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A BRIEF OVERVIEW- MOLECULAR BASIS OF ANTIMICROBIAL RESISTANCE

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ABSTRACT

Antibiotics play a vital role in the treatment of bacterial infections in both humans and animals. As a result antibiotic resistance develops, posing serious health problems in both humans and animals. This has led to concerns about the public health implications of antibiotic use in animal husbandry. To avoid elimination by antibiotic substances, bacteria develop resistance by mutation or by acquisition of genes from other bacteria. Horizontal gene transfer between bacteria is a common event and an important factor in microbial evolution. These antibiotic resistant bacteria get into human food chain posing a potential risk. Transmission of resistant gene between different species occurs. Resistance genes can persist in the environment both in their original bacterial hosts and in transferred organisms. Resistance becomes a problem when a bacterium causing a disease withstands antibiotic therapy. Increased resistance to antibiotics may therefore cause clinical problems and may shorten the useful life span of some antibiotics. The present paper highlights their mode of entry into food chain, various biochemical methods by which bacteria develops resistance, possible health problems and measures to control antibacterial resistance.

KEY WORDS: **Horizontal gene transfer, Resistance genes, Antibiotics and microbial evolution**

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INTRODUCTION

The discovery and clinical application of antibacterial agents represent one of the outstanding achievements of medical science in the 20th century. In the early 20th century, ethyl hydrocupreine (optochin) was tried for the treatment of pneumococcal pneumonia and the treatment trial in 1917 was terminated because of drug toxicity. Meanwhile, the emergence of resistance to the drug being used during therapy was also noted. This was found to be the first documentation of the emergence of resistance to an antimicrobial agent in humans. With the discovery of each new class of antimicrobial agents like sulfonamides and penicillin, resistance has developed and now this has become a worldwide problem. Initially, scientists were combatting the problem of resistance by discovering and developing new classes of antimicrobial agents that were effective against resistant organisms. However, in the past twenty years, this particular developmental chain link has been drying up progressively. Thus no new classes of antimicrobial agents have been discovered for more than two decades.This article provides a brief insight on the responsible and judicial use of antimicrobials in veterinary medicine, with the aim of protecting both animal and human health. The use of an antimicrobial for any infection, in any dose and over any time period, causes a “selective pressure” on microbial populations. Most of the time majority of microbes will be taken care of by the body’s immune system, however few mutant strains elusive to the immune system do exist under selective pressure and when the treatment is insufficient or the patient is immunocompromised, the mutants can flourish. Thus the treatment may fail. The use of antimicrobial agents irrationally can cause the emergence, prevalence and propagation of antimicrobial resistance in bacteria. Antimicrobial resistant bacteria, arising from the use of veterinary antimicrobial agents, were transmitted to humans through livestock products and further reduced the efficacy of antimicrobial drugs in humans.

Antimicrobial resistance issues have been addressed by coveted international organizations including the Food and Agriculture Organization (FAO), International Committee of Office International des Epizooties (OIE), World Health Organization (WHO) and Codex[1]. There are certain factors that are included in the relationship between the usage of antimicrobials and the presence of antimicrobial resistance. The factors affecting the occurrence, prevalence, and dissemination of resistance should be identified in each country to implement corrective measures to contain the emergence, prevalence and dissemination of antimicrobial resistance and the betterment of human and animal health.

1. CAUSES FOR ANTIMICROBIAL RESISTANCE

Bacteria are skilled enough to counteract the effects of antimicrobials. By understanding what drives bacteria to develop resistance, we may be able to limit its impact on the treatment of infections. Some of the general factors that may increase the risk of bacterial resistance are discussed below.

- Previous exposure to antibiotics
- Overuse of antibiotics
- Inadvertent use of broad spectrum antibiotics
- Use of similar antibiotics

The causes for antimicrobial resistance are dealt in detail taking in to account its molecular level of action and are discussed below:

1.1 Constitutive and Inducible resistance:

Pathogens overcome antimicrobial action through 2 strategies: constitutive resistance versus inducible resistance. A constitutive (passive) mechanism of resistance refers to inherent properties of an organism that confer resistance and are normally expressed even in the absence of antimicrobial exposure. Whereas, inducible (adaptive) resistance mechanisms include those triggered in response to the antimicrobial peptide or the due to the stress it causes to the target cell. These two mechanisms go hand in hand and provide pathogens with the greatest likelihood of survival in diverse contexts containing antimicrobial agents.

