pH turns the pH indicator (bromthymol blue) from green to blue. This is a positive result (the tube on the right is citrate positive). *Klebsiella pneumoniae* and *Proteus mirabilis* are examples of citrate positive organisms. *Escherichia coli* and *Shigella dysenteria*e are citrate negative.

Spirit Blue Agar
This agar is used to identify organisms that are capable of producing the enzyme lipase. This enzyme is secreted and hydrolyzes triglycerides to glycerol and three long chain fatty acids. These compounds are small enough to pass through the bacterial cell wall. Glycerol can be converted into a glycolysis intermediate. The fatty acids can be catabolized and their fragments can eventually enter the Kreb's cycle. Spirit blue agar contains an emulsion of olive oil and spirit blue dye. Bacteria that produce lipase will hydrolyze the olive oil and produce a halo around the bacterial growth. The Gram-positive rod, *Bacillus subtilis* is lipase positive (pictured on the right) The plate pictured on the left is lipase negative.

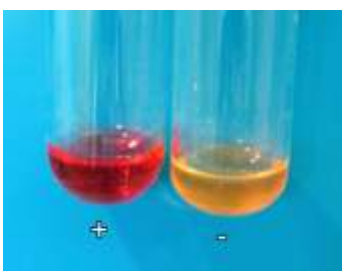


Lipase negative ***Lipase positive***

Starch hydrolysis test
This test is used to identify bacteria that can hydrolyze starch (amylose and amylopectin) using the enzymes a-amylase and oligo-1,6-glucosidase. Often used to differentiate species from the genera *Clostridium* and *Bacillus*. Because of the large size of amylose and amylopectin molecules, these organisms can not pass through the bacterial cell wall. In order to use these starches as a carbon source, bacteria must secrete a-amylase and oligo-1,6-glucosidase into the extracellular space. These enzymes break the starch molecules into smaller glucose subunits which can then enter directly into the glycolytic pathway. In order to interpret the results of the starch hydrolysis test, iodine must be added to the agar. The iodine reacts with the starch to form a dark brown color. Thus, hydrolysis of the starch will create a clear zone around the bacterial growth. *Bacillus subtilis* is positive for starch hydrolysis.

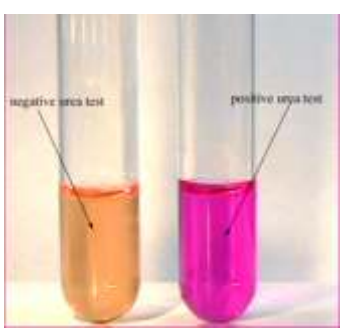
Methyl Red / Voges-Proskauer (MR/VP)

This test is used to determine which fermentation pathway is used to utilize glucose. In the mixed acid fermentation pathway, glucose is fermented and produces several organic acids (lactic, acetic, succinic, and formic acids). The stable production of enough acid to overcome the phosphate buffer will result in a pH of below 4.4. If the pH indicator (methyl red) is added to an aliquot of the culture broth and the pH is below 4.4, a red color will appear (first picture, tube on the left). If the MR turns yellow, the pH is above 6.0 and the mixed acid fermentation pathway has not been utilized (first picture, tube on the right). The 2,3butanediol fermentation pathway will ferment glucose and produce a 2,3 butanediol end product instead of organic acids. In order to test this pathway, an aliquot of the MR/VP culture is removed and a-naphthol and KOH are added. They are shaken together vigorously and set aside for about one hour until the results can be read. The Voges-Proskauer test detects the presence of acetoin, a precursor of 2,3butanediol. If the culture is positive for acetoin, it will turn "brownish-red to pink" (tube on the left in the second picture). If the culture is negative for acetoin, it will turn "brownish-green to yellow" (tube on the left in the second picture). Note: A culture will usually only be positive for one pathway: either MR+ or VP+. *Escherichia coli* is MR+ and VP-. In contrast, *Enterobacter aerogenes* and *Klebsiellapneumoniae* are MR- and VP+. *Pseudomonas aeruginosa* is a glucose nonfermenter and is thus MR- and VP-.



