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Review

Updates on microbial resistance to drugs

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The treatment of bacterial infections and other microbial infections is increasingly complicated by the ability of bacteria and other microbes to develop resistance to antimicrobial agents. Microbial resistance to antimicrobial agents or chemotherapeutic agents had been attributed to their ability to bypass or overcome the various mechanisms by which the said antimicrobial agents exhibit their activities against them. These mechanisms include interference with cell wall synthesis (for example, β-lactams and glycopeptide agents), inhibition of protein synthesis (macrolides and tetracyclines), interference with nucleic acid synthesis (fluoroquinolones and rifampin), inhibition of a metabolic pathway (trimethoprim-sulfamethoxazole), and disruption of bacterial membrane structure (polymyxins and daptomycin). This is because antibiotics exhibit their activities on microbes through any of these methods. Bacteria may be intrinsically resistant to first- class of antimicrobial agents, or may acquire resistance by *de novo* mutation or via the acquisition of resistance genes from other organisms. Acquired resistance genes confer on bacteria the ability to carry out any of the mechanism of resistance earlier stated. Acquisition of new genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria may occur through conjugation, transformation, or transduction, with transposons often facilitating the incorporation of the multiple resistance genes into the host's genome or plasmid. Similarly, several cases of resistance to antifungal agents had been identified in clinical cases. The persistence nature of resistance to chemotherapeutic agents in both fungi and bacteria stimulates this write up. This paper tries to analyze the various mechanisms by which microorganism develop resistance in recent time and suggest the alternative solution such as the synergy between prebiotics and probiotics and the use of phytomedicine to combat this problem; including public health measures.

Key words: Prebiotics, phytomedicine, fungi, bacteria, resistance.

INTRODUCTION

Microorganisms remain the major enemy of humans, animal and plants. Microbial infections is a serious menace, which continuously engage man, animals, and plants in a continuous war. Microorganisms are responsible for most of the known diseases in the world. The presence of microorganisms in various disease conditions was first identified with bubonic plague, about 200 million years ago. Subsequently, several other microbial infections such as tuberculosis, gonorrhea, malaria, and the most recent human immune deficiency virus acquired immune deficiency syndrome, have been identified. These diseases have implicated a number deaths and morbidity in man, animal and plant (Tenover, 2006). The increasing death toll arising from microbial infection had remained a major concern since its identification in the health sector, particularly during the period of gonorrhea in the early century.

However, the discovery of antibiotics in the early 20th century and other means of infection control helped to turn down the course of event with respect to the health of man and animals. This development however, barely

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managed to survive, when resistance to the said antimicrobial agent were discovered; with the emergence of Methicillin resistant strains of *Staphylococcus aureus*. From this period several cases of microbial resistance have been reported and are being recorded in clinical practices from day to day among various species of bacteria and other microbes.The continuous emergence of microorganisms to the available methods of controls for microbial infections is a serious threat to public health, with an impending danger of the emergence of some disease conditions that may defy the available remedy in the near future even though there have been records of such in recent times. This observation may account for the increase in morbidity and mortality witnessed in the health sector in recent time. It may also lead to an increase in the cost of treatment. Having identified the danger associated with microbial resistance, scientists had been involved in various research activities with the aim of finding solution to remedy this long lasting enemy of health (Gerard, 2005). Some of such activities include the development of new drugs that are thought efficient to combat this problem, synergistic use of antibiotics, development of vaccine and the like. This paper therefore examined the current updates on the mechanism of microbial resistance and the possible means of combating this problem.

CAUSES OF MICROBIAL RESISTANCE AND PROBLEMS ASSOCIATED WITH RESISTANCE IN MICROBES

The uncontrollable use of antibiotics in livestock contributes significantly to the high incidence of microbial resistance being witnessed in antibiotics. This indiscriminate use of antibiotics and antifungal agents in livestock often stimulate the microbial floras in their guts to develop resistance to such antimicrobial agents. Hence, may transfer such ability to other pathogenic species that may find their way into the host, either through conjugation transformation or transduction. Such pathogen when they get to human host may pose a serious threat to administered drugs. Another major reason for the high incidence of resistance is the indiscriminate use of antibiotics among the people in the community.This haphazard use of antibiotics often encourages microbes to develop resistance andalso transfer this ability to other groups of microorganism either by conjugation, transformation, or transduction.

The incidence of microbial resistance remains a serious menace to the public health in most part of the world as it has led to the discovery of some diseases conditions which have defied clinical chemotherapy. It is also responsible for the emergence of chronic cases from infections which were initially considered less harmful. A recent report from the European Union revealed that a new strain of *Gonococcus* resistance to almost antibiotics was isolated from a clinical condition. This is thus sending a warning signal to the treatment of infections globally. The presence of resistant among microbes in the community may not pose any significant threat but it has been found to have the possibility of causing serious infection problem to the health care sector (Gerard, 2005). Although, MRSA isolated from community acquired infection have been identified to show less resistant to anti-microbial agent they have the possibilities of producing clinically serious toxins such as Panton–Valentine leukocidin, which may be spread through horizontal gene transfer among pathogens (Fred, 2006).

Microbial resistance is a subject of hassle to physicians, health workers, and scientist, due to the continuous emergence of resistance species of micro-organisms, being isolated in clinical infectious cases. For Instance, several resistant species of bacteria particularly members of the *Staphylococci, Enterococci*, *Klebsiella pneumoniae*, and *Pseudomonas* spp, is now a common phenomenon in healthcare institutions (Chamber, 2001; Francis et al., 2005; Leslie et al., 1988; Mahmound and Luis, 1999). Several species of these microorganisms have been isolated from one clinical condition or the other.There have been several reports associated with treatment failure due to the presence of resistant species or strain of a particular microorganism in such clinical condition.A very good example of this had been reported in the following bacteria *Pseudomonas aeruginosa*, *S. aureus*, *K. pneumoniae*, *E. coli*, *Enterobacter* spp, coagulase-negative staphylococci, and enterococci (Julian, 1979; Krause, 2004). Resistant may not only result in treatment failure but also spread among normal microbial flora in the affected patient resulting in a wider or broader infection problem, such as is found in MRSA *Staphylococci isolated* from community related infections and extended spectrum β-lactamase (ESBL)–producing *E. coli* (Chamber, 2001; Rouviex, 2007; Wiese et al., 1999).

