Role played by Gene Factor in Initiation of Bacterial Antibiotic Resistance

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Abstract: The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antibiotics. Antibiotics have different mechanisms of action; interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of a metabolic pathway, and disruption of bacterial membrane structure. Antibiotic resistance bacteria may be intrinsic or acquired. In the case of intrinsic resistance, bacterial strains are inherently resistant to a certain compound while acquired resistance occurs by mutation and/or horizontal gene transfer events. The main mechanisms of horizontal gene transfer are conjugation, transformation, and transduction. Acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibiotics, to express efflux systems that prevent the drug from reaching its intracellular target, to modify the drug's target site, or to produce an alternative metabolic pathway that bypasses the action of the drug. This review article focused on some resistant pathogens such as, the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) and methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* resistance to third-generation cephalosporins, the resistant *Pasteurella multocida* is due to enzymes conferring β-lactamase resistant, multidrug resistance of *Pseudomonas aeruginosa* that occurs by different ways, as well as the intrinsic drug resistance of *Mycobacterium tuberculosis*.
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Introduction

Antibiotic resistance describes the ability of certain bacteria to resist the effect of treatment with antibiotics, and this resistance is associated with the use of an antibiotic that inhibits or destroys susceptible bacteria within a population. **(WHO, 2011)**. The extensive use of antibiotics in human and veterinary medicine for their prophylactic and growth promoting use in agriculture and aquaculture have lead to a huge rise of antibiotic resistant bacteria and an increase of antibiotic resistant genes in the horizontal gene pool **(Walsh and Fanning , 2008).**

Antibiotic resistance bacteria may be intrinsic or acquired. In the case of intrinsic resistance, bacterial strains are inherently resistant to a certain compound and the resistance cannot be transferred horizontally **(Fajardo *et al*., 2008)**.

Acquired resistance occurs by mutation and/or horizontal gene transfer events. The main mechanisms of horizontal gene transfer are conjugation (mobile genetic elements are being transferred from a donor to a recipient cell), transformation (uptake of naked DNA), and transduction (bacteriophages as transporters of genetic information). Conjugation is considered as the principal mode for antibiotic resistance transfer since many antibiotic resistance genes are situated on mobile elements, such as plasmids and conjugative transposons. Conjugation of broad-host-range plasmids enables DNA to be transferred over genus and species borders, whereas transformation and transduction are usually more limited to the same species **(Mathur and Singh, 2005)**.

There is evidence that naturally occurring antibiotic resistance is common. The genes that confer this resistance are known as the 'environmental resistum'. These genes may be transferred from non pathogenic bacteria to pathogenic bacteria, leading to clinically significant antibiotic resistance **(Wright, 2010).** Conventional antimicrobials are increasingly ineffective due to the emergence of multidrug-resistance among pathogenic microorganisms. The need to overcome these deficiencies has triggered exploration for novel and unconventional approaches to controlling microbial infections **(Kourtesi *et al.*, 2013)**. Despite the need for new antibiotic therapies, there has been a continued decline in the number of newly approved drugs. Antibiotic resistance therefore poses a significant problem. **(Donadio *et al.*, 2010).**

Why is resistance a concern

There are a number of reasons why bacterial resistance should be a concern.

Antibiotic use in nonhuman niches is an important reason for the spread of resistant bacteria

(Martínez and Baquero, 2002). It is known that the use of antimicrobial agents in animal food is related to bacterial resistance, for example, *Salmonella*, *Campylobacter* and *Enterococcus* acquire resistance to antibiotics and transfer genes of antibiotic resistance to natural human flora, also high *Escherichia coli* (*E. coli*) resistance to ciprofloxacin is associated with the use of fluoroquinolones in aviculture **(Rice *et al*., 2003; Džidic *et al*., 2008)**. Resistant bacteria particularly *Staphylococcus*, *Enterococcus*, *Klebsiella pneumoniae*, and *Pseudomonas* spp. are becoming common-place in healthcare institutions, as bacterial resistance often results in treatment failure, which can have serious consequences, especially in critically ill patients **(NNIS, 2004)**. Resistant bacteria may also spread and become broader infection-control problems, not only within healthcare institutions, but in communities as well. Clinically important bacteria, such as MRSA and extended-spectrum B–lactamase (ESBL)–producing *E. coli* are increasingly observed in the community **(Chambers, 2001; Pitout *et al*., 2004 and Stevenson, 2005)**. There has been extensive use of antibiotics in animals, these antibiotics can affect the meat, milk, and eggs produced from those animals and can be the source of superbugs (highly antibiotic resistant bacteria). For example, farm animals, particularly pigs, harbor MRSA strains which are asymptomatic for them and can seriously harm humans **(Joint scientific report, 2009)**.

