# REVIEW

*Medicina (Kaunas) 2011;47(3):137-46*

# Antibiotic Resistance Mechanisms of Clinically Important Bacteria

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***Key words:*** *bacteria; antibiotics; resistance mechanisms.*

***Summary.*** *Bacterial resistance to antimicrobial drugs is an increasing health and economic problem. Bacteria may be innate resistant or acquire resistance to one or few classes of antimicrobial agents. Acquired resistance arises from: (i) mutations in cell genes (chromosomal mutation) leading to cross-resistance, (ii) gene transfer from one microorganism to other by plasmids (conjugation or transformation), transposons (conjugation), integrons and bacteriophages (transduction). After a bacterium gains resistance genes to protect itself from various antimicrobial agents, bacteria can use several biochemical types of resistance mechanisms: antibiotic inactivation (interference with cell wall synthesis, e.g., β-lactams and glycopeptide), target modification (inhibition of protein synthesis, e.g., macrolides and tetracyclines; interference with nucleic acid synthesis, e.g., fluoroquinolones and rifampin), altered permeability (changes in outer membrane, e.g., aminoglycosides; new membrane transporters, e.g., chloramphenicol), and "bypass" metabolic pathway (inhibition of metabolic pathway, e.g., trimethoprim-sulfamethoxazole).*

## Introduction

Bacterial resistance is closely associated with the use of antimicrobial agents in clinical practice. Prolonged therapy with antibiotics can lead to the development of resistance in a microorganism that initially is sensitive to antibiotics, but later it can adapt gradually and develop resistance to antibiotics. When an antibiotic attacks bacteria, bacterial cells susceptible to it will die, but those that have some insensitivity will survive. The emergence of a phenotype resistant to antimicrobial agents depends on various factors of a host: degree of resistance expression, capability of a microorganism to tolerate resistance mechanism, initial colonization site, and other factors. When resistance determinants are on plasmids, they will spread quickly within the genus and even unrelated bacterial genera. When resistance is associated with genes on chromosomes, resistant microorganisms will spread more slowly (1, 2).

An important cause of the spread of antimicrobial resistance is a failure to apply infection control measures in a hospital and outside it. It has been established that methicillin-resistant *Staphylococcus aureus* (*S. aureus*, MRSA) in a hospital and MRSA in the community are often genetically related. Resistant bacteria are transmitted by aerosol transmission, especially during periods of viral upper respiratory infections, frequent hand-nose contacts, and poor hand washing among health care workers (3).

Antibiotic use in nonhuman niches is another important reason for the spread of resistant bacteria (4). It is known that the use of antimicrobial agents in animal food is related to bacterial resistance; for example, *Salmonella* and *Campylobacter* acquire resistance to antibiotics and transfer genes of antibiotic resistance to natural human flora, for example, *Enterococcus*. High *Escherichia coli* (*E. coli*) resistance to ciprofloxacin is associated with the use of fluoroquinolones in aviculture (1, 3).

Over the years, the continued use of various antibacterial/antimicrobial agents has led microorganisms to develop resistance mechanisms, which are the cause of resistance to one or more drugs (multidrug resistance, MDR) (5). Resistance mechanisms probably have evolved from genes present in organisms that produce antibiotics (6). Multidrug resistance has been demonstrated in *Pseudomonas aeruginosa (P. aeruginosa), Acinetobacter baumannii* (*A. baumannii*), *E. coli,* and *Klebsiella pneumoniae* (*K. pneumoniae*), producing extended-spectrum β-lactamases (ESBL), vancomycin-resistant enterococci *Enterococcus faecium* (*E. faecium*) (VRE), MRSA, vancomycin-resistant *S. aureus* VRSA, extensively drug-resistant (XDR) *Mycobacterium tu-*

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*berculosis* (*M. tuberculosis*) (5), *Salmonella enterica* (*S. enterica*) serovar *Typhimurium*, *Shigella dysenteriae* (*S. dysenteriae*), *Haemophilus influenzae* (*H. influenzae*), *Stenotrophomonas,* and *Burkholderia* (1). Antibiotic resistance can be acquired as a chromosomal mutation, but usually resistance to antibiotics is associated with mobile extrachromosomal DNA elements – plasmids, transposons, and integrons – acquired from other bacteria. Efflux pumps are recognized as the main multidrug resistance mechanism in bacteria (5).

**Genetics of Antibiotic Resistance**

Bacterial resistance to antibiotics can be intrinsic or innate, which is characteristic of a particular bacterium and depends on biology of a microorganism (*E. coli* has innate resistance to vancomycin), and acquired resistance (2). Acquired resistance occurs from (i) acquisition of exogenous genes by plasmids (conjugation or transformation), transposons (conjugation), integrons and bacteriophages (transduction), (ii) mutation of cellular genes, and (iii) a combination of these mechanisms (3, 6–8).

