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Antibiotic resistance

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"Penicillin brought more curative power to a barefoot, itinerant care provider in the deepest reaches of Africa than the collective powers of all the physicians in New York City" [1]. Along with public sanitation and immunization, antibiotic control of infectious diseases has contributed greatly to the doubled human lifespan in developed countries over the past century. No longer are we subjected to the ravages of cholera, typhus, typhoid fever, yellow fever, diphtheria, whooping cough, rheumatic fever, smallpox, and other mass communicable diseases. In 1967, the United States Surgeon General declared that: "The time has come to close the book on infectious diseases."

The Surgeon General was echoing the "prevailing wisdom" of the 1960s and a beginning age of optimism regarding antibiotics. After the panic of the late 1950s resulting from the advent of highly antibiotic resistant staphylococci and the realization that bacteria could transfer the mechanisms of antibiotic resistance between themselves, the medical world was blessed with a seemingly endless parade of new antibiotics: methicillin, cephalosporins, clindamycin, metronidazole, vancomycin, and new aminoglycosides and tetracyclines. This led to the arrogance that the inventiveness of the human mind was profoundly superior to such inferior microbial life forms. Little did anyone realize that no entirely new antibiotic would become available for another 40 years (until the year 2000). As stated so clearly by Murphy: "Optimism indicates that the situation is not clearly understood."

In 1993, 17 million people died of infectious diseases with 11.4 million due to diarrhea and bacterial pneumonia, mostly in children. In the same year, the worldwide mortality rate for cardiovascular disease and cancer combined was 15.6 million. Today the four primary infectious disease killers are the same as in 1900: diarrhea, pneumonia, tuberculosis, and malaria [2].

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Each year, *Streptococcus pneumoniae* kills 3 to 5 million people and malaria accounts for another 1.5 to 2.7 million deaths. During World War II, 55 million people were killed; by 2010, 65 million will have died of AIDS.

Microbial resistance in the general population

The United States Centers for Disease Control and Prevention estimates that 65,000 to 90,000 people die each year in United States from hospital-acquired (nosocomial) infections. This is almost surely a significant underestimation, and the correct number is likely closer to 200,000 or more as infectious disease deaths may be commonly misclassified as renal, respiratory, or cardiac failure instead of the underlying cause: pneumonias, septicemias, septic shock, and disseminated intravascular coagulation. In 1977, 100,000 germ-negative bacteremic deaths were estimated annually; today it is 150,000 [3]. If an intensive care unit (ICU) patient acquires a bacteremia, the mortality rate may be as high as 50%; in children and neonates, ICUs present at least a 12% risk of a life-threatening infection. Nosocomial blood stream infections, which account for only 15% of all nosocomial infections, have been variously estimated to be the fourth to the eighth leading cause of death in the United States [4,5].

Hospitals are currently plagued by vancomycin-resistant enterococci (VRE), beta-lactam (penicillins and cephalosporins), and vancomycin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*, multiply antibiotic-resistant *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacteriaceae*, and extended-spectrum-beta-lactamase–producing *Klebsiella pneumoniae*. Approximately 50% to 60% of all nosocomial pathogens are resistant to multiple antibiotics with a 27% to 70% chance of acquiring an infection to these microbes in an ICU [6]. The community is increasingly subjected to the ravages of methicillin-resistant *Staphylococcus aureus* (once thought only to reside in hospitals), penicillin and macrolide-resistant *Streptococcus pneumoniae*, beta-lactamase–producing *Haemophilus influenzae* and *Moraxella catarrhalis*, and increasing fluoroquinolone resistance in many micro-organisms. The oral cavity is now home to viridans group streptococci (VGS) with a 30% to 50% resistance rate to penicillins and macrolides (erythromcin, azithromycin, clarithromycin) and beta-lactamase production in 25% or more of *Porphyromonas* and *Prevotella* isolates. The first fully resistant isolate of *Staphylococcus aureus* to vancomycin was detected in a Michigan hospital [7] and other reports have followed. If at some future time Group A streptococci (*Streptococcus pyogenes*) develop penicillin resistance (already being highly resistant to the macrolides), then every single human microbial pathogen will exhibit multiple resistance to common antibiotics.

