Review on Mechanism of Antimicrobial Drug resistance in Animal and Its Public Health Significance

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Abstracts: Antimicrobials are used in livestock production as therapeutics, prophylactics, and growth promoters. These drugs assist in sustaining livestock production and in controlling bacterial pathogens that may be transferred to humans. Bacteria exhibit a number of well characterized mechanisms of resistance to antimicrobials that include: modification of the antimicrobial; alteration of the drug target; decreased access of drug to target; and implementation of an alternative metabolic pathway not affected by the drug. The mechanisms of resistance are complex and depend on the type of bacterium involved (e.g. Gram–positive or Gram–negative) and the class of drug. Some bacterial species have accumulated resistance to nearly all antimicrobial classes due to a combination of intrinsic and acquired processes. This has and will continue to lead to clinical failures of antimicrobial treatment in both human and animal medicine. The development of resistance can be minimized provided that a number of measures are observed to prolong the useful life of all antibiotics in both human and veterinary medicine. Antibiotic use should be limited to situations where they are needed and the selection of the right antibiotic should take a number of factors into account.

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Introduction

An antimicrobial is an agent that kills bacteria or suppresses their multiplication or growth. Fleming discovered penicillin in 1928 and soon after other classes of antimicrobials were also identified. The first therapeutic use of penicillin however was not until 1940. Consequently, for more than 50 years the world has enjoyed a tremendous decrease in mortality and morbidity from bacterial diseases. Shortly after antibacterial agents were discovered, it was determined that some microorganisms were resistant to their effects (Furuya and Lowy, 2006). During the past decade, the threat of antimicrobial resistance has become increasingly real and its global dimensions have been increasingly recognized. Antimicrobial resistance is a property of bacteria that confers the capacity to inactivate or exclude antibiotics, or a mechanism that blocks the inhibitory or killing effects of antibiotics, leading to survival despite exposure to antimicrobials (Institute of Medicine, 1998).

The intrinsic ability of some organisms to resist antimicrobials was clearly present prior to the clinical use of antimicrobials. In addition, antimicrobial resistance can occur as a result of random genetic mutations in bacteria, leading to variation in susceptibility within any bacterial population. More commonly, resistance is not due to a chromosomal change event, but to the presence of extra chromosomal DNA (plasmid) which was acquired from other bacteria. Use of antimicrobials however, selects for these resistant organisms, leaving them to multiply more freely after the susceptible bacteria have been eliminated. This phenomenon is called selective pressure (D'Costa et al., 2006).

Major factor in the increasing problem of resistance is the overuse, misuse, and injudicious use of antimicrobials use in food animals antimicrobials are used in livestock production as therapeutics, prophylactics, and growth promoters. These drugs assist in sustaining livestock production and in controlling bacterial pathogens that may be transferred to humans (Alexander et al., 2011; Sparo et al., 2012). Many of the antimicrobial resistant bacteria transmitted to humans via food chain and direct contact from environment (Bywater 2005; Hauser et al., 2010; Gomes et al., 2012; Merchant et al., 2012). Antimicrobial resistant infections in humans lead to increased morbidity, mortality, and longer hospitalizations. Increased health care costs are associated particularly with longer hospital stays and use of more expensive antimicrobials necessary to fight resistant pathogens (Institute of Medicine, 1998).

Limiting availability of antimicrobials, enhanced surveillance, and on-farm interventions (including prudent antimicrobial use and management practices) have been proposed as key strategies to reduce antimicrobial resistance in food animal agriculture. Improved, rapid diagnostic methods and accelerated development and approval of new antimicrobial drugs can also play an important role in preventing and

controlling antimicrobial resistance (Bailey et al., 2010; Davies and Davies, 2010; Sommer et al., 2010).

An antimicrobial resistance is a global problem including our county Ethiopia, which causes morbidity, mortality and serious economic crisis, in spite of this no more research is conducted on antimicrobial resistance public health impacts and its control measures (National Research Council, 2010).

Therefore, the objectives of this paper are:

- To review on mechanism of antimicrobial resistance and its control measures.
- To highlight public health significance of antimicrobial resistances.