*Mutations. Spontaneous Mutations.* Chromosomal mutations are quite rare (one in a population of $10^6$–$10^8$ microorganisms) and commonly determine resistance to structurally related compounds (3). These mutations occur as errors of replication or an incorrect repair of damaged DNA. They are called spontaneous mutations or growth-dependent mutations. Resistance to quinolones in *E. coli* is caused by changes in at least seven amino acids in the *gyrA* gene or three amino acids in the *parC* gene (1, 6, 9), whereas only a single point mutation in the *rpoB* gene is associated with a complete resistance to rifampin (3). A chromosomal mutation in dihydropteroate synthetase results in a reduced affinity for sulfonamides (7). Some biochemical resistance mechanisms are the result of mutations. Antibiotic uptake or efflux system can be modified by mutations (10).

*Hypermutators.* According to the "hypermutable state" model, a small bacterial population during a prolonged nonlethal selection of microorganisms may achieve a short-term state when the population mutates at a very high rate (hypermutable strains or mutators) (1). These cells can increase the rate of mutations from 10 to 50 up to 10 000 times (11). Most hypermutators are found in populations of *E. coli, S. enterica*, *Neisseria meningitides* (*N. meningitides*), *H. influenzae, S. aureus, Helicobacter pylori* (*H. pylori*), *Streptococcus pneumoniae* (*S. pneumoniae*), and *P. aeruginosa* (1).

*Adaptive Mutagenesis.* Most mutations occur in dividing cells. However, they can also arise in nondividing or slowly dividing cells. Mutations occur only during nonlethal selection of microorganisms and are called "adaptive mutations." This adaptive process is the only and main source of the antibiotic-resistant mutants to originate under normal conditions. Streptomycin causes a hypermutable phenotype in *E. coli*, and some antibiotics (quinolones) can induce the SOS mutagenic response and increase the rate of emergence of resistance to antibiotics (1, 12, 13).

*Horizontal Gene Transfer.* A transfer of resistance genes from one bacterium to another is called a horizontal gene transfer (14). The main mechanisms of resistance gene transfer in a bacterium are plasmid transfer, transfer by viral delivery, and transfer of free DNA (Fig. 1). Genes can be transferred by three main ways: transduction (via bacteriophages and integrons), conjugation (via plasmids and conjugative transposons), and transformation (via incorporation of chromosomal DNA, plasmids into a chromosome) (mobile genetic elements are described in Table 1). Then genes are incorporated into the recipient chromosome by recombination or transposition and may have one or several changes in gene sequence (1, 5, 15).

Most plasmids are double-stranded circular DNA whose size may vary from 2–3 kb to plasmids, which encode up to 10% of the host cell chromosome. The transfer of resistance genes is more effective than chromosomal mutation (5). Plasmids encode genes that confer resistance to main classes of antimicrobial agents (cephalosporins, fluoroquinolones, and aminoglycosides) (14), toxic heavy metals (mercury, cadmium, silver), and virulence determinants that help a cell to survive in the environment of lethal antibiotic doses (15, 16).

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" (6). Transposons can be integrated into plasmids or the host's chromosome, encompass small elements called insertion sequences (IS elements), transposons, and transposing bacteriophages. They have terminal repeat sequences that play a role in recombination and recognize a protein (for example, transposase or recombinase) that is necessary to insert or remove a transposon from specific genome regions (5, 14, 16). Transposons are transferred by conjugation, transformation, or transduction (e.g., *mec*A gene is found in MRSA) and spread quicker than genes in chromosomes. Conjugative transposons have characteristic features of plasmids and can help to transfer endogenic plasmids from one microorganism to another (8, 15, 17).

Bacterial integrons are gene capture systems (Fig. 2) that instead of transposition use a specific recombination mechanism (14, 15). Integron encodes three main components in the 5′ conserved segment: an enzyme integrase (gene *int*) that serves as a specific recombination system to insert or to

*Table 1.* Mobile Genetic Elements (5)

| Genetic Element | General Characteristic | Resistance Determinants Specified/Examples |
|---|---|---|
| Plasmid | Variable size (1–>100 kb), conjugative, and mobilizable | R factor: multiple resistance |
| Insertion sequence | Small (<2.5 kb), contains terminal inverted repeats, and specifies a transposase | IS1, IS3, IS4 |
| Composite (compound) transposon | Flanked by insertion sequences and/or inverted repeats | Tn5: Kan, Bleo, Str |
| Complex transposon | Large (>5 kb), flanked by short terminal inverted repeats, and specifies a transposase and recombinase | Tn1 and Tn3: β-lactamase<br>Tn7: Tmp, Str, Spc<br>Tn1546: glycopeptides |
| Conjugative transposon | Promotes self-transfer | Tn916: Tet and Mino<br>Tn1545: Tet, Mino, Ery, and Kan |
| Transposable bacteriophage | A bacterial virus that can insert into the chromosome | Mu |
| Other transposable elements | Other than composite, complex, and conjugative transposons | Tn4: Amp, Str, Sul, and Hg<br>Tn1691: Gen, Str, Sul, Cm, and Hg |
| Integron | Facilitates acquisition and dissemination of gene cassettes; specifies an integrase, attachment sites, and transcriptional elements to drive expression of multiple resistance genes | Class 1: multiple single determinants and MDR efflux pump (Qac)<br>Class 2: Tmp, Strp, Str, and Spc (Tn7)<br>Class 3: carbapenems<br>Class 4: *Vibrio* spp. super-integron |