Mode of action of antibiotics:

Table (1): Mechanisms of action of antibacterial agents

Mode of action	Antibiotic
Interference with cell wall synthesis	1-β-lactams: penicillins, cephalosporins, carbapenems, monobactams 2-Glycopeptides: vancomycin, teicoplanin
Protein synthesis inhibition	1-Bind to 50S ribosomal subunit: macrolides, chloramphenicol, clindamycin, quinupristin-dalfopristin, linezolid 2-Bind to 30S ribosomal subunit: aminoglycosides ,tetracyclines
Interference with nucleic acid synthesis	1-Inhibit DNA synthesis: fluoroquinolones 2-Inhibit RNA synthesis: rifampin
Disruption of bacterial membrane structure	polymyxins**,** daptomycin
Inhibition of metabolic pathway	sulfonamides, folic acid analogues

Genetic Aspect of Antibiotic Resistance

Bacterial resistance to antibiotics can be intrinsic or acquired or combination of both of them.

Intrinsic resistance:

Intrinsic resistance is characteristic of a particular bacterium and depends on biology of a microorganism "*E. coli* has innate resistance to vancomycin" **(Vaičiuvėnas, 2005).**

Acquired resistance:

Acquired resistance occurs by mutation and/or horizontal gene transfer.

Mutation:

Mutation susceptible bacteria can acquire resistance to an antimicrobial agent via mutation which includes two main types spontaneous mutation and chromosomal mutation. These mutations occur as errors of replication or an incorrect repair of damaged DNA. Chromosomal mutation is quite rare and commonly determines resistance to structurally related compounds **(Rice, 2003).** Some biochemical resistance mechanisms are the result of mutation which:

(1) Altering the target protein to which the antibacterial agent binds by modifying or eliminating the binding site (e.g., change in penicillin-binding protein in pneumococci, which results in penicillin resistance)

(2) Up-regulating the production of enzymes that inactivate the antimicrobial agent (e.g., erythromycin ribosomal methylase in staphylococci).

(3) Down-regulating or altering an outer membrane protein channel that the drug requires for cell entry (e.g., *Omp*F porin in *E coli*).

(4) Up-regulating pumps that expel the drug from the cell (efflux of fluoroquinolones in *S. aureus*), **(Hooper, 2001)**.

Horizontal Gene Transfer:

A transfer of resistance genes from one bacterium to another is called a horizontal gene transfer and may occur between strains of the same species or between different bacterial species or genera **(Bennett, 2008).**

Mechanisms of genetic exchange include conjugation, transduction, and transformation. During conjugation (via plasmid and conjugative transposons) a Gram-negative bacterium transfers plasmid-containing resistance genes to an adjacent bacterium, often via an elongated proteinaceous structure termed a *pilus*, which joins the two organisms. Conjugation among Gram-positive bacteria is usually initiated by production of sex

pheromones by the mating pair, which facilitate the clumping of donor and recipient organisms, allowing the exchange of DNA. During transduction, resistance genes are transferred from one bacterium to another via bacteriophage (bacterial viruses). Finally, transformation, i.e.; the process where bacteria acquire and incorporate DNA segments from other bacteria that have released their DNA complement into the environment after cell lysis, can move resistance genes into previously susceptible strains. Then genes are incorporated into the recipient chromosome by recombination or transposition and may have one or several changes in gene sequence **(Džidic, 2008; Alekshun , 2007)**.

a) Plasmid transfer; b) transfer by viral delivery; c) transfer of free DNA**.**

Mobile genetic elements (MGE) play a major role in horizontal gene transfer (HGT) in bacteria. Among them, integrative and conjugative elements (ICEs) (also called conjugative transposons) are self-transmissible elements that are wide spread in bacteria and are characterized by both integrative and conjugative properties **(Frost *et al*., 2005)**.