CHEMOTHERAPEUTIC AGENTS OR ANTIBIOTICS

Chemotherapeutic agent is any substance derived from microorganism, either naturally, semi-synthetic modification of microbial metabolite, or chemical synthesis of already known antibiotics which have the capability or tendency to inhibit microbial growth or kill an organism causing infection but causes little or no harm to the host. These agents can be obtained from microbial metabolites or the modification of such metabolite either synthetically or semi-synthetically. Chemotherapeutic agentscould be an antibacterial agent or antibiotics, antifungal agents, antiviral agents, and drugs active against protozoan. Their mode of administration could be systemic or topical.

Antibiotics are more effective against actively growing bacteria, than non-growing persisters or spores. When two antibiotics are used in combination, their effect could be additive, synergistic, or antagonistic. Antibiotics could

be broad-spectrum and narrow-spectrum; For instance, Tetracycline, a broad spectrum antibiotic, is active against both Gram-positive and Gram-negative bacteria, and even against mycobacteria; whereas penicillin, which has a relatively narrow spectrum, can be used mainly against G- positive bacteria. Other antibiotics, such as Pyrazinamide, have an even narrower spectrum, and can be used only against *Mycobacterium tuberculosis*. Antibiotics employ various mechanisms to carry out their activities against microorganisms.These mechanisms include (i) cell wall inhibition, for example, Penicillin and Vancomycin, (ii)Inhibition of nucleic acid synthesis, for example, Fluoroquinolones, which inhibits DNA synthesis, and Rifampin, which inhibits RNA synthesis, (iii)Protein synthesis inhibitors, such as Aminoglycoside, (iv) Anti-metabolites, such as the sulfa drugs, Antibiotics that can damage the membrane of the cell, such as Polymyxin B, Gramicidin and Daptomycin.

MICROBIAL RESISTANCE

Antimicrobial resistance is the reduction in the susceptibility of pathogenic microorganism to one or more of the chemotherapeutic agents administered in clinical medicine. It is usually manifested by the failure of drugs to cure an initially susceptible disease. Microbial resistance is as old as the advent of antimicrobial agents; Sir Alexander Fleming reported in the New York Times of 1945 those microorganisms that are trained to resist penicillin, following the discovery of Methicillin resistant strains of *S. aureus*. Since the discovery of the Methicillin resistant strains of *S. aureus,* microbial resistance had been a continuous occurrence both in the hospital and the community. Resistance in microorganisms could be intrinsic or acquired.

Mechanism of resistance in microorganism

The ability of microorganism to resist antibiotics could be natural (that is, intrinsic, in which case, it is develop spontaneously) or acquired (this is usually due to mutation).

Acquired resistance usually takes place suddenly in microorganisms. It involves chromosomal mutation which may be due to microbial ability to acquire certain transferable gene such as plasmid, transposon, and integrons which confer on them resistance to antimicrobial agents. Once a microorganism had acquired resistance to a particular drug, it is possible for such ability to be transferred to other organism via either one of horizontal gene transfer system or vertical gene transfer method.

Vertical gene transfer occurs when an offspring acquire resistance from its parent. This ability to resist antibiotics in the parent may be due to chromosomal mutation or natural selection. Horizontal gene transfer involves such genetic process such as conjugation, transformation and transduction. Horizontal gene transfer is the medium through which resistant genes are transferred from an organism which had acquired resistance to an antibiotics either spontaneously or through induced mutations, to another that is not of the same parent (Oliver et al., 2000). A good example for this was found in *E. coli* which transferred β- lactamases resistant gene to *Haemophillus influenza* through a horizontal gene transfer process.

Conjugation

This is a process of unilateral transfer of genetic material among bacteria of the same or different species. It involves direct cell to cell contact among bacteria. It involves the transfer of R-plasmid through a cytoplasmic bridge from a donor cell to a recipient cell. It is mediated by a plasmid or a transposon. It is a common phenomenon among different genera of Gram- negative bacteria. Transfer of plasmid also occurs among Gram positive bacteria. A single plasmid can confer resistance on bacteria to a wide variety of antibiotics simultaneously that is, cross resistance.

Transformation

Transformation is a process by which bacteria pick up pieces of a naked DNA from an environment and add them up to their own chromosome. The process may occur spontaneously in which the bacterial chromosome leak out from the donor cell to the recipient cell or it may occur by an artificial means as a result of extraction by chemical procedures. It may also be due to cellular break after lysis by bacteriophages. Transfer only takes place between competent cells. Transfer occurs only in bacteria and it is commonly found in Gram positive bacteria which are capable of taking up high molecular weight DNA from the aqueous environment. Many bacteria species commonly Gram positive *Haemophilus* spp, *Bacillus* spp *and E. coli are* capable of acquiring resistance by this method.

Transduction

Transduction is a process of genetic transfer in which a small portion of the DNA is transferred from a donor cell to the recipient cell through temperate bacteriophages. The phage which acts as the agent of genetic transfer becomes integrated in the genome of the donor cell, replicating along with the host genome. It also transcribed at the expense of the host genome. Once this has occurred, the phage DNA is passed along to the daughter cell alongside the mother cell. Under stressed condition,

either by heat or chemical the phage may be lysed loosing part of its DNA to the host cell or carry part of the host chromosome upon excision. Subsequent, infection of another bacterium by the phage which had just been lysed, from the other host and carrying the additional DNA picked up from the host may influence the introduction of the former DNA segment to its new host. This process is known as transduction. It occurs in bacterial genera such as *Salmonella, Shigella, Staphylococcus, Pseudomonas, Vibrio, Proteus, Escherichia,* and *Bacillus*.