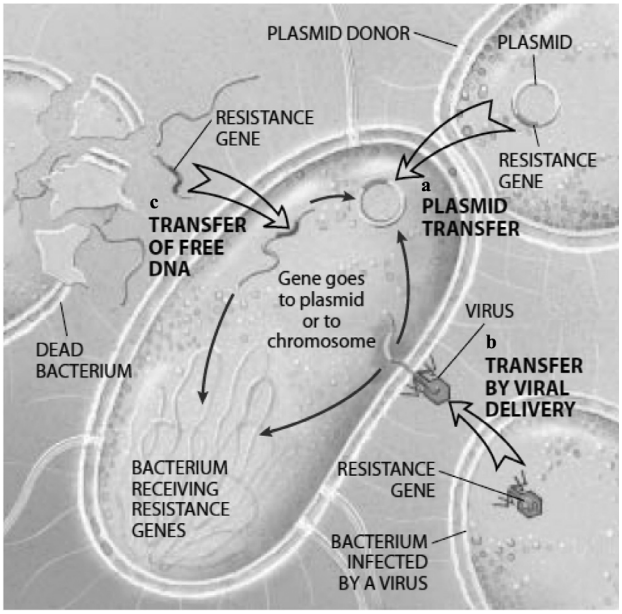


*Fig. 1.* Three main mechanisms of resistance gene transfer in a bacterium (9)

a, plasmid transfer; b, transfer by viral delivery; c, transfer of free DNA.

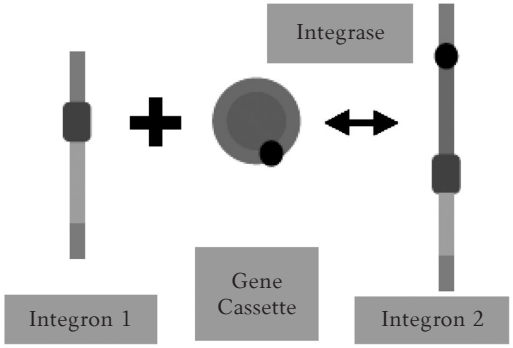


*Fig. 2.* Simplified scheme of gene cassette capture by a bacterial integron (14)

remove a new gene cassette, specific recombination site (*attI* site), and a promoter that starts gene transcription. Most integrons of class I in the 3′ conserved segment have an additional gene *suII* responsible for resistance to sulphonamides (10, 18, 19).

## Biochemical Resistance Mechanisms

The main types of biochemical mechanisms that bacteria use for defense are as follows: decreased uptake, enzymatic modification and degradation, altered penicillin-binding proteins (PBPs), efflux, altered target, and its overproduction (Table 2) (3, 20, 21). Below we will describe main types of different biochemical mechanisms that are found in clinically important bacteria.

### *Antibiotic Inactivation or Modification*

There are three main enzymes that inactivate antibiotics: β-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferases (7).

*Antibiotic Modification by Hydrolysis.* β-Lactamases are broadly prevalent enzymes that are classified using two main classification systems: Ambler and Bush-Jacoby-Medeiros (5). It is known about 300 different β-lactamases. The most clinically important are produced by gram-negative bacteria (22) and are coded on chromosomes and plasmids. Genes that encode β-lactamases are transferred by transposons but also they may be found in the composition of integrons (23). β-Lactamases hydrolyze nearly all β-lactams that have ester and amide bond, e.g.,

*Table 2*. Biochemical Resistance Mechanisms (3, 20, 21)