1.1.1 *Constitutive (Passive) Resistance*:
Serratia, Proteus and Providencia species often prove to be refractory to inhibition or killing by cationic antimicrobials[2]. Burkholderia (formerly Pseudomonas) species also exhibit broad resistance to antimicrobial peptides in vitro[3]. These examples suggest the likelihood of certain microbial pathogens being inherently resistant to antimicrobial agents due to stable structural or functional properties or pathogenesis strategies. Several intriguing observations may provide insights into molecular basis of inherent resistance.

1.1.1.1 *Inherent Mechanisms of Resistance to Antimicrobial agents*:
Antimicrobial agents interact with the outermost surface of the target pathogen at some point in their mechanism of action. At that point, such surfaces inherently lack electrostatic affinity for or may even repel cationic antimicrobial agents. Certain staphylococcus species constitutively express membranes with reduced negative charge. In Enterococcus, resistance to antimicrobial agents has been attributed with unusual susceptibility and resistance patterns to conventional antibiotics that target their cell membrane or cell wall. Cashman *et al* demonstrated that Enterococcus species exhibit broad resistance to a class of cationic antimicrobial agents and identified an inverse correlation between glycopeptide susceptibility and resistance to cationic antimicrobial agents[4]. Nahaie *et al* examined the constitutive phospholipid composition of a group of Staphylococcus species[5]. Most species displayed polar lipid profiles consisting predominantly of Phospoglycerides and di-Phosphoglycerides. However, among the organisms screened, *S. aureus* was unique in having a lipid composition rich in unsaturated menaquinones with eight isoprene units and lysyl-Phosphoglyceride, a derivative of Phosphoglyceride that is considerably less electronegative[6]. Analogous modifications in the outer membrane of some Gram-negative bacteria are also hypothesized to preserve membrane integrity in the presence of antimicrobial agents[7].

1.1.1.2 *Altered Membrane Energetics*:
The resistance to several types of antimicrobial agents has been shown to be influenced by transmembrane potential. For example, the type-I (highly cationic) defensins appear to exert equivalent antimicrobial potency against metabolically energetic or quiescent bacteria[8]. However, type-II defensins exert maximal antimicrobial activity against highly energized cells. These distinctions illustrate the concept that antimicrobial peptides may have significantly reduced potencies against organisms with inherently low or that have the capability to adapt to such a status.

1.1.1.3 *Electrostatic Shielding*:
Capsule production is an important virulence factor particularly among microorganisms that colonize or infect the mammalian bloodstream, respiratory tract and gastrointestinal mucosa. Probable reason for the role of capsule in antimicrobial resistance is attributed to the composition of the glycocalyx of many microbial pathogens which is an anionic complex of carbohydrate and phosphate[9]. Thus, it is reasonable to hypothesize that matrices such as these sequester cationic antimicrobial peptides, preventing them from accessing their intended targets.

1.1.1.4 *Niche-Specific Resistance*:
Some pathogens likely exploit specific tissues or physiologic microenvironments to subvert the host defense contributions of antimicrobial agents.

1.1.2 *INDUCIBLE (ADAPTIVE) RESISTANCE*
1.1.2.1 *Coordinate Microbial Responses to Antimicrobial agents stress*:
In bacteria, inducible resistance to antimicrobials is largely controlled through sensor-transducer response systems. Fields *et al* for the first time determined that a specific genetic locus of the pathogen *S. typhimurium* is integral to intracellular survival in macrophages and virulence in mice by conferring resistance to a defensin peptide[10]. Recent discovery reveals that activation of the PhoP/PhoQ regulon yields globular protein, phospholipid and lipopolysaccharide modifications in Gram-negative pathogens that mitigate actions of antimicrobials[11]. For

example, numerous tests have shown that the PhoP/PhoQ system plays key role in activating transcription of genes in *Salmonella* that encode inducible surface and secretory proteins, enzymes that modify lipopolysaccharide, lipid and protein constituents of the outer membrane, and proteases that likely degrade certain antimicrobials. Gunn and Miller have shown the PhoP/PhoQ system to regulate phoP-activated genes (*pag*) and phoP-repressed genes (*prg*) [12]. The combined effects of these gene clusters coordinately upregulate expression of pathways that actively defend against antimicrobials.

1.1.2.2 *Adaptive Mechanisms of Resistance to Antimicrobials*:

This mechanism of inducible resistance involves modifications of the pathogen envelope or extracellular facet of the cytoplasmic membrane directly offsetting lethal action of antimicrobials.