Transposition

The discovery of transposons (or transposable element) often referred to as jumping gene has provided explanation for frequent occurrence of drug resistance in bacteria. Transposons are units of DNA that move from one molecule of DNA to another, inserting themselves nearly at random (Pelczar et.al., 1993). Transposons transfer is not restricted like plasmid which is based on close relatedness of host genome. It can insert between one plasmid and another, or between a plasmid and a portion of bacterial chromosome within a bacteria cell without reliance on DNA sequence homology.Complex transposons usually have genes that code for many antibiotics and it is their activities that have resulted in R-Plasmid with resistance markers to the antibiotics (Russell, 2004).

Integrons

Integrons are gene capture system found in plasmids, chromosomes and transposons. They recongnise and capture multiple gene cassettes. A gene cassette may encode genes for antibiotic resistance, although, most genes in integrons are uncharacterized. Therefore, integrons have been identified as a primary source of resistant genes within microbial population and were suspected to serve as reservoir of antimicrobial resistance gene within microbial population (Xu et al., 2009). The cassette has a specific recombination sites that confer mobility because it is recognized by recombinase encoded by the integrons that catalyses its integration into specific site within the integrons (Smith, 2004).

Four classes of integrons have been identified with only one member of class 3 described and class 4 integrons are limited to *Vibrio cholera* (Smith**,** 2004).The class 1 is found in both Gram-negative and Gram- positive bacteria while the class 2 integrons are frequently associated with the members of the family of *Enterobacteriaceae* such as *Salmonella*, *E. coli*. The same class 2 was recently detected in *P. aeruginosa* in which multi- drug resistant rates of integrons positive and negative strains were reported as 93.2 and 18.2% respectively (Xu et al., 2009).

Plasmids

Plasmids also known as R- factor are extra chromosomal factor which confer resistance on bacteria to antibiotics. Plasmids are like chromosome because they are capable of independent replication but they differ from bacterial chromosome because they are smaller with an approximate size of between 0.1 to 10% of the chromosome in bacteria. Plasmids do not play any role in the normal functioning of bacteria cell but may confer on the bacteria character that can give the bacteria some survival advantage in certain condition such as the ability to survive in an unfavourable condition like in the presence of an antibiotic. For example the ability of *S. aureus* to resist the activity of Methicillin is associated with the presence of a beta lactamase plasmid which made this possible. Similarly, an R- factor, pSH6, found in *S. aureus* encodes resistance to gentamicin, trimethoprim and Kanamycin (Lambert, 2004). Plasmid may also code for other properties in bacteria such as the ability to produce toxin, utilize or ferment unusual sugar or food source for example, camphor, production of pili for the attachment of a cell to substrate (for example, intestinal epithelial).

Biochemical basis of resistance

Changes in target site

Most antibiotics exhibit their activities on microorganisms by acting on a particular site on the microbes referred to as the drug target site on the microorganisms. These sites are usually enzymes on the microbes. The inhibitors that is, the antibiotics usually have a high affinity for the sites on the microbes and can out-compete any other substrate that may also depend on this site. However, if this site is modified or altered it may lead to reduced or loss of affinity for the antibiotics and the bacteria with such altered site becomes resistant to the inhibitor. This kind of resistance has been found in some organisms that showed resistant to sulphonamides.

Sometimes it is the target proteins in the microbial cell that are altered. The altered penicillin binding protein in the cell wall of a particular strain of *S. aureus* confer on it resistance to β-lactam and on *S. pneumoniae* resistance to penicillin. Alteration of target site had also been identified as the biochemical basis for the resistance in *Enterococcus species* to Aminoglycosides; in this group of bacteria, the ribosomal binding site has been altered. A number of target sites can be affected by mutation, for example, ribosome, RNA polymerases, penicillin-binding proteins and enzymes such as dihydropteroate synthase. These sites are known as the receptor site of the

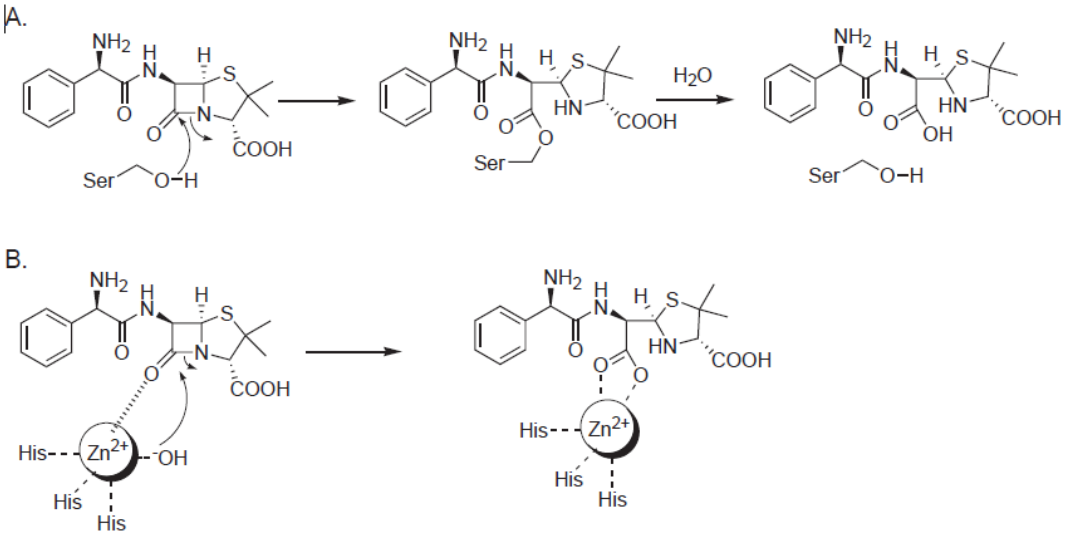

Figure 1. The hydrolysis of the penicillin amoxicillin is shown to be catalyzed by Ser-b-lactamase (A) and a metallo-b-lactamase (B). Source: Adapted from Wright (2005).