| Antibiotic Class | Resistance Type | Resistance Mechanism | Common Example(s) |
|---|---|---|---|
| Aminoglycosides | Decreased uptake | Changes in outer membrane permeability | *P. aeruginosa* |
| | Enzymatic modification (AMEs) | Phosphotransferase | Wide range of enteric negative bacteria |
| | | Adenyltransferase | Wide range of enteric negative bacteria |
| | | Acetyltransferase | Wide range of enteric negative bacteria |
| | | Bifunctional enzyme | *S. aureus, E. faecium* and *E. faecalis aac(6´)-aph(2´´)* |
| $\beta$-lactams | Altered PBPs | PBP2a (additional PBP) | *mecA* in *S. aureus* and coagulase-negative staphylococci<br>*S. pneumoniae* |
| | | PBP2x, PBP2b, PBP1a | *E. faecium* |
| | | PBP5 (point mutation) | TEM-1 *in E. coli*, *H. influenzae,* and *N. gonorrhoeae* |
| | Enzymatic degradation ($\beta$-lactamases) | Ambler class A | SHV-1 in *K. pneumoniae*<br>K-1 (OXY-1) in *K. oxytoca*<br>Extended-spectrum $\beta$-lactamases (TEM – 3+, SHV – 2+, and CTX-M types) *K. pneumoniae* and *E. coli*<br>BRO-1 in *M. catarrhalis*<br>PC1 in *S. aureus*<br>PSE-1 in *P. aeruginosa*<br>$\beta$-lactamases of *C. koseri* and *P. vulgaris*<br>L-1 in *S. maltophilia* |
| | | Ambler class B | Ccr-A in *B. fragilis*<br>Amp C in *E. cloacae*, *C. freundii* |
| | | Ambler class C | *S. marcescens*, *M. morganii*,<br>*P. stuartii* and *P. rettgeri* |
| | | Ambler class D | OXA-1 in *E. coli* |
| Chloramphenicol | Enzymatic degradation | CAT | CAT in *S. pneumonia* |
| | Efflux | New membrane transporters | *cmlA* and *flo*-encoded efflux in *E. coli* and *Salmonella* spp. |
| Glycopeptides | Altered target | Altered peptidoglycan cross-link target (D-Ala-D-Ala to D-Ala-D-Lac or D-Ala-D-Ser) encoded by complex gene cluster | *vanA* and *vanB* gene clusters in *E. faecium* and *E. faecalis* |
| | Target overproduction | Excess of peptidoglycan | Glycopeptide "intermediate" strains of *S. aureus* |
| Fosfomycin | Enzymatic degradation | Thioltransferase | *fosA* in negative bacteria and *P. aeruginosa;*<br>*fosB* in staphylococci and *B.subtilis* |
| Fusidic acid | Altered target | Mutation leading to reduced binding to active site(s) | Mutation in *fusA* in *S. aureus* |
| | Decreased permeability | Chloramphenicol acetyltransferase | Mutation in *fusB* in *S. aureus* |
| Macrolides-lincosamides-streptogramins B | Altered target | Methylation of ribosomal active site with reduced binding | *erm*-encoded methylases *in S. aureus*, *S. pneumoniae*, and *S. pyogenes* |
| Macrolides | Efflux | Mef type pump | *mef*-encoded efflux in *S. pneumoniae* and *S. pyogenes* |
| Oxazolidinones | Altered target | Mutation leading to reduced binding to active site | G2576U mutation in rRNR in *E. faecium* and *S. aureus* |
| Streptogramins Streptogramin A | Enzymatic degradation | Acetyltransferase | *vatA, vatB*, and *vatC* in *S. aureus*<br>*E. faecium vatD* and *vatE* |
| Quinolones | Altered target | Mutation leading to reduced binding to active site(s) | Mutations in *gyrA* in enteric gram-negative bacteria and *S. aureus* |
| | Efflux | New membrane transporters | Mutations in *gyrA* and *parC* in *S. pneumoniae*<br>NorA in *S. aureus* |
| Rifampin | Altered target | Mutations leading to reduced binding to RNA polymerase | Mutations in *rpoB* in *S. aureus* and *M. tuberculosis* |
| Tetracyclines | Efflux | New membrane transporters | *tet* genes encoding efflux proteins in gram-positive and gram-negative bacteria |
| | Altered target | Production of proteins that bind to the ribosome and alter the conformation of the active site | *tet*(M) and *tet*(O) in diverse gram-positive and gram-negative bacteria species |

*Table 2.* Biochemical Resistance Mechanisms (3, 20, 21) (continuation)

| Antibiotic Class | Resistance Type | Resistance Mechanism | Common Example(s) |
|---|---|---|---|
| Sulfonamides | Altered target | Mutation or recombination of genes encoding DHPS | Found in a wide range of species: *E. coli*, *S. aureus*, *S. pneumoniae* |
| | | Acquisition of new low-affinity DHPS genes | *sul*I *and sul*II in enteric gram-negative bacteria |
| Trim-ethoprim | Altered target | Mutations in gene encoding DHFR | *S. aureus*, *S. pneumoniae*, *H. influenzae* |
| | | Acquisition of new low-affinity DHFR genes | *dhfrI* and *dhfrII* encoded, found in a wide range of species *E. coli* |
| | Overproduction of target | Promoter mutation leading to overproduction of DHFR | |

penicillins, cephalosporins, monobactams, and carbapenems. Serine β-lactamases – cephalosporinases, e.g. AmpC enzyme – are found in *Enterobacter* spp. and *P. aeruginosa* and penicillases in *S. aureus* (5, 24–27). Metallo-β-lactamases (MBLs) found in *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *Proteus mirabilis* (*P. mirabilis*), *Enterobacter* spp. have the same role as serine β-lactamases and are responsible for resistance to imipenem, new-generation cephalosporins and penicillins. MBLs are resistant to inhibitors of β-lactamases but sensitive to aztreonam (24, 28). Specific *A. baumannii* carbapenem-hydrolyzing oxacillinase (OXA) enzymes that have low catalytic efficiency together with porin deletion and other antibiotic resistance mechanisms can cause high resistance to carbapenems (24). The resistance of *K. pneumoniae* carbapenamases (KPC-1) to imipenem, meropenem, amoxicillin/clavulanate, piperacillin/tazobactam, ceftazidime, aztreonam, and ceftriaxone is associated with the nonconjugative plasmid-coded *bla* gene (29).

