Chapter

Antimicrobial Agents: Antibacterial Agents, Anti-biofilm Agents, Antibacterial Natural Compounds, and Antibacterial Chemicals

Yaw Duah Boakye, Newman Osafo, Cynthia Amaning Danquah, Francis Adu and Christian Agyare

Abstract

The surge in antimicrobial resistance coupled with the decline in the antimicrobial drug pipeline calls for the discovery and development of new agents to tackle antibiotic resistance and prevent a return to a post-antibiotic era. Several factors account for resistance of microbes; some are natural and others are acquired. Natural selection, presence of efflux pumps, impermeable cell wall, biofilm formation and quorum sensing are some of the factors. Though it is difficult to outwit the pathogens, the discovery and development of compounds with pleiotropic modes or mechanisms of action different from the conventional drugs currently being used can help us tackle antimicrobial resistance. Natural products have been known to be a rich source of bioactive compounds with diverse structures and functional group chirality. Various reports indicate medicinal plants with antibacterial, anti-biofilm, efflux pump inhibition, wound healing effects or properties and others used for upper respiratory and urinary tract infections. There is an urgent need to research into natural products particularly plants for antimicrobial agents including antibacterial agents, anti-biofilm agents, antibacterial natural compounds and antibacterial chemicals. This chapter throws more light on such antimicrobials.

Keywords: antimicrobials, biofilm inhibitors, natural products, antibacterials

1. Antibacterial agents

1.1 Introduction

The last decade has seen in a dramatic fashion, an accelerated microbiological evolution and resistance to antimicrobial agents. There is therefore the need to optimize appropriate stewardship of infection control in the light of an apparent

stagnation in the development of novel antimicrobial agents. This chapter therefore considers current anti-infective agents of the various classes that are clinically used in treating infections.

1.2 Beta lactam derivatives

The beta lactam derivatives stand as the oldest class of antibiotics used. The beta lactam ring has proven to be the major weapon in the fight against bacterial infections. Several novel molecules modeled after Alexander Fleming's penicillin and its derivatives have been developed. These drug moieties share the common characteristic of the beta lactam ring being an integral part of the structural make up and its effectiveness. These include the cephalosporins, monobactams, cephamycins, and the carbapenems (imipenem and meropenem). Beta lactams are indicated for a varied number of bacterial infections ranging from respiratory and urinary tract infections, ear and eye infections and gonorrhea to more life-threatening conditions like meningitis, septicemia and pneumonia. It is also widely adopted for prophylactic use in bacterial endocarditis, surgical site infections and in immuno-compromised situations [1].

Beta lactam antibiotics are bactericidal in their action. They inhibit the building of bacterial cell wall by interfering with the synthesis of peptidoglycan. Penicillin binding proteins which are bacterial enzymes which are essentially for bacterial cell wall synthesis are usually the targets of beta lactams [1]. Beta lactam antibiotics are generally available for parenteral administration with some also showing good absorption from the gastrointestinal tract. In patients with intact renal function, most beta lactams have a serum half-life of 1–2 h. Ceftazidime and temocillin break off this usual norm with a half-life of 4–6 h and an even higher half-life of 8–10 h for ceftriaxone. Penicillins and cephalosporins are eliminated primarily through glomerular filtration with varying levels of active transport across the renal tubules as well as the hepatobiliary system [2].

Owing to the general abuse of antibiotics, the beta lactams have suffered the challenge of the development of resistance in target pathogenic organisms. The production of beta lactamases has been a major determinant in the resistance observed especially in Gram-negative pathogens. Alterations in the beta-lactam targets, the penicillin binding proteins, are also important in Gram-positive pathogens. Efflux mechanisms and/or exclusion of these agents also contribute more often in conjunction with the other two mechanisms [3]. New agents of the beta-lactam group that have been approved following development have come from the cephalosporin class.

1.3 Cephalosporins

Cephalosporins are usually classified based on spectrum, generation, chemical structure, clinical pharmacology and resistance to beta-lactamases. The first cephalosporins were assigned first-generation cephalosporins; later, more expanded spectrum cephalosporins were designated as second-generation cephalosporins. Each more current generation has altogether more prominent activity against Gram-negative bacteria than the preceding generation and much of the time with diminished action against Gram-positive bacteria. Fourth-generation cephalosporins, however, have true broad-spectrum activity. The recent addition to the block, the fifth generation cephalosporins has become very crucial due to its activity against multidrug-resistant *Staphylococcus aureus* (MRSA) [4].

The drug of choice in this latest generation is ceftaroline. It is the only beta-lactam with MRSA activity. Ceftaroline, which is available as a pro-drug, ceftaroline prosamil is again unique for its expanded and extensive Gram-positive activity beyond all presently available cephalosporins. Ceftaroline active against the

Gram-positive organisms (*Streptococcus pneumoniae*, *Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram-negative species (*Haemophilus influenzae* and *Moraxella catarrhalis*), including resistant phenotypes. Data for its approval proved its effectiveness in the treatment of acute bacterial skin and skin structure infections and community acquired bacterial pneumonia [5]. Ceftaroline demonstrates dose-proportional pharmacokinetics similar to other renally excreted cephalosporins after intravenous administration. It is half-life after dose is 2.53 h with protein binding of approximately 20%. Ceftaroline also showed in clinical trials to have positive attributes with regards to antibacterial stewardship by having a low potential for development of resistance as well as favorable tolerability and safety profile [5].

Ceftolozane is a novel beta-lactam cephalosporin combined with beta-lactamase inhibitor, tazobactam for the management of complicated urinary tract and intra-abdominal infections. The peculiar chemistry and dosing accounts for its extensive coverage of Gram-negative organisms including multidrug-resistant *Pseudomonas aeruginosa* as well as extended-spectrum beta-lactamase producing organisms and some anaerobes. Its efficacy can be compared to levofloxacin in patients with complicated urinary tract infections, including pyelonephritis, and comparable to that of meropenem against complicated intra-abdominal infections. Ceftolozane-tazobactam has shown to be indispensable due to the lack of susceptibility to the usual mechanisms of resistance mostly by Gram-negative organisms such as the production of beta-lactamases, efflux pumps, alterations in penicillin binding proteins as well as porin loss [6].

1.4 Glycopeptides

Glycopeptide antibiotics are complex and rigid molecules that repress a late stage in bacterial cell wall peptidoglycan synthesis. The selective toxicity of glycopeptides is attributable to the fact that its 3D structure harbors a cleft into which peptides of a specific configuration found only in bacterial cell walls can fit. Glycopeptide has assumed a special role in the face of the general threat of antimicrobial activity resistance since its unique mechanism involving the attachment of a bulky inhibitor to a substrate with the goal that the active sites enzymes are unable to align themselves correctly, therefore renders resistance to glycopeptides more difficult to achieve than other antimicrobial agents [7].

A good number of glycopeptides have gone through development in recent years and have been approved for clinical use. Oritavancin, a lipoglycopeptide obtained from the naturally occurring chloroeremomycin of the eremomycin class of glycopeptides is very similar to vancomycin but possess two 4-epi-vancosamine monosaccharides, one supplanting vancosamine and the other connected to ring-6 via an amino acid residue. Based on the features of its pharmacophore and its stereochemistry, it has enhanced antimicrobial against Gram-positive organisms including those possessing both VanA- and VanB-mediated vancomycin-resistance [8]. Oritavancin is transcendently cleared by means of the reticuloendothelial system, accumulating most notably in macrophages of the liver (Kupffer cells), kidney, spleen and lungs, as well as in the intestinal mucosa, thymus, and lymph nodes. Subsequent release and elimination from these tissues does not occur readily and thus only trace amounts are recouped from urine and feces. Dosage adjustments are not required in hepatic and renal insufficiency [9].

Telavancin also derivative of vancomycin and a lipoglycopeptide has been shown to have a dual mechanism of action by causing an inhibition of the peptidoglycan synthesis and through membrane depolarization. Telavancin is reliably active against *Staphylococcus aureus*, including MRSA, vancomycin-intermediate-resistant *Staphylococcus aureus*, linezolid-resistant *Staphylococcus aureus*, and daptomycin-susceptible strains and therefore effective for the treatment of complicated skin and skin-structure infections. It has additionally proven effective in the treatment of

Gram-positive bacterial infections especially pneumonia. Its non-inferiority is compared with vancomycin, in the treatment of complicated skin and skin-structure infections and pneumonia. Telavancin is excreted by the kidneys, and thus, dosage adjustments are required in cases of renal failure. Telavancin is related with higher rates of renal events, changed taste, nausea and vomiting but however lesser rates of pruritus and infusion related events relative to vancomycin [10].