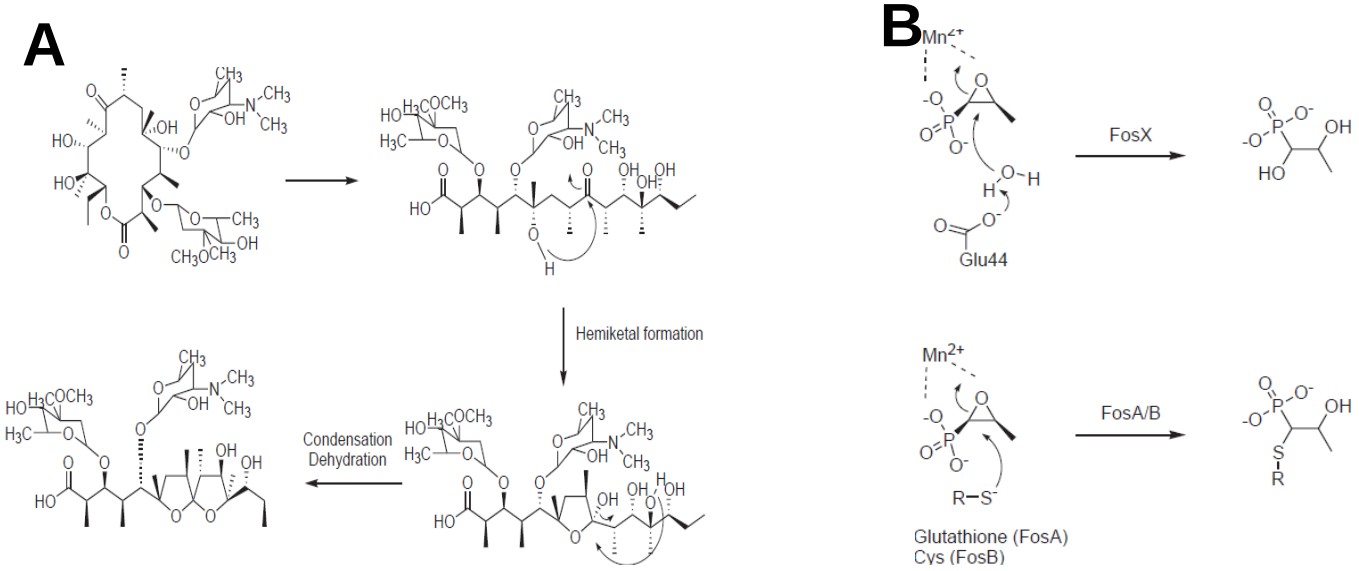

Figure 2. The equations above represent the ring opening attempt catalyzed by fosfomycin resistant enzyme. Source: Wright (2008).

microorganism and are usually protein molecule.

The mycolic nature of the microbial cell wall and the phospholipid bilayer of the cell membrane are such that they pose tough obstruction to the ingress of materials into the microbial cell. However, the nutrient required by the cell for its growth are all located outside the cell, and therefore the cell must device a means by which these molecules get into the cell. Microbes have developed various mechanisms for the transport of these materials some of which include facilitated diffusion, and active transport. These mechanisms are responsible for the transport of antimicrobial agents into the cell (Cloutier, 1995). The ability of materials to move across the cell membrane of bacteria for example is largely controlled by the presence of small water filled channels(porins) or opening (Non-water filled porins) within the phospholipids' bilayer of the bacteria cell. Mutation in any of the genes responsible for these porins or opening on the cell membrane could affect the influx of material into the microbial cell (Micheal, 1995) for example Neisseria gonorrhea porin may acquire mutation and become resistant to penicillin and Tetracycline. Enterobacter

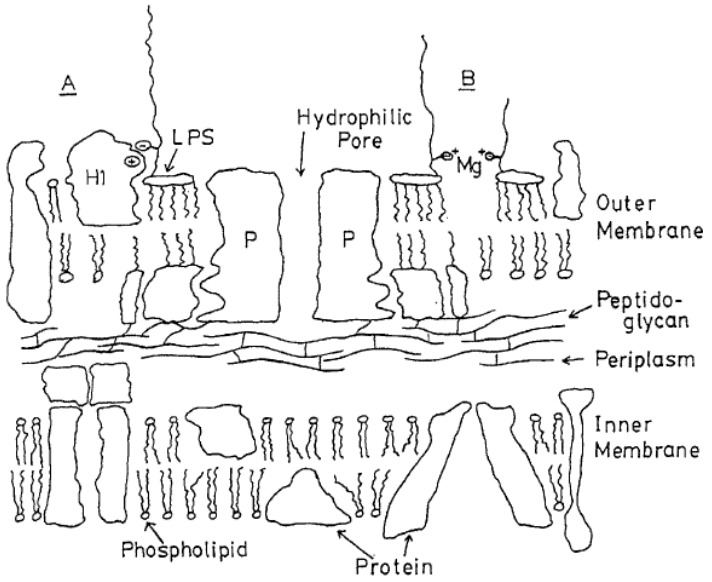

Figure 4. Schematic representation of cross section of the cell envelope of Gram-ve bacteria P rep porin protein involved in the uptake of hydrophilic antibiotics. LPS represents lipopolysaccharide, I represents other membrane protein. A represent site at which uptake is block by protein HI in *Pseudomonas aeruginosa*. B represent site at which poly cation and chelators can displace divalent cation from LPS resulting in self-promoted uptake: Alteration in the nature of B site, for example, reduction of in the affinity of LPS divalent cation might result in non porin pathway for antibiotic that are poly cation (including hydrophobic antibiotics). Source: Hancock and Bell (1986).

