MECHANISMS OF PATHOGENESIS AND ANTIBIOTICS RESISTANCE IN ESCHERICHIA COLI

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Summary
Pathogenicity is the bacteria capacity to produce disease in the host, expressed by virulence, which refers to pathogenicity degrees of a particular bacterium. The virulence determinants can be any structural, biochemical or genetical characteristics of pathogen which are capable to produce disease. The relation between host and pathogen is a dynamic one because each modifies the other activity and functions and the result is in dependence of pathogen virulence and the degree of susceptibility or resistance of the host. There are many pathogen bacteria in humans, but only a few are so versatile as Escherichia coli both by the mechanisms of pathogenicity and antibiotic resistance. Besides, alarming increase of antibiotic resistance of certain pathogenic strains of Escherichia coli has become a real public health problem. Therefore, this is a review of the several aspects of the molecular mechanisms involved in pathogenesis and emergence of antibiotic resistance in Escherichia coli.

Keywords: pathogenicity, virulence, antibiotic resistance, Escherichia coli, molecular mechanisms

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Introduction
Gram-negative bacteria like pathogenic Escherichia coli can cause disease through the ability to invade tissues and toxigenesis. The mechanisms of colonization include: adhesion, initial proliferation, extracellular production of substances that facilitate invasion (invasins), followed by the ability to bypass or overcome host defense mechanisms. Adhesion of bacteria to eukaryotic cell or a tissue surface requires a receptor and a ligand. Receptors are usually carbohydrate or peptide chain of eukaryotic cell surface. The bacterial ligand called adhesin is a macromolecular component of bacterial cell surface that specifically interacts with the receptor. The bacterial cell surface has properties determined by the molecular structure of the cell membrane and the cell envelope which includes the capsule or glycocalyx, S layers, peptidoglycans, lipopolysaccharides, flagella, pili and fimbriae. The bacterial surface serves as a permeability barrier, contains: adhesins involved in adhesion, enzymes that catalyze important reactions for survival and protective structures against phagocytosis; antigens involved in bypassing activation of host defense mechanisms and endotoxins that cause inflammatory reactions in host. Cell surface also has some “sensory proteins” which trigger mechanisms of cellular response to temperature changes, osmolarity, salinity, light, oxygen or nutrient content. They cause an intracellular molecular signal acting at the genome level by activating the gene expression of virulence factors.
Toxigenesis is the ability to produce toxins (exotoxins and/or endotoxins). Exotoxins are secreted by bacterial cells, acting on the host tissue. Endotoxins are constituent molecules of the bacterial cell, often the term refers to the lipopolysaccharides which are constituents of the outer membrane of Gram-negative bacteria. Endotoxins can be released only when the bacterial cell is affected by defense mechanisms of host cells or the activity of antibiotics (penicillins or cephalosporins). Both types of bacterial toxins—soluble and cell associated—may produce cytotoxic effects on some host tissue sites distant from the original point of invasion and multiplication. Some toxins have an important role even in colonization and invasion.

The *Escherichia coli* species include pathogenic and opportunistic pathogenic strains, the latter producing infections in immunocompromised hosts.

Strains are subgroups of the same species with a particular characteristic, expressed physiologically or in cell cycle, often detectable only at molecular level, which prints unicity to a strain. One such feature is the pathogenicity or the resistance to certain antibiotics [Ryan & Ray, 2004, Schaechter et al, 2006, Todar, 2008].

Evaluation of an antibiotic spectrum of activity is defined by the organisms with natural resistance to that antibiotic class, by organisms which have acquired resistance to antibiotic and by susceptible organisms. The most important resistance mechanisms are triggered by clinical use of antibiotic. Antibiotic resistance is a form of evolution due to the process of natural selection, under pressure exerted by the presence of antibiotic in the life environment of bacteria. Contrary to their versatile nature, bacteria have a limited set of mechanisms for acquiring resistance to various antibiotics: modification of the target molecule, which is no longer recognized by the antibiotic, production of bacterial enzymes that modify the antibiotic molecule or decrease in antibiotic takeover by reducing the permeability of bacterial cell or by emergence of active efflux systems. A phenotype resistant to certain classes of antibiotics may be the result of simultaneous use of the above mechanisms. However, the emergence of antibiotic-resistant strains is the result of mutations in chromosomal DNA (vertical evolution) or the acquisition of foreign genetic material (horizontal evolution) [Hawkey, 1998, Tenover, 2006].

