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The Prescriptions' Epidemiology of the Challenges for Overcoming Antibiotic Resistance in Hospitals

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Abstract
Understanding the mechanisms of antibiotic resistance is important in the development of strategies to solving the problem. Active efflux of drugs, alteration of target sites and enzymatic degradations are the strategies by which pathogenic bacteria acquire or develop intrinsic resistance to antibiotics. Multi-drug resistance (MDR) pumps, capable of recognizing and expelling a variety of structurally unrelated compounds from the bacterial cell and conferring resistance to a wide range of antibiotics have since been characterized in many gram positive and gram negative pathogens like Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and, more recently, in mycobacteria. The ability of some chemical compounds to modify the resistance phenotype in bacteria by working synergistically with antibiotics in vitro has since been observed. The search for such compounds which can be combined with antibiotics in the treatment of drug resistant infections may be an alternative to overcoming the problem of resistance in bacteria.
Keywords: Antibiotic resistance, resistance modifying agents.

1.1 Introduction

Since the discovery of antibiotics and their uses as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eradication of infectious diseases. However diseases and disease agents that were once thought to have been controlled by antibiotics are returning in new forms resistant to antibiotic therapies (Levy and Marshall, 2004). Incidents of epidemics due to such drug resistant microorganisms are now a common global problem posing enormous public health concerns. The global emergence of multi-drug resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections (Hancock, 2005). Examples include methicillin-resistant staphylococci, pneumococci resistant to penicillin and macrolides, vancomycin-resistant enterococci as well as multidrug resistant gram-negative organisms (Norrby et al., 2005). As resistance to old antibiotics spreads, the development of new antimicrobial agents has to be expedited if the problem is to be contained. However, the past record of rapid, widespread and emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates et al., 2002).

Confronted with a possible shortage of new antimicrobials, there is need to ensure a careful use of our available drugs. This has led to calls for controlled use of antibiotics through the reduction of dosage used per regime of treatment or by regulating prescriptions in areas such as animal husbandry and aquaculture (Hernandez, 2005). While reduced use could lead to delayed resistance development, the emergence of resistant strains is from an evolutionary viewpoint inevitable. It becomes imperative therefore that alternative approaches are explored. Targeting and blocking resistance processes could be an attractive approach. The presence of efflux pumps and multidrug resistance (MDR) proteins in antibiotic resistant organisms contribute significantly to the intrinsic and acquired resistance in these pathogens. The discovery and development of new compounds that either block or circumvent resistance mechanisms could improve the containment, treatment, and eradication of these strains (Oluwatuyi et al., 2014).

A few studies such as Gibbons et al. (2003), Dickson et al. (2006) and Braga et al. (2005) have reported that plant extracts can enhance the in vitro activity of certain antibiotics against strains of MDR Staphylococcus aureus and other pathogens. These studies have prompted the search for such MDR Pump or Efflux Pump inhibitors from medicinal plants. This paper reviews the mechanisms of resistance to antibiotics by pathogenic bacteria and how such processes can be curtailed by the use of plant extracts and plant derived compounds in a bid to highlighting the importance of this untapped resource in the fight against the spread of antibiotic resistant pathogens. The development of resistance in bacteria is one of the mechanisms of natural adaptation to the presence of an antimicrobial agent that inhibits susceptible organisms and selects the resistant ones. Under continued selection pressure, the selected resistant organisms multiply and spread to other geographic locations as well as to other microbes by transfer of resistance genes (Levy and Marshall, 2004). Selection of resistant strains occurs so rapid for some bacteria that clinical usefulness of the antibiotics is lost within a 5 year period (Bush, 2004). The emergence and spread of microbes that are resistant to cheap and effective first-choice drugs has become a common occurrence. The problem is even more evident in bacterial infections which contribute most to the global infectious disease burden such as diarrheal, respiratory tract, meningitis, sexually transmitted infections, and tuberculosis (WHO, 2015).