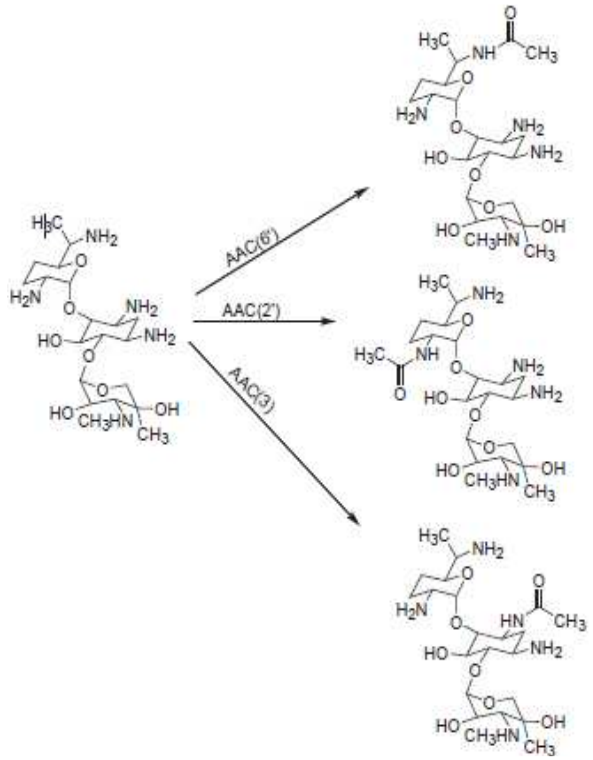

Figure 4. Source: Wright (2005).

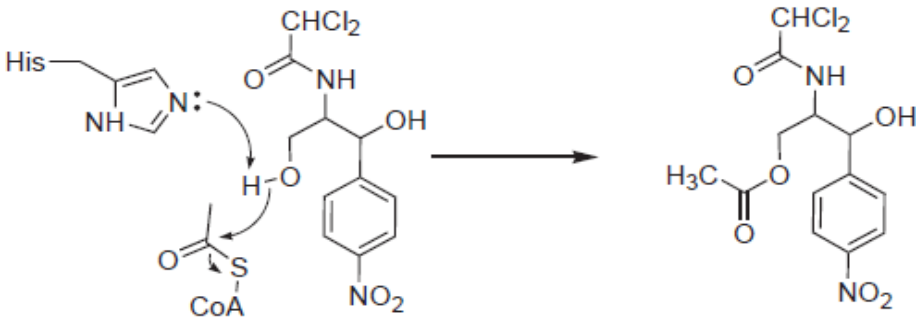

Figure 5. Mechanism of chloramphenicol acetylation by chloramphenicol acetyl transferases. Source: Wright (2005).

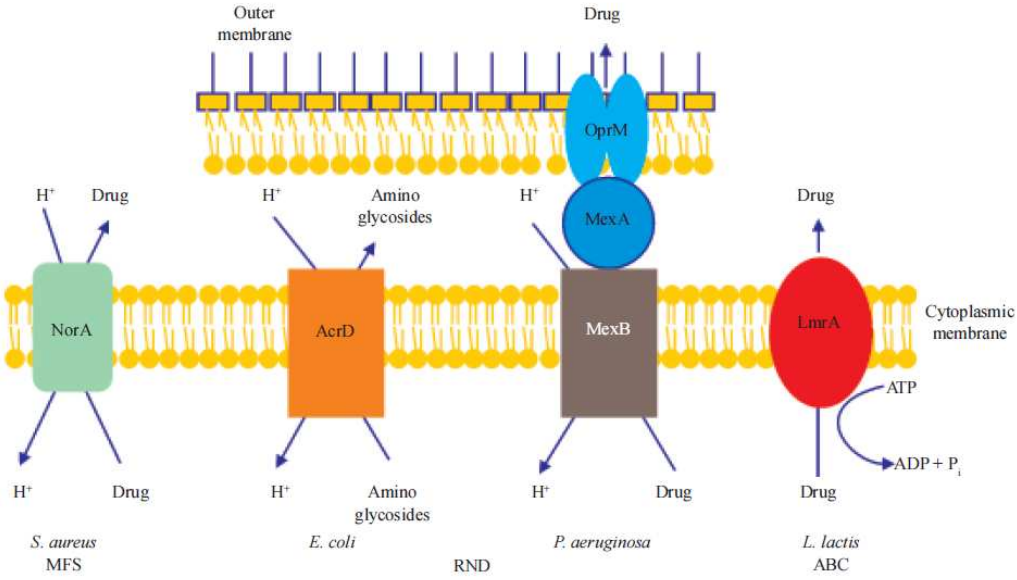

Figure 6. Source: Schweizer (2003).

aerogene has also been found to exhibit resistance to Cephalosporine as a result of the ability of their porin to acquire mutation. Mutation in any of the gene responsible for the protein present on cell membrane surface could result in their loss hence resistance due to the absence of receptor site. This mutation may be plasmid such that the energy rich ATP required for the synthesis of adenosine 5'-triphosphate is affected and in turn the transport of the antimicrobial agent across the cell membrane of the bacteria is impeded. This is a common mechanism among Gram-negative bacteria; they possess an outer membrane consisting of an inner layer phospholipid, and outer layer containing lipid, (A moiety of lipopolysaccharide) (Davies, 2007). The composition of this outer membrane enables it to slow down rate of antibiotics influx to the cell (Davies, 2007). The chemical composition of a drug will greatly influence its movement into the cell; antimicrobial agents that are hydrophobic in nature will move with a greater ease into the cell than those with hydrophilic characteristic because of its close nature with the plasma membrane concentration (Russell, 2008). Specialized mechanisms are often employed for the transport of hydrophilic bounded drug; such specialized mechanisms include the presence of porin on the surface of the cell membrane.