Long-term-care facilities have become septic reservoirs for highly and multiply antibiotic resistant micro-organisms: methicillin-resistant

Staphylococcus aureus and *epidermidis*, VRE, *Escherichia coli*, *Enterobacter*, and *Citrobacter* [8]. Between 20% and 60% of children attending day-care centers are carriers of antibiotic-resistant *Streptococcus pneumoniae* [9]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is now a community pathogen with the number of children hospitalized with community-acquired MRSA rising from 10 per 100,000 hospital admissions in 1988–1990 to 259 per 100,000 in 1993–1995 [10]. Possibly 1 billion tons of animal (chicken, pig, and cattle) waste (manure) are excreted on United States soil annually (Wisconsin alone provides enough to fill its Camp Randall football stadium daily) with antibiotic resistance genes in staphylococci, *Campylobacter*, *Salmonella*, *Enterococcus*, *Escherichia coli*, and *Yersinia enterocolitica* [11]. The identical genes are present in both animal and humans indicating transfer between these species [11]. The evidence is unequivocal that agricultural use of antibiotics as growth promoters (providing an average increase in animal weight by 4% to 6%) has selected for multiple antibiotic resistance to ampicillin, amoxicillin, tetracyclines, erythromycin, aminoglycosides, sulfonamides, methicillin/oxacillin, vancomycin, and chloramphenicol [12].

Streptococcus pneumoniae is annually responsible for 3000 cases of meningitis, 50,000 bacteremias, 500,000 pneumonias, and 2 million cases of otitis media in the United States and 3 to 5 million deaths per year worldwide [13]. High level ($\geq$2 μg/mL) resistance to penicillin is seen in 14% of United States isolates, 6.8% in Canada, 10.4% in Europe, and 17.8% in the Asia Pacific region [14]. Resistance in *Streptococcus pneumoniae* to the macrolides ranges from <3% to 77% depending on the area of the world (all antibiotic resistance is local and depends on the usage patterns of the antibiotic in the area) [15]. In Taiwan, where macrolides are over-the-counter drugs, resistance rates are the highest in the world: MRSA (80%), methicillin-sensitive *Staphylococcus aureus* (MSSA) (30%), *Streptococcus pneumoniae* (58%), and *Streptococcus pyogenes* (42%) [16]. In 1999 in a study of more than 10,000 bloodstream isolates in 49 United States hospitals, 29% of *Staphylococcus aureus* isolates were methicillin resistant as were 80% of the coagulase-negative staphylococci [17]. Currently 17% of all enterococci in United States hospitals are vancomycin resistant [18]. Resistance in the United States in *Helicobacter pylori* is 10.1% for clarithromycin, 1.4% for amoxicillin, and 36.9% for metronidazole [19] with rising tetracycline resistance due to a triple base pair substitution in the 16SrRNA gene [20].

Microbial resistance in the oral cavity

Increasing attention is being directed toward antibiotic resistance in pathogenic oral micro-organisms because viridans group streptococci (VGS) are a major cause of morbidity and mortality associated with bloodstream infections in neutropenic patients undergoing cancer chemotherapy or bone

marrow transplantation (BMT). Increasing amoxicillin resistance in VGS may reduce the efficacy of antibiotic prophylaxis for bacterial endocarditis, and multidrug resistance in many oral pathogens may result in treatment failures of acute orofacial infections.