Dalbavancin has proven to be a valuable addition to the armamentarium of antimicrobial agents as it is the first once a week antibiotic with activity against a broad range of Gram-positive pathogens. Dalbavancin's uniqueness is its novel pharmacokinetic profile with a half-life of 170–210 h, which makes the once-weekly dosing optimal. Forty percent is eliminated via the renal route. Most of the drug is excreted as intact drug. Concentration was unchanged in patients with mild renal impairment. No adjustments are needed in hepatic insufficiency, as concentrations of the drug do not increase with severe hepatic impairment. It is still unknown if the drug penetrates the cerebrospinal fluid, or whether the drug is removed during hemodialysis. However, the high protein binding of dalbavancin would suggest both of these scenarios to be unlikely [11].

1.5 Oxazolidinones

Oxazolidinones are synthetic antimicrobial agent which inhibit bacterial protein synthesis. Linezolid, the first oxazolidinone to be approved for clinical use, has bacteriostatic activity against many important resistant pathogens including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and penicillin-resistant *Streptococcus pneumoniae* as seen from *in vitro* studies [12]. Tedizolid which is available as a prodrug (tedizolid phosphate) is the first in the class to be dosed once daily as it has a half-life of 12 h. It also has a profound oral bioavailability of about 90% and no dosage adjustment is required between intravenous and oral administration, nor is dosage adjustment needed based on hepatic or renal impairment. Its activity covers Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus.* It is indicated for the management of acute bacterial skin and skin structures infections. Tedizolid appears to depart from linezolid in the incidence of gastrointestinal and hematologic side effects and the apparent lack of drug interaction with selective serotonin reuptake inhibitors [13].

1.6 Fidaxomicin (macrocyclic antibiotics)

Fidaxomicin is the first in the new class of narrow spectrum macrocyclic antibiotics. It is derived from the organism *Dactylosporangium aurantiacum* as a fermentation product. Fidaxomicin's action leads to an inhibition transcription process by interfering with RNA polymerase. Fidaxomicin acts at a distinct site and step of RNA synthesis to that of the rifamycins and streptolydigin, and thus no overlapping antibiotic resistance has been identified. Its narrow spectrum of activity is against most Clostridial spp., including *Clostridium difficile*, and exhibits moderate activity against other Gram-positive organisms, such as staphylococci and enterococci. Systemic absorption is very limited with serum concentrations remaining generally low [14].

1.7 Aminoglycosides

Aminoglycosides are exceptionally potent, broad-spectrum antimicrobial agents with numerous alluring properties for the treatment of hazardous diseases. The antibacterial potency of the aminoglycosides is attributable to one or several

aminated sugars linked by glycosidic bonds to a dibasic cyclitol in their chemical structures. Aminoglycosides act primarily by impairing bacterial protein synthesis through binding to prokaryotic ribosomes. Widespread resistance against these agents including semi-synthetic additions such as amikacin and netilmicin has prompted the need for development of alternative agents. The three main resistance mechanisms identified include a decreased cell permeability; alterations at the ribosomal binding sites; and production of aminoglycoside modifying enzymes [12]. Plazomicin is a next-generation semisynthetic aminoglycoside derived from sisomicin, a naturally occurring aminoglycoside antibiotic. Plazomicin is active against Gram-negative and selected Gram-positive bacteria and it is active against enterobacteriaceae, multidrug-resistant enterobacteriaceae (MDR-EC), aminoglycoside-resistant enterobacteriaceae (AR-EC), carbapenem-resistant Enterobacteriaceae (CR-EC), colistin-resistant enterobacteriaceae (CRE), tigecycline-resistant enterobacteriaceae (TR-EC). It has no nephrotoxic and ototoxic effects that characterize agents in this class. Its superior pharmacokinetic profile also supports a more convenient once daily IV dosing [15].

1.8 Pleuromutilins

Pleuromutilins are antimicrobial agents that selectively restrain bacterial translation and derivatives of the naturally occurring tricyclic diterpenoid pleuromutilin. The interest in pleuromutilins has resurged following the resistance in more prominent classes like the beta-lactams. Pleuromutilins inhibit bacterial protein synthesis by binding to the central part of domain V of the 50S ribosomal subunit at the peptidyl transferase center ultimately affecting peptide bond formation [16]. Retapamulin used as a topical agent for the treatment of impertigo has bacteriostatic effect against *Staphylococcus aureus* and *Streptococcus pyogenes*. Retapamulin is well tolerated with the most commonly reported adverse effect being pruritus at the application site. Although comparative efficacy has not been established with mupirocin, it is an effective alternative with dosing advantage of twice a day application.

1.9 Tetracyclines

The tetracyclines are another old class of natural product antibiotics. Until the development of doxycycline, they were used topically. However subsequent systemic application allowed for its use in the treatment in respiratory tract infections. Tetracyclines also inhibit protein synthesis by inhibiting acyl-tRNA transfer on the bacterial 30S ribosome. Tetracyclines have a fused linear tetracyclic structure and form chelation complexes with divalent cations such as calcium and thus its use has been limited to adults albeit with side effects. They usually serve as alternative to patients who are intolerant of macrolides or macrolide-resistant pathogens [12].

Tigecycline is the foremost drug in the new glycycline subclass of antibiotics. In spite of the fact that it is structurally identifies with minocycline, modifications to the molecule has brought about an expanded spectrum of activity and decreased susceptibility to the development of resistance when compared with other tetracycline antibiotics. Tigecycline has a broad spectrum of activity, including activity against drug-resistant Gram-positive organisms [17].

1.10 Macrolides

Macrolides which have erythromycin as its first member inhibit protein synthesis by binding to the 23S RNA of the bacterial 50S ribosomal subunit at the exit of the peptide synthesis tunnel. Macrolides are most widely adopted for respiratory

tract infections as they have targeted activity against respiratory pathogens. Macrolide antibiotics achieve high tissue and intracellular concentrations, which helps to address bacteria that are intracellular. In addition, they have strong anti-inflammatory properties [12]. Telithromycin belongs to a class of drugs described as the ketolides which varies slightly from the existing class of the macrolides. The characteristic feature of the ketolides as opposed to other macrolides is the removal of the neutral sugar, L-cladinose from the three position of the macrolide ring and the subsequent oxidation of the 3-hydroxyl to a 3-keto functional group. Telithromycin is indicated for the treatment of upper respiratory tract infections such as community acquired pneumonia and sinusitis. Introduction of telithromycin was opportune due to the rise of microbial resistance in the existing macrolides as it appears to be effective against macrolide-resistant bacteria such as macrolide-resistant *Streptococcus pneumoniae* [18].

1.11 Quinolones

The quinolones although not obtained from microbial source, are derived indirectly from natural products, i.e., as a by-product of chloroquine synthesis, which is in itself an analogue of the plant alkaloid quinine. It has established itself as useful in the treatment of urinary tract infections. The introduction of fluorine group to the core structure, give rise to the fluoroquinolones like ciprofloxacin and moxifloxacin yielded products of an improved spectrum and better pharmacokinetic profiles. However, resistance to the class of antibiotics whose mechanism of action is to inhibit bacterial DNA gyrase and topoisomerase IV and even newer fluoroquinolones has prompted the need for the development of new and effective agents [12]. Delafloxacin is a new anionic fluoroquinolone used for the treatment of acute bacterial skin and skin structure infections caused by Gram-positive and Gram-negative organisms including MRSA and *Pseudomonas aeruginosa*. As with all fluoroquinolones, resistance is mediated through mutations in the target enzymes and drug efflux. However, delafloxacin has greater stability against target enzyme mutations in Gram-positive bacteria relative to other fluoroquinolones. Its availability in infusion and oral formulations, stability and enhanced antibacterial potency in acidic environments and overall tolerability gives a potentially better antimicrobial agent in the treatment of other infections [19].

2. Anti-biofilm agents

2.1 Biofilms

Microorganisms have a strong tendency to become associated with surfaces [20]. Bacteria, thus, live in communities, adhering to surfaces of implanted medical devices or damaged tissues. On these surfaces they encase themselves in a hydrated matrix of polysaccharide and protein forming a slimy layer known as biofilms [21]. Biofilm is a microbial culture which is identified as cells permanently bound to an interface or to other cells and are firmly attached to matrix consisting of polymers produced as a result of phenotypic alteration due to growth rate or transcription of genes. Bacteria tend to form biofilms in environments with rapid flow of matter. Planktonic bacteria can adhere to surfaces and initiate biofilm formation in the presence of shear forces that are higher than those of heart valves and exceed Reynolds numbers of 5000. The Reynolds number has no dimension and describes the turbulent flow of a liquid. If it is high, turbulent flow exists but if it is low then laminar flow conditions prevail. It is speculated that turbulent flow enhances bacterial adhesion to surfaces and biofilm formation by impinging the planktonic cells on the surface [22].