Beta lactam antibiotics, chloramphenicol and flouroquinolone for example depend on porins for their movement into the cell (Davies, 2007). Any change in the porin copy number, copy size, will selectivity affect the permeability of the bacteria cell membrane to an antibiotic of this category (Wisse et al., 1999, Vaara et al.,1993).The resistance of *P. aeruginosa*, to Imipenem has been linked with the lack of the specific D2 porin on the surface of their cell membrane. Imipenem therefore

cannot penetrate the cell of this microorganism (Peter, 1998) (Figure 4).

Reduction in cellular permeability

There is a need for antibiotics to overcome certain mechanical barrier presented by the host cell membrane before they can elicit their activities. Mutants in bacteria have been identified to produce protective coat in the bacteria which reduce or inhibit the cellular uptake of antibiotics such as aminoglycosides and beta-lactams, chloramphenicol, bacitracin, and Isoniazid. Decreased uptake of flouroquinolone compound has been found associated with the changes in the porin protein of the bacterial cell membrane.

Conversion of active drug into inert products

Some bacteria develop resistance to antibiotics by producing enzymes which inactivates the antibiotics of choice either by:

1. Destroying the antibiotics in use: These enzymes act by breaking one or more molecular covalent bonds in the antibiotics molecule which are central to the activities of the antibiotics. This mechanism is typical of Staph aureus which produces beta lactamases that breaks the bond of the beta lactam ring in penicillin and Cephalosporine rendering the drug inactive. It is responsible for the resistance in staphylococci to Benzyl Penicillin. The presence of β- lactamase gene on plasmid which can be exchanged among many bacterial species has conferred penicillin resistance on many non- beta- lactamase producing which were initially susceptible to such penicillins. Some Gram-negative bacteria such as *Serratia, Enterobacter,* and *Pseudomonas* have developed resistance to penicillin through this mechanism.

2. Inactivating an active product may be by the inhibition of a vital moiety in the antibiotics: Most Gram-negative bacteria usually inactivate antibiotics by producing phosphorylating and adenylating enzymes which destroys the activity of the antibiotics. Resistance to chloramphenicol is observed in bacteria that produce chloramphenicol acetyl transferases, which acts in the presence of acetyl Co A to catalyze the acetylation of 3'-OH group in the chloramphenicol molecule. The compound formed from the catalysis is inactive. Example of this can be found in the enzymatic inactivation of various drugs such as discussed thus:

3. Enzymatic inactivation: This refers to an enzyme mediated in inactivation of antimicrobial agents such that the active ingredient acting on the organisms had been deleted or deactivated, hence the loss of activity by the antimicrobial agents (Wright, 2002). Several organisms with this mechanism of resistance have been isolated from clinical conditions. Some strains of *S. aureus* with this ability have been isolated from clinical conditions. Enzymatic inactivation of drug could involve any of these under-listed strategies; hydrolysis, group transfer, and redox transfer.

Hydrolysis

In hydrolysis, microorganisms usually inactivate antibiotics through hydrolysis by cleaving the ester and amides group attached to their rings.The nature of these bonds determines the effectiveness and the biological activities of these antibiotics.Various enzymes with these properties have been isolated from both Gram-positive and Gram-negative bacteria (Wright, 2005). Enzymes in this category are the Amidases (for example, beta lactamase that cleave the beta lactam ring of penicillin and Cephalosporin class of drugs), Esterases, and Epoxidases (which is responsible for ring opening in fosfomycin thus, conferring resistance to fosfomycin). These enzymes require only water as co substrate for its activities. In the presence of Water, microorganisms can release these enzymes and thereby intercept the activity of the antibiotics before they come in contact with the microbes.

Group transfer

Group transfer is another method of inactivation employed by bacteria to resist antibiotics. Group transferases are enzyme in microorganism which inactivate antibiotic through structural modification of the target binding site. These enzymes inactivate the drugs through O - acylation, and N acetylation, O – phosphrylation, O – ribosylation, O – glycosylation and thiol transfer. This is the mechanism employ by many organisms to resist the activities of some drugs such as Amino glycosides (for example, gentamycin, and kanamycin,), and chloramphenicol. Examples of group transferases are Acyl transferases, phosphotransferases, Thiol transferases, nucleotodyl transferases, ADP-ribosyltransferases, and glycosyl transferases.

Increased production of biochemical intermediate

Bacteria may also develop resistance to an antibiotic by increasing the production of a metabolite which competes with the active site of the antibiotics. This mechanism has been described associated with *Staph aureus* resistance to sulphonamide because sulphonamide is an analogue to paraamino-benzoic acid (PABA) in *S. aureus,* which develops resistance to sulphonamide by increasing the

production of PABA that would competitively displace sulphonamide. This unusual increased production of PABA has been found to be due to mutation in the regulatory gene of phosphate biosynthetic pathway.

Enhanced drug efflux mechanism of resistance

Efflux mechanism involves the removal of materials from the microbial cell in an energy dependent process linked to proto-motive force or ATP (Stuart, 1992). This was first identified with the transport of some cationic compounds such as Na^+, K^+ Ca^{2+}. These cations are transported in an energy mediated process linked to the activities of Proto-motive force or ATP (Stuart, 1992). This was later identified as the basis for the resistance to tetracycline by some bacteria species in the early 1970s (Rouveix, 2007). Bacteria with this mechanism have efflux pumps through which they selectively force out specific antibiotics such as macrolides, lincosamides and/or streptogramins and tetracyclines.This mechanism has been described as the basis for the multi-drug resistance witnessed in a mutant strain of *Bacillus subtilis* (Neyfakh et al., 1991).There are five families of bacteria efflux system which have been identified (Putman et al., 2000): they include (i) the small multidrug efflux family (chang and saier, 2001). (ii) Resistance Nodulation division (RNd) efflux family (Zgurskaya and Nikardo, 2000; Schweze, 2003) (iii) The ATP binding cassette efflux family, (Van and Koning, 1998) (iv). The major facilitator family (Pao et al., 1998), (v) The multidrug and Toxic compound extrusion family (Brown et al., 1999). These efflux mechanisms are energy dependent and therefore depend on either proto motive force or ATP (Paulsen et al., 1996).