1.2 Challenge of Antibiotic Resistance

The development of resistance in bacteria is one of the mechanisms of natural adaptation to the presence of an antimicrobial agent that inhibits susceptible organisms and selects the resistant ones. Under continued selection pressure, the selected resistant organisms multiply and spread to other geographic locations as well as to other microbes by transfer of resistance genes (Levy and Marshall, 2004). Selection of resistant strains occurs so rapid for some bacteria that clinical usefulness of the antibiotics is lost within a 5 year period (Bush, 2004). The emergence and spread of microbes that are resistant to cheap and effective first-choice drugs has become a common occurrence. The problem is even more evident in bacterial infections which contribute most to the global infectious disease burden such as diarrheal, respiratory tract, meningitis, sexually transmitted infections, and tuberculosis (WHO, 2015). Resistance to penicillin in S. aureus first appeared in 1942 immediately following its clinical use. By the late 1960s, more than 80% of both community- and hospital-acquired staphylococcal isolates were resistant to penicillin (Lowy, 2003).

1.3 Mechanism of resistance

The use of antibiotics should have created a catastrophic situation for microbial populations but the genetic flexibility allowed bacteria to survive and multiply under the antibiotic pressure. Bacteria can resist antibiotics as a result of chromosomal mutation or by exchange of genetic materials, which carry resistance genes, through transformation, transduction or conjugation by plasmids. The mechanism of resistance to antimicrobial agents can be due to (Rice & Bonomo, 1996):

(i) Impermeability of the drug: This is the most frequent cause of intrinsic resistance. Resistance in Enterococcus sp. and Pseudomonas aeruginosa is a good example of such mechanisms;

(ii) Alteration in target molecules—This is one of the most important mechanisms of resistance to clinically used antibacterial drugs, for example, methicillin resistant S. aureus with altered penicillin binding proteins;

(iii) Enzymatic drug modifications—b-lactamase enzymes currently account for most of the resistance to penicillins and cephalosporins. b-lactamases affect a common drug site i.e., b-lactam ring. Penicillins, cephalosporins, monobactams and carbapenems can all be hydrolyzed by multiple members of the beta lactamase family of enzymes, resulting in a microbiologically ineffective compound. The other important class of antibiotics, which are destroyed by enzymes are aminoglycosides due to the action of aminoglycoside - modifying enzymes produced by the bacteria;

(iv) Efflux—The role of efflux of drug from the bacterial cell as a resistance mechanism is comparatively less common in clinical practice.

1.3.1 Alteration of target site

Chemical modifications in the antibiotic target may result in reduced affinity of the antibiotic to its binding site (Lambert, 2005). This is a mechanism employed by a number of pathogenic bacteria in evading the effect of antibiotics. Modifications are usually mediated by constitutive and inducible enzymes. Resistance to macrolides, lincosamide and streptogramin B antibiotics (MLSB resistance) in pathogenic Streptococcus species is a result of methylation of the N^6 amino group of an adenine residue in 23S rRNA. This is presumed to cause conformational changes in the ribosome leading to reduced binding affinity of these antibiotics to their binding sites in the 50S ribosomal subunit (Seppala et al., 1998). Beta-lactams antibiotics function by binding to and inhibiting the biosynthetic activity of Penicillin Binding Proteins (PBPs), thereby blocking cell wall synthesis. In S. aureus and S. pneumoniae, resistance to -lactams can be a result of mutations leading to the production of PBP2a and PBP2b respectively. The two proteins have a reduced affinity for -lactams and yet they take over the functions of normal PBPs in the presence of inhibitory levels of -lactams (Golemi-Kotra et al., 2003; Grebe and Hakenbeck, 1996). This mechanism of resistance is also responsible for -lactam resistance in non- lactamase producing Haemophillus influenza (Matic et al., 2013).