Literature Review

Mechanisms of action of antimicrobial agents

Antimicrobial is any agent that kills or inhibits growth of susceptible organisms. It acts selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. The understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways how resistance against them develops. Broadly, antimicrobial agents may be described as either bacteriostatic or bactericidal. Bacteriostatic antimicrobial agents only inhibit the growth or multiplication of the bacteria giving the immune system of the host time to clear them from the system. Complete elimination of the bacteria in this case therefore is dependent on the competence of the immune system. Bactericidal agents kill the bacteria and therefore with or without a competent immune system of the host, the bacteria will be dead and also antimicrobial agents may be narrow spectrum or broad spectrum based on range of effectiveness. However, the mechanism of action of antimicrobial agents can be categorized further based on the structure of the bacteria or the function that is affected by the agents. These include: Inhibition of the cell wall synthesis, Inhibition of ribosome function, Inhibition of nucleic acid synthesis, Inhibition of folate metabolism and Inhibition of cell membrane function (Neu *et al*., 1996).

Table 1: Summary of the mode of action for the major classes of antibiotics.

Group of antimicrobial agents	Effect on bacteria	Mode of action in general
Penicillins	Bactericidal	Inhibition of cell wall Synthesis
Cephalosporins	Bactericidal	Inhibition of cell wall synthesis
Carbanepems	Bactericidal	Inhibition of cell wall synthesis
Polypeptide antibiotics	Bactericidal	Inhibition of cell wall synthesis
Quinolones	Bactericidal	Inhibits DNA synthesis
Metronidazole	Bactericidal	Inhibits DNA synthesis
Rifamycins	Bactericidal	Inhibitions of RNA transcription
Lincosamides	Bactericidal	Inhibition of protein synthesis
Aminoglycosides	Bactericidal	Inhibition of protein synthesis
Macrolides	Bacteriostatic	Inhibition of protein synthesis
Tetracyclines	Bacteriostatic	Inhibition of protein synthesis
Chloramphenicol	Bacteriostatic	Inhibition of protein synthesis
Sulfonamides	Bacteriostatic	Competitive inhibition of para aminobenzoic acid

Adopted from www.pvj.com.pk,2013

Development and spread of mechanisms of antimicrobial resistance

Resistance can be an intrinsic property of the bacteria itself which is possessed by all members of the genus, and renders it unaffected by a specific mechanism of an antimicrobial. Resistance can also develop as the result of a single or multiple step mutation, for example, which changes a ribosomal protein that was a target of an aminoglycoside antimicrobial. More commonly, resistance is not due to a chromosomal change event, but to the presence of extra chromosomal DNA which was acquired from other bacteria. This type of resistance is plasmidmediated (Smillie *et al.*, 2010). Bacteria can transfer chromosomal or plasmid DNA- containing resistance genes to another bacteria by conjugation, transduction, and transformation.

A plasmid is a circular body of double stranded DNA which is separate from the chromosome and carries genes that encode various traits such as virulence and antimicrobial resistance boundaries (Carattoli, 2009).

There are two types of plasmids based on their ability to transfer from one bacterium to another. Conjugative plasmids can transfer to other bacteria via sex pili, and nonconjugative plasmids cannot. Cell-to-cell contact is necessary for conjugation to occur and both donor and recipient end up with a copy of the

plasmid. R-factors are plasmids that have traits for both conjugation and antimicrobial resistance (Smillie *et al.*, 2010). The transfer of plasmids by conjugation is an extremely important mechanism because transfer can occur in a broad range of bacterial species and can extend to highly unrelated organisms. A single plasmid can contain genes conferring resistance to multiple classes of antimicrobials. Transduction occurs when chromosomal or plasmid DNA is transferred from one bacterium to another by bacteriophages.

Bacteria can pick up free or "naked" DNA from their environment by a process called transformation. The presence of free DNA is common after cell lysis, but the range of compatibility between the free DNA and the intact recipient bacteria is narrow. Therefore, transformation is not an important method of resistance gene transfer. A transposon is a gene which contains an insertion sequence at each end. The insertion sequences allow the gene to jump to different locations on chromosomal DNA, from plasmid to plasmid or from chromosome to plasmid (Hall and Collis, 2010). The movement of a transposon is called transposition. Transposons are important because they can move resistance genes from a nonconjugative plasmid or chromosome to a conjugative plasmid, which can then be easily transferred to other bacteria. Another genetic element, called an integron, may be located on a plasmid or transposon. An integron contains one or more resistance genes (called gene cassettes) between two conserved DNA regions.

Mechanisms of antimicrobial resistance

Resistance to antimicrobials fall into two broad categories: intrinsic or acquired. Intrinsic resistance refers to tolerance of an antimicrobial due to the natural physiology of that particular genus or species with regard to the chemical structure of the drug. In some cases this occurs if a bacterial genus or species does not possess the metabolic or structural target for inhibition (McDermott *et al.*, 2003). For example, Gram–negative bacteria are intrinsically resistant to glycopeptides (e.g. vancomycin) and macrolides (e.g. tylosin) because these drugs are structurally too large to penetrate the outer membrane that exists among all Gram–negative bacteria. Alternatively, *Enterococci* are intrinsically resistant to cephalosporins due to insufficient binding affinity to penicillin binding proteins (PBPs) (Williamson *et al.*, 2005).