RND Efflux mechanism has been found to be associated in *Pseudomonas* multi drug resistance behavior (Poole, 2000, Poole and Srikumar, 2001).

Synergy between the outer membrane permeability and the active efflux family plays significant role on the ability of efflux pump to confer resistance. Efflux mediated resistance usually increases the determined MIC of antibiotics (Rouveix, 2007).

Figure 6 shows the main types of efflux pump that can be found in *S. aureus, E. coli, P. aeruginosa, and L. lactis*. The figure illustrates NorA (which is an efflux pump of the major facilitator super family), AcrD and Mex AB-OPRN which are member of the resistance nodulation family (RND)), and Lmr, which is a member of the ATP-binding cassette efflux family (ABC).

Loss of enzyme involve in drug activation

Plasmids are capable of providing a cell with a replacement enzyme, in which the enzyme retains the function of the replaced enzyme but it's structurally different from it. The structural composition of the new cell put the antimicrobial agent into confusion, thus rendering it ineffective while the microbial cell continues with its metabolic activities. This is because the new enzyme or replacement enzyme is not compliant with the antimicrobial agent. An enzyme coded for by the chromosome of the bacteria may be susceptible while the replaced enzyme by the plasmid is resistant. This mechanism of resistant has been found in some bacteria resistant to sulphonamide and Trimethoprim, in which the chromosomal coded dihydropteroate synthetases and dihydrofoliate reductases had been replaced with those coded by plasmid.

Acquired resistance are usually due to alteration in the genetic make-up of the organism, hence, it is said to be a genetic process. Resistance in microorganisms may also be phenotypic. Phenotypic resistance is due to changes in the bacterial physiological state, such as the stationary phase, antibiotic persisters, and the dormant state.

Genetic basis of resistance

Resistance in bacteria can be an intrinsic property of the bacteria or may be acquired. Acquired antibiotic may be due to mutation of cellular genes or the acquisition of foreign resistance genes from other species or the same species, or a combination of these two mechanisms. There are two ways by which microorganisms can acquire resistance to an antimicrobial agent. These are; mutation in different chromosomal loci and horizontal gene transfer (Aminov et al., *2007*).

Mutation could be spontaneous or induced; Spontaneous mutation usually occurs randomly as a replication error or incorrect repair in an actively dividing cell. A point mutation in a particular nucleotide could result in an antibiotic resistance (Woodford and Ellington, 2007). For instance, quinolone resistance phenotype in *E. coli* is due to changes in at least seven positions in the *gyrA* gene, but in only three positions in the *parC* gene (Hooper, 1999; Nakamura et.al., 2001). A variety of gene mutation could confer antibiotic resistance on micro-organism because several metabolites in microorganisms essential for antimicrobial activities on bacteria are products of gene expression.

A large proportion of resistance to antibiotics in bacteria are due to mutation at one stage of their life or another; such as mutations in the sequences of genes encoding the target site required for the binding/activities of some antibiotics. Resistance to rifamicins and fluoroquinolones are caused by mutations in the genes encoding the targets of these two molecules, RpoB and DNA-topoisomerases, respectively (Ruiz 2003; Martinez and Baquerot 2000). The variation in the expression of antibiotic uptake or of the efflux systems may also be modified- owing to gene mutation ([S. D@IDI*et*al]: 2005 Wolter et al., 2004). Some resistances associated with the uptake and efflux systems are caused by mutations in regulatory genes or their promoter regions (Depardieu et

al., 2004; Piddock, 2006). Similarly, mutations leading to increased expression of the efflux systems, may also be due to mutation of regulatory gene; for example, mutation of the *E. coli mar* gene affect the expression of about 60 different genes, including down-regulation of OmpF and up-regulation of AcrAB (Barbosa, 2000). AcrAB is involved in the efflux of b-lactams, fluoroquinolones, chloramphenicol, and tetracycline. In *P. aeruginosa*, mutation in *mexR*up-regulates the mexA-mexB- -oprM operon and raises resistance to most beta lactams, fluoroquinolones, tetracyclines, chloramphenicol and macrolides (Adewoye et al., 2002). The overproduction of antibiotic-inactivating enzymes may also be achieved be due to mutation. Many Gram-negative microorganisms produce chromosomal b-lactamases at low levels and mutations producing up-regulation of their expression may cause resistance to most cephalosporin. Similarly, resistance in some strains of clinically relevant pathogens such as *Mycobacterium tuberculosis* has been identified to be exclusively due to mutation (Rasmasaramy et al., 2000).

In conclusion genotypic resistance may be due to chromosomal mutation in which the bacteria intrinsically acquired resistance from the environment or due to a failure of one or metabolic process. It may howeveron the other hand bedue to an acquired gene from other species of bacteria, which have developed resistant ability and could pass to other organisms.

Phenotypic resistance

Phenotypic resistance is a kind of resistance associated with the behavior of the bacteria or microorganisms. It has to do with their growth pattern; some bacteria resist the activities of antimicrobial agent by remaining in the lag phase or stationary phase of growth. They also develop other mechanisms which reduces their pattern of susceptibility to antimicrobial agent such as the formation of biofilms. Biofilms are colonies of bacteria suspension that have attached themselves to the surfaces through the production of slimming substances which kept them in tact on the surfaces (Costerton et al., 1995). This mechanism of resistance had been found in some species of *M. tuberculosis, S. aureus* and *Staphylococcus epidermidis* (Ying, 2010). Salycylic acid is the active ingredient in Aspirin induced resistance. This has been demonstrated in a mouse model where Aspirin was found to induce resistance to the activity of isoniazide in an *in vitro* experiment. A similar situation was also observed in *E. coli* where Salicylic acid induces resistance by binding to the MarR operon and hence preventing the binding of the *E. coli* to antibiotics. This in turn brings about the suppression of another operon in the *E. coli*; the MarAB which encodes a transcription factor that activates the transcription of efflux pump acrAB as well as, the membrane channel required for the functioning the efflux pump (Ying, 2010).