**Enterotoxigenic *Escherichia coli* (ETEC)**

ETEC adheres to enterocytes by adhesive fimbriae and produces two types of enterotoxins: a) heat-labile enterotoxin (LT), consisting of a subunit A and five subunits B that make up the holotoxin, with structure and function similar to the cholera toxin. The LTB subunits bind irreversibly to GM1 ganglioside, and the A subunit activates adenylate cyclase, which results in increases in cyclic AMP in enterocytes, which stimulates secretion of water and electrolytes (Na⁺, K⁺, Cl⁻, HCO₃⁻) in bowel lumen; b) heat-stable enterotoxin (ST), whose mechanism of action is intracellular accumulation of cGMP. Chloride secretion by the crypt cells is then increased and inhibition of neutral sodium chloride absorption occurs, leading to outpouring of diarrheal stool. ETEC strains are not invasive, they do not leave the intestinal lumen [Sakellaris et al, 1996, Karmali, 2004, Qari et al, 2005, Schmitt & Schaffrath, 2005, Khan et al, 2006].

**Enteropathogenic *Escherichia coli* (EPEC)**

EPEC not possess fimbriae, but the expression of toxins similar to those of the genus *Shigella* causes severe forms of bleeding diarrhea disease associated with complications like hemorrhagic colitis or renal failure. EPEC adheres to host intestinal cells by intimin, a bacterial outer membrane protein. EPEC interaction with host cell induces rearrangements of actin
cytoskeleton into “pedestal-like” structure. EPEC strains are non-invasive, localized outside of enterocytes, molecular virulence factor being injected through a “molecular syringe” called type III secretion system. “Translocated intimin receptor” or Tir is a key factor in the virulence of this bacterial strains, also translocated by type III secretion system. Tir insertion occurs in target cell membrane where Tir acts as receptor for bacterial adhesion protein called intimin. Phosphorylation at serine residues in the C-terminus of Tir by a cAMP-dependent kinase is involved in mediating structural and electrostatic changes, which explain Tir insertion in host cell membrane and subsequent intermolecular interactions, including the interaction with specific ligand, intimin, leading to formation of “pedestal-like” structure. Adherence to the intestinal mucosa leads to the local loss of microvilli, so-called A/E lesions (attaching/effacing lesions) and rearrangements of actin cytoskeleton in enterocytes. Cellular signaling pathways are activated, whose purpose is to break the intercellular junctions by virulence factors such as EspF, to alter the electrolytes secretion and to produce immune response. EPEC have a number of virulence factors similar to those of the genus Shigella, known as verotoxin. E. coli EHEC and EPEC, related pathogenic strains, possess a homologous DNA region called LEE (Locus of Enterocyte Effacement) containing all the genes required for synthesis of virulence factors involved in the formation of A/E lesions. This locus has a low GC (38%) content compared to Escherichia coli chromosome, which shows the acquisition of this region by horizontal gene transfer. LEE genes encode factors involved in the construction of type III secretion system, secreted virulence factors, effectors: EspB, EspD, EspF, Map, intimin and proteins with regulator role. The targets of Map (Mitocondrial associated protein) are the host cell mitochondria because these proteins interfere with the ability to maintain membrane potential. The pathogens that induce increase of mitochondrial membrane permeability can trigger the programmed cell death by proapoptotic factors released from mitochondria and consequent activation of caspases. The infections with EPEC in vivo did not lead to detection of an increased rate of apoptosis of the infected host cells, by contrast, it has been found a decrease of apoptosis normal rates. Map should have an antiapoptotic role associated to a function of altering ATP synthesis, as reflected in the functioning of ATP-dependent cellular proteins [Goosney et al, 2000, Brendan, 2002, Paul & Brendan, 2004].