Extended-spectrum β-lactamases (ESBL) – TEM, SHV, OXA, PER, VEB-1, BES-1, GES, IBC, SFO and CTX – mainly are encoded in large plasmids. They can be transferred in connection of two plasmids or by transposon insertion. ESBL are resistant to penicillins (except temocillin), third-generation oxyimino-cephalosporins (e.g., ceftazidime, cefotoxime, ceftriaxone), aztreonam, cefamandole, cefoperazone, byt they are sensitive to methoxy-cephalosporins, e.g., cephamycins and carbapenems, and can be inhibited by inhibitors of β-lactamases, e.g., clavulanic acid, sulbactam, or tazobactam (23, 30–34). Strains producing ESBL are commonly resistant to quinolones but their resistance depends not on multiple resistance plasmids but on mutations in *gyr*A and *par*C genes (35). Such strains are found among *E. coli*, *K. pneumoniae,* and *P. mirabilis* (1). The number of known ESBLs reaches 200 (32, 36).

Hydrolysis of antibiotics can be run by other enzymes, e.g., esterases. *E. coli* gene *ere*B encodes erythromycin esterase II that hydrolyzes a lactone ring of erythromycin A and oleandomycin. *ere*B gene is prevalent in *Enterobacteriaceae* strain and is responsible for resistance to erythromycin A and oleandomycin (37). Ring-opening epoxidases cause resistance of bacteria to fosfomycin (1).

*Antibiotic Inactivation by Group Transfer.* The group of enzymes inactivating aminoglycosides, chloramphenicol, streptogramin, macrolides, or rifampicin is called transferases. Inactivation is made by binding adenylyl, phosphoryl, or acetyl groups to the periphery of the antibiotic molecule. These modifications are achieved in the process of transport across the cytoplasmic membrane (co-substrate ATP, acetyl-CoA, $NAD^+$, UDP-glucose, or glutathione) (1, 16). Aminoglycosides are neutralized by specific enzymes: phosphoryltransferases (APHs), nucleotidyltransferases or adenylyltransferases (ANTs), and acetyltransferases (AACs). These aminoglycoside-modifying enzymes (AMEs) reduce affinity of a modified molecule, impede binding to the 30S ribosomal subunit (38), and provide extended-spectrum resistance to aminoglycosides and fluoroquinolones (39). AMEs are identified in *S. aureus*, *Enterococcus faecalis* (*E. faecalis*), and *S. pneumoniae* strains. Presumably, they evolved from actinomycetes (*Streptomyces* spp. and *Micromonospora* spp.) that produce AMEs. Most AMEs are transferred by transposons (4).

Gram-positive and gram-negative bacteria and some of *H. influenzae* strains are resistant to chloramphenicol and they have an enzyme chloramphenicol transacetylase that acetylates hydroxyl groups of chloramphenicol. Modified chloramphenicol is enable to bind to a ribosomal 50S subunit properly (17).

*Antibiotic Inactivation by Redox Process.* Oxidation and reduction reactions are used by pathogenic bacteria as a resistance mechanism against antibiotics. *Streptomyces virginiae* produces type A antibiotic virginiamycin $M_1$ and protects itself from its own antibiotic by substituting a ketone group to an alcohol residue at position 16 (1, 6).

## Target Modification

An interaction between an antibiotic and a target molecule is very specific so even small changes in a target molecule can influence antibiotic binding to a target. Sometimes, in the presence of a modification

in a target, other changes in the cell are needed to compensate an altered target (1, 40).

*Peptidoglycan Structure Alteration.* Inhibition of cell wall synthesis is performed by β-lactams, e.g., penicillins, cephalosporins, carbapenems, monobactams, and glycopeptides, e.g., vancomycin and teicoplanin. The presence of mutation in PBPs leads to a reduced affinity to β-lactam antibiotics. It results in resistance of *E. faecium* to ampicillin and *S. pneumoniae* to penicillin. *S. aureus* resistance to methicillin and oxacillin is associated with integration of a mobile genetic element – "staphylococcal cassette chromosome mec" (SCC*mec*) – into the chromosome of *S. aureus* that contains resistance gene *mec*A. *mec*A gene encodes PBP2a protein, a new penicillin-binding protein, that is required to change a native staphylococcal PBP (1, 5, 41). PBP2a shows a high resistance to β-lactam antibiotics (they do not bind to β-lactams) and ensures cell wall synthesis at lethal β-lactam concentrations (6, 42). *S. aureus* strains resistant to methicillin can be cross resistant to all β-lactam antibiotics, streptomycin, and tetracycline and in some cases to erythromycin (43). When lesions in membrane proteins are present, cross-resistance between β-lactam antibiotics and fluoroquinolones is possible (44). Cell wall synthesis in gram-positive bacteria can be inhibited by glycopeptides, e.g., vancomycin or teicoplanin, by their binding to acyl-D-alanyl-D-alanine (acyl-D-Ala-D-Ala) residues of peptidoglycan precursors. Resistance to glycopeptides can be innate (*VanC*-type resistance) or acquired (1, 43). *E. faecium* and *E. faecalis* strains have high resistance to vancomycin and teicoplanin (*VanA*-type resistance). *VanA*-type resistance to glycopeptides is transferred from *E. faecalis* to *E. faecalis*, *S. pyogenes*, *S. sanguis*, and *Listeria monocytogenes* (*L. monocytogenes*) by conjugation. *E. faecium* and *E. faecalis* strains that have *VanB*-type resistance show resistance to vancomycin, when its minimal inhibitory concentration (MIC) varies from 4 to 1024 μg/mL, and are sensitive to teicoplanin. *Enterococcus gallinarum, Enterococcus casseliflavus*, and *Enterococcus flavescens* have low innate resistance to vancomycin and are sensitive to teicoplanin (*VanC*-type resistance). This type of resistance depends on a chromosomal gene (8, 17, 45). β-Lactams (piperacillin, ceftazidime, imipenem, meropenem, and aztreonam) inhibit peptidoglycan-assembling transpeptidases that are located on the outer side of cytoplasmic membrane, whereas polymyxins (colomycin, colistin) bind to phospholipids (27).