Leuconostoc, *Pediococcus*, *Enterococcus gallinarum* and Lactobacilli are Gram–positive bacteria that possess an alternative pathway for cell wall construction and are resistant to glycopeptides.

However, some of gene located on the bacterial chromosome, or when the bacterium acquires exogenous genes on mobile DNA elements acquired resistance whereby a naturally susceptible microorganism acquires ways of not being affected by the drug. This is exemplified by two general mechanisms: when a mutation has occurred in a gene, often located on the bacterial chromosome, or when the bacterium acquires exogenous genes on mobile DNA elements cross resistance occurs when one mechanism confers resistance to multiple antibiotics or classes of antibiotics (Alexander *et al.*, 2011).

Modification of the antimicrobial

Drug modification occurs when an enzyme catalyzes a structural change of the drug such that its mechanism of action is no longer effective for bacterial inhibition. Antibiotic classes that are inhibited by this mechanism include: beta (β)–lactams, aminoglycosides, chloramphenicol, streptogramins and macrolides (McDermott *et al.*, 2003). β–lactam antimicrobials act by binding to cell wall synthesizing proteins PBPs which effectively inhibits cell growth. The most commonly encountered mechanism of resistance for β–lactam antibiotics in Gram–negative bacteria is hydrolysis of the β–lactam ring by β–lactamase enzymes (Rice and Bonomo, 2011).

There are three mechanisms of aminoglycoside inactivation, these include drug modification by: acetylation, adenylylation and phosphorylation. Each mechanism is represented by a family of multiple enzymes (Shaw *et al.*, 1993; Davies and Wright, 1997). Among these are ATP–dependent *O*–phosphorylation by phosphotransferases, ATP–dependent *O*– adenylation by adenylytransferases or nucleotidyl transferases and acetyl CoA–dependent *N*– acetylation by acetyltransferases. These enzymes are widespread and have been identified in most Gram– negative and Gram–positive bacteria (Shaw *et al.*, 1993).

Modification of the drug target:

Structural modification of the antimicrobial drug target may render the drug ineffective, particularly if binding of the drug to the target is necessary. The interaction between the target molecule and antimicrobial is very specific and small structural changes, induced by point mutations that encode a different amino acid, may affect the binding affinity of the antimicrobial to its target (McDermott *et al.*, 2003; Giedraitiene *et al.*, 2011).

A post–translational modification of the target molecule by an enzyme may also reduce the efficacy of the drug. Antimicrobials that are inhibited by target modification include: β–lactams, aminoglycosides, macrolides, lincosamides, streptogramins, quinolones, rifampicin, trimethoprim, tetracyclines, and mupirocin (McDermott *et al.*, 2003). Hydrolysis of the β–lactam ring of β–lactam antimicrobials is a common resistance mechanism among Gram–negative bacteria;

for Gram– positive bacteria, target modification is the most commonly encountered mechanism of resistance for β– lactam antibiotics. Bacteria that produce PBPs that have a reduced binding affinity to β–lactam antibiotics display a resistant phenotype (Rice and Bonomo, 2011). In contrast, it has been long known that enterococci are intrinsically resistant to penicillin due to the production of low–affinity BPBs (Williamson *et al.*, 1985).

Aminoglycosides primarily act by binding to the 16S rRNA that recognizes the aminoacyl–tRNA; this action inhibits bacterial protein synthesis. Target modification by ribosomal mutations or enzymatic modifications of ribosomal components inhibit the action of aminoglycosides (Davies and Wright, 1997).

Tetracyclines inhibit bacterial protein synthesis by preventing the attachment of t–RNA to the ribosome (Chopra and Roberts, 2001). Tetracycline resistance due to target modification is mediated by ribosomal protection proteins that represent a widely distributed class of resistance genes (Thaker *et al.*, 2010).

Fluoroquinolones are broad spectrum antibiotics that act by inhibiting bacterial DNA replication by binding to two essential enzymes, DNA topoisomerase IV consisting of two subunits of each ParC and ParE and DNA gyrase composed of two subunits of each GyrA and GyrB (Hopkins *et al.*, 2005). Accumulation mutations in *parC* and *parE* and/or *gyrA* and *gyrB* genes, primarily in the quinolone resistance–determining regions, may confer resistance to fluoroquinolones by reducing the binding affinity of the drug (Rice and Bonomo, 2011).