**Enteroinvasive Escherichia coli** (EIEC)

EIEC strains produce diseases in humans and contain only one group of organisms that determine disease similar enteritis generated by Shigella species. EIEC have the ability to invade intestinal epithelial cells, multiply intracellularly, and produce the disease difficult to distinguish from shigellosis. The virulent EIEC strains isolated from patients possess in high proportion a plasmid called 140 MDa characteristic to Shigella. Both bacteria require the expression of genes located in the chromosome, but also plasmid to invade intestinal cells. EIEC possess products of *ipaC, mxiG, mxij, mxiM, mxiD, invX* genes in cell membrane, required for invasion. 140 MDa plasmid of Shigella flexneri species contains the regulon *vir* genes consisting of *ipa* and *mwcI-spa* genes. *ipa* genes encode proteins with a role of adhesins /invasins preserved in EIEC and a hemolysin for preventing the phagocytosis of bacteria. The *mxi* and *spa* genes are required for export of Ipa protein. Product of other gene from *ipa* operon is necessary for multiplication and intra- extracellular spread. Clinically, the disease caused by Shigella, respectively EIEC is difficult to distinguish, but while for the shigellosis 500 of bacterial cells are sufficient to trigger the disease, in case of EIEC it takes
10^6 cells. All plasmidic and chromosomal determinants of invasion existing in \textit{Shigella}, are present also in \textit{EIEC}, but the difference may be determined by the resistance to gastric acidity of \textit{Shigella} cells [Parsot, 2005].

**Enterohemorrhagic \textit{Escherichia coli} (EHEC)**

EHEC are pathogenic strains which constitute a subset of verotoxin producing (VTEC) or Shiga-like toxin producing (STEC) \textit{E. col}. EHEC strains contain enterohemolysin A coding gene in a 60 MDa plasmid and induce A/E lesion (attaching/effacing). Verocytotoxins and shigatoxins (VT/Stx) have a A-B type structure: consist of five subunits B that surround an active component A, which is transported into the host cell by receptor-mediated endocytosis after B subunits recognize and bind to a glycolipid receptor Gb3 or Gb4 (globotriaosylceramide). This receptor is located in renal or blood vessels endothelial cells membrane, in epithelial cells membrane from the intestinal tract, in smooth muscle cell membrane, in red blood cells and B lymphocytes membranes. Toxins are actively transported into intestinal epithelial cells and release into circulation occurs passively through the intestinal epithelial cell injury. The active subunit A is a RNA N-glycosidase: it breaks a specific N-glycosidic link of 28S rRNA molecules that mediate peptide bond formation during the elongation phase of protein synthesis (preventing the dependent eEF-1 binding of aminoacyl-tRNA to ribosomal 60S subunit). By blocking protein synthesis is exerted the cytotoxic effect on renal endothelial cells, with impaired glomerular function and microvascular occlusion. Pathological effects are characterized by decreasing glomerular filtration rate, haematuria and acute renal failure known as hemolytic uremic syndrome (HUS). To the intestinal mucosa, the ulcerations cause initially watery diarrhea and ulcer bleeding diarrhea, due to lesions, characterizing the hemolytic colitis known also as ischemic colitis (HC). VT/Stx are subdivided in two families: VT1/Stx1 identical with Stx from \textit{Shigella} and VT2/Stx2, a heterogeneous group of toxins [Kaper, 1998, Karch \textit{et al}, 2005, Doughari \textit{et al}, 2009].

**Enteroaggregative \textit{E. coli} (EAggEC)**

EAggEC adheres by fimbriae to the intestinal mucosa and possess ST hemolysin and enterotoxins coding genes, similar to those of ETEC. It is a heterogeneous group, with type I fimbriae (AAF/I), autoaggregation, haemagglutination and biofilm formation features [Law \\& Chart, 1998, Villaseca \textit{et al}, 2005].

**Uropathogenic \textit{E. coli} (UPEC)**

UPEC strain of \textit{Escherichia coli} is the most common pathogen isolated from the patients with urinary tract infections (UTI), often from the patient’s own intestinal flora. Strain-specific virulence factors and host susceptibility give them the ability to colonize the urinary tract epithelia selectively, ascending through the urethra, bladder, possibly kidney tissue and cavities, triggering inflammatory reactions. The bacterial adhesion to urinary tract mucosa is a critical virulence factor. Adhesins are in great majority of fimbrial nature. Type I fimbriae mediate adhesion to host cells through recognition of the mannose type oligosaccharide residues of cell surface glycoproteins and mediate erythrocyte haemagglutination. FimH adhesins from type I fimbriae structure link to epitopes like Tamm Horsfall protein from bladder mucosa structure or uroplakin from the surface of uroepithelial cells, type I fimbriae having an important role as bladder colonization factor. Type P fimbriae mediate adhesion through recognition of digalactoside type residues (Galβ1→Gal1β) of P-type antigens of blood groups. These receptors are expressed in epithelial cells of the urinary tract. Type P fimbriae are heteropolymers formed from
different protein subunits encoded by *pap* operon. PapG adhesin localized on top of this type of fimbriae is the crucial ligand for adhesion by antigen P receptors. *pap* gene was identified in 20% of fecal *E. coli* strains, in 60% of the strains that cause cystitis, in 80% of the strains that produce pyelonephritis and in 100% of isolates from patients with pyelonephritis and septicemia. The presence of *pap* gene confers advantage in regard to uropathogenicity. Moreover, individuals with P1 antigen and increased expression of receptors Galα1→Galβ compared to individuals with P2 antigen, have a much higher rate of UTI and pyelonephritis.