Most plasmids are double-stranded circular DNA which encode up to 10% of the host cell chromosome. The transfer of resistance genes is more effective than chromosomal mutation, **(Alekshun and Levy, 2007)**. Plasmids encode genes that confer resistance to main classes of antimicrobial agents (cephalosporins, fluoroquinolones, and aminoglycosides), **(Bennett, 2008).**

MDR bacteria are able to counteract antibiotics treatment by acquiring resistance genes, spontaneous mutation, and disseminating resistance genes via mobile genetic elements (i.e., plasmid, transposon, and insertion sequences**.** MDR genes are located in a DNA sequence that is transferred from one plasmid to another or to the genomes, which are called transposons or "jumping gene systems**" (Stokes *et al*., 2011).** Transposons can be integrated into plasmids or the host's chromosome and are transferred by conjugation, transformation, or transduction (e.g., *mec*A gene is found in MRSA) and spread quicker than genes in chromosomes **(Tolmasky, 2000; Raghunath, 2008).**

Staphylococcus aureus:

S. aureus isolates from worldwide are increasingly resistant to a greater number of antimicrobial agents**.**

Table (2): *S. aureus* resistance mechanisms to selected antibiotics **(Franklin, 2003)**.

Antibiotic	**Resistance gene(s)**	**Gene product(s)**	**Mechanism(s) of resistance**
β Lactams	*blaZ*	Lactamase	Enzymatic hydrolysis of lactam nucleus
	*mec*A)	PBP2a	Reduced affinity for PBP
Glycopeptides	*van*A	Altered peptidoglycan	Trapping of vancomycin in the cell wall
		D-Ala-D-Lac	Synthesis of dipeptide with reduced affinity for vancomycin
Quinolones	*par*C	*Par*C (or *Grl*A) component of to poisomerase IV	Mutations in the QRDR region, reducing affinity of enzyme-DNA complex for quinolones
	*gyr*A or *gyr*B 2)	*Gyr*A or *Gyr*B components of gyrase	
Aminoglycosides (e.g., gentamicin)	Aminoglycoside-modifying enzymes (e.g., aac, aph)	Acetyltransferase, phosphotransferase	Acetylating and/or phosphorylating enzymes modify aminoglycosides
Trimethoprim-sulfamethoxazole	Sulfonamide: *sul*A *dfr*B	Dihydropteroate synthase Dihydrofolate reductase (DHFR)	Overproduction of p-aminobenzoic) Reduced affinity for DHFR cid by enzyme
Oxazolidinones	rrn	23S rRNA	Mutations in domain V of 23S rRNA component of the 50S ribosome. Interferes with ribosomal binding
Quinupristin-dalfopristin (Q-D)	Q:*erm*A,*erm*B, *erm*C	Ribosomal methylases	Reduce binding to the 23S ribosomal subunit
	D:*vat*, *vat*B	Acetyltransferases	Enzymatic modification of dalfopristin

Methicillin resistant *Staphylococcus aureus* (MRSA):

Staphylococcus aureus has a strong adaptive capacity and thus acquired various types of resistance to several classes of antibiotics. More than 90% of isolates produce a penicillinase. MRSA is due to penicillin-binding protein (PBP2a) with low affinity for beta-lactams encoded by mecA gene which is carried by a chromosomal element which also carry other resistance genes to other antibiotics thus explaining the multi-resistant profile of hospital associated MRSA **(Dumitrescu *et al*., 2010)**.