Biofilms do not form only between same species of microorganism but there can be inter-species adhesions. Interspecies binding outside the oral cavity have been described as well, most notably between pathogenic and commensal microorganisms of the urinary tract. Co-aggregation between aquatic bacteria has been reported, with *Micrococcus luteus* being mentioned as a bridging organism in the development of aquatic biofilms owing to its ability to co-aggregate with many aquatic heterotrophs [20, 23].

2.2 Bacterial biofilm formation

Genes are responsible for noted biochemistry of living things and biofilm production is no exception. Changes in microbial colony formation and organization may be due to mutations in one or more of certain genes. Mutations in a gene called *wspF*, which is part of a putative chemosensory signal-transduction operon result in cell aggregation and altered colony morphology. The phenotypic characteristics of *WspF* depend on the presence of *WspR*, which is a member of a family of signal transduction proteins known as response regulators. *WspR* contains a glycine-glycine-aspartic acid-glutamic acid-phenylalanine (GGDEF) domain known to catalyze formation of a cytoplasmic signaling molecule cyclic diguanylate (c-di-GMP). Mass sequencing of genomes in bacteria detected the highly abundant protein domains GGDEF and Glutamic acid-Alanine-Leucine (EAl) [24]. These two protein domains are involved in the turnover of c-diGMP *in vivo*.

The GGDEF domain stimulates c-diGMP production whereas EAL stimulates its degradation. Increased cellular levels of c-diGMP has been observed to correspond to increased biofilm formation in a *wspF* mutant while increased levels of EAL catalyze degradation of c-diGMP and reversed the phenotypes of a *WspF* mutant and inhibited biofilm initiation by wild-type cells, indicating that the presence of c-diGMP is necessary for biofilm formation. The *psl* and *pel* operons, which are involved in exopolysaccharide production and biofilm formation, were expressed at high levels in a *WspF* mutant [25].

2.3 Stages of biofilm formation

The process of biofilm formation is complex, but generally identified as consisting of five stages.

2.3.1 The conditioning film

The conditioning layer is the foundation on which a biofilm grows, and can be composed of many particles, organic or inorganic. Via gravitational pull or direction of flow of the bulk fluid, particles rest and become integral component of conditional layer. Hence, the conditioning layer which is basically made up of organic matter provides anchorage and nutrients for bacterial growth [26].

2.3.2 Reversible adhesion

When plankton bacteria come into close proximity with the conditioning layer, it attaches using bacteria appendages such as flagella, fimbriae, and pili. A fraction of the cells reaching the surface reversibly adheres. However, the ability of the plankton to adhere is dependent on factors such as available energy, bacteria orientation, temperature and pressure in the immediate environment. If repulsive forces are greater than the attractive forces, the bacteria will detach from the surface [26].

2.3.3 Irreversible adhesion

A number of the reversibly adsorbed cells remain immobilized and become irreversibly adsorbed. It has been reported that the physical appendages of bacteria overcome the physical repulsive forces. Henceforth, the attachments do come in close proximity with the bulk lattice of the conditioning layer culminating in oxidation and hydration which strengthen the bacteria–surface bond. There is a data supporting the assertion that microbial adhesion intensely relies on the hydrophobic–hydrophilic nature of interacting surfaces [26].

2.3.4 Cell growth

With the adhered cells undergoing binary division, the resulting clonally expanded cells spread from the point of adhesion leading to clusters formation. Ideally, such growth occurring in the biofilm yields a mushroom-like arrangement. Nutrient supply to the bacteria present within the biofilm is made possible through the utilization of this mushroom-like assemblage. As the cells increase, they produce polysaccharide intercellular adhesion polymers which facilitate stronger attraction between adjacent cells [26, 27].

2.3.5 Final stages of biofilm development

As the bioburden increases, organisms enter into the stationary phase of growth cycle where the rate of cell division equals the rate of cell death. At this level of high population density, the bacteria cells interact with each other via signaling mechanisms called quorum sensing, a good explanation for the role of auto inducers in the stimulation of the mechanical and enzymatic procedures through genetic expression.

Death phase entails the collapse of the formed biofilm. The bacteria culture produce enzymes which catalyze the catabolism of the structural polysaccharides within the biofilm leading to exposure of the bacteria within the matrix to colonize new substrates [26, 28].

2.4 Antibiotic resistance due to biofilm formation

Inside biofilms, organisms resist antibiotic action by multicellular strategies, rather than the known genetic processes that involve plasmids, transposons and mutations that make individual cells resistant [29]. Biofilms are the root cause of many persistent chronic infections due to bacteria. Several organisms have been known to produce biofilms. In *P. aeruginosa*, it has been shown that the gene (*alg*C) that controls phosphomannomutase involved in alginate (exopolysaccharide) synthesis is up-regulated within minutes of adhesion to a solid surface. It has also been shown that *alg*D, *alg*U, *rpo*S and the genes controlling polyphosphokinase synthesis are all up-regulated during biofilm formation [22]. Multi-system that includes poor antibiotic penetration, nutrient limitation and slow growth, adaptive stress responses, and formation of persister cells are hypothesized to constitute the organisms' resistance to antibiotics in biofilms [29]. Organisms that have shown resistance to antibiotics as a result of biofilm production include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Pseudomonas pseudomallei*, and *Streptococcus sanguis* [22].

Susceptibility tests with *in vitro* biofilm models have shown the survival of bacterial biofilms after treatment with antibiotics at concentrations hundreds or even a thousand times the minimum inhibitory concentration of the bacteria measured in a suspension culture indicates a state of a high resistance to antibiotics [30]. Antibiotics used for the treatment of such bacterial infections suppress symptoms

of infections by killing free-floating bacteria shed from the attached population, but fail to eradicate those bacterial cells still embedded in the biofilm. The biofilm, thus, offers protection to the organisms from the effects of the antibiotics. After the treatment course of the infection, the biofilm can act as a microbial repository for recurrence of infection. Biofilm infections can linger on for months, years, or even a lifetime as long as the colonized surface is not removed from the body [29].

In biofilms, the familiar mechanisms of antibiotic resistance such as efflux pumps, modifying enzymes, and target mutations do not account for the resistance of the organism and protection of bacteria in a biofilm. Even sensitive bacteria that do not have a known genetic basis for resistance can have profoundly reduced susceptibility when they form a biofilm. The fact that all these antibiotic resistance mechanisms are inherently multicellular helps to explain why bacteria dispersed from biofilms rapidly revert to a susceptible phenotype [31]. Although they are less common, fungal biofilms are also found on implanted medical devices. *Candida albicans* biofilms are drastically very resistant to most antifungal drugs, and are a major cause of morbidity in blood-stream infections [32, 33].

2.5 Other effects of biofilms

Biofilms can be both beneficial and detrimental. They are beneficial in the degradation of environmental hazardous substances in the soil, in a bioreactor and as bio-flocculants in the separation of coal particles from associated mineral matter. They are detrimental on food and slaughterhouse equipment, ship hulls, biomaterials implants, and in the oral cavity [20]. They are implicated in otitis media [34], otolaryngologic infections [35], osteomyelitis [36], bacterial endocarditis [37], cystic fibrosis and nonhealing wounds [38].

When organisms are in biofilms, they tend to cause infections with similar features although there could be significant variations in the causative organisms. Importantly, bacterial biofilms tend to bypass host defense mechanism and can withstand drug treatment irrespective of the competence of the individual's immune system. Actually, tissues adjacent to the biofilm might undergo collateral damage by immune complexes and invade the neutrophils [29]. Biofilms have also been documented as the major sources of infection by *Candida albicans*, specifically in view of the vast number of biomaterials that are now being used in the medical industry. Biomaterials serve as ideal substrates for microbial adhesion and eventual biofilm formation. Such materials include stents, catheters, and orthopedic joints [39].