*Protein Synthesis Interference.* Antibiotics (aminoglycosides, tetracyclines, macrolides, chloramphenicol, fusidic acid, mupirocin, streptogramin, and oxazolidinones) can interfere with protein synthesis at its different stages; for example, during transcription via RNA polymerase, rifamycins modify a specific target (46). Aminoglycosides (gentamicin, tobramycin, amikacin) bind to the 30S ribosomal subunit (27) while chloramphenicol binds to the 50S ribosomal subunit and suppresses protein synthesis (47).

Macrolides, lincosamides, and streptogramin B (MLS antibiotics) block protein synthesis in gram-negative bacteria by binding to the 50S ribosomal subunit. Then the 50S subunit undergoes a post-transcriptional modification (methylation). RNA methyltransferase involves RNA that is close to or in the binding place of antibiotics. Mutations in 23S rRNA, the same as nonmethylated rRNA, are associated with resistance to MLS (1). Nonmethylated 23S rRNA and 16S rRNA at U2584 position in *Haloarcula marismortui* cause resistance to kasugamycin and sparsomycin. A nonreactive *rluC* gene is responsible for resistance to clindamycin, linezolid, and tiamulin. Oxazolidinones interfere with proteins synthesis at several stages: (i) inhibit protein synthesis by binding to 23S rRNA of the 50S subunit and (ii) suppress 70S inhibition and interaction with peptidyl-tRNR (5, 7).

*DNA Synthesis Interference.* The mechanism of resistance is a modification of two enzymes: DNA gyrase (also known as topoisomerase II) (genes *gyrA* and *gyrB*) (37) and topoisomerase IV (*parC* and *parE*). Mutations in genes *gyrA* and *parC* are followed by replication failure, and then quinolones/fluoroquinolones cannot bind. The most common mutation in *E. coli gyrA* causes a reduced drug affinity for modified-DNA complex, and MIC is higher (3, 5, 44, 48). Quinolones (ciprofloxacin) bind to DNA gyrase A subunit (26). Usually resistance to quinolones is associated with mutations in chromosomes, but plasmid-mediated (49–51) and point mutation-related (in genes *gyrA* and *parC*) resistance to quinolones (52) was reported as well.

### *Efflux Pumps and Outer Membrane Permeability*

Membrane proteins that export antibiotics from the cell and maintain their low intracellular concentrations are called efflux pumps (Fig. 3). Reduced outer membrane (OM) permeability results in reduced uptake of antibiotics (1).

*Efflux Pumps.* In analyzing resistance to antibiotics, identification and characterization of efflux pumps is one of the most actual problems. Single-component efflux systems transfer their substrates across the cytoplasmic membrane. Multicomponent pumps found in gram-negative bacteria and together with a periplasmic membrane synthesis protein (MFP) component and an OM protein (OMP) component transfer substrates across the cell envelope (1, 5, 6, 46). Antibiotics of all classes except

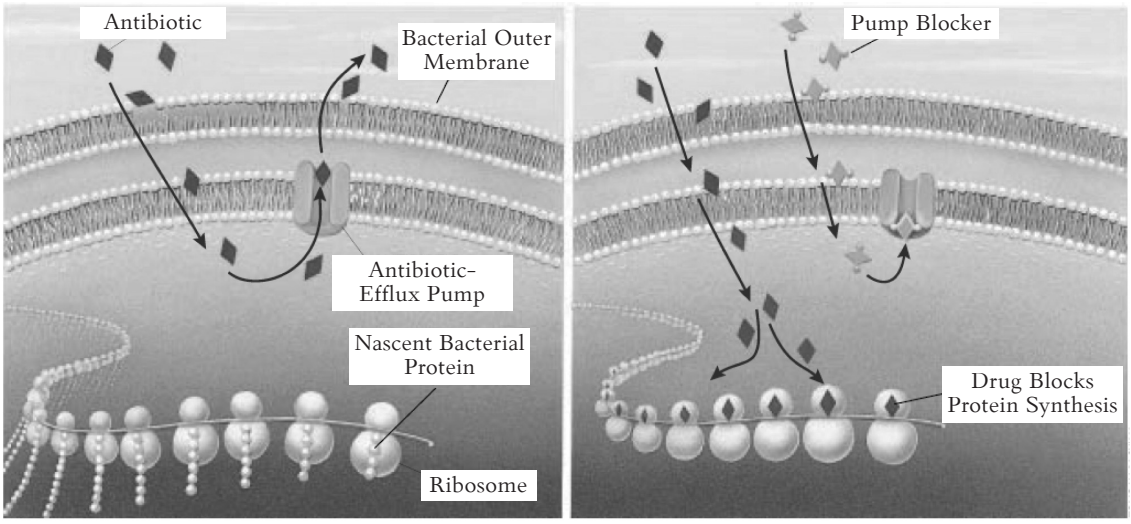


*Fig. 3.* Bacterial efflux system

A, system for antibiotic pumping out of the cell; B, antibiotic interfering with ribosomes in protein biosynthesis (9).