Adhesion mediated by P fimbriae, through their attachment to oligosaccharide receptors, induces epithelial cell activation and production of IL-6 and IL-8. Signaling pathway is activated by binding the fimbrial proteins to the receptor, followed by the release of ceramide which is the anchoring domain of the receptor and also the secondary messenger of signaling pathway. Type P fimbriae uses TLR-4 ("toll like receptor 4") as co-receptor in epithelial cells activation and activates transcription of TLR-4 coding gene in uroepithelial cells. Lipopolysaccharide (LPS) activation is independent in these cells and are CD14 negative. LPS bound to a LPS-binding protein are presented to the CD14 receptors from de surface of sensitive cells as monocytes or macrophages and initiate the cascade of events that can lead to septic shock, activate NF-kB gene transcription in immune cells and synthesis of proinflammatory cytokines. LPS are known as polyclonal mitogens of B-cells, enhancing the humoral immune response against several bacterial antigens. Uroepithelial cells stimulation in other ways than those involving the release of ceramide is mediated by type 1 fimbriae and secretion of chemokines at the finale. Type S and type F1C fimbriae efficiently bind to specific receptors on the surface of epithelial and endothelial cells of the kidney or urinary tract. The proteins of the thin fimbriae, called curli are optimal expressed at ambient temperature (about 50%). These proteins promote colonization of the perineal area, which may favor the onset of UTI. A surface virulence factor, capsule, protects against the phagocytosis by macrophages and against bactericidal effect mediated by the complement. Certain capsular antigen molecules mimic host cell surface components, preventing the onset of an appropriate immune response from the host.

Serine protease autotransporters of UPEC called Sat have the capacity to induce vacuolation of the cytoplasm of epithelial cells in glomerulus and proximal tubules. Kidney cells are a more sensitive target to Sat compared to bladder cells. This virulence factor is not necessary in colonization, but the destruction of the glomerular and proximal tubules epithelium promotes the dissemination of pyelonephritis-associated strains in circulation.

α-hemolysin is a toxin with a broad spectrum of target cells: erythrocytes, leukocytes, renal endothelial or epithelial cells. Cytotoxic necrosis factor 1 (CNF-1) interferes with phagocytosis of bacteria and apoptosis of bladder epithelial cell. Cytolethal distending toxin CDT and cytolsyn A are expressed in UPEC. CDT mediate limited DNA damage of the host cell chromosome, which triggers the response of the cell cycle checkpoint that brings to the arrest of the cells in G2 and also induces apoptotic cell death of lymphocytes. Cytolysin A induces proinflammatory responses by recognition receptors (TLR) or by alteration of the cellular Ca\(^{2+}\) homeostasis. Virulence factors are encoded by genetic elements called pathogenicity islands (PAI), which are mobile DNA segments flanked by repeated inverted and inserted near tRNA genes. Horizontal transfer of PAI is an important evolutionary mechanism in UPEC virulence, because PAI encodes adhesins, toxins, iron acquisition systems, mechanisms of secretion and bacterial
components of the bacterial capsule. UPEC strains purchase several PAI, with the possibility that genetic information for the same phenotypic character to be present in several PAI, in the same bacterial cell. A discrete difference of homology in coding sequences or regulatory sequences of PAI can influence quantitatively the expression of virulence genes or product functionality.