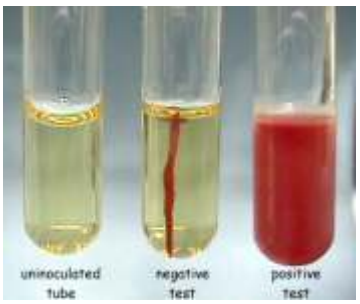
Urease test

This test is used to identify bacteria capable of hydrolyzing urea using the enzyme urease. It is commonly used to distinguish the genus *Proteus* from other enteric bacteria. The hydrolysis of urea forms the weak base, ammonia, as one of its products. This weak base raises the pH of the media above 8.4 and the pH indicator, phenol red, turns from yellow to pink. *Proteus mirabilis* is a rapid hydrolyzer of urea. The tube on the far right was inoculated with a urease negative organism and the tube on the left was uninoculated.



Motility agar

It is a differential medium used to determine whether an organism is equipped with flagella and thus capable of swimming away from a stab mark. The results of motility agar are often difficult to interpret. Generally, if the entire tube is turbid, this indicates that the bacteria have moved away from the stab mark (are motile). The organisms in the two tubes pictured on the right are motile. If, however, the stab mark is clearly visible and the rest of the tube is not turbid, the organism is likely nonmotile (Center picture).



Triple sugar iron medium

Used to differentiate among the different groups of *Enterobacteriaceae* based on their ability to ferment glucose, lactose and/or sucrose. Also differentiates between groups capable of reducing sulfur to hydrogen sulfide (Sodium Thiosulfate -> Hydrogen sulfide).

The phenol red in medium is a pH indicator. If the medium becomes acidic, then the phenol red turns yellow. If the medium becomes alkaline, then the phenol red turns purple. If an organism can only ferment glucose, then the medium initially will turn yellow. Because there is so little glucose in the medium, however, the bacteria quickly will exhaust the glucose supply and begin to oxidize amino acids for energy. The oxidation of amino acids produces ammonia as a by-product. The ammonia will cause an increase in pH and a return to a red or purple color on the surface of the slant. Therefore, organisms that can only ferment glucose will produce a slant with a red surface and yellow butt. If the organism being tested can ferment lactose and sucrose, then the entire tube will turn, and remain, yellow. The production of gas can be detected in TSI slants by the presence of bubbles within the agar. Furthermore, if the produced gas is H_2S, it will react with the ferrous sulfate to produce ferrous sulfide, a black precipitate.

Red slant/Red butt = no fermentation
Red slant/Yellow butt = only glucose fermentation
Yellow slant/yellow butt = lactose and/or sucrose fermentation
Dark color (Black Colour) = Hydrogen Sulfide produced Sodium thiosulfate reduced

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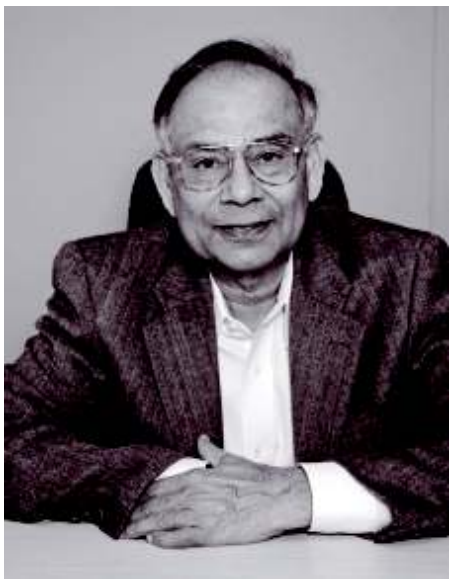
Anand Mohan Chakrabarty



Born : 4 April 1938 (age 76) Sainthia, Birbhum
Nationality : Indian-American
Fields : Microbiology
Known for : Genetically engineering a Pseudomonas bacteria

Scientific work

Prof. Chakrabarty genetically engineered a new species of *Pseudomonas* bacteria ("the oil-eating bacteria") in 1971 while working for the Research & Development Center at General Electric Company in Schenectady, New York.