In the 1970s, VGS and anaerobic streptococci were universally sensitive to the beta-lactam antibiotics with 90% to 99% also sensitive to erythromycin and clindamycin. In 1983, a high rate of penicillin resistance was detected in the oral flora of children in South Africa and an equally high resistance rate in *Streptococcus pneumoniae* heralding the transfer of resistance genes among these species [21]. In children with acute otitis media treated with antibiotics who had samples taken of their subgingival plaque, 60% of *Streptococcus sanguis* isolates were resistant to one antibiotic, 28% to two antibiotics with 32% resistant to amoxicillin, 24% to penicillin V, and 20% resistant to both penicillin V and amoxicillin [22]. Reports of 23% to 81% resistance rates of VGS to amoxicillin in both community- and hospital-acquired isolates are not uncommon. In a cohort of Japanese children at high risk for bacterial endocarditis, 31.7% of oral VGS had high level resistance with MICs between 4 and 16 μg/mL [23]. Children on long-term penicillin prophylaxis for the prevention of rheumatic fever may have penicillin resistance rates in VGS as high as 81%. In the United States, 40% to 50% of oropharyngeal VGS may be resistant to penicillin at MICs of $\geq$0.25 μg/mL [24]. A study of 43 United States medical centers in 1993 to 1994 of 352 VGS blood cultures found high level resistance to penicillin (MIC $\geq$4 μg/mL) in 13.4% and intermediate resistance ($\geq$ 0.25 to 2 μg/mL) in 42.9% [25].

Viridans group streptococcal bacteremias in neutropenic patients on cancer chemotherapy or receiving a BMT may result in serious morbidity and mortality. Approximately 18% to 21% of bacteremias experienced by these immunocompromised patients may be due to VGS with a 3.2% to 21% resistance rate to penicillin G or cephradine [26]. The VGS shock syndrome is manifested by rash, hypotension, palmar desquamation, adult respiratory distress syndrome, and eventual respiratory failure in up to 25% of adults and 13% to 21% of children after BMT [27]. The mortality rate may reach 60% to100% even with treatment.

Another serious concern is that oral streptococci resistant to the penicillins are much more likely to harbor resistance genes to other antibiotics (cephalosporins, macrolides, clindamycin, tetracyclines). Additionally the selection of oral penicillin–resistant micro-organisms by antibiotics results in the greater selection of multidrug-resistant pathogens, their clonal spread, and the fostering of the nasopharyngeal carriage of these multidrug-resistant pathogens with eventual resistance gene transfer to susceptible species [15,28,29].

Approximately 25 studies have detected beta-lactamase production in oral *Prevotella* and *Porphyromonas* species isolated from acute orofacial infections or periodontitis lesions. Most studies document a 25% or greater

median/mean prevalence of beta-lactamase in these organisms. At present they remain susceptible to beta-lactam/beta-lactamase inhibitors, metronidazole, and azithromycin. Beta-lactamase is also produced by oral *Veillonella*, *Fusobacterium*, *Capnocytophaga*, *Pseudomonas aeruginos*a, and *Bacteroides fragilis*. Antibiotic resistance in *Prevotella*, *Porphyromonas*, and *Fusobacterium* is promoted by both long and repeated antibiotic exposures [30,31].

Resistance to the macrolide antibiotics also is increasing in the oral cavity. In a study of 769 oropharyngeal microbial isolates resistant to the macrolides, 63% were VGS at MICs $\geq$2 μg/mL [32]. In 191 blood isolates of VGS collected across Canada, 36% were resistant to penicillin, 42% to erythromycin, 10% to clindamycin, and 8% to ciprofloxacin [33]. In a survey of antibiotic sensitivities in VGS across Asia, Europe, Latin America, and North America, 31.4% were resistant to penicillin and 35.5% to erythromycin [34].

Resistance to the fluoroquinolones is increasing in VGS and it appears that the antibiotic resistance determinants to these agents can be transferred between VGS and *Streptococcus pneumoniae*. Methicillin-resistant *Staphylococcus aureus* may be present in the mouths of children for longer than five years [35]. In a study of gingival crevicular fluid micro-organisms from periodontitis patients and their resistance to seven antibiotics at two different time periods (1980–1985 and 1991–1993), the resistance rate increased by 172% for tetracycline, 193% for doxycycline, 133% for penicillin G, 238% for amoxicillin, 116% for erythromycin and 108% for clindamycin [36]. Acute orofacial infections appear less susceptible to routine antibiotics than in the past, placing even greater emphasis on timely and adequate incision and drainage. Amoxicillin resistance in VGS will merit serious consideration in the formulation of the new American Heart Association guidelines for endocarditis prevention.