1.1.2.3 *Proteases and Peptidases*:

Proteases and peptidases coded by specific genes confer resistance by cleaving the specific sites in the antimicrobials. To illustrate this PgtE protein was recently demonstrated by Guina *et al* to be an outer membrane endopeptidase in *Salmonella*[13]. These proteases target peptides between paired basic residues and at the carboxy-terminal aspect of basic amino acid residues that precede a non polar residue. Thus, a variety of amphipathic and cationic antimicrobial peptides are potential substrates of PgtE protease. Osapay *et al* recently identified a synthetic modified form of indolicidin termed X-indolicidin and X-indolicidin was found to be more resistant to trypsin and chymotrypsin digestion[14].

1.1.2.4 *Extracellular Structural Modifications*:

Antimicrobials generally target and interact with microbial structures exterior to the cytoplasmic membrane. Thus, microbial pathogens have evolved mechanisms by which these targets may be modified to resist peptide targeting and circumvent the ensuing antimicrobial mechanisms.

1.1.2.5 *Efflux-Dependent Resistance Mechanisms*:

Efflux has also played a major role in evading antimicrobials by microbial pathogens. In *Neisseria gonorrhoeae*, Shafer *et al* have shown that resistance to antibacterials of diverse structure is mediated in part by an energy-dependent efflux system termed mtr[15]. Evidence also indicates the MtrCDE complex ejects antibiotics, dyes, and detergents, suggesting this mechanism protects the pathogen against mucosal or other endogenous or exogenous antimicrobials[16].

1.1.2.6 *Modification of Intracellular Targets*:

Another way of microbial cell death is attributed to targeting intracellular targets by antimicrobials. Accordingly, new data indicate the existence of complex mechanisms that specifically modify these intracellular targets to confer resistance. Del castillo *et al* have identified a mutation in the *gyrB* gene that is associated with a significant reduction of *E. coli* susceptibility to microcin B17, an antimicrobial believed to inhibit DNA replication[17]. This mutation yields replacement of tryptophan 751 by arginine in the GyrB polypeptide; ostensibly reducing microcin B17 targeted inhibition of DNA gyrase. These above supposed causes and studies represent areas of research focusing on the growing awareness that antimicrobials exert mechanisms of action which transcend their initial interaction with phospholipid bilayers.

2. CONTROL OF ANTIMICROBIAL RESISTANCE

There's an urgent need to alert the problem and take appropriate action. Medical or Veterinary practitioners, pharmacists, consumers, government, professional societies and international agencies have to make a step ahead in combating antimicrobial resistance. WHO took right step towards planning a strategy needed to curb trends which have health implications. Moreover, in view of the global nature of the antimicrobial resistance problem, the efforts of any nation to implement the WHO Global Strategy are likely to be felt worldwide. To counteract the antimicrobial resistance some of the following steps have to be followed:

- Control of the therapeutic efficacy: Following proper pharmacodynamics and the establishment of the activity of antimicrobial agents towards the targeted bacteria and also proper follow up of pharmacokinetics and the establishment of the dosage regimens allowing maintenance of effective antimicrobial levels.
- Assessment of the potential antimicrobials selectively for resistant bacteria.
- Establishment of acceptable daily intake, maximum residue limit and withdrawal periods for antimicrobial compounds.
- Establishment of a summary of product characteristics for each veterinary medicinal product.
- Training of antibiotic users.
- Marketing authorization of veterinary medicinal products: The veterinary pharmaceutical industry has responsibility to supply all the information requested by the national regulatory authority in order to establish objectively the quality, safety and efficacy of veterinary medicinal products.
- Use of antimicrobials when necessary.
- Determination of choice of an antimicrobial.

CONCLUSION

Antimicrobial agents are very important tools for controlling a great number of bacterial diseases in both animals and humans. It is vital that all countries implement the appropriate systems to ensure that antimicrobials are manufactured, marketed, distributed, prescribed, supplied and used responsibly so that these systems are adequately monitored. Global solution is the need of the hour to combat such a devastating consequence of antimicrobial resistance. Global leader, WHO launched the global strategy to curb the antimicrobial resistance known as *WHO Global Strategy for Containment of Antimicrobial Resistance*, which recommends a large number of interventions that can be used to slow the emergence and reduce the spread of resistance in a diverse range of settings. A more thorough understanding of the balance between the opposing mechanisms of action and resistance among antimicrobial peptides will further reveal how these molecules function to defend against infection. These insights may provide novel strategies or templates from which novel agents may be developed to improve the prevention or treatment of infections, particularly those caused by pathogens resistant to conventional antibiotics. Thus, pharmacologic agents may be discovered and developed that target strategic microbial structures or functions, suppress pathogen resistance to host defenses, and restore or potentiate the activities of conventional antibiotics against drug-resistant pathogens. From these perspectives, the mechanisms of antimicrobial peptide action and resistance may hold many secrets yet to be uncovered or fully appreciated.