At the time, four known species of oil-metabolizing bacteria were known to exist, but when introduced into an oil spill, competed with each other, limiting the amount of crude oil that they degraded. The genes necessary to degrade oil were carried on plasmids, which could be transferred among species. By irradiating the transformed organism with UV light after plasmid transfer, Prof. Chakrabarty discovered a method for genetic cross-linking that fixed all four plasmid genes in place and produced a new, stable, bacteria species (now called pseudomonas putida) capable of consuming oil one or two orders of magnitude faster than the previous four strains of oil-eating microbes. The new microbe, which Chakrabarty called "multi-plasmid hydrocarbon-degrading *Pseudomonas*," could digest about two-thirds of the hydrocarbons that would be found in a typical oil spill.

The bacteria drew international attention when he applied for a patent—the first U.S. patent for a genetically modified organism. (U.S. utility patents had been granted to living organisms before, including two pure bacterial cultures, patented by Louis Pasteur. Chakrabarty's modified bacterium was granted a patent in the U.K. before the U.S. patent came through.) He was initially denied the patent by the Patent Office because it was thought that the patent code precluded patents on living organisms. The United States Court of Customs and Patent Appeals overturned the decision in Chakrabarty's favor.

Pseudomonas putida is a Gram-negative, rod-shaped, saprotrophic soil bacterium. Based on 16S rRNA analysis, *P. putida* has been placed in the *P. putida* group, to which it lends its name.

It is the first patented organism in the world. Because it is a living organism, the patent was disputed and brought before the United States Supreme Court in the historic court case Diamond v. Chakrabarty which the inventor, Ananda Mohan Chakrabarty, won. It demonstrates a very diverse metabolism, including the ability to degrade organic solvents such as toluene. This ability has been put to use in bioremediation, or the use of microorganisms to biodegrade oil. Use of *P. putida* is preferable to some other *Pseudomonas* species capable of such degradation, as it is a safe species of bacteria, unlike *P. aeruginosa*, for example, which is an opportunistic human pathogen.

Current work

Currently, his lab is working on elucidating the role of bacterial cupredoxins and cytochromes in cancer regression and arresting cell cycle progression. These proteins have been formerly known for their involvement in bacterial electron transport. He has isolated a bacterial protein, *azurin*, with potential antineoplastic properties. He has expanded his lab's work to include multiple microbiological species, including *Neisseria*, *Plasmodia*, and *Acidithiobacillus ferrooxidans*.

In 2001, Prof. Chakrabarty founded a company, CDG Therapeutics, (incorporated in Delaware) which holds proprietary information related to five patents generated by his work at the University of Illinois at Chicago. The University of Illinois owns the rights to the patents but has issued exclusive licences to CDG Therapeutics.

In 2008, Prof. Chakrabarty co-founded a second bio-pharmaceutical discovery company, Amrita Therapeutics Ltd., registered in Ahmedabad, Gujarat, to develop therapies, vaccines and diagnostics effective against cancers and/or other major public health threats derived from bacterial products found in the human body. Amrita Therapeutics Ltd. received initial funding in late 2008 from GVFL, and more recently received a grant for a 2-year research program in 2010 from the Indian Department of Biotechnology under the Biotechnology Industry Promotion Program (BIPP).

Awards

Dr. Chakrabarty has received many awards, including

- the 'Scientist of the Year' award in 1975 by Industrial Research Organization of the United States,
- the Distinguished Scientist Award from the United States Environmental Protection Agency,
- the MERIT Award from NIH,
- the Distinguished Service Award given by the U.S. Army
- the Public Affairs Award awarded by the American Chemical Society, and
- the Procter & Gamble Environmental Biotechnology Award given by the American Society for Microbiology.
- the Golden Eurydice Award for contributions in Biophilosophy in 2007.