Antibiotic mechanisms of action

Before determining the means by which microorganisms evade the growth-inhibiting or killing effects of antibiotics, it is useful to review how antibiotics work. Antimicrobials affect microorganism viability by five major processes: (1) inhibition of cell wall synthesis, (2) alteration in cell membrane permeability, (3) inhibition of ribosomal protein synthesis, (4) suppression of nucleic acid (DNA) synthesis, and (5) inhibition of folic acid synthesis. Beta-lactam and glycopeptide (vancomycin, teicoplanin) cell wall inhibitors suppress the formation of the rigid bacterial cell wall to prevent micro-organisms from maintaining their internal osmotic pressure and eventually resulting in their rupture. Polymyxin B and our natural cationic peptides disrupt cell membrane integrity with the cationic peptides literally putting holes in the membrane. Ribosomal proteins synthesis inhibitors

(macrolides, clindamycin, tetracyclines, streptogramins and oxazolidinediones) interrupt protein synthesis at various ribosomal receptor sites. Metronidazole and the fluorquinolones inhibit microbial DNA synthesis and the sulfonamides and trimethoprim block successive stages in the synthesis of folic acid necessary for the survival of certain bacteria.

Transposable antibiotic resistance

Although antibiotic resistance can be acquired by chromosomal mutations, this is relatively rare (one per 1 billion cell divisions). Rather the major mechanism for resistance is now the transfer of antibiotic-resistance genes between microorganisms and then the health and food policies that promote such transfer. Microorganisms possess three mechanisms for genetic variation: (1) local nucleotide changes in the genome, (2) rearrangement of genomic sequences, and (3) horizontal acquisition of DNA from other microorganisms [37]. They acquire new genetic information by transformation, transduction, and conjugation, using a number of transposable elements: bacteriophages, plasmids, transposons, and integrons [38].

During transformation, bacteria acquire "naked" DNA from their surrounding environment—usually from disintegrated or lysed bacteria and incorporate it into their own genome [39]. Such transformations are uncommon and require critical interaction microbial gene binding, uptake, and integration, yet at least 50 bacteria (including VGS and *Streptococcus pneumoniae*) are "competent" to acquire environmental genes from their fellow microbes, plants, yeasts, and animals [39]. Transduction is the movement of DNA from one bacterium to another via bacteriophages (bacterial viruses).

Conjugation is the self-transfer of genetic information via plasmids or transposons to other microorganisms commonly using a direct protoplasmic extension (a sex pilus) stimulated by various pheromones (small peptides). Mobile elements commonly require "site-specific" DNA combinations, but not all DNA segments need this specificity allowing for very broad DNA movement among microbial species [40]. Transposons are DNA segments that cannot self-replicate but can self-transfer between plasmids, bacteriophages and chromosomes [40]. Transposons are "tailor-made gene haulers" that can recruit as many genes as necessary for their purposes [40].

Plasmids may be conjugative (self-transmissible) or nonconjugative (unable to cause their own transfer) and may be narrow range (replicate in only one or a few hosts) or broad range (replicate in many hosts) [41]. Plasmids may also be constitutive (continuous formation in the bacterium) or inducible (formed only when stimulated/induced by a foreign chemical). Plasmids and transposons carry antibiotic resistance genes but also virulence genes or "pathogenicity islands" that contain all the components

necessary to damage the host directly or via host-induced inflammatory responses.

Microbial resistance is further enhanced by integrons: a genetic element that captures and disseminates genes via a site-specific integration of DNA (gene cassettes) that may mediate antibiotic resistance, virulence, and other biochemical functions [42,43]. Integrons possess three distinct genes encoding an integrase enzyme, a recombinant site, and a "promoter" element [42,43]. Integrons resemble the bundling of products with an operating system: integron-packaged resistance determinants available for widespread gene dissemination [41]. Each gene is a cassette, and generally up to five genes are present in one integron. Now super-integrons have been isolated in *Vibrio cholerae* that contain hundreds of gene cassettes that encode many more bacterial functions than resistance and virulence [44]. Integrons cannot self-transfer as they lack transposable genes but are commonly associated with transposons and conjugative plasmids.