2.6 Biofilm formation inhibitors

Prevention of biofilm formation or countering the resistance mechanisms due to biofilms may simplify the treatment of infections caused by biofilm producing organisms and bring back the usefulness of antibiotics that are out of use due to biofilm resistance [29]. Many substances, both natural and synthetic, have been found to inhibit biofilm formation. For example, silver nanoparticles (AgNPs) effectively prevent the formation of biofilms and kill bacteria in established biofilms produced by clinical strains including *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococcus mutans*. Anti-biofilm formation activity of AgNPs was more pronounced on Gram-negative than Gram-positive bacteria although both groups exhibit equal antibacterial activity to the substance. AgNPs also inhibit biofilm production by *Candida albicans* [40]. The helical human cathelicidin LL-37 exhibits effective antimicrobial, anti-attachment, and anti-biofilm activity against *Staphylococcus aureus* [41]. Some plants have also been found to inhibit biofilm formation. Extracts of the sticks of *Salvadora persica*

has been reported for its antibacterial and anti-biofilm activities against *Streptococcus mutans* [42]. Essential oils of lemon grass have biofilm inhibition activity against *S. aureus* [43] while the essential oils of Boswellia spp. (*B. papyrifera* and *B. rivae*) are active against staphylococcal and *C. albicans* biofilms [44].

2.7 Detection of biofilms

Various methods have been used to determine the presence of biofilms produced by bacteria. The methods include tissue culture plate method (TCP), tube method (TM), Congo red agar method (CRA), bioluminescent assay, piezoelectric sensors, and fluorescent microscopic examination. In the tube method a loopful of overnight culture of the test organism is inoculated into trypticase soy broth containing 1% glucose and incubated. The bacterial cultures are poured out of the solution and the tubes washed using phosphate buffer saline and dried. The dried tubes are stained with 0.1% crystal violet with the excess stained washed with deionized water. The tubes are dried while inverted with observation made for biofilm formation which confirmed by the presence of visible film lining the wall as well as the bottom of the tube. If there is an observed ring-like development at the liquid interface it does not indicate biofilm formation [45]. In the Congo red agar method, the test organism is grown in red Congo agar on Brain heart infusion broth containing Congo red dye (0.8 g/L). The appearance of black colonies with a dry crystalline consistency indicated biofilm formation [45, 46]. In the tissue culture plate method a 24-h broth culture of the test organism is inoculated into trypticase soy broth (TSB). This primary inoculum is then inoculated into TSB with 1% glucose prepared in different dilutions (1:20, 1:40, 1:80 and 1:100) and loaded into 96 wells flat bottom microtiter plate, which is an abiotic surface. The plate is then incubated at 37°C for 24 h. The content of the wells are decanted and washed with phosphate buffer, fixed with methanol for 15 min and decanted. They are then stained with crystal violet (0.5%) for 20 min, decanted and washed with distilled water. Glacial acetic acid is then added to extract the crystal violet and the optical density determined at 490 nm using an ELISA plate reader [45].

3. Antibacterial natural products

3.1 Introduction

Natural products play a significant role in the discovery of lead compounds for the development of drugs for the treatment of human diseases. The importance to medicine of natural product molecules lies not only in their pharmacological or chemotherapeutic effects but also in their role as template molecules for the production of new drug substances. Nature in one way or another continues to influence the design of small molecules and most of the antibacterial drugs in clinical usage are naturally derived.

Natural products have the ability to provide diversity, complexity, novelty and new scaffolds with various chiral centers, rings, bridges and functional groups in the molecule [47]. They differ from synthetic compounds by having more oxygen atoms and stereochemical elements such as polycycle (often bridged) carbon skeletons [48, 49]. Some of the most valuable products and promising leads in oncology were naturally derived or naturally inspired. For instance paclitaxel a chemically established drug came from natural sources. Doxorubicin, camptothecins, and tamoxifen derived from natural product leads, steroid hormones. Most of the promising pipeline candidates in oncology all arose from natural products screening followed by synthetic modifications.

3.2 Examples of antibacterial natural products

Antibacterial natural compounds include secondary metabolites isolated from plants, bacteria, fungi, marine organisms and algae. These compounds are categorized based on their chemical type as terpenes (sesquiterpenes, diterpenes, sesterterpenes, and triterpenes), steroids (sterols), alkaloids (indole, quinoline, pyridoacridone, and amine alkaloids), aromatics (flavonoids, chalcones, coumarins, lignans, xanthones, anthracenes, anthraquinones, naphthalene), polyketides (acetylenic fatty acids, polycyclic esters and quinones), and peptides. Sometimes, the categorization tends to put together structurally relevant natural products with low bioactivity and also those synthetic analogues with remarkable antibacterial activity [47].

Since there is a continual need for a pipeline of new agents to combat multidrug-resistant bacteria, it is important the search goes on especially from plant materials. Microbially derived products, of which there are many first class drug examples which can be readily fermented with few re-supply issues [50]. The value of natural products as a screening resource has recently been highlighted and it is likely the focus is on plants, microbes and marine organisms [51].

Examples of antibacterial natural products include cranberry juice *Vaccinium macrocarpon* (family: Ericaceae) which is used in the management of urinary tract infections and the prevention of recurrent cystitis. Berberine has antibacterial activity against various strains of methicillin resistant *Staphylococcus aureus* (MRSA). Thymol is used as an antiseptic. Bearberry, *Arctostaphylos uva-ursi* L. (family: Ericaceae) is an antimicrobial agent used particularly for urinary tract infections against several organisms including *Bacillus subtilis, Escherichia coli, Mycobacterium smegmatis, S. aureus* and *Shigella* spp. and the antibacterial activity is attributed to arbutin. Lemon balm, *Melissa officinalis* (family: Lamiaceae) and tea tree, *Melaleucae alternifolia* (family: Myrtaceae) are taken as herb teas and tea tree oil is applied as ointment [51]. Garlic, *Allium sativum* (family: Alliaceae) have antimicrobial and antiseptic properties and is used for respiratory tract infections [48, 49] .

Tannins and resins produced by plants have antimicrobial and wound-healing properties. The essential oil constituents of plants also possess antimicrobial activity. Plants produce phytoalexins in response to infections caused by fungi viruses and bacterial that may infect them. Resveratrol is an example of an antifungal phytoalexin, which has anticancer, antioxidant and cardioprotective benefits for humans. Plant metabolites with antibacterial properties include anti-staphylococcal activities of the acylphloroglucinols and terthiophenes [47].

The quest for newer and potent antimicrobial agents has ventured into studies on plants in this research driven direction. It, however, makes a cogent argument since plants are known to produce varied chemicals for defense purposes against microorganisms. Also they produce cytotoxic compounds some of which have been successfully utilized as chemotherapeutic agents and hence give a laudable reason to continue with search for new treatment protocols for man. There is also an ecological rationale for the production of natural products that modify microbial resistance. Plants may have evolved compounds which evade MDR mechanisms and that plant antimicrobials might be developed into broad-spectrum antibiotics in combination with inhibitors of MDR [52]. These MDR proteins are commonly found in nature as efflux pumps for foreign toxic substances, as they are in clinical isolates of resistant pathogens.

In the food industry, herbs, spices and essential oils are chiefly employed as preservatives of foods. A number of plants and spices are used as antimicrobial agents for killing and decreasing pathogenic bacterial load in foods hence improving its quality. These plant-based antimicrobials are produced from various extraction

techniques using the flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots of various plants [50, 51].

4. Non-medicinal antibacterial chemicals

An ideal antibacterial agent (medicinal or non-medicinal) tends to have characteristics, which include ability to effectively inhibit or kill bacteria. Also it must have appreciable solubility either in water or any suitable solvent [53]. Antibacterial chemicals should be stored for a reasonable period of time with no significant loss of antibacterial action [54]. Such preparation must also be homogeneous with the active ingredients present in each application of the non-medicinal chemical against bacteria. An antibacterial chemical should be minimally inactivated when exposed to extraneous material such as proteins and other organic materials found in substances they are being utilized in treating. This decreases the amount of the agent the bacteria are exposed to [55, 56]. Antibacterial chemicals should not necessarily require elevated temperatures beyond that of the environments they are being used [57]. Again, such ideal antibacterial chemicals do possess cleansing properties, must be either odorless or have pleasant odor [58] and should be physically safe on surfaces they are used on.

The antimicrobial activities of these chemicals are often strongly influenced by the biocide affinity for the structural or molecular components of the cell, which can, in turn, depend on the attraction of dissimilar charges or hydrophobic interactions [53, 59]. It is henceforth convenient to consider the modes of action based on biocides target on the bacteria. Some probable drug target to be exploited include the cell wall, cell membrane and the cytoplasm. The precise mechanism of antimicrobial action of some of these agents will be discussed in this section as well as unraveling the concentration-dependent multiplicity in action of some of these agents [57].