For his work in genetic engineering technology, he was awarded the civilian Padma Shri by the Government of India in 2007.

Funny Quotes

Postman: I have to come 5 miles to deliver you this packet.
Santa: Why did u come so far? Instead U could have posted it.

" Women won't play football not coz they aren't gud at it..
But coz its against their ego to b dressed up exactly like 10 other women in front of 10,000 people.."

Saw It With My Eyes But Couldn't Understand It
Took It In My Hands, But Couldn't Understand It
Keep Thinking For A Long Time, But Again Couldn't Understand It
It was Not A Dream,
It was Is Not A Love,
It was Not Even Friendship,
Then I Realized: "It Was Question Paper."

Santa giving exam while standing at the door.
A man asked "Why are you standing at the door?"
Santa: "Idiot, I am giving entrance test."

Question by a student !!
If a single teacher can't teach us all the subjects,
Then…
How could you expect a single student to learn all subjects?

Women are like Fruits.
Every Woman has her own unique taste and colour…
But
The problem is the Men.
They seem to love Fruit salad..!!

A pizza and an apple were thrown down from the 15th floor.
Which will reach down first?
Ans: The Pizza, as it's fast food!

One million copies of a new book sold In just two days due to typing error of one alphabet in title.
"An idea, that can change your WIFE"
While real word was (LIFE).

Teacher: Why are you late?
Santa: Because of the sign.
Teacher: What sign?
Santa: The one that says, "School Ahead, Go Slow."

New Teacher: anybody who thinks he is stupid, stand up
Pappu stoodup
Teacher: R U stupid?
Pappu: "nhi, Aap akeli khari theen mujhe acha nhi lag raha tha."

Words of Wisdom

Shakespeare	....	*Never play with the feelings of others. Because you may win the game but the risk is that you will surely loose the person for a lifetime.*
Napoleon	....	*The world suffers a lot. Not because of the violence of bad people, but because of the silence of good people.*
Einstein	....	*I am thankful to all those who said NO to me, it's because of them I did it myself.*
Abraham Lincoln	....	*If friendship is your weakest point then you are the strongest person in the world.*
Martin Luther King Jr	....	*We must learn to live together as brothers or perish together as fools.*
Mahatma Gandhi	....	*The weak can never forgive. Forgiveness is the attribute of the strong.*
Dr Abdul Kalam	....	*It Is Very Easy To Defeat Someone, But It Is Very Hard To Win Someone.*

Listeria monocytogenes

Introduction:

Listeria monocytogenes are rod-shaped proteobacteria that cause the food borne illness listeriosis. Although listeriosis is rare, it is a potentially deadly disease. The bacteria are found in water, soil and in animals, and they can grow even in cold temperature environments, including your refrigerator. You can get infected by eating contaminated foods, such as raw vegetables, meat and unpasteurized milk. Listeria can be serious, especially for those with a weakened immune system or women who are pregnant.

Classification:

L. monocytogenes is a Gram-positive, nonspore-forming, motile, facultatively anaerobic, rod-shaped bacterium. It is catalase-positive and oxidase-negative, and expresses a beta hemolysin, which causes destruction of red blood cells. This bacterium exhibits characteristic tumbling motility when viewed with light microscopy. Although *L. monocytogenes* is actively motile by means of peritrichous flagella at room temperature (20- 25 °C), the organism does not synthesize flagella at body temperatures (37 °C).

The genus *Listeria* belongs to the class, *Bacilli*, and the order, *Bacillales*, which also includes *Bacillus* and *Staphylococcus*. The genus *Listeria* includes six different species (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*). Both *L. ivanovii* and *L. monocytogenes* are pathogenic in mice, but only *L. monocytogenes* is consistently associated with human illness. There are 13 serotypes of *L. monocytogenes* that can cause disease, but more than 90 percent of human isolates belong to only three serotypes: 1/2a, 1/2b, and 4b. *L. monocytogenes* serotype 4b strains are responsible for 33 to 50 percent of sporadic human cases worldwide and for all major foodborne outbreaks in Europe and North America since the 1980s.