It was once thought that the presence of resistance genes would pose such a "fitness" problem for bacteria (require too much energy) and that these genes would be quickly lost as soon as the microorganism was removed from the antibiotic. However, resistance genes can become so important to a number of bacterial functions that they may become a permanent member of the bacterial chromosome. Tetracycline efflux pumps can become necessary for bacterial survival by functioning in sodium-potassium ion exchange across the bacterial cell membrane [45]. The problem is further compounded when the resistance gene for a particular antibiotic becomes integrated into a multiple resistance gene array. Eliminating this antibiotic from the environment may do nothing to reduce resistance until all the resistance genes for all the antibiotics are removed.

The most certain way to foster the development of microbial resistance and to promote the expression and transfer of resistance genes is with "sub-therapeutic" doses of antibiotics that do not kill the microorganism or suppress its growth but rather allow it to perceive the chemical as a threat to its survival and react either by chromosomal mutation, the acquisition or transfer of resistance or virulence genes, or the induction (expression) of such genes [46,47]. We are dealing with living entities that will use any means available to ensure their survival as they have for more than 3.5 billion years.

Mechanisms of microbial resistance to antibiotics

Microorganisms have developed seven major mechanisms to evade the bactericidal or bacteriostatic actions of antimicrobial agents: (1) enzymatic inactivation, (2) modification/protection of the target (receptor) site, (3) limiting access to the target site (altering cell wall or membrane permeability), (4) active drug efflux from the cell, (5) failure to activate the antibiotic within the cell, (6) use of alternate growth requirements, and (7) overproduction of target sites (see following list) [12,46,48].

Enzymatic antibiotic inactivation
Beta-lactamases: beta-lactams (penicillins, cephalosporins)
Acetyltransferases: aminoglycosides, chloramphenicol, streptogramins

Modification/protection of target sites
Modified penicillin binding proteins: beta-lactams
Altered DNA gyrase and topoisomerase IV: fluoroquinolones
Altered RNA polymerase: rifampin
Methylation of an adenine of 23SrRNA: erythromycin, clindamycin, streptogramins
Alteration of 16SrRNA: tetracyclines
Altered tetrahydrofolate and dihydrofolate reductase: sulfonamides and trimethoprim
Substitution of terminal peptidoglycan alanine with lactate: vancomycin and teicoplanin

Limiting antibiotic access to microbial cell
Altered outer membrane porins/reduced membrane transport: most antibiotics

Active efflux
Antibiotic efflux proteins: tetracyclines, fluoroquinolnes

Failure to activate antibiotic
Deceased flavodoxin production: metronidazole

Development of alternate growth requirements
Production of auxotrophs: enterococci

Overproduction of target sites
Hyper-beta-lactamase production: enteric bacilli

Enzymatic inactivation of the antibiotic is a common mechanism of resistance often seen with the beta-lactamases inactivating the penicillins and cephalosporins, acetyltransferases altering chloramphenicol and aminoglycosides and to a minor extent enzymes that metabolize tetracyclines and the macrolides. The beta-lactam antibiotics are inactive once their beta-lactam ring is opened, and beta-lactamases hydrolyze these agents to form a linear molecule incapable of binding to their receptors (penicillin-binding proteins, PBPs) [49].

A single beta-lactamase enzyme is capable of hydrolyzing 1000 beta-lactam molecules per second, and if 10,000 enzymes are present per resistant bacterium then 10 million beta-lactam molecules can be hydrolyzed per second [50]. There are more than 340 beta-lactamases both chromosomally and plasmid mediated—some of which are of the "extended spectrum" variety that metabolize all the beta-lactam antibiotics except the carbapenems (imipenem) and cephamycins. Metallo-beta-lactamases have evolved to be capable of metabolizing imipenem. The most pressing problems with

beta-lactamases are their: widespread dissemination throughout the microbial ecology, ready ability to move genes between widely disparate bacteria, tendency to rapidly inhibit new antibiotic agents, and increasing resistance to beta-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam).