Decreased access of drug to target

Decreased access to the intracellular drug target is primarily a consequence of active drug efflux. This involves the extrusion of noxious substances out of the cell resulting in sub toxic intracellular concentrations of the antimicrobials. This process may be very broad or narrow in substrate specificity depending on the type of efflux pump. Broad spectrum activity includes the expulsion of dyes and other inhibitory compounds (Nikaido and Pages, 2012). For this reason, expression of efflux proteins often confers resistance to multiple drugs simultaneously. Drug efflux is a highly prevalent mechanism of resistance among bacteria and there are primarily five types. These include: the major facilitator super family, the small multidrug resistance family; the resistance nodulation cell division family, the ATP binding cassette super family and the multidrug and toxic compound extrusion family.

In some cases multiple types of efflux proteins are present in one bacterial strain resulting in high–level resistance when neither protein alone confers resistance (Rice and Bonomo, 2011). Efflux may also enhance other mechanisms of resistance leading to clinically relevant resistance (Nikaido and Pages, 2012). Efflux pumps are usually encoded chromosomally and gene expression is activated by environmental signals or by mutations in regulatory genes that control expression (Levy, 2002). Efflux is active against all clinically relevant antimicrobial classes (Nikaido and Pages, 2012).

Alternative metabolic pathway

Some bacteria possess or acquire a different metabolic pathway that by–passes the pathway the antimicrobial inhibits. In enterococci the peptidoglycan component of the cell wall is formed when two molecules of D–Ala–D–Ala are added to UDP–*N*– acetylmuramyl–tripeptide to form the UDP–*N*– acetylmuramyl–pentapeptide. This is subsequently incorporated into the nascent peptidoglycan providing the structure for formation of cross–bridges in the peptidoglycan layer (Cetinkaya *et al.*, 2000). Glycopeptide antibiotics inhibit cell wall synthesis in Gram–positive by binding to the D–Ala–D–Ala precursor, thus, blocking their addition to the nascent peptidoglycan chain (Cetinkaya *et al.*, 2000). An example of low level intrinsic vancomycin resistance, VanC resistance, is exhibited in motile *Enterococcus casseliflavus, E. gallinarum and E. flavescens*. This is due to the ability to substitute D–Ala–D–Ala with D–Ala–D–Ser at the carboxyl terminus of the peptidoglycan precursor analogues this in turn lowers the affinity for vancomycin (Arthur *et al.*, 1996).

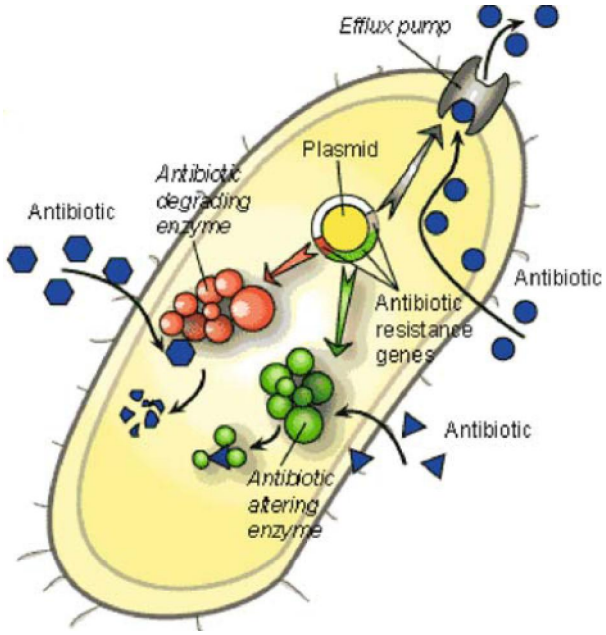

Fig. 1. Illustration of how some antimicrobial agents are rendered ineffective (Adopted from http://www.chembio.uoguelph.c)

Detection of resistance

Resistance among microorganisms can generally be detected either phenotypically or genotypically. For clinically important bacteria, diagnostic laboratories perform phenotypic-based analyses using standardized susceptibility testing methods, usually in accordance with those published by the Clinical and Laboratory Standards Institute. Determination of resistance via phenotype uses growth inhibition assays performed in broth or by agar disc diffusion. In a dilution-based growth inhibition assay, the minimal inhibitory concentration (MIC) can be calculated for each bacterial isolate and antimicrobial drug, and then interpreted as susceptible, intermediate, or resistant. This type of assay enables the practitioner to more readily choose the antibiotic that is most appropriate for clinical use because a susceptible interpretation conveys likely favorable clinical outcome, whereas resistant conveys likely treatment failure (www.clsi.org, 2014).