4.1 Alcohols

Two water-soluble alcohols, i.e., ethyl alcohol and isopropyl alcohol, are normally employed as disinfectants due to their high germicidal activity [60]. They have rapid bactericidal and bacteriostatic activity against vegetative forms of bacteria. Although not active against bacterial spores, their tuberculocidal, fungicidal and virucidal activity are also apparent. Their cidal activity drops sharply when diluted below 50% concentration, and the optimum bactericidal concentration is 60–90% v/v solutions in water [60, 61]. Protein denaturation is the most likely cause of the alcohol action. This assertion is affirmed by the realization of the reduced bactericidal activity of absolute ethyl alcohol when compared with hydroalcohol due to the requirement of water to facilitate the protein denaturation process [62, 63]. This observation is also in conformation with the observed destruction of dehydrogenases of *Escherichia coli* by alcohol [64] and also the enhancement of the lag phase of *Enterobacter aerogenes* by alcohol [65] which is however reversed with some amino acids. Blockade of metabolites required for cell division has been linked to the bacteriostatic action of alcohol.

The role of hydrophobic alkyl groups of long-chain alcohols in antibacterial activity against *Staphylococcus aureus* and *Propionibacterium acnes* has been established [66]. The maximum activity was found to be dependent on the chain length from the hydrophilic hydroxyl group, and also the test bacteria. The antimycobacterial activities of alcohols with chain length ranging from C_5 to C_{13} against *Mycobacterium smegmatis* mc^2155 and *M. tuberculosis* $H_{37}R_v$ have been established, with best activity found with alcohol with C_{10} chain length [67]. This bactericidal activity is attributed

to decanol's potential to harm the robust and complex cell envelope of *Mycobacteria* and its ability to reduce biofilm formation by *M. smegmatis* [67].

4.2 Aldehydes

The aqueous solutions of some aldehydes, such as formaldehyde and glutaraldehyde, have been found to be bactericidal, tuberculocidal, fungicidal, virucidal, and sporicidal [68–70]. Formaldehyde inactivates microorganisms by alkylating the amino and sulfhydryl groups of proteins and ring nitrogen atoms of purine bases [71]. The biocidal activity of glutaraldehyde results from its alkylation of sulfhydryl, hydroxyl, carboxyl, and amino groups of microorganisms, which alters RNA, DNA, and protein synthesis [72].

Studies have established that different concentrations of formaldehyde have deleterious effect on a lot of microorganisms. It has been realized that 8% formalin can inactivate poliovirus in 10 min although other viruses are inactivated by as low as 2% formalin [68]. Tuberculocidal potential can be seen with 4% formalin with the agent being able to inactivate 10^4 *Mycobacterium tuberculosis* cells with 2 min [69]. Also, about 10 million *Salmonella typhi* cells can be inactivated by 2.5% formaldehyde within 10 min even in the presence of organic matter [70]. However, formaldehyde has significantly reduced sporicidal activity than glutaraldehyde as seen from experimental studies [69].

Some aliphatic saturated and unsaturated aldehydes are produced by enzymatic cleavage of unsaturated fatty acids when plants undergo microbial attack; thus they may be one of the multichemical defense mechanisms used by several fruits to resist invasion by microorganisms [73]. In this direction, Trombetta and colleagues established the mechanism of the antimicrobial activity of aliphatic α,β-unsaturated aldehydes which included (E)-2-hexenal **(1)**, (E)-2-eptenal **(2)**, (E)-2-octenal **(3)**, (E)-2-nonenal **(4)**, (E)-2-decenal **(5)** and (E,E)-2,4-decadienal **(6)** (**Figure 1**). Their findings suggested that the 2E-alkenals tested elicit, very likely, a gross perturbation of the lipidic fraction of plasma membranes and are able to penetrate into bacterial cells.

The mechanisms of antimicrobial action of other aldehydes, such as *o*-phthalaldehyde **(7)**, are likely to involve interaction with the cytoplasmic membrane and increase in its permeability [74, 75]. *o*-Phthalaldehyde also appears to kill spores by blocking the spore germination process [76]. Although membrane functional proteins are generally supposed to be the potential targets toward which aldehydic antimicrobial agents are directed, other mechanisms of action/interaction can help explain their antimicrobial activity [73].

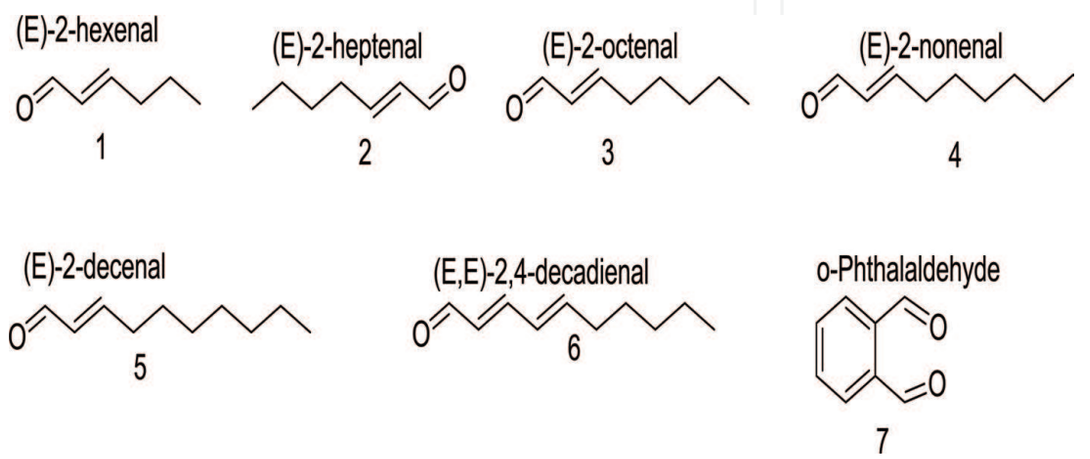

Figure 1.
Aldehydes with antibacterial properties.

4.3 Halogens

Over the years, tinctures and solutions of iodine have been used mainly as antiseptic agents on skin surfaces and other tissues. However, iodophors such as povidone iodine, are employed mainly as antiseptics and disinfectants. Such iodophors tend to possess germicidal ability but generally do not stain surfaces as well as do possess less toxic and irritant potential [77]. Iodine has the potential to penetrate the cell wall of microorganisms rapidly and tend to compromise proteins and nucleic acids as well as the synthesis of proteins.

Chlorine and products of chlorine do have broader spectrum of activity but they also leave behind toxic residues. They however are not affected by hardness of water, are less expensive and do possess faster onset of action [78]. They tend to possess activity on organisms fixed to surfaces and biofilms [79] with accompanied low risk of toxicity [80]. Its mechanism of antimicrobial action is associated with hypochlorous acid (HOCl) it yields. When HOCl dissociates, it produces hypochlorite ion (OCl^-) which has reduced antimicrobial activity and the process is pH-dependent [81]. It has not been mechanistically established how chlorine specifically kills microorganisms. However, chlorine tends to cause oxidation of sulfhydryl enzymes and amino acids leading to loss of intracellular contents with reduced nutrition uptake by cells. Also, chlorine tends to reduced adenosine triphosphate production as well as inhibition of DNA synthesis and increased DNA destruction [81]. A number of these process may account for the mechanism of antimicrobial action of chlorine [82].

4.4 Oxidizing agents

Peroxyacetic acid **(1)**, chlorine dioxide **(2)** (**Figure 2**), and hydrogen peroxide have similar mechanism of antimicrobial action (chemical oxidation of cellular components), but they do vary greatly in their efficacy against microorganisms [83, 84]. Biochemically, there is significant variations in these agents with resultant variation in their outcomes on macromolecules. This attests to the variations in their biocidal activity, most especially between liquid and gas peroxide [84].

Administration of hydrogen peroxide results in the production of hydroxyl free radicals which attack lipid membranes, DNA and essential cell components leading to cell death. Aerobic organisms and facultative anaerobes tend to produce catalase and these enzymes offer protective benefits from hydrogen peroxide by catalyzing its conversion to water and oxygen. Such protective benefits are compromised by the concentrations of oxidizing agents administered as disinfectant [85]. Peracetic acid, which has rapid action on all microorganisms, does not yield any harmful products when it is broken down and is easily cleared from organic materials without leaving behind any residues [86]. In addition to its potent activity in the presence of organic matter, it retains sporicidal activity even at low temperatures.