Entry, Multiplication, and Spread:

L. monocytogenes initially gains access to the body through the gastrointestinal tract but is capable of infecting the blood through monocytes, macrophages and polymorphonuclear leukocytes. The bacterium is also capable of infecting hepatocytes, endothelial cells, and epithelial cells. *L. monocytogenes* lives as an intracellular pathogen inside these host cells, using the cell's own machinery to survive. Pathogenic *L. monocytogenes* go through an intracellular life cycle involving early escape from the phagocytic vacuole, rapid intracytoplasmic multiplication, bacterially induced actin-based motility, and direct spread to neighboring cells, in which they reinitiate the cycle. The bacterium is first phagocytosed by these cells and secretes a pore-forming toxin called listeriolysin, which allows the bacterium to escape from the phagosome. All virulent strains of *L. monocytogenes* synthesize and secrete listeriolysin. Phospholipase A and B are other virulence factors that facilitate escape of *L. monocytogenes* from the phagosome. Once out of the phagosome *L. monocytogenes* is capable of rapid division in the cytoplasm, evading the immune response and moving throughout the cytoplasm from cell to cell.

L. monocytogenes is well known for its ability to propel itself like a rocket through the cell cytoplasm. This is the result of the bacterium's ability to polymerize actin filaments at its tail end. Actin is arranged in subunits to form microfilaments that are capable of directing cell movement. *L. monocytogenes* accomplishes cell motility through a virulence factor called ActA that takes advantage of normal actin polymerization going on in the cell. The ActA protein shares sequence homology with a protein called WASP that is found in virtually all eukaryotic cells. WASP is responsible for recognizing and binding to the Arp2/3 complex. Once they are bound actin filament polymerization is initiated locally because the Arp2/3 complex allows nucleation of actin into long chain filaments. ActA is therefore present only at one end of the bacterium, called the tail end, to direct site-specific actin polymerization. The rocketing movement of *L. monocytogenes* enables the bacterium to protrude out of one cell and into an adjacent one, evading the immune response.

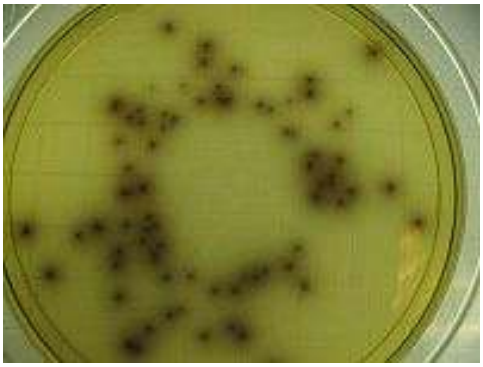
Symptoms:

Symptoms appear following an incubation period of 1-8 weeks, usually 31 days. It is therefore difficult to contain the spread of *L. monocytogenes* because incubation may be as long as 8 weeks, resulting in numerous asymptomatic individuals. Symptoms of listeriosis include fever, muscle aches, stiff neck, and gastrointestinal symptoms such as nausea or diarrhea. Infection may also spread to the nervous system resulting in symptoms such as headache, stiff neck, confusion, loss of balance, or convulsions. Pregnant women may experience flu-like symptoms while infants may experience pneumonia. Complications from listeriosis are very serious, including mortality rates of 70% from septicemia and 80% from perinatal/neonatal infections. About 20% of individuals infected with listeriosis die from the infection.

Risk:

Those at high risk for infection with *L. monocytogenes* are primarily pregnant women, newborns and immuno-compromised individuals, including the elderly, patients diagnosed with cancer, AIDS, and diabetes. An infected mother is capable of transmitting listeriosis to her newborn through the placenta. It is believed that healthy adults and children may become infected but rarely develop symptoms of listeriosis or become seriously ill unless contamination is very high. Specifically individuals younger than one year and older than 60 years have the highest incidence rate of infection with *L. monocytogenes*, 3.9 per 100,000 and 1.7 per 100,000 respectively.