1.3.2 Enzymatic inactivation

The production of hydrolytic enzymes and group transferases is a strategy employed by a number of pathogens in evading the effect of antibiotics (Wright, 2005). Genes that code for antibiotic degrading enzymes are often carried on plasmids and other mobile genetic elements. The resistance to -lactam antibiotics by both gram negative and gram positive bacteria has long been attributed to -lactamases (Frere, 1995). These enzymes confer significant antibiotic resistance to their bacterial hosts by hydrolysis of the amide bond of the four membered lactam ring (Wilke et al., 2005). Resistance to aminoglycosides in gram-negative bacteria is most often mediated by a variety of enzymes that modify the antibiotic molecule by acetylation, adenylating or phosphorylation (Over et al., 2011).

1.3.3 Antibiotic efflux

It is now widely recognized that constitutive expression of efflux pump proteins encoded by house-keeping genes that are widespread in bacterial genomes are largely responsible for the phenomenon of intrinsic antibiotic

resistance (Lomovskaya and Bostian, 2006). Several studies have shown that active efflux can be a mechanism of resistance for almost all antibiotics (Li et al., 1994a; Gill et al., 1999; Lin et al., 2002). The majority of the efflux systems in bacteria are non-drug-specific proteins that can recognize and pump out a broad range of chemically and structurally unrelated compounds from bacteria in an energy-dependent manner, without drug alteration or degradation (Kumar and Schweizer, 2005). The consequence of this drug extrusion is that, it leads to a reduced intracellular concentration of the antimicrobial such that the bacterium can survive under conditions of elevated antimicrobial concentration (Marquez, 2005). The MIC of the drug against such organisms will be higher than predicted.

1.3.4 Some characterized efflux proteins of pathogenic bacteria

The NorA protein of S. aureus is the best studied chromosomally encoded pump in pathogenic grampositive bacteria (Hooper, 2005). It is present in S. epidermidis but appears to be absent in Enterococcus faecalis or in gram-negative organisms, such as E. coli and K. pneumoniae (Kaatz et al., 1993). Overexpression of the NorA gene in S. aureus confers resistance to chloramphenicol and hydrophilic fluoroquinolone antimicrobials (Hooper, 2005; Kaatz and Seo, 1995). QacA is a member of the major facilitator super-family of transport proteins, which are involved in the uniport, symport, and antiport of a wide range of substances across the cell membrane (Mitchell et al., 1998). The QacA multidrug exporter from S. aureus mediates resistance to a wide array of monovalent or divalent cationic, lipophilic, antimicrobial compounds. QacA provides resistance to these various compounds via a proton motive force-dependent antiport mechanism (Brown and Skurray, 2011).

1.4 The Use of Resistance Modifying Agents in Combination with Antibiotics to Overcome Resistance

The selection pressure exerted by the continued presence of bactericidal or bacteriostatic agents facilitates the emergence and dissemination of antibiotic resistance genes. Over generations, the genotypic makeup of bacterial populations is altered (Taylor et al., 2002). The clinical implications of this are that many infections become untreatable resulting in serious morbidity and mortality. Although the introduction of new compounds into clinical use has helped to curtail the spread of resistant pathogens, resistance to such new drugs, has developed in some cases. For instance, resistance to the lipopeptide, daptomycin among clinical isolates of Enterococcus faecium has already been detected (Pankey et al., 2005). This is despite the fact that the drug was first licensed in 2003 (Norrby et al., 2005). It has been observed by several studies that antibiotic combinations can have synergistic benefits and interactions between existing antibiotics (Bayer et al., 1980; Hooton et al., 1984; Cottagnoud et al., 2000; Hallander et al., 1982). Several current therapeutic regimes are based on synergistic interactions between antibiotics with different target sites. As new antimicrobial compounds are discovered, there is need to assess their potentials in combination therapies with old antibiotics that have been rendered ineffective by the development of resistant strains, even when such compounds are not directly evidently inhibitory. Taylor et al., (2002) suggested that the use of agents that do not kill pathogenic bacteria but modify them to produce a phenotype that is susceptible to the antibiotic could be an alternative approach to the treatment of infectious disease. Such agents could render the pathogen susceptible to a previously ineffective antibiotic, and because the modifying agent applies little or no direct selective pressure, this concept could slow down or prevent the emergence of resistant genotypes. The inhibition of resistance expression approach was successfully used in the production of Augmentin, a combination of amoxycillin and clavulanic acid (Reading and Cole, 1977). In this case, clavulanic acid is an inhibitor of class-A -lactamases which is coadministered with amoxicillin. The combination has been used clinically since the late 1970s (Neu et al., 1993). A similar approach can be used for target-modifying enzymes and for efflux systems. A number of in vitro studies have reported the use of plant extracts in combination with antibiotics, with significant reduction in the MICs of the antibiotics against some resistant strains (Al-hebshi et al., 2006; Darwish et al., 2002; Betoni et al., 2006). The curative effect of plant extracts in this combination study has been variably referred to as resistance modifying/modulating activity (Gibbons, 2004). This ability of plant extracts to potentiate antibiotics has not been well explained. It is speculated that inhibition of drug efflux, and alternative mechanisms of action could be responsible for the synergistic interactions between plant extracts and antibiotics (Lewis and Ausubel, 2006; Zhao et al., 2001).