The acquisition of antibiotic resistant phenotype *Escherichia coli* by spontaneous and adaptative mutations

Some mutations that confer antibiotic resistance occur randomly. They are the consequence of errors during DNA replication or inefficiency in repair mechanisms of DNA damage in bacterial cell division and are known as spontaneous mutations. *Escherichia coli* quinolone-resistant phenotype is a result of changes in at least seven positions of the *gyrA* gene and in three positions of the *parC* gene. Resistance to rifampicin is determined by mutations in the genes encoding the target proteins of these antibiotics, called RpoB [Nakamura et al, 1989, Hooper, 1999, Martinez & Baquero, 2000, Ruiz, 2003]. Mechanisms of antibiotic resistance involving efflux or import systems are genetically determined by mutations in regulatory regions of genes and even in promoter regions [Piddock, 2006, Depardieu et al, 2007]. Certain bacterial strains resistant to ampicillin and chloramphenicol are the beneficiaries of an altered promoter that allows a more efficient expression and synthesis of an appreciable amount of chromosomal β-lactamase [Jacoby & Archer, 1991].

Some mutations occur in non-dividing cells or in cells that have a low rate of division and are related with the non-lethal selection pressure that favors bacterial cells. Such mutations are named adaptive and represent the main source of emergence of antibiotic-resistant phenotypes in natural conditions. The main pions in this processes are DNA polymerase V prone-errors (*umuCD*) and DNA polymerase IV (*dinB*) which increase transitory the rate of mutations. Some antibiotics are capable to produce bacterial DNA damage and trigger the mutagenic SOS response, enhancing the rate of occurrence of antibiotic-resistant phenotypes of *Escherichia coli*. Accumulation of single-stranded DNA because of lesions that blocks replication of the bacterial chromosome leads to the formation of RecA nucleoprotein complexes, with single-stranded DNA. Under these conditions, RecA has a coprotease activity (RecA*) and facilitates the autocleavage of repressor protein LexA, leading to a derepression of sos genes. Another role of coprotease RecA* is to process UmuD to UmuD’, a required step for assembling mutagen- dependent DNA polymerase V UmuD’C. UmuC is a type of DNA polymerase with a weak polymerase activity, but capable of translesional replication. In the presence of UmuD’, RecA and single-stranded DNA binding protein (SSB), UmuC is highly activated and performs a “bypass” of the lesion continuing DNA synthesis, so it is the main responsible for the SOS response induced mutagenesis. It allowed DNA replication to continue in this way, the cost being the loss of fidelity and the appearance of mutations [Rosche & Foster, 2000, Sutton et al, 2000, Biedov et al, 2003, Janion, 2008].
Quinolones are able to introduce mutations in the gyrA, gyrB, parC genes which encode DNA gyrase and topoisomerase IV, blocking bacterial DNA replication and cell division. The derepression of sos genes is triggered (more than 40 independent genes). Most of these genes encode proteins involved in repair mechanisms of DNA lesions, replication and mutagenesis. SOS response consecutive to DNA damage introduced by antibiotic, has various effects: in repair DNA lesions or mutagenic. Mutagenesis induced by SOS response triggered by antibiotic can be even the mechanism that causes antibiotic-resistant mutant phenotype [Ysern et al, 1990, Piddock & Wise, 1997].

**Hipermutator strains can acquire antibiotic resistance phenotypes**

Both in natural environments and in laboratory conditions, there are strains that have an increased rate of mutations. The frequency of such strains in natural or clinical isolates is higher than expected, which coincides with the idea that these strains would be advantaged in terms of selection. Under environmental pressure, in the presence of an antibiotic, a small bacterial population may adopt a high rate of mutations.

Given this status, the likelihood that the population can acquire a favorable mutation is high, and then the cells grow and divide and leave this status. In *Escherichia coli*, hipermutations in presence of antibiotic can produce inactivation of more than 20 different genes. These strains have a defective repair mechanism of DNA damage due to inactivation of *mutS* and *mutL* genes involved in the mechanism called MMR or “methyl-directed mismatch repair system” which, normally, controls the fidelity of DNA replication and horizontal gene transfer, preventing recombinations between non homologous DNA sequences. Such a phenotype, with a high frequency of mutations increases the probability of acquisition resistance to one or more antibiotics. Such a mutation, considered favorable, is the resistance to antibiotics and can be fixed in the population in conditions of the antibiotic presence in the environment [Horst et al, 1999, Harfe & Jinks-Robertson, 2000].