Identifying resistance versus susceptibility to food antimicrobial agents and/or sanitizers may be problematic because there are no standardized testing methods or accepted breakpoint values for these substances. Bacterial isolation techniques are often highly selective and may miss the majority of bacteria in a sample that are not the study target and the less predominant strains. These techniques will also miss the bacteria that cannot grow in the laboratory (Chopra, 2001).

Molecular detection techniques, such as polymerase chain reaction or DNA–DNA hybridization, are standard techniques used to determine the presence of specific resistance genes. Microarrays13 have been used to test for the presence of a number of genes from a given bacterial isolate (Call *et al*., 2003; Yu *et al*., 2004).

Public health significance of antibiotic resistance in animal

The emergence of antibiotic-resistant microorganisms in human medicine is primarily the result of the use of antibiotics in humans, although the use of antibiotics in animals is also part responsible. The resistant bacteria in animals due to antibiotic exposure can be transmitted to humans via three pathways, those being through the consumption of animal products (milk, meat, eggs, etc.), from close or direct contact with animals or other humans, or through the environment (WHO, 2002).

Antimicrobial resistant infections in humans lead to increased morbidity, mortality, and longer hospitalizations. Increased health care costs are associated particularly with longer hospital stays and use of more expensive antimicrobials necessary to fight resistant pathogens. Often the antimicrobials used to combat resistant organisms are more toxic, with more serious side effects. Other associated costs to society include lost work days and value of lives lost due to deaths. The cost of antimicrobial resistant hospital acquired infections was estimated by the National Foundation for Infectious Disease to be as high as four billion dollars annually. In 1995, the Office of Technology Assessment produced a minimum estimate of 1.3 billion (1992 dollars) yearly in-hospital costs related specifically to six species of antibiotic resistant bacteria and only one antibiotic (Institute of Medicine, 1998).

Control of antibiotic resistance

A number of approaches can be taken to limit the development and spread of antibiotic resistance. All our efforts should be directed towards reducing the selection pressure as much as possible antibiotics should be administered at therapeutic doses only short periods, prolonged use may select resistant strains (WHO; 2002). Slight decrease use or withdrawal of certain drugs followed by dramatic reduction in resistance to these and other antibiotics, it is commonly assumed that misuse and inapproprat use of antibiotics is the main cause of resistance. Thus, the control of antibiotics resistance depends on the carful and appropriate use of antibiotics. The following measures used to control resistance (www.pvj.com.pk, 2013).

Limiting the spread of drug resistant bacteria

Several measures could be used to prevent the spread of drug resistant bacteria (CDC; 2006). First, we could use better treatment strategies; better immunization programmes; improved hygiene and nutrition; and initiatives targeting the poor populations. Second, it might be useful to establish antibiotic resistance surveillance programmes. Third, better education of health care professionals is required to prevent the prescription of unnecessary antibiotics.

Development of new antibiotics

Another possibility is to develop new antibiotics however that is not an easy task. The sad irony is that many pharmaceutical companies have decided to abandon their antibiotic development programmes when new antibiotics are needed most, since 99% of the drug candidates fail, and antibiotics are not as profitable as other, more commonly used, drugs (WHO; 2002).

Phage therapy

Bacteriophages or "phages" are viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse. Phage therapy is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections. This approach had already been used by the Russians during the Second World War, and has been gaining popularity again in recent years. Phage can be applied

on the wounds of a patient to kill the bacteria, and has proven to be quite effective. Of course, it cannot be used for internal infections, and the bacteria might also develop phage resistance (Masco *et al*., (2006).

Conclusion And Recommendations

Antimicrobials are used in livestock production as therapeutics, prophylactics, and growth promoters. These drugs assist in sustaining livestock production and in controlling bacterial pathogens that may be transferred to humans. Bacteria exhibit a number of well characterized mechanisms of resistance to antimicrobials that include: modification of the antimicrobial; alteration of the drug target; decreased access of drug to target; and implementation of an alternative metabolic pathway not affected by the drug. These can be manifested by prolonged duration of illness, increased frequency of blood stream infections, increased hospitalization, or increased mortality and cause economical as well as psychological crisis. based on this conclusion the following recommendations are forwarded:

- Create national systems to monitor antimicrobial usage in food animals.
- Monitor resistance to identify emerging health problems and take timely corrective actions to protect human health.
- Develop guidelines for veterinarians to reduce overuse and misuse of antimicrobials in food animals.
- Avoid group medication by feed or water wherever possible.

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