Vancomycin resistant *Staphylococcus aureus* (VRSA):

VRSA strains are resistant to vancomycin because of the acquisition of the vanA operon from an enterococcus that allows synthesis of a cell wall precursor that ends in D-Ala-D-Lac dipeptide rather than D-Ala-D-Ala. The new dipeptide has dramatically reduced affinity for vancomycin. In the presence of vancomycin, the novel cell wall precursor is synthesized, allowing continued peptidoglycan assembly **(Murray, 2000).**

Escherichia coli:

E. coli are a common cause of urinary tract infections and bacteremia in humans, and are frequently resistant to amino-penicillins, such as amoxicillin orampicillin, and narrow spectrum cephalosporins. Resistance is typically mediated by the acquisition of plasmid-encoded B -lactamases, such as TEM-1, TEM-2, or SHV-1, which hydrolyze and inactivate these drugs **(Landgren *et al*., 2005)**. Some *E. coli* strains develop resistance to third-generation cephalosporins and monobactams (eg: aztreonam) through the acquisition of extended-spectrum beta lactamase (ESBLs), commonly arising through mutation of TEM-, SHV-, or CTX-M–type enzymes **(Bradford, 2001; Rupp and Fey, 2003)**. The ESBLs are not active against cephamycins, such as cefoxitin and cefotetan; however, resistance to cephamycins and other β -lactams may arise as a result of changes in the porins in the outer membrane, such changes decrease or eliminate the flow of small molecules like β -lactam drugs across the membrane **(Ananthan and Subha , 2005)**.

The main reservoir for *E. coli* is the bowel and there is a large turnover of the bacterium every day. Studies show that a large proportion of *E. coli* carried by people is acquired via food, and especially from poultry. This is particularly the case for antibiotic-resistant bacteria **(Johnson *et al*., 2006)**. In developed countries *E. coli* remains largely sensitive to third-generation cephalosporins, fluoroquinolones and/or aminoglycosides, and these agents can generally still be used to treat those with serious infections, however, this is not the case in developing countries **(Walsh *et al*., 2008)**.

Salmonella spp.:

Salmonella enterica serovar *typhimurium* is considered as the main food-borne pathogen responsible for causing human disease, **(Voetsch *et al*. 2004)**. Fluoroquinolones are the main drugs for the treatment of salmonellosis, however the emergence of MDR *S. enterica* serovar *typhimurium* has led to the failure of the treatment, **(Cosgrove, 2006)**.

The resistant mechanisms of *S. enterica* serovar *typhimurium* to fluoroquinolone mainly include target site mutations in quinolone resistant determining regions (QRDRs), decreased fluoroquinolone uptake and plasmid-mediated fluoroquinolone resistance, **(Chen *et al*., 2003)**. A single point mutation in the QRDR of *gyr*A can mediate high-level resistance to nalidixic acid and reduce susceptibility to fluoroquinolones. The *par*C mutations always occurred with mutations in *gyr*A and lead to high-level fluoroquinolone resistance, **(Katie *et al*., 2005)**. The genome of *S. enterica* serovar *typhimurium*, has five MDR efflux pumps which could extrude quinolones when over expressed **(Nishin *et al*., 2006)**. Most recently, active MDR efflux pump has been recognized as a primary fluoroquinolone resistant mechanism in clinical *S. enterica* serovar *typhimurium*, **(Escribano *et al*., 2004)**. *Salmonella* containing ESBL can develop from the use of third-generation cephalosporins in poultry. In Canada a close association has been found between the use of a third-generation cephalosporin (ceftiofur), ESBL *Salmonella* and ESBL *E. coli*, **(CIPARS, 2007)**.