Altered target sites (receptors) also are a common resistance mechanism and include ribosomal point mutations for tetracyclines, macrolides, clindamycin, streptogramins, and oxazolidindiones, mutations in DNA gyrase and topoisomerase IV for the fluoroquinolones, and altered PBPs in staphylococci, VGS, and *Streptococcus pneumoniae*. Single point mutations in PBP2x or PBP2b account for the resistance of VGS and *Streptococcus pneumoniae* to the penicillins and likely developed in VGS possibly by transformation. The gene was then passed to *Streptococcus pneumoniae* in the oropharynx [51]. Staphylococci become resistant to methicillin and all other beta-lactamase–resistant penicillins via an altered PBP2 conferred through a *mec* gene, resulting a greatly diminished binding affinity for methicillin. Enterococci have become resistant to vancomycin by substituting a D-lactate for the terminal D-alanine in bacterial cell wall peptidoglycan, thereby reducing the affinity of vancomycin by 1000 fold [52]. Major resistance to the macrolide antibiotics is accomplished by target site modification via several *erm* (erythromycin methylase) genes that alter the A2058 region of the peptidyl transferase loop in domain V of 23SrRNA, thereby reducing the binding of the macrolides, lincosamides (clindamycin), and the streptogramins (MLS_B resistance) [15].

Most microorganisms have developed ways to alter their cell wall or membrane permeability either by deleting outer membrane pores or by closing these membrane channels. This mechanism usually confers only low level resistance but when coupled with other more efficient resistance mechanisms can add significantly to the defenses of the microorganism.

Multidrug antibiotic efflux pumps have been adapted by microorganisms from their original purpose (to expel waste products or toxins) to a very efficient means of antibiotic resistance [53]. More than 50 such efflux systems (multidrug efflux pumps, cytoplasmic membrane efflux proteins) have been described operating in many microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa*, enterococci, staphylococci, and streptococci. These efflux pumps are the major route to resistance against the tetracyclines and are highly regulated by repressors to prevent their accidental overproduction [54]. The tetracyclines themselves can derepress this system, leading to an overproduction of efflux proteins and resulting in increased microbial resistance to themselves and other antibiotic agents [55].

A few bacteria have developed a means by which the required activation of metronidazole within the cell does not occur. Enterococci can evade destruction by developing alternate growth requirements (auxotrophs), and sulfonamide resistance may result from the overproduction of para-aminobenzoic acid. Some enteric bacilli evade the beta-lactams by

hyperproducing beta-lactamases, and a novel resistance mechanism to erythromycin has been detected whereby the bacterial ribosome produces small peptides about the size of the erythromycin molecule that displace the drug from the ribosome [56]. Although not strictly a resistance mechanism, antibiotic tolerance is a means by which antibiotics no longer kill the microorganism but merely prevent its replication, rendering the antibiotic bacteriostatic instead of bactericidal. Tolerance usually is due to the loss of bacterial autolysin activity via the failure to create or mobilize autolysin enzymes [57].

Two chemicals (tetracycline and heavy metals) used in dentistry have received particular attention regarding their abilities to promote the expression and transfer of mobile antibiotic resistance genes. Tetracycline downregulates a repressor gene controlling drug efflux mechanisms with only nanomolar amounts of the antibiotic required for this purpose [58]. Tetracyclines promote gene transfer by increasing the frequency of bacterial conjugation, and colonic *Escherichia coli* may only express resistance genes to tetracycline and other antibiotics when the drug is present [12].

Dental amalgam mercury may promote microbial resistance in the oral cavity and colon [59], but this appears to be a transient effect for a short period after the restoration is placed [60]. Several clinical studies have not detected a difference in levels of mercury-resistant microorganisms in the oral cavity or colon or any increased resistance to tetracycline, chlorhexidine, cefuroxime, or penicillin [61–63].