*Table 3.* Multidrug Resistance Efflux System of Clinically Important Bacteria (5)

| Bacterium | Efflux System | Representative Antibiotic Resistance |
|---|---|---|
| *P. aeruginosa* | MexAB-OprM | β-lactams, fluoroquinolones |
| | MexCD-OprJ | fourth-generation cephalosporins |
| | MexEF-OprN | fluoroquinolones, chloramphenicol, trimethoprim, triclosan |
| | MexHI-OprD | ethidium bromide |
| | MexJK-OprM | ciprofloxacin, tetracycline, erythromycin, triclosan |
| | MexVW-OprM | fluoroquinolones, chloramphenicol, tetracycline, erythromycin, ethidium bromide, acriflavine |
| | MexXY-OprM | aminoglycosides, tigecycline |
| *A. baumannii* | AdeABC | aminoglycosides, fluoroquinolones, tetracycline, cefotaxime, chloramphenicol, erythromycin, trimethoprim |
| *S. maltophilia* | SmeABC | aminoglycosides, β-lactams, fluoroquinolones |
| | SmeDEF | macrolides, tetracycline, fluoroquinolones, carbapenems, chloramphenicol, erythromycin |
| *B. cepacia* | CeoAB-OpcM | chloramphenicol, ciprofloxacin, trimethoprim |
| *B. pseudomallei* | AmrAB-AprA | macrolides, aminoglycosides |
| *E. coli* | AcrB-Tolc | fluoroquinolones, β-lactams, tetracycline, chloramphenicol, acriflavine, trimethoprim |
| *K. pneumoniae* | AcrB-TolC | fluoroquinolones, β-lactams, tetracycline, chloramphenicol |
| *S. aureus* | MepA | tigecycline, minocycline, tetracycline, ciprofloxacin, norfloxacin ethidium bromide, tetraphenylphosphonium bromide |
| *E. faecalis* | EmeA | norfloxacin, ethidium bromide, clindamycin, erythromycin, novobiocin |
| | Lsa | clindamycin, quinupristin-dalfopristin |
| *S. pneumoniae* | PmrA | fluoroquinolones, acriflavine, ethidium bromide |

polymyxins are susceptible to the activation of efflux systems (27). Efflux pumps can be specific to antibiotics. Most of them are multidrug transporters (Table 3) that are capable to pump a wide range of unrelated antibiotics – macrolides, tetracyclines, fluoroquinolones – and thus significantly contribute to MDR (1). Bacteria resistant to tetracyclines often produce increased amounts of membrane proteins that are used as export or efflux pumps of antimicrobial drugs (53). To eliminate toxic compounds from the cytoplasm and periplasm, *P. aeruginosa* uses more than four powerful MDR efflux pumps (Mex) (38, 54, 55).

MexV-MexW-OprM MDR efflux pumps are responsible for resistance to fluoroquinolones, tetracyclines, chloramphenicol, erythromycin, ethidium bromide, and acriflavine (38). Increased expression of MexAB-OprM efflux pumps results in higher inhibitory concentration against penicillins, broad-spectrum cephalosporins, chloramphenicol, fluoroquinolones, macrolides, novobiocin, sulfonamides, tetracycline and trimethoprim, dyes and detergents

(24, 56). β-Lactam antibiotics in gram-negative bacteria can penetrate through a membrane protein filled with water named porin. Absence of *P. aeruginosa*-specific OprD2 porin results in resistance to imipenem, whereas resistance to meropenem occurs due to changes in MexAB-OprM efflux system (1, 57). Overexpression of OprM, production of acquired β-lactamase, and overexpression of AmpC cephalosporinase are attributed to *P. aeruginosa* resistance to ticarcillin (58). MexZ, a transcriptional regulator of the *mexXY* multidrug transporter operon, confers resistance to aminoglycosides (59). Loss of 29 kDa OMP is responsible for *A. baumannii* resistance to imipenem and meropenem. Loss of *K. pneumoniae* OMP together with ampC β-lactamase ad new generation carbapenemase A, KPC, results in resistance to carbapenems (24), whereas overexpression of AdeABC efflux pumps – resistance to aminoglycosides and reduced sensitivity to fluoroquinolones, tetracyclines, chloramphenicol, erythromycin, trimethoprim, ethidium bromide, netilmicin, and meropenem. Chloramphenicol, lipophilic β-lactams, fluoroquinolones, tetracyclines, rifampin, novobiocin, fusidic acid, nalidixic acid, ethidium bromide, acriflavine, bile salts, short-chain fatty acids, SDS, Triton X-100, and triclosan serve as substrates for *E. coli* AcrAB-TolC efflux system. The MtrCDE efflux pump of penicillin-resistant *Neisseria gonorrhoeae* (*N. gonorrhoeae*) strains interacts with porins (*penB*) and low-affinity PBPs, and stimulates resistance to β-lactams. Homologues of Mex and Acr efflux systems are found in *Enterococcus aerogenes*, *Klebsiella* spp., *P. mirabilis*, *Serratia marcescens* (*S. marcescens*), *Morganella morganii*, *H. influenzae*, and *H. pylori* (60). The main elimination system for macrolides that is encoded by *mef* gene is prevalent in gram-positive bacteria and can be used for the elimination of fluoroquinolones and aminoglycosides from the cell (61). An elimination system of tetracyclines and chloramphenicol that is encoded by *ramA* gene is found in *E. coli* and *K. pneumoniae*. This also might result in resistance to norfloxacin (43). Resistance to tetracyclines might be encoded by *tetK* gene that is found in gram-positive bacteria – *Enterobacteriaceae*, *Haemophilus*, *Vibrio*, *Aeromonas*, and *Moraxella* strains, whereas *tetL* gene - in *Streptococcus* spp. and *Enterococcus* spp. Gram-positive cocci have both these genes: *tetL* and *tetK* (61).