Concentration of the selection factor plays a particularly important role in the mutation rate determining an antibiotic-resistant phenotype. Resistance to high concentrations of antibiotic is the result of mutations affecting different genes, which may confer different levels of resistance to an antibiotic. At a low concentration of antibiotic, mutations in either of the genes related to antibiotic resistance mechanisms can protect the bacteria of the antibiotic action. Higher concentration of antibiotic decreases the number of resistant phenotypes selected, because at a certain concentration of antibiotic, mutations which affect more than one gene are required to provide an antibiotic resistant phenotype. Such a mutant phenotype can reach a maximum frequency in the population at a concentration close to the MIC (minimum inhibitory concentration) of antibiotic. A specific concentration of antibiotic may be sufficient to reduce population growth rate and even to suppress the ancestral population (the wild type population), without being sufficient to affect resistant mutant population. A higher concentration of antibiotic can suppress both the susceptible and the resistant population, so that the selection of resistant phenotype can not occur. So a very narrow range of antibiotic concentrations allow the selection of resistant strains, that range is known as the selection window. Mutation rate is very sensitive to changes in the concentration of antibiotic, a discontinuous spectrum of mutations rates and phenotypes of antibiotic resistance can be obtained in the range of variation in concentration of antibiotic. Time of exposure to an antibiotic action is critical in intensifying the rate of mutations, as well as the dynamic of an antibiotic action on the bacterial cell wall. If it is not used a correct administration period and
also a correct concentration of the antibiotic, the cells maintained under conditions of stress increase their mutation rate. Moreover, the existence of a mutation is not necessarily sufficient for the appearance of resistant phenotype, but an insufficient concentration for suppress, instead gives ideal conditions for the emergence of other mutations in the genes involved in the mechanisms of resistance and, consequently, for the appearance of phenotype resistant to antibiotics [Martinez & Baquero, 2000].

**Increasing concentrations of transcription factors may lead to antibiotics resistance in Escherichia coli cells**

There are some transcription factors in *Escherichia coli* cells, known as MarA, Sox and Rob, which activate a set of 40 promoters belonging to marA / soxS / rob regulon, whose function includes antibiotic resistance, superoxide resistance and tolerance to organic solvents. Synthesis of each transcriptional factor is the result of a particular signal: the weak organic acids (salicylic acid) enhance marA gene transcription, the superoxide radicals increase soxS gene transcription, the bile salts and dipyridyl increase rob gene transcription. Increasing the concentration of these transcription factors results in enhancement of gene transcription involved in the growth of antibiotic efflux (acrAB, tolC), in the lowering of outer membrane permeability (micF), in increasing resistance to superoxide radicals (zwf, fpr, soda), in the substitution of proteins sensitive to superoxide with some resistant proteins (acnA, fumC) or in DNA repair processes (nfo).

The synthesis level of protein F (OmpF) from the outer membrane, representing some of the major porins which confer permeability to antibiotics is dependent on the concentration of MarA. Multiple antibiotic resistance is genetically determined by locus mar, located at 34 minutes on the chromosome of *Escherichia coli*, by controlling the intrinsic susceptibility of this bacterial strains to several structurally unrelated antibiotics: tetracycline, chloramphenicol, beta-lactams and quinolones. Mar phenotype existence depends on two transcription factors: MarR, transcription repressor and MarA, activator of transcription. MarCRAB locus consists of four genes marC, marR, marA and marB arranged in two transcriptional units (TU1 and TU2), which are transcribed divergent from a promoter region marO. TU1 contains marC, which contributes to full expression of multiple antibiotic resistance phenotype. TU2 contains marRAB operon. Singular expression of marB has no effect on antibiotic resistance and expression along with marA results in an intensification of the effect of antibiotic resistance. mar operon is widespread in enteric bacteria, including *Salmonella*, *Shigella*, *Klebsiella*, *Citrobacter*, *Hafnia* and *Enterobacter*. mar locus appears to mediate the cellular response to various stimuli and is able to affect distant genes from this locus, that form mar regulon, involved in *Escherichia coli* response to oxidative stress and to action of weak acids. In fact, the first promoter activated by MarA is even marRAB promoter, which has the effect of increasing their own synthesis. MarR inactivation by mutations or small molecules, activates marRAB transcription and determines antibiotic resistance phenotype. Thus, bacterial cells may adopt different defensive strategies depending on the type of signal, signal amplitude and duration.[Barbosa & Levy, 2000, Alekshun & Levy, 2004]

**Emergence of antibiotic resistance by horizontal gene transfer**

Mobility of antibiotic resistance genes involves mechanisms that allow the DNA transfer between species or between the individuals of the same species. The transfer takes place from one bacterial cell to another by conjugation, transduction and transformation or from a DNA molecule to
another through various types of recombination mechanisms. Classical recombination, RecA-dependent, requires an extensive homology between DNA molecules involved in recombination. Transposition, by discrete transposable elements, does not require homology between the sequences that recombine.