REFERENCES

1. Dehaumont, OIE international standards on antimicrobial resistance, Journal of Veterinary Medicine Series B, 51: 411–414 (2004).
2. Viljanen P, Vaara M, Susceptibility of gram-negative bacteria to polymyxin B nonapeptide. Antimicrob. Agents Chemother. 25: 701–705 (1984).
3. Manniello JM, Heymann H, Adair FW, Resistance of spheroplasts and whole cells of Pseudomonas cepacia to polymyxin B. Antimicro. Agents Chemother 14: 500–504 (1978).
4. Cashman KA, Bayer AS, Yeaman MR, Diversity and susceptibility to antibiotics and cationic peptides among Enterococcus faecalis or Enterococcus faecium isolates of diverse clinical or geographic origin. 98th Genreal Meeting for the American Society for Microbiology: May 17–21: Atlanta GA, (1998).
5. Nahaie MR, Goodfellow M, Minnikin DE, Hajek V, Polar lipid and isoprenoid quinone composition in the classification of Staphylococcus. J. Gen. Microbiol., 130: 2427–2437 (1984).

6. Sievers S, Ernst CM, Geiger T, Hecker M, Wolz C, Becher D, Peschel A,Changing the phospholipid composition of Staphylococcus aureus causes distinct changes in membrane proteome and membrane-sensory regulators. Proteomics. 10(8):1685-93 (2010).
7. Guo L, Lim KB, Poduje CM, Daniel M, Gunn JS, Hackett M, Miller SI, Lipid A acylation and bacterial resistance against vertebrate antimicrobial peptides. Cell, 95: 189–198 (1998).
8. Lehrer RI, Ganz T, Endogenous vertebrate antibiotics: Defensins, protegrins and other cysteine-rich antimicrobial peptides. Ann. NY. Acad. Sci., 797: 228–239 (1996).
9. Michael Dunne W, Bacterial Adhesion: Seen Any Good Biofilms Lately,Clin Microbiol Rev, 15(2): 155–166 (2002).
10. Fields PI, Groisman E, Heffron FA, Salmonella locus that controls resistance to microbicidal proteins from phagocytic cells. Science (Wash DC) 243: 1059–1062 (1989).
11. Hiroshi N, Molecular Basis of Bacterial Outer Membrane Permeability Revisited Microbiol. Mol. Biol. Rev. 67 (4): 593-656 (2003).
12. Gunn JS, Miller SI, PhoP-PhoQ activates transcription of pmrAB, encoding a two-component regulatory system involved in Salmonella typhimurium antimicrobial peptide resistance. J. Bacteriol., 178: 6857–6864 (1996)
13. Guina T, Yi EC, Wang H, Hackett M, Miller SI, A PhoP-regulated outer membrane protease of Salmonella enterica serovar typhimurium promotes resistance to alpha- helical antimicrobial peptides, J Bacteriol. 182(14): 4077-86 (2000).
14. Osapay K, Tran D, Ladokhin AS, White SH, Henschen AH and Selsted M Formation and Characterization of a Single Trp-Trp Cross-link in Indolicidin That Confers Protease Stability without Altering Antimicrobial Activity. J. Biol. Chem., 275: 12017–12022 (2000).
15. Shafer WM, Qu X, Waring AJ and Lehrer RI, Modulation of Neisseria gonorrhoeae susceptibility to vertebrate antibacterial peptides due to a member of the resistance/nodulation/division efflux pump family. Proc. Natl. Acad. Sc.i USA, 95: 1829–1833 (1998).
16. Michael RY, Nannette Y, Yount, Mechanisms of antimicrobial peptide action and resistance, Pharmacol Rev, 55: 27–55 (2003).
17. Del Castillo FJ, Del Castillo I, Moreno F, Construction and characterization of mutations at codon 751 of the Escherichia coli gyrB gene that confer resistance to the antimicrobial peptide microcin B17 and alter the activity of DNA gyrase. J Bacteriol 183: 2137–2140 (2001).