As a note of optimism regarding microbial antibiotic resistance, the role of minimal inhibitory concentrations (MICs) should be addressed as well as the ongoing attempts to develop new approaches to attacking multi–antibiotic-resistant micro-organisms. The MIC is the lowest concentration of the antibiotic that prevents the growth of microorganisms after an 18- to 24-hour incubation period and serves as a surrogate marker in blood for the assumed drug concentration in the infected tissues unable to be measured. Antibiotic resistance is defined as a rise in the microorganism MIC or reduced clinical efficacy of the antibiotic even to outright clinical failure of the antibiotic. The MIC breakpoint for resistance is the concentration of the antibiotic above which the organism is unaffected by the antibiotic. This breakpoint must be compatible with the blood levels of the antibiotic that can be reasonably attained with commonly used clinical doses. Many studies do not adequately state the breakpoint used to define resistance, whereas others use a breakpoint so high that no resistance is possible under their definition. Choosing a resistance breakpoint of 0.04 μg/mL when 2.0 μg can be obtained without difficulty can vastly overstate microbial resistance just as choosing 16 μg/mL for resistance when only 4 μg/mL can be obtained in the blood will greatly underestimate the magnitude of resistance.

The current practice of formulating a ''new'' antibiotic that is merely a modification of an existing drug and which confers only a slightly better clinical profile will not solve our problems of microbial resistance. New

approaches must be developed attacking unique vulnerabilities in the microbial armor. Efforts are underway to: inhibit microbial enzymes specific to only a single microbial species, use bacteriophages as anti-infective agents, explore the use of our own natural defense mechanisms (cationic antimicrobial peptides of our skin and mucosa) as therapeutic agents, inhibit enzymes that control bacterial membrane lipopolysaccharide synthesis, use antisense RNA inhibition, sequester the iron necessary for microbial survival, sequence the bacterial genome to better detect points of attack, develop more narrow spectrum antibiotics that will be less likely to disturb the microbial ecology and interfere with microbial "quorum sensing" (the ability of microorganisms to "talk" to each other) so that bacteria misread signals for the expression of resistance, virulence, attachment to surfaces, and uninhibited growth [64–67].

Another approach is the "recycling" of antibiotics that have not been widely used in a particular environment as now typified by the tetracyclines. Microbial resistance to the tetracyclines is commonly thought to be widespread, inducible, transposable and often permanent. Transposable elements with only a tetracycline resistance gene are rare, and resistance to the drugs is most often combined with the resistance genes for several other antibiotics in a transposable element. This is the normal course of events. Such a scenario may not be entirely true as the prevalence of tetracycline-resistant microorganisms in hospitals and even communities can be remarkably limited and is associated with extremely low MICs for these organisms: 0.04 to 0.25 μg/mL [12] versus the 0.79 μg/mL that can be attained with "sub-therapeutic" doses of these agents [68]. Tetracyclines have been successfully used in the treatment of life-threatening VRE and MRSA nosocomial infections [12] and are now becoming a therapy of choice for *Helicobacter pylori* infections, pneumonia due to *Streptococcus pneumoniae* and other respiratory tract pathogens, and as chemoprophylaxis of highly drug-resistant malaria.

Microbes will leave us alone if we leave them alone and stop forcing them to invent new ways to survive (after all, they have had 3.5 billion years of practice). This can be accomplished by reducing our use of antimicrobials to the level where they are necessary for our survival and not merely for doctor and patient comfort.

Dentistry and antibiotic resistance

Dentists prescribe between 7% and 11% of all common antibiotics (beta-lactams, macrolides, tetracyclines, clindamycin, metronidazole), and abuse can be substantial [69]. Inappropriate antibiotic use in dentistry includes: (1) postsurgical "prevention" of an infection not likely to occur and not demonstrated clinically to respond to "after the fact" prophylaxis, (2) use in endodontics as "analgesics," (3) failure to adhere to principles established for use of prophylactic antibiotics, (4) overuse to prevent metastatic "focal"

infections, (5) treatment of chronic adult periodontitis, which is almost totally amenable to mechanical therapy, (6) using antibiotics instead of mechanical periodontal therapy, (7) chronic long-term antibiotic therapy for periodontal disease, (8) antibiotic therapy instead of appropriate incision and drainage, (9) use of antibiotics to prevent negligence claims, and (10) antibiotics used in inappropriate situations, dosages, and durations of therapy [12].