1.4.1 Efflux pump inhibition in combination with antibiotics as a strategy for overcoming resistance

The discovery and development of clinically useful Efflux Pump Inhibitors (EPIs) that decrease the effectiveness of efflux pumps represents a significant advance in the development of therapeutic regimes for the treatment of MDR-related conditions. This approach termed the EPI strategy (Lomovskaya and Bostian, 2006), is based on blocking the activity of the pumps, resulting in the accumulation of the antibiotic inside the bacterial cell, consequently increasing access to its target sites. In addition, this will lead to increased susceptibility of the bacterium, thus implying that the therapeutic effect of the drug is achieved with low concentrations. Combining broad spectrum efflux pump inhibitors with current drugs that are pump substrates can recover clinically relevant activity of those compounds and thus may provide new dimensions to the ever increasing need for development of new antimicrobial agents (Kaatz, 2012). This approach will in addition lead to the preservation and

improvement of the usefulness of old and cheap antibacterial agents. Ultimately this could reduce the appearance and spread of resistant mutants (Kaatz, 2012).

1.4.2 Multiple targets and mutual interference strategies

A combination of antimicrobials with different target sites and mechanisms of action can be beneficial in reducing resistance development. The likelihood that a pathogen could simultaneously develop resistance against more than one drug is low (Dryselius et al., 2005). Other combinations may involve antibiotics and other compounds that are not antimicrobial but can enhance the activity of the antibiotics. Combinations between antibiotics and known or new antimicrobial compounds might uncover some beneficial potential that might be useful in curbing resistance to antibiotics. Some drug formulations in current use are already based on the concept of dual targets or mutual interference (Rossolini and Mantengoli, 2005). For instance, the combination of trimethoprim and sulphamethoxazole, (cotrimoxazole) involves a mutual interference of two sequential steps in the bacterial folate biosynthesis pathway. Sulphamethoxazole competitively inhibits bacterial dihydropteroate synthetase, an enzyme involved in the first step in the reaction leading to folic acid synthesis. Trimethoprim, inhibits the enzyme dihydrofolate reductase, involved in the next step in the folic acid pathway (Jerry and Smilack, 1999). Beta-lactamase inhibitors, clavulanic acid and sulbactam have been used to enhance the activity of beta lactam antibiotics against beta lactamase producing organisms (Moosdeen et al., 1988; Maddux, 1991). The synergy between epigallocatechin gallate (EGCg) in tea catechins (the main compounds responsible for the antimicrobial activity of tea) and oxacillin observed by Zhao et al. (2001) was attributed to the combined action of EGCg and Oxacillin on the biosynthesis of the cell wall thereby bypassing the resistance mechanism resulting from the reduced affinity of Penicillin Binding Proteins (PBP) to Oxacillin.