*Changes in Outer Membrane Permeability*. The OM in gram-negative bacteria contains an inner layer that has phospholipids and an outer layer that has the lipid A. Such OM composition reduces drug uptake to a cell and transfer through the OM (through porin proteins, e.g., OmpF in *E. coli* and OprD in *P. aeruginosa*). Drug molecules to a cell can be transferred by the following mechanisms: (i) diffusion through porins, (ii) diffusion through the bilayer, and (iii) by self-promoted uptake. A type of entry depends on chemical composition of a drug molecule (1). Acquired resistance to all antibiotic classes in *P. aeruginosa* is due to low OM permeability. Small hydrophilic molecules (β-lactams and quinolones) can cross the OM only through porins. Aminoglycosides and colistin cannot be transferred to the cell through porins; therefore, self-promoted uptake to the cell is initiated by binding to lipopolysaccharides of the outer side of the OM (27). Acquired resistance is characteristic of high resistance to almost all aminoglycosides (especially to tobramycin, netilmicin, and gentamicin) (62).

***Bypass of Antibiotic Inhibition***

The fourth mechanism of bacterial resistance to antibiotics is specific. Bacteria produce an alternative target (usually an enzyme) that is resistant to inhibition of antibiotic (for example, MRSA produces an alternative PBP). At the same time, bacteria produce a native target too, which is sensitive to antibiotics. An alternative target allows bacteria to survive by adopting the role of a native protein. Resistance to trimethoprim and sulphonamides is caused by reduced sensitivity and affinity of altered enzymes dihydropteroate synthetase (DHPS) and dihydropteroate reductase (DHFR) to trimethoprim and sulphonamides (16, 23).

## Conclusions and recommendations

Massive usage of antibiotics in clinical practice resulted in resistance of bacteria to antimicrobial agents. Bacteria use innate and acquired resistance mechanisms to protect themselves. Acquired resistance arises from mutations, gene transfer by conjugation or transformation, transposons, integrons, and bacteriophages. The following biochemical types of resistance mechanisms are used by bacteria: antibiotic inactivation, target modification, altered permeability, and "bypass" metabolic pathway.

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.

## Statement of Conflict of Interest

The authors state no conflict of interest.

# Kliniškai svarbių bakterijų antimikrobinio atsparumo mechanizmai

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**Raktažodžiai:** bakterijos, antibiotikai, atsparumo mechanizmai.

**Santrauka.** Bakterijų atsparumas antimikrobiniams vaistams yra didėjanti sveikatos ir ekonomikos problema. Bakterijos gali turėti įgimtą atsparumą arba įgyti atsparumą vienai arba kelioms antimikrobinių vaistų klasėms. Įgytas atsparumas antibiotikui atsiranda, kai įvyksta: 1) mutacijos ląstelių genuose (chromosominės mutacijos), sąlygojančios kryžminį atsparumą; 2) genų perkėlimas iš vieno mikroorganizmo į kitą plazmidėmis (konjugacija arba transformacija), transpozonais (konjugacija), integronais ir bakteriofagais (transdukcija). Įgijusi atsparumo genų, apsaugai nuo įvairių antimikrobinių preparatų bakterija gali naudoti keletą biocheminio atsparumo mechanizmo tipų: antibiotiko inaktyvaciją (antibiotiko sąveika su ląstelės sienelės sinteze, pvz., β-laktamai ir glikopeptidai), taikinių modifikaciją (baltymų sintezės inhibicija, pvz., makrolidai ir tetraciklinai; interferencija su RNR sinteze, pvz., fluorokvinolonai ir rifampinas), pakitusį pralaidumą (pokyčiai išorinėje membranoje, pvz., aminoglikozidai; nauji membraniniai pernešėjai, pvz., chloramfenikolis) ir nuosruvio metabolinį kelią (metabolinio kelio inhibicija, pvz., trimetoprim-sulfametoksazolis).

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*Received 11 May 2010, accepted 17 March 2011*
*Straipsnis gautas 2010 05 11, priimtas 2011 03 17*