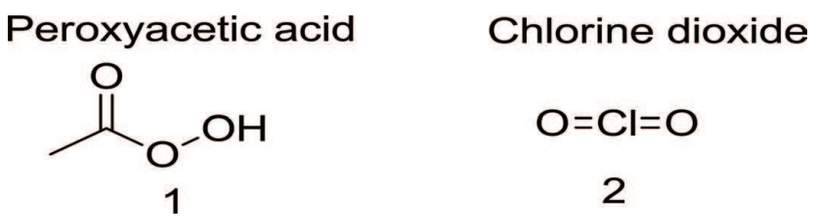

Figure 2.
Oxidizing agents with antibacterial activity.

However, its specific mechanism of antimicrobial action has not been unraveled although postulated to be working in the same manner as other oxidizing agents. Hence possibly through oxidation of sulfhydryl and sulfur bonds in proteins, enzymes and other cellular metabolites [85].

4.5 Phenolic compounds

Phenolic compounds are produced from structural modification of phenol by addition of alkyl, phenyl, benzyl groups, and halogen as substituent on the aromatic ring. *o*-Phenylphenol **(1)** and *o*-benzyl-*p*-chlorophenol **(2)** are commonly employed phenol derivatives in disinfectants. Phenolic compounds tend to have enhanced antimicrobial potential than phenol [87].

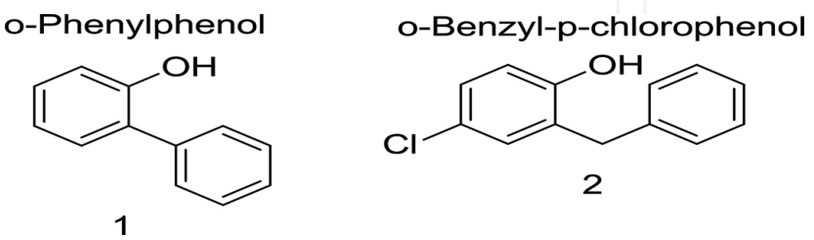

Figure 3.
Phenolic antibacterial chemicals.

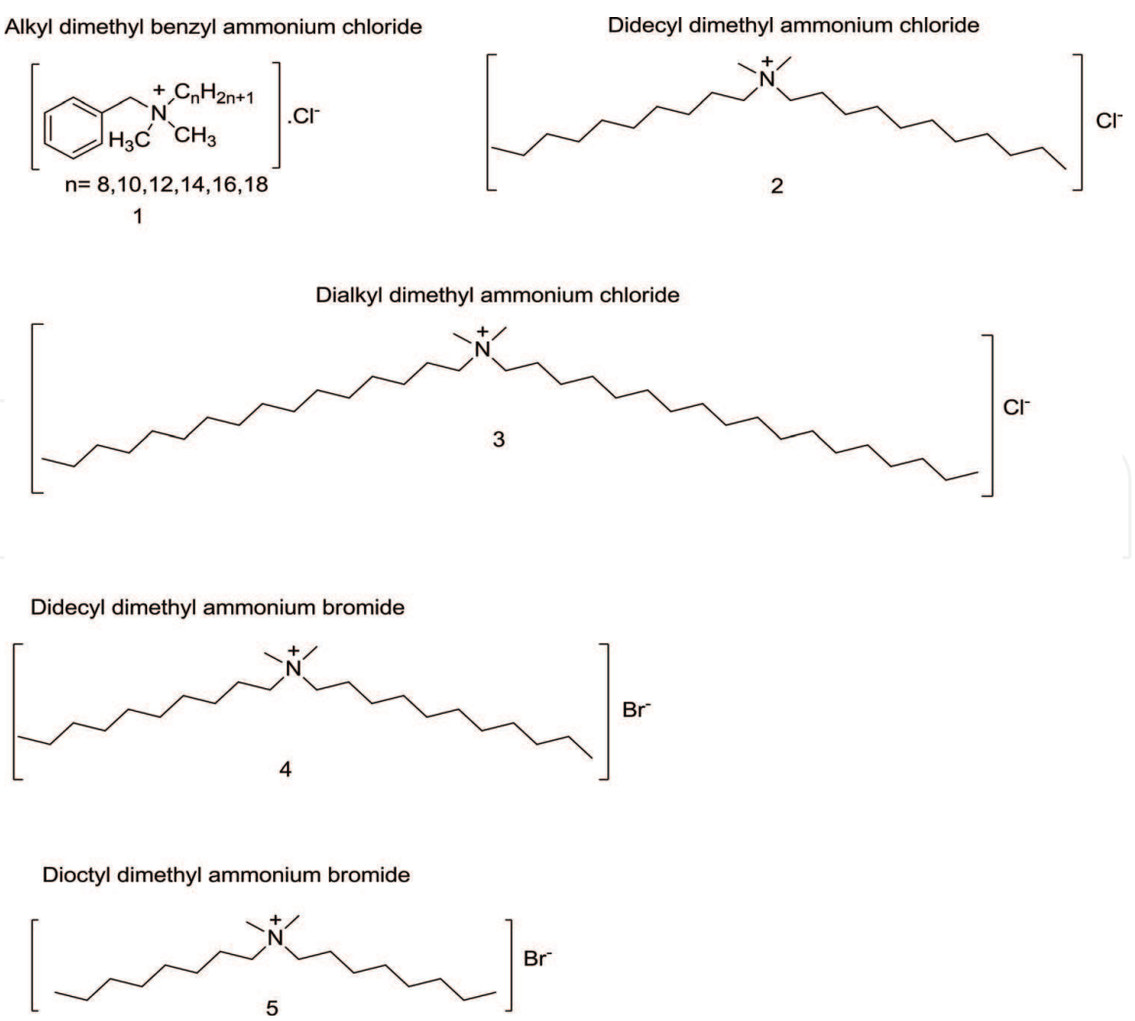

Figure 4.
Quaternary ammonium compounds with antibacterial activity.

Phenol acts as a penetrating and disrupting molecule on cell walls of organisms as well as increase precipitation in important proteins within cells leading to cell death at high concentrations. However, phenol and high molecular weight phenolic compounds tend to kill bacteria cells via enzyme inactivation and compromising of cell wall at lower concentration (**Figure 3**) [88].

4.6 Quaternary ammonium compounds

One of the most used disinfectants is the quaternary ammonium compounds. When contaminated quaternary ammonium compounds are employed in patient-care supplies, they contribute to significant proportion of its associated infections [89]. Gram-negative bacteria are documented to survive in this group of disinfectants [90].

The quaternary compounds are ammonium-derived moieties with nitrogen atom with a valence of 5. There are four alkyl or heterocyclic radical substitutes and a fifth halide, sulfate or similar radical substitute in the structure [91]. Some of these quaternaries include alkyl dimethyl benzyl ammonium chloride **(1)**, alkyl didecyl dimethyl ammonium chloride **(2)**, and dialkyl dimethyl ammonium chloride **(3)** (**Figure 4**). The innovative quaternary ammonium compounds (i.e., fourth generation), referred to as twin-chain or dialkyl quaternaries (e.g., didecyl dimethyl ammonium bromide **(4)** and dioctyl dimethyl ammonium bromide **(5)** (**Figure 4**)), have been reported to be active even in hard water and can withstand anionic residues [92]. Quaternaries are believed to be bactericidal due to their ability to inactivate energy-producing enzymes and also denature and disrupt cell proteins and membrane respectively [91, 92].

5. Conclusion

A multidisciplinary approach to antimicrobial drug discovery, involving the generation of novel molecular diversity from natural product sources, combined with total and combinatorial synthetic methodologies, and including the manipulation of biosynthetic pathways will continue to provide the best approach to antibiotic discovery and development and also overcome the challenges associated with antimicrobial resistance.

Conflict of interest

Authors declare no conflict of interest.