1.5 Plants as Sources of new Antimicrobials and Resistance Modifying Agents

Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being (Iwu et al., 1999). Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent (Betoni et al., 2006; Shibata et al., 2005). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Lewis and Ausubel, 2006; Cowan, 1999). Literature is awash with compounds that have been isolated from a variety of medicinal plants. Despite this abundant literature on the antimicrobial properties of plant extracts, none of the plant derived chemicals have successfully been exploited for clinical use as antibiotics (Gibbons, 2004). A significant part of the chemical diversity produced by plants is thought to protect plants against microbial pathogens. Gibbons (2004), observes that a number of plant compounds often classified as antimicrobial produce MIC ranges greater than 1,000 µg/ml which are of no relevance from a clinical perspective. Tegos et al. (2002) suggests that a vast majority of plant compounds showing little in vitro antibacterial activity are not antimicrobial but are regulatory compounds playing an indirect role in the plant defense against microbial infections. The observation that plant derived compounds are generally weak compared to bacterial or fungal produced antibiotics and that these compounds often show considerable activity against gram-positive bacteria than gram-negative species has been made by many (Nostro et al., 2000; Gibbons, 2004). This led to Tegos et al. (2002) hypothesizing that; Plants produce compounds that can be effective antimicrobials if they find their way into the cell of the pathogen especially across the double membrane barrier of Gram negative bacteria. Production of efflux pump inhibitors by the plant would be one way to ensure delivery of the antimicrobial compound. This hypothesis has been supported by the findings of Stermitz et al. (2000 a,b), who observed that Berberis plants which produce the antimicrobial compound, berberine, also make the MDR inhibitors 5-methoxyhydnocarpin D (5-MHC-D) and pheophorbide A. The MDR inhibitors facilitated the penetration of berberine into a model gram-positive bacterium, S. aureus. In testing their hypothesis, Tegos et al. (2002), showed that two MDR inhibitors (INF271 and MC207110) dramatically increased the effectiveness of thirteen putative plant antimicrobial compounds against gram-negative and gram positive bacteria including isolates known to express efflux pumps.

1.5.1 Resistance modifying activities of plants crude extracts: the basis for isolation of potentially useful compounds

If the isolation of resistance modifying compounds from plants is to be realistic, screening for such activities in crude extracts is the first step in identifying leads for isolation of such compounds, and some plants have provided good indications of these potentials for use in combination with antimicrobial therapy. Typical examples are as follows: Aqueous extracts of tea (Camellia sinensis) have been shown to reverse methicillin resistance in MRSA and also, to some extent, penicillin resistance in betalactamase-producing Staphylococcus aureus (Stapleton et al., 2004). Forty to one hundred fold dilutions of tea extracts was able to reduce the MICs of high- level resistant MRSA (256 µg/ml) to less than 0.12 µg/ml for methicillin and penicillin (Yam et al., 1998; Stapleton et al., 2004). Aqueous crude khat (Catha edulis) extracts of Yemen showed varying antibacterial activities with a range of 5-20 mg/ml -1 against periodontal bacteria when tested in isolation. Addition of the

extracts at a sub- MIC (5 mg/ml) resulted in a 2 to 4-folds potentiation of tetracycline against resistant strains Streptococcus sanguis TH-13, Streptococcus oralis SH-2, and Fusobacterium nucleatum (Al-hebshi et al., 2006). Betoni et al. (2006), observed synergistic interactions between extracts of guaco (Mikania glomerata), guava (Psidium guajava), clove (Syzygium aromaticum), garlic (Allium sativum), lemongrass (Cymbopogon citratus), ginger (Zingiber officinale), carqueja (Baccharis trimera), and mint (Mentha Pieria) from Brazil and some antibiotics which represented inhibitors of protein synthesis, cell wall synthesis, nucleic acid synthesis and folic acid synthesis against Staphylococcus aureus. Darwish et al., (2002) reported that sub-inhibitory levels (200 µgml −1) of methanolic extracts of some Jordanian plants showed synergistic interactions in combination with chloramphenicol, gentamicin, erythromycin and penicillin G against resistant and sensitive S. aureus. The methanolic extract of Punica granatum (PGME) showed synergistic interactions with chloramphenicol, gentamicin, ampicillin, tetracycline, and oxacillin. The bactericidal activity of the combination of PGME (0.1×MIC) with ampicillin (0.5×MIC) by time-kill assays, reduced cell viability by 99.9 and 72.5% in MSSA and MRSA populations, respectively (Braga et al., 2005). The ethanol extracts of the Chinese plants, Isatis tinctoria and Scutellaria baicalensis in combination with ciprofloxacin had synergistic activities against antibiotic resistant S. aureus (Yang et al., 2005).