The phenotypic mechanism of resistance though reversible plays significant influence in the failure of antibiotics in the treatment of microbial infections.

Mechanism of drug resistance to antifungal agent

Antifungal agents has a different mode of action against fungi when compare with antibacterial agents in their activities against bacteria. However in the mechanism of resistance display by fungi, there is a little similarity with those found in bacteria. The mechanisms employed include alteration/modification of target, reduction in the intercellular concentration of target enzyme, over-expression of antifungal drug target, alteration in membrane composition.

Alteration/modification of target

Modification of target: Several lines of evidence implicate a modification in the quantity or quality of 14a-demethylase in the expression of resistance to azole antifungal agents. A recent study examined the biochemical mechanisms for resistance to fluconazole by comparing sterol composition, fluconazole accumulation, and inhibition of 14a-demethylase by fluconazole in two clinical *C.krusei* strains (expressing intrinsic resistance to fluconazole) and a susceptible *C. albicans* isolate (101). No significant differences in the sterol content of *C. krusei* and *C. albicans* were detected (ergosterol was the majorsterol in both species). Studies performed on cell extracts indicatedthat the concentration of fluconazole required to inhibitthe synthesis of ergosterol by 50% was approximately 24-to 46-fold higher in *C. krusei* than in *C. albicans*, suggestingthat affinity of the target enzyme is different in the two species (101). A comparison of fluconazole accumulation by *C. albicans* and *C. krusei* indicated that fluconazole accumulation in the first 60 min was similar in all study strains. However, analysis after 90 min of incubation revealed that *C. krusei* accumulated 60% less fluconazole than did *C. albicans*; implicating active efflux in the fluconazole resistance expressed by these *C. krusei* strains.

The potential coexistence of two resistance mechanisms precludes a precise calculation of the level of resistance contributed by the low-affinity 14a-demethylase.

The mechanism of resistance of fungi has not been fully studied as most the antifungal agent has remain very effective over the years.

DISCUSSION AND CONCLUSION

Bacteria resistance had become a popular phenomenon

in the health care delivery system and it is gaining more attention of the public. There is an increase in the rate of microbial emergence to antibiotics; this in turn had resulted in the emergence of incurable infectious diseases, high cost of health management to those affected, increase morbidity and mortality. The problem is getting worse and treatment options for combating bacteria resistant to multiple drugs are narrowing (Matthew, 2005). Owing to the problem associated with drug resistance several methods had been put in place to combat microbial resistance to drug, particularly at a time like this when drugs are being discovered. These methods are based on thorough knowledge of the mechanism of microbial resistance. These mechanisms include 2) co-inhibitor antibiotics, 3) co administration of antibiotics, 4) the use of probiotics in place of antibiotics, 5) Phage therapy, 6) Inhibition of Efflux pump.

Other strategy to overcome resistance is to improve the delivery or otherwise enhance the accessibility of antibiotics to their sites of action. For example, liposomal preparations of hydrophobic antibiotics such as ethambutol for treatment of mycobacterial infections have been reported.Another approach that has been explored is the linking of two different classes of antibiotics (for example, Beta lactams and quinolones or b-lactams and oxazolidinones).

These examples used stable or labile linking bonds to together the antibiotics, although a clever alternative strategy that harnesses the enzymatic action of b-lactamases to release tethered antibiotics has been reported. For example, a cephalosporin–b-chloro-Ala adduct released the Alaracemase inhibitor h-chloro-Ala upon h-lactamase action, and cephalosporin–triclosanhybrids have been designed to release triclosan upon cleavage of the h-lactam bond by beta-lactamases.

Prudent use of antimicrobial drugs using the appropriate drug and the appropriate dosage and for the appropriate duration is one important means of reducing the selective pressure that helps resistant organisms emerge. The other vital aspect of controlling the spread of multidrug-resistant organisms is providing sufficient personnel and resources for infection control in all healthcare facilities. New antibacterial agents with different mechanisms of action are also needed.

It is also essential that plants product be given adequate consideration in the combat of varieties of microbial infection. A recent study by Idowu *et al* in Nigeria 2011reveals the potency of Carica papaya seed extract against resistant strains of *S. aureus* isolated from wound pus. This shows how significant they can be in combating microbial resistance.

A good Public health measure in combating microbial infection is the practices of good public hygiene, such as hand washing among other factors after every contact with an infected patient or animals. This was identified to reduce the transfer of pathogenic organisms among hospital workers in the US as a result of different contact with fomites (Anderson, 2009).

In conclusion, microbial resistance had done a great deal of harm to adequate health care delivery, and therefore require an adequate attention from both the health care expert and the public involved, Bacteria resistances usually arise as a result of mutation which could be phenotypic or genotypic. The misuse of antibiotics by veterinarian and in animal husbandry also plays significant role in microbial resistance to drugs.

The problem of resistance can better be overcome by having thorough knowledge of the mechanism by which microorganisms resist drugs. It also requires discipline among patient, health care workers;this does not exclude the dairy farmers who continually have encounter with livestock.

Although, there are several strategies which are in place to combat microbial resistance, the continuous emergence of resistance to even the newly developed drugs requires a better method of combating microbial resistance to drugs, such as the development of newer drugs through phytomedication, use of probiotics, and combination therapy. It is more important that there is proper utilization of antimicrobial agents to avoid subsequent resistance to the newly developed drugs, and to be able to combat resistant which already exist.

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