DOI: http://dx.doi.org/10.5772/intechopen.82560

Author details

Yaw Duah Boakye[1], Newman Osafo[2], Cynthia Amaning Danquah[2], Francis Adu[1] and Christian Agyare[1*]

1 Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

2 Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

*Address all correspondence to: cagyare.pharm@knust.edu.gh

References

[1] Becker DE. Antimicrobial drugs. Anesthesia Progress. 2013;**60**(3):111-123

[2] Bergand T. Pharmacokinetics of beta lactam antibiotics. Scandinavian Journal of Infectious Diseases. Supplementum. 1984;**42**:83-98

[3] Kuriyama T, Karasawa T, Nakagawa K, Yamamoto E, Nakamura S. Incidence of β-lactamase production and antimicrobial susceptibility of anaerobic Gram-negative rods isolated from pus specimens of orofacial odontogenic infections. Oral Microbiology and Immunology. 2001;**16**(1):10-15

[4] Mehta D, Sharma AK. Cephalosporins: A review on imperative class of antibiotics. Inventi Rapid/Impact: Molecular Pharmacology. 2016;**1**:1-6

[5] Laudano JB. Ceftaroline fosamil: A new broad-spectrum cephalosporin. Journal of Antimicrobial Chemotherapy. 2011;**66**(3):11-8

[6] Cluck D, Lewis P, Stayer B, Spivey J, Moorman J. Ceftolozane-tazobactam: A new-generation cephalosporin. 2015;**72**:2135-2146

[7] Reynolds P. Structure biochemistry and mechanism of action of glycopeptide antibiotics. European Journal of Clinical Microbiology & Infectious Diseases. 1989;**8**(11):943-950

[8] Stein G. Oritavacin: A long-half life lipoglycopeptide. Clinical Infectious Diseases. 2015;**61**(4):627-632

[9] Karrine D, Ryback J. Oritavancin: A new lipoglycopeptide antibiotic in the treatment of Gram-positive infection. Infectious Disease and Therapy. 2016;**16**:1-15

[10] Saravolatz LD, Stein GE, Johnson LB. Telavancin: A novel lipoglycopeptide. Clinical Infectious Diseases. 2009;**49**(12):1908-1914

[11] Chen AY, Zervos MJ, Vazquez JA. Dalbavancin: A novel antimicrobial. International Journal of Clinical Practice. 2007;**5**(May):853-863

[12] Fernandes P, Martens E. Antibiotics in late clinical development. Biochemical Pharmacology. 2017;**133**:152-163

[13] Burdette SD, Trotman R. Tedizolid: The first once-daily oxazolidinone class antibiotic. Agents. 2018;**61**(June):1315-1321

[14] Mullane K, Gorbach S. Fidaxomicin: First-in-class macrocyclic antibiotic. Expert Review of Anti-Infective Therapy. 2011;**9**(7):767-778

[15] Gupta A. Plazomicin: A step toward next generation aminoglycosides. Review. Asian Journal of Research in Pharmaceutical Sciences. 2017;**7**(3):1-8

[16] Paukner S, Riedl R. Pleuromutilins: Potent drugs for resistant bugs—Mode of action and resistance. Cold Spring Harbor Perspectives in Medicine. 2017;**7**(1).pii:a027110

[17] Greer ND. Tigecycline (Tygacil): The first in the glycylcycline class of antibiotics. Proceedings (Baylor University, Medical Center). 2006;**19**(2):155-161

[18] Ackermann G, Rodloff AC. Drugs of the 21st century: Telithromycin (HMR 3647)—The first ketolide. The Journal of Antimicrobial Chemotherapy. 2003;**51**:497-511

[19] Mogle BT, Steele JM, Thomas SJ, Bohan KH, Kufel WD. Clinical review of delafloxacin: A novel anionic fluoroquinolone. The Journal

of Antimicrobial Chemotherapy. 2018;**73**(6):1439-1451

[20] Bos R, Van Der MHC, Busscher HJ. Physico-chemistry of initial microbial adhesive interactions its mechanisms and methods for study. FEMS Microbiology Reviews. 1999;**23**(2):179-230

[21] Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001;**358**(9276):135-138

[22] Donlan RM, Costerton JW, Donlan RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clinical Microbiology Reviews. 2002;**15**(2):167-193

[23] Reid G, Cook L, McGroarty JA, Angotti R. Lactobacillus inhibitor production against *Escherichia coli* and coaggregation ability with uropathogens. Canadian Journal of Microbiology. 1988;**34**(3):344-351

[24] Simm R, Morr M, Kader A, Nimtz M, Römling U. GGDEF and EAL domains inversely regulate cyclic di-GMP levels and transition from sessility to motility. Molecular Microbiology. 2004;**53**(4):1123-1134

[25] Hickman JW, Tifrea DF, Harwood CS. A chemosensory system that regulates biofilm formation through modulation of cyclic diguanylate levels. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(40):14422-14427

[26] Banerjee P, Singh M, Sharma V. Biofilm formation: A comprehensive review. 2015;**3**(2):556-560

[27] Hall-Stoodley P. Developmental regulation of microbial biofilms. Current Opinion in Biotechnology. 2002;**13**:228-233

[28] Dunne WM. Bacterial adhesion: Seen any good biofilms lately? 2002;**15**:155166

[29] Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. International Journal of Medical Microbiology. 2002;**292**(2):107-113

[30] Ceri H, Olson ME, Stremick C, Read RR, Morck D. The calgary biofilm device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. Journal of Clinical Microbiology. 1999;**37**(6):1771-1776

[31] Walsh C. Molecular mechanisms that confer antibacterial drug resistance. Nature. 2000;**406**:775-781

[32] Douglas LJ. Candida biofilms and their role in infection. Trends in Microbiology. 2003;**11**(1):30-36

[33] Wesenberg-Ward KE, Tyler BJ, Sears JT. Adhesion and biofilm formation of Candida albicans on native and pluronic-treated polystyrene. Biofilms. 2005:63-71

[34] Bakaletz LO. Biofilms in otitis media: Evidence and relevance. The Pediatric Infectious Disease Journal. 2007;**26**(10):S17-S19

[35] Post JC, Stoodley P, Hall L, Ehrlich GD. The role of biofilms in otolaryngologic infections. Current Opinion in Otolaryngology & Head and Neck Surgery. 2004;**12**(3):185-190

[36] Brady RA, Leid JG, Calhoun JH, Costerton JW, Shirtliff ME. Osteomyelitis and the role of biofilms in chronic infection. FEMS Immunology and Medical Microbiology. 2008;**52**(1):13-22

[37] Høiby N. Understanding bacterial biofilms in patients with cystic fibrosis: Current and innovative approaches to potential therapies. Journal of Cystic Fibrosis. 2002;**1**:249-254

[38] James GA, Swogger E, Wolcott R, Pulcini E, Secor P, Sestrich J,

et al. Biofilms in chronic wounds. Wound Repair and Regeneration. 2008;**16**(1):37-44

[39] Ramage G, Saville SP, Wickes BL, López-ribot JL. Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. Applied and Environmental Microbiology. 2002;**68**(11):5459-5463

[40] Martinez-Gutierrez F, Boegli L, Agostinho A, Sánchez EM, Bach H, Ruiz F, et al. Anti-biofilm activity of silver nanoparticles against different microorganisms. Biofouling. 2013;**29**(6):651-660

[41] Dean SN, Bishop BM, Van HML. Natural and synthetic cathelicidin peptides with anti-microbial and anti-biofilm activity against *Staphylococcus aureus*. BMC Microbiology. 2011;**11**:114

[42] Al-sohaibani S, Murugan K. Anti-biofilm activity of *Salvadora persica* on cariogenic isolates of *Streptococcus mutans*: *In vitro* and molecular docking studies. Biofouling. 2012;**28**(1):29-23

[43] Adukwu EC, Allen SCH, Phillips CA. The anti-biofilm activity of lemongrass (*Cymbopogon flexuosus*) and grapefruit (*Citrus paradisi*) essential oils against five strains of *Staphylococcus aureus*. Journal of Applied Microbiology 2012;**113**(5):1217-2

[44] Schillaci D, Arizza V, Dayton T, Camarda L, Stefano VD. *In vitro* anti-biofilm activity of Boswellia spp. oleogum resin essential oils. Letters in Applied Microbiology. 2008;**47**(5):433-438

[45] Deka N. Comparison of tissue culture plate method , tube method and congo red agar method for the detection of biofilm formation by coagulase negative Staphylococcus isolated from non-clinical isolates. International Journal of Current Microbiology and Applied Sciences. 2014;**3**(10):810-815

[46] Kaiser TD, Pereira EM, Dos Santos KR, Maciel EL, Schuenck RP, Nunes AP. Modification of the Congo red agar method to detect biofilm production by *Staphylococcus epidermidis*. Diagnostic Microbiology and Infectious Disease. 2013;**75**(3):235-239

[47] Gibbons S. Anti-staphylococcal plant natural products. Natural Product Reports. 2004;**2**:263-277

[48] Danquah CA, Kakagianni E, Khondkar P, Maitra A, Malkinson J, Bhakta S, et al. Analogues of disulfides from *Allium stipitatum* demonstrate potent anti-tubercular activities through drug efflux pump and biofilm inhibition. 2018, 1150;**8**(1):18