The combinations of pencillin with ethanolic extracts of Paederia scandens and Taraxacun monlicum showed a strong bactericidal activity on two strains of S. aureus (Yang et al., 2005). When Ciprofloxacin was incorporated at sub-inhibitory concentrations (1/8MIC) to the crude chloroform extracts of Jatropha elliptica and the mixture assayed against NorA expressing S. aureus, the activity of the extract was enhanced. This suggests the presence of an inhibitor of the pump which could restore the activity of Ciprofloxacin (Marquez et al., 2005). In another study, Ahmad and Aqil, (2006) observed that crude extracts of Indian medicinal plants, Acorus calamus, Hemidesmus indicus, Holarrhena antidysenterica and Plumbago zeylanica showed synergistic interactions with tetracycline and ciprofloxacin against Extended Spectrum -lactamase (ESL), producing multidrug-resistant enteric bacteria with ciprofloxacin showing more synergy with the extracts than tetracycline. from a variety of medicinal plants. Some of the compounds which have been observed to have direct antimicrobial activity have also been shown to be potentiate against the activity of antibiotics when used at low MIC levels.

1.6 Future Direction

While there is an abundance of published data validating the antimicrobial activity of medicinal plants commonly used in folk medicine, this has not resulted in the identification of commercially exploitable plant derived antibacterial agents (Lewis and Ausubel, 2006). The majority of plant derived antimicrobial compounds generally have higher MICs than bacterial or fungal produced antibiotics, thus limiting their therapeutic potential (Gibbons, 2004). The findings of Tegos et al. (2002) have provided a foundation for a rationale on the potential actions of plant derived antimicrobial compounds and other compounds with no intrinsic antimicrobial value. It has already been established that crude extracts of some medicinal plants and some pure compounds from such plants can potentiate the activity of antibiotics in vitro (Marquez et al., 2005; Smith et al., 2007). This search for antibiotic resistance modulators in plants represents a new dimension to addressing the problem of antibiotic resistance. The chemical diversity available in plants still remains largely uninvestigated for potentials in improving the clinical efficacy of antibiotics. Most interestingly are medicinal plants and food plants which are inadvertently used with antibiotics in common community practices providing opportunities for interactions. As many medicinal plants still remain unexplored, there are enormous opportunities for the discovery of novel resistance modifying compounds of plant origins. Screening of antibiotic resistance modifying compounds from plants sources are expected to provide the basis for identifying leads for the isolation of therapeutically useful compounds. This could in future be followed by in vivo assessments to determine the clinical relevance of such compounds. This represents a potential area of future investigation.

1.7 Conclusion

The quest for solutions to the global problem of antibiotic resistance in pathogenic bacteria has often focused on the isolation and characterization of new antimicrobial compounds from a variety of sources including medicinal plants. This has seen several medicinal plants being screened for antimicrobial activities. Investigations into the mechanisms of bacterial resistance have revealed that active efflux plays a significant role in the development of bacterial acquired and intrinsic resistance. Overcoming efflux has therefore been seen as an attractive alternative to circumventing the problem. Bacterial efflux pump inhibitors have since been isolated from some plants. The combination of such MDR inhibitors with antibiotics in vitro has shown that the activities of some antibiotics can be dramatically increased even against antibiotic resistant strains of bacteria. The large varieties of compounds produced by plants have proved to have therapeutic potentials as antimicrobials and as resistance modifiers.

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