Pasteurella multocida

Pasteurella multocida is a worldwide-distributed pathogen responsible for a broad range of diseases in animals. In humans, this pathogen produces pneumonia, meningitis, urinary tract infections, sepsis, and peritonitis (**Guillet *et al*. 2007).** Human infections are treated primarily with penicillin, ampicillin, and cephalosporins. Although the frequency of resistance to β-lactams remains low in this species, ROB-1 is the most frequent enzyme conferring β -lactam resistance, whereas TEM-1 has been reported from a human isolate in France, BlaP1 conferring resistance to ampicillin and carbenicillin, has been described in a *P. multocida* strain of avian origin in Taiwan. In Spain, a study showed a shift from 1.6 to 14.4% in oxytetracycline resistance between 1987 and 2004 in *Pasteurella multocida* strains isolated from pigs **(Lizarazo *et al*., 2006).** Antimicrobial resistance in *Pasteurella multocida* has been related to small, non-conjugative plasmids encoding determinants conferring resistance to

ampicillin, tetracycline, streptomycin, or florfenicol **(Alvaro *et al*., 2009).**

Pseudomonas aeruginosa

The intrinsic and acquired resistance of *P. aeruginosa* to many structurally-unrelated antibiotics is due to several adaptations, including active efflux systems, reduced cell wall permeability, plasmid acquisition, expression of various enzymes, or by biofilm formation **(Deplano *et al*., 2005).**

The genetically mobile metallo-β-lactamases (MBLs) are able to hydrolyze all β- lactams except monobactams. Genes encoding for MBL were shown to be carried on large transferable plasmids or were associated with transposons, allowing horizontal transfer of these MBL genes among different bacterial genera and species, there are five types of acquired MBL genes (*im*P, *vi*M, *sp*M, *gi*M, and *si*M) have been identified based on their divergent protein molecular structures **(Yalda *et al*., 2012)**.

Antibiotic molecules, which pass through the cell wall, may then be removed by efflux pumps. Four different efflux systems dependent on the genes mexAB-oprM (beta-lactams), mexXY-oprM (aminoglycosides), mexCD-oprJ and mexEF-oprN (carbapenems and quinolones) are known to exist allowing extrusion of all classes of antibiotics except the polymixins. Genes for these efflux systems are found in all strains of *P. aeruginosa* but are expressed at relatively low levels, under the control of regulatory genes. Mutations in these regulators can lead to high level expression and confer enhanced antibiotic resistance, **(Poole, 2001)**.

A biofilm is microcolonies of bacteria embedded in a self-produced polymer matrix consisting of polysaccharide, protein and extracellular DNA and are resistant to antibiotics, disinfectant chemicals and to phagocytosis **(Bjarnsholt *et al*., 2009)**.

***Mycobacterium tuberculosis (TB)*:**

Unlike the situation in other bacteria where acquired drug resistance is generally mediated through horizontal transfer by mobile genetic elements, such as plasmids, transposons or integrons, in *M. tuberculosis*, acquired drug resistance is caused mainly by spontaneous mutations in chromosomal genes, producing the selection of resistant strains during sub-optimal drug therapy, **(Kochi *et al*., 1993).**

Intrinsic drug resistance of *M. tuberculosis* has traditionally been attributed to the unusual structure of its mycolic acid-containing cell wall that gives the bacteria a low permeability for many compounds such as antibiotics and other chemotherapeutic agents **(Jarlier and Nikaido, 1994).**

The role of efflux mechanisms has also been recognized as an important factor in the natural resistance of mycobacteria against antibiotics such as tetracycline, fluoroquinolonesand aminoglycosides, among others. **(De Rossi *et al*., 2006**).

With the discovery of several other drugs with anti-TB activity, multidrug therapy became fundamental for the control of the disease by promoting the cure of the patients and interrupting the chain of transmission. Moreover, new forms of antibiotic resistance have emerged. MDR-TB is caused by a strain of *M. tuberculosis* are resistant to rifampicin and isoniazid. Also, extensively drug-resistant TB (XDR-TB), caused by *M. tuberculosis*, threaten adequate control of the disease. In addition of being MDR, they are also resistant to any fluoroquinolone, kanamycin, capreomycin and amikacin. **(Dye, 2009)**.

Conclusion

It is necessary to determine bacterial resistance to antibiotics of all classes (phenotypes) and mutations that are responsible for bacterial resistance to antibiotics (genetic analysis). Better understanding of mechanisms of antibiotic resistance, location of genes in a chromosome and their expression would allow us to develop screening and control strategies that are needed to reduce the spread of resistant bacteria and their evolution.

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