In England between 33% and 87% of various antibiotics were judged to have been prescribed inappropriately according to the recommendations of the Dental Practitioners Formulary [70]. In a survey of general Canadian dentists, 17.5% did not use the 1997 American Heart Association (AHA) guidelines for the prevention of bacterial endocarditis, 66% used antibiotic prophylaxis for patients with a history of rheumatic fever without rheumatic heart disease, 25% for patients with HIV/AIDS, 70% for patients with prosthetic joints, and 66% for restorative dentistry not associated with significant bleeding in endocarditis-risk patients even though this is not recommended by the AHA [71]. Twenty percent of specialists did not use the AHA guidelines for patients with cardiac valve prostheses [71]. In a survey of members of the American Academy of Endodontists, 12.5% used antibiotics as analgesics for postoperative pain, 37.3% as antibiotic "prophylaxis" after surgery, 44.8% after incision and drainage in patients without systemic involvement, and between 12% and 59% for various endodontic procedures where antibiotics are not effective [72].

Antibiotics for the management of acute orofacial infection are often used in too low a dosage and for too long a time. Both these practices encourage the development and expression of microbial resistance [12,46,73]. The principle of antibiotic dosing is still the same as that stated by Paul Ehrlich in 1913: "Hit hard and hit fast." With an acute infection it is imperative that if possible the area be surgically drained to decrease the inoculum size and remove tissue barriers to antibiotic penetration. A loading dose (two to four times the maintenance dose) is advisable to rapidly attain therapeutic blood levels, and the dosing intervals should be appropriate to attain relatively constant antibiotic blood levels. Generally, antibiotics used in dentistry are most effective when the organism is consistently exposed to the agent (time-dependent rather than concentration-dependent activity) [73].

The old adage that antibiotics should be used for a certain number of required days to "kill the resistant strains" is an oxymoron, because resistant bacteria are by definition unresponsive to the antibiotic. Although some bacteria may occasionally mutate chromosomally in a "stepwise" fashion over several generations, the vast majority acquire their resistance via transposable elements that are preferentially transferred when antibiotics are used in sub-therapeutic doses or for long durations [12,46,73].

Another difficulty with antibiotic dosing leading to increased microbial resistance is the common admonition to "finish the course of antibiotics." In

certain situations (fungal, respiratory, urinary tract infections) where "rebound infections" are common (recur once the antibiotic is terminated), such an approach is appropriate. However in many cases this advice is based on an erroneous assumption that the practitioner knows beforehand precisely how long the infection will last. Given the many variables associated with any infection [73], such advice is likely to lead to a longer duration of antibiotic therapy than necessary. Antibiotics should be used aggressively and for as short a period as is compatible with patient remission of disease [73]. The ideal antibiotic duration is the shortest time that will prevent both clinical and microbiologic relapse. The only practical guide to the effectiveness of antibiotic therapy and hence the duration of therapy is clinical improvement of the patient as determined by remission of the disease [73–75]. As a practical matter, the dentist should prescribe a reasonable amount of antibiotic (3 to 5 days) with the initial loading dose given in the office if the patient is present. The dentist can then reevaluate the patient's clinical course and progress periodically at which time additional antibiotic can be advised if indicated.

Finally, antibiotics are "societal drugs" that affect microbial resistance not only in the person taking the drug but also everyone else, because resistance genes are easily passed via personal contact, fomites, and human and animal refuse [46]. Any health practitioner must appreciate that misuse of antibiotics likely occurs millions of times daily and that the cumulative toll of these abuses is precisely what has gotten us into our present predicament. With antibiotics, no person is an island.

Summary

Through billions of years of evolution, microbes have developed myriad defense mechanisms designed to ensure their survival. This protection is readily transferred to their fellow life forms via transposable elements. Despite very early warnings, humans have chosen to abuse the gift of antibiotics and have created a situation where all microorganisms are resistant to some antibiotics and some microorganisms are resistant to all antibiotics.

When antibiotics are used, six events may occur with only one being beneficial: when the antibiotic aids the host defenses to gain control and eliminate the infection. Alternatively, the antibiotic may cause toxicity or allergy, initiate a superinfection with resistant bacteria, promote microbial chromosomal mutations to resistance, encourage resistance gene transfer to susceptible species, or promote the expression of dormant resistance genes.

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