Detection:



Colonies of typical *Listeria monocytogenes* as they appear when grown on *Listeria*-selective agar

PALCAM Agar is a selective medium used for the differentiation and isolation of *Listeria monocytogenes* from milk and cheese, as well as in other food products, even highly contaminated.

Anton Test : A test used in the identification of *Listeria monocytogenes*; instillation of a culture into the conjunctival sac of a rabbit or guinea pig causes severe keratoconjunctivitis within 24 hours.

Culture Characteristics: Listeria grows on media such as Mueller-Hinton agar. Identification is enhanced if the primary cultures are done on agar containing sheep blood, because the characteristic small zone of hemolysis can be observed around and under colonies. Isolation can be enhanced if the tissue is kept at 4 °C for some days before inoculation into bacteriologic media. The organism is a facultative anaerobe and is catalase-positive and motile. Listeria produces acid but not gas in a variety of carbohydrates. The motility at room temperature and hemolysin production are primary findings that help differentiate listeria from coryneform bacteria.

A new selective medium (Al-Zoreky-Sandine listeria medium [ASLM]) was formulated to recover *Listeria monocytogenes* from food specimens; the medium completely inhibited common food microflora. Recognition of Listeria colonies is evident by black discoloration of the medium due to esculin hydrolysis without need for special illuminating equipment. The medium contains acriflavin, ceftazidime, and moxalactam as selective agents. Compared with Listeria Selective Agar, ASLM was equally effective in recovering *L. monocytogenes*. However, ASLM inhibited micrococci, enterococci, and gram-negative bacteria, especially a strain that mimicked *L. monocytogenes* on Listeria Selective Agar. The new medium was able to recover heat injured cells with only 15% less count than the nonselective medium.

Prevention and Treatment:

Many preventative measures can be taken to avoid infection with *L. monocytogenes*. It is most important to thoroughly cook raw meat and thoroughly wash raw vegetables before consumption. Those at high risk for listeriosis can avoid the following foods: hot dogs, soft cheeses, unpasteurized products, refrigerated meat spreads and refrigerated smoked seafood. Antibiotics can be used to treat *L. monocytogenes* and those that are most effective include ampicillin, gentamicin, penicillin, and trimethoprim/sulfamethoxizole (Bactrim®, Septa®). Antibiotics, if given early, to the pregnant woman can often prevent infection of the fetus. Babies with listeriosis receive the same antibiotics as adults. Even with prompt treatment, some infections result in death. This is particularly likely in those with central nervous system involvement, the elderly and in persons with other serious medical problems.

***Use as a* Cancer vaccine:**

The facultative intracellular bacterium *Listeria monocytogenes* is being developed as a cancer vaccine platform because of its ability to induce potent innate and adaptive immunity.

Listeria monocytogenes is a facultative intracellular bacterium that enters professional antigen-presenting cells by active phagocytosis. As a live bacterium, it induces antigen-presenting cell maturation and strong innate immunity which may assist in the immune response to poorly immunogenic antigens, such as tumor-associated antigens. Listeria produces virulence factors that allow it to escape from the phagolysosome and colonize the cytosol of the host cell. It is thus a potent vaccine vector for the presentation of passenger antigens to the major histocompatibility complex class I and II pathways of antigen processing and presentation. Recent progress in developing this bacterium as a vaccine vector for tumor-associated antigens is reviewed. In mouse models, recombinant Listeria carrying a number of such antigens has provided therapeutic immunity directed towards established tumors.

A live attenuated *L. monocytogenes* cancer vaccine, ADXS11-001, is under development as a possible treatment for cervical carcinoma.

Use as a transfection vector:

Because *L. monocytogenes* is an intracellular bacterium, some studies have used this bacterium as a vector to deliver genes in vitro. Current transfection efficiency remains poor. One example of the successful use of *L. monocytogenes* in *in vitro* transfer technologies is in the delivery of gene therapies for cystic fibrosis cases.