Site-specific recombination is recombination between short homologous sequences, mediated by recombination enzymes specific for the site of recombination. This type of recombination allows direct insertion of the antibiotic resistance gene or genes and carries out the spread of resistance genes by elements like integrons or gene cassettes. Many antibiotic resistance genes are located on large transferable DNA elements called plasmids, that can contain other mobile genetic elements called transposons. Some transposons contain a gene for resistance to a single antibiotic: β-lactamase (Tn3), kanamycin (Tn5) or tetracycline (Tn10). Plasmids and transposons that encode resistance to multiple antibiotics often contain another mobile genetic element called the integron [Hall, 1997, Bennett & Howe, 1998].

Integron possesses an attI site where takes place gene cassette insertion by site-specific recombination. The attI site encodes the enzyme called integrase that mediates recombination. Gene cassettes are genetic elements that can exist as circular and free DNA molecules, unable of self-replication when the genetic transfer is taking place from one site to another, but normally found like constituent sequences of a large molecule of plasmid or chromosomal DNA. Gene cassettes have a length of 500-1000 bp, usually contain a single gene and an additional short sequence of 59 bp, which is the attC specific recombination site. attC sites are nucleotide sequences that serve as recognition sites for integrase, without their own promoter, so their transcription requires a promoter that belongs to integron [Hall et al,1991, Collis & Hall, 1992].

There are five distinct classes of integrons with a role in the dissemination of antibiotic resistance genes, each with characteristic features: a gene that encodes integrase, the recombination site where gene cassettes will integrate and a promoter for the transcription of gene/genes from the cassette. All of the five classes are physically linked in a mobile DNA element such as insertion sequences (ISs), transposons or plasmids, which can serve as carriers for transferring genetic material between species or between individuals of a species.

Class 1 integrons consist of a variable region delimited by conserved regions 5' and 3'. The region 5' consists of intI, attI genes and Pant promoter involved in transcription of genes from the variable region. The region 3' consists of a locus for resistance to ethidium bromide qacEAl, a truncated version of the gene for resistance to detergents qacE, a gene for resistance to sulphonamide sulI and two transcriptional units orf5 and orf6, encoding proteins with unknown function. The initial class 1 integron is a structure consisting of region 5' and region 3', without a integrated gene cassettes. The acquisition of one or more gene cassettes through insertion at attI site determines the appearance of a particular type of integron, assigned with a number. _In1_ confer resistance to aminoglycosides and beta-lactam antibiotics mediated by products of resistance genes _oxa2_ and _aadA1b_. _In2_ contains a single antibiotic resistance gene _aadA1a_, which encodes an enzyme that catalyse the aminoglycosides modifications. Class 1 integrons has the next formal structure: intIattI(r59b)_n_ qacE1sul1orf5orf6 where _r59b_ represents the antibiotic resistance gene cassette and _n_ is the number of the inserted cassettes [Recchia & Hall, 1995, Bennett, 1999, Ploy et al, 2000, Mazel, 2006].

Class 2 integrons are associated exclusively to Tn7 derivatives, and class 3 integrons are located in transposons inserted into plasmids that are not characterized. Class 4 integrons are
components of a subset of SXT genetic elements from Vibrio cholerae and class 5 integrons were identified in a transposon, component of a plasmid Both 4 and 5 classes are involved in resistance to trimethoprim of Vibrio genus.

Class 1 integrons were highlighted in clinical isolates and most of the gene cassettes that confer resistance to antibiotics are belonging to this class. They were described over 80 different gene cassettes in class 1 integrons, containing resistance genes to β-lactam antibiotics, to all aminoglycosides, to chloramphenicol, trimethoprim, streptomycin, rifampicin, erythromycin, fosfomycin, lincomycin and to antiseptics with quaternary ammonium group. Only six gene cassettes encoding antibiotic resistance were identified in class 2 integrons, this reduction in diversity being due to a mutation in the gene that probably encodes the integrase in this class of integrons [Bennett, 1999, Recchia & Sherratt, 2002]. The new integrons are generated by the insertion of gene cassettes at attI site or by the deletion of one or more gene cassettes from an integron, that are taking place also by site-specific recombination between any element consisting of 59 bp and attI or another attC element.