[49] O'Donnell G, Poeschl R, Zimhony O, Gunaratnam M, Moreira JBC, Neidle S, et al. Bioactive pyridine-N-oxide disulfides from *Allium stipitatum*. Journal of Natural Products. 2009;**72**(3):360-365

[50] Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products. 2012;**75**(3):311-335

[51] Heinrich M, Barnes J, Gibbons S, Williamson EM. Fundamentals of Pharmacognosy and Phytotherapy. Edinburgh: Churchill Livingstone/ Elsevier; 2012

[52] Tegos G, Mylonakis E, editors. Antimicrobial Drug Discover: Emerging Strategies. Wallingford: CABI; 2012

[53] McDonnell G, Russel AD. Antiseptics and disinfectants: Activity, action, and resistance. . 1999;**12**(1):147179

[54] Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews. 2010;**74**(3):417-433

[55] Marriott NG. Principles of Food Sanitation. P. 143. Gaithersburg, MD: Aspen Publishers, Inc; 1999. p. 364

[56] Cords BR, Burnett SL, Hilgren J, Finley M, Magnuson J. Sanitizers: Halogens, surface-active agents, and peroxides. In: Davidson PM, Sofos J, Branen AL, editors. Antimicrobials in Food. 3rd ed. Boca Raton: Taylor and Francis; 2005

[57] Kahrilas GA, Blotevogel J, Stewart PS, Borch T. Biocides in hydraulic fracturing fluids: A critical review of their usage, mobility, degradation, and toxicity. Environmental Science & Technology. 2015;**49**(1):16-32

[58] Lambert D. Topical deodorant. In: Baran R, Maibach HI, editors. Textbook of Cosmetic Dermatology. 2nd ed. London, UK: Martin Dunitz Ltd; 1998. p. 172

[59] Denyer SP, Hodges NA, Gorman SP. Non-antibiotic antibacterial agents: Mode of action and resistance. In: Hugo and Russell's Pharmaceutical Microbiology. 7th ed. Oxford, UK: Blackwell Publishing Company; 2008. p. 309

[60] Spaulding EH. Alcohol as a surgical disinfectant: Pros and Cons of a much dicussed topic. AORN Journal. 1964;**2**:67-71

[61] Morton HE. The relationship of concentration and germicidal efficiency of ethyl alcohol. Annals of the New York Academy of Sciences. 1950;**53**(1):191-196

[62] Ali Y, Dolan MJ, Fendler EJ, Larson EL. Alcohols. In: Block SS, editor. Disinfection, Sterilization, and Preservation. Philadelphia: Lippincott Williams & Wilkins; 2001. pp. 229-254

[63] Morton HE. Alcohols. In: Block SS, editor. Disinfection, Sterilization, and Preservation. Philadelphia: Lea & Febiger; 1983. pp. 225-239

[64] Sykes G. The influence of germicides on the dehydrogenases of Bact. coli. Part I. The succinic acid dehydrogenase of Bact. coli. (London). 1939;**39**(4):463-469

[65] Dagley S, Dawes EA, Morrison GA. Inhibition of growth of Aerobacter aerogenes: The mode of action of phenols, alcohols, acetone and ethyl acetate. Journal of Bacteriology. 1950;**60**:369-378

[66] Kubo I, Muroi H, Himejima M, Kubo A. Antibacterial activity of long-chain alcohols: The role of hydrophobic alkyl groups. Bioorganic & Medicinal Chemistry Letters. 1993;**3**(6):1305-1308

[67] Mukherjee K, Tribedi P, Mukhopadhyay B, Sil AK. Antibacterial activity of long-chain fatty alcohols against mycobacteria. FEMS Microbiology Letters. 2013;**223**(2):177-183

[68] Klein M, DeForest A. The inactivation of viruses by germicides. Chemical Specialties Manufacturers Association Proceedings. 1963;**49**:116-118

[69] Rubbo SD, Gardner JF, Webb RL. Biocidal activities of glutaraldehyde and related compounds. Journal of Applied Microbiology. 1967;**30**:78-87

[70] McCulloch EC, Costigan S. A comparison of the efficiency of phenol, liquor cresolis, formaldehyde, sodium hypochlorite and sodium hydroxide against *Eberthella typhi* at various temperatures. The Journal of Infectious Diseases. 1936;**59**:281-284

[71] Favero MS, Bond WW. Chemical disinfection of medical and surgical materials. In: Block SS, editor. Disinfection, Sterilization, and

Preservation. Philadelphia: Lea & Febiger; 1991. pp. 617-641

[72] Scott EM, Gorman SP. Glutaraldehyde. In: Block SS, editor. Disinfection, Sterilization, and Preservation. Philadelphia: Lippincott Williams & Wilkins; 2001. pp. 361-381

[73] Trombetta D, Saija A, Bisignana G, Arena S, Caruso S, Mazzanti G, et al. Study on the mechanisms of the antibacterial action of some plant α,β-unsaturated aldehydes. Letters in Applied Microbiology. 2002;**35**:285-290

[74] Simons C, Walsh SE, Maillard JY, Russell AD. A note: Ortho-phthalaldehyde: Proposed mechanism of action of a new antimicrobial agent. Letters in Applied Microbiology. 2000;**31**:299-302

[75] Tsuchiya H. Biphasic effects of acetaldehyde-biogenic amine condensation products on membrane fluidity. The Journal of Pharmacy and Pharmacology. 2001;**53**:121-127

[76] Cabrera-Martinez RM, Setlow B, Setlow P. Studies on the mechanisms of the sporicidal action of ortho-phthalaldehyde. Journal of Applied Microbiology. 2002;**92**:675-680

[77] Gottardi W. Iodine and iodine compounds. In: Block SS, editor. Disinfection, Sterilization, and Preservation. Philadelphia: Lippincott Williams & Wilkins; 2001. pp. 159-184

[78] Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health-care facilities. Clinical Microbiology Reviews. 1997;**10**:597-610

[79] Merritt K, Hitchins VM, Brown SA. Safety and cleaning of medical materials and devices. 2000;**53**:131-136

[80] Heidemann SM, Goetting MG. Treatment of acute hypoxemic respiratory failure caused by chlorine exposure. 1991;**7**:87-88

[81] Dychdala GR. Chlorine and chlorine compounds. In: Block SS, editor. Disinfection, Sterilization, and Preservation. Philadelphia: Lippincott Williams & Wilkins; 2001. pp. 135-157

[82] Gerba CP, Rusin P. Relationship between the use of antiseptics/disinfectants and the development of antimicrobial resistance. In: Rutala WA, editor. Disinfection, Sterilization and Antisepsis: Principles and Practices in Healthcare Facilities. Washington, DC: Association for Professional in Infection Control and Epidemiology; 2001. pp. 187-194

[83] Denyer SP, Stewart GSAB. Mechanisms of action of disinfectants. International Biodet and Biodegrad. 1998;**41**:261-268

[84] Finnegan M, Linley E, Denyer SP, McDonnel G, Simons C, Maillard J-Y. Mode of action of hydrogen peroxide and other oxidizing agents: Differences between liquid and gas forms. The Journal of Antimicrobial Chemotherapy. 2010;**65**(10):2108-2115

[85] Block SS. Peroxygen compounds. In: Block SS, editor. Disinfection, Sterilization, and Preservation. Philadelphia: Lippincott Williams & Wilkins; 2001. pp. 185-204

[86] Tucker RC, Lestini BJ, Marchant RE. Surface analysis of clinically used expanded PTFE endoscopic tubing treated by the steris process. ASAIO Journal. 1996;**42**:306-313

[87] Kahn G. Depigmentation caused by phenolic detergent germicides. Archives of Dermatology. 1970;**102**:177-187

[88] Prindle RF. In: Block SS, editor. Phenolic compounds, Disinfection, Sterilization, and Preservation. Philadelphia: Lea & Febiger; 1983. pp. 197-224

[89] Ehrenkranz NJ, Bolyard EA, Wiener M, Cleary TJ. Antibiotic-sensitive *Serratia marcescens* infections complicating cardiopulmonary operations: contaminated disinfectant as a reservoir. Lancet. 1980;**2**:1289-1292

[90] Rutala WA, Cole EC. Antiseptics and disinfectants—Safe and effective? Infection Control. 1984;**5**:215-218

[91] Sykes G. Disinfection and Sterilization. London: E & FN Spon Ltd; 1965

[92] Merianos JJ. Surface-active agents. In: Block SS, editor. Disinfection, Sterilization, and Preservation. Philadelphia: Lippincott Williams & Wilkins; 2001. pp. 283-320