Statistics:

- About 2,500 people in the U.S develop *Listeriosis* each year.
- 5 out of every 100 people carry *Listeria Monocytogenes* in their intestines.
- About 20% of people die from the infection.
- In 1989, there were 1,965 cases of Listeriosis with 481 deaths.
- In 1993, there were 1,092 cases of Listeriosis with 248 deaths.
- *Listeria Monocytogenes* reached the blood and cerebrospinal fluid in 89% of cases.
- *Listeriosis* results is a higher number of hospitalizations than any other food-born illness.
- Pregnant women account for 27% of cases, people with immunodeficiency disorders account for 70% of cases.
- AIDS patients are 280 time more likely to contract *Listeriosis* than others.

Are You a Mosquito Magnet? Here's What You Can Do

If you've ever felt that you're irresistible to pesky mosquitoes, it probably isn't just paranoia. Numerous scientific studies have revealed that mosquitoes really do prefer to bite some people over others when given the choice.

In fact, one study out of Japan found that the tiny critters are even attracted to one blood type over all others. Researchers revealed that people with Type O blood were found to be twice as attractive to mosquitoes than those with Type A blood. Mosquitoes can even taste via skin secretions what blood type we are before sucking it down, enabling them to find their preferred type.

Mosquitoes are also drawn to certain other particularities, like smelly feet and people with high concentrations of steroids or cholesterol on the surface of their skin. The color of clothing also makes a difference, as the bugs prefer dark colors that don't reflect much light. Of course, after the sun goes down it really doesn't matter what color your clothes are.

Fortunately, there is some good news for those who feel they might be a "mosquito magnet" as there are a number of ways to repel them naturally, without turning to potentially harmful chemical sprays.

Wear lighter-colored clothes

When you plan to be outside, wear lighter colored clothing like white, khaki or pastels, which all happen to be popular during the summer months anyway. Avoid dark colors like navy blue and black.

Stay in a screened-in area at dawn and dusk

If you plan to go camping, consider investing in a screened room or shelter that can be placed over a picnic table to keep you protected, especially at dawn and dusk when mosquitoes are most active. When you're at home, avoid being outside during those times, or stay in a screened-in porch.

Don't kill those spiders

Although many people have an aversion to spiders, arachnids love dining on mosquitoes and are a great way to reduce them in your area as they easily get stuck inside their webs. Leave spiders alone and their webs intact, and you're likely to notice a significant reduction in the numbers of skeeters you see.

Make your own mosquito repellent

There are lots of wonderfully smelling natural, essential oils that help to repel mosquitoes, such as a combination of lavender, tea tree oil and a dash of citronella.

Eat more garlic

You've probably heard that garlic repels vampires, but did you know that this pungent herb can keep mosquitoes away? While there hasn't been much research conducted to back up this claim, many people swear that it works.

Not only that, but garlic is filled with powerful antioxidants and is well known to help fight off infection. If you're worried about the smell, try chewing a sprig of fresh parsley after eating it.

Lemon eucalyptus oil

The Centers for Disease Control recommends lemon eucalyptus oil and says that it offers protection that is similar to low concentration DEET products. A 40 percent or higher concentration is recommended for fighting off mosquitoes as well as ticks.

Using fire

If you've ever sat around a campfire, you've probably noticed that mosquitoes seem to stay away, even from those they're most attracted to. It really does work because they don't like being around smoke. You can use standard candles or citronella candles – or, just enjoy sitting around the fire on a beautiful summer night.

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 triclosan-impregnated storage boxes were effective at reducing the

numbers of various challenge inocula and that the susceptibility of the strains was unaffected after repeated exposure on these treated items.

By way of contrast, Reverdy et al. showed that antibiotic-sensitive *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 ciprofloxacin-sensitive 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 schoolchildren 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 *qacE*and were part of transposon Tn*21*. The selection pressure for Tn*21*, 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-to-day 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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