Integrons can recruit gene cassettes directly from the chromosomal superintegrons, these gene cassettes can be substrate for integrase gene of the class 1 integrons. Superintegrons contain a large number of gene cassettes, are located in the host bacterial chromosome and are not associated with a mobile genetic element. They have a high degree of identity between attC sites. First discovered in the Vibrio cholerae species, they were subsequently identified in the genomes of many species belonging to the Gammaproteobacteria class. Thus, two gene cassettes encoding carbenicillinases (CARB-7 and CARB-9) have been identified in environmental isolates of Vibrio cholerae, being associated with type VCR attC sites. These gene cassettes belong to a class of carbenicillinases known as RSG carbenicillinases, characterized by presence of an RSG triad of amino acids in 234-236 position [Mazel et al, 1998, Rowe-Magnus et al, 2002, Mazel, 2006].

The existance of mobile integrons containing more than two gene cassettes, would be due to the recombinations between different mobile integrons and not to successive recruitments of gene cassettes from the superintegrons of different species. The mobility of gene cassettes in and out from integrons is a random process, rearranging that persists and those lost are the result of circumstances and of natural selection. If genetic recombination brings genes involved in a specific antibiotic resistance in a bacterial cell and at the same time gives the advantage to bacteria, that bacterial clone will be selected and that integron will be amplified by clonal amplification [Bennett, 1999, Boucher et al, 2002, Mazel, 2006].

The antibiotic resistome

Studies on antibiotic resistance are generally focused on pathogenic bacteria because their clinical significance. A term like “antibiotic resistome” defines in literature a new point of view and means all the antibiotic resistance genes spread in pathogenic and non-pathogenic bacterial strains, and even in antibiotic producing strains.

An important but underestimated source of genes involved in resistance to antibiotics is the microorganisms from soils. D’Costa and his collaborators publish the results of a interesting study about the resistance to 21 antibiotics of natural, semi-synthetic or synthetic origin of ~ 500 actinomycete strains isolated from different soil types (urban, agricultural and forestry). Each strain has resistance to multiple antibiotics: from a minimum of 2 antibiotic resistance to a maximum of 15 antibiotic resistance, with an average of resistance at 7-8 antibiotics. Horizontal transfer of genes involved in antibiotic resistance from environmental non-pathogenic strains to
pathogenic strains is a matter which does not to be overlooked [D’Costa et al., 2006, Wright, 2007].

Many of the bacterial strains resident in soils are producing antibiotics. Since 1940, Selman Waksman, discoverer of streptomycin, believed that the purpose of antibiotic production in some strains is inhibiting the growth of other bacterial strains as part of the competition for a particular niche. The antibiotic producing strains own also the mechanisms for protection against the antibiotic produced by themselves and the coexisting strains developed their resistance mechanisms or acquired them by horizontal gene transfer. At the incidence of antibiotic resistance in non-producing antibiotic bacteria has probably contributed the uncertainty of achieving the minimum inhibitory concentration of the antibiotic that was secreted in environment by the producing strain. This favored the emergence of hipermutators or of some modifications in genes transcription and consequently, of some bacterial phenotypes with new features [Benvesite & Davies, 1973, Linares et al, 2006 ].

Conclusions

Using the appropriate drug at the appropriate dosage and for the appropriate duration is one important means of reducing the selective pressure that helps resistant organisms emerge. New antibacterial agents with different mechanisms of action are needed and also specific new targets like cell wall biosynthesis, aromatic amino acid biosynthesis, cell division, two component signal transduction, fatty acid biosynthesis, isoprenoid and tRNA biosyntheses. Strategies could be developed to target virulence factors of pathogens instead of whole bacteria (develop drugs that target the plasmids containing resistance genes or drugs that target the adhesion of virulent bacteria to a tissue). All the alternative strategies to overcome resistance require expanded knowledge of the molecular mechanisms of virulence and antibiotic resistance, their origins and evolution, and their distribution throughout bacterial populations and genomes.

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