

Laboratory diagnosis of antibiotic resistance

by Real-Time PCR



The acquisition of **antibiotic resistance** is caused by the presence of a genetic determinant of resistance. PCR diagnostic kits are intended for the detection of these genetically determined determinants of resistance of microorganisms to antibiotics. Today, antibiotic resistance goes beyond a purely medical problem, it has great socio-economic importance and is considered a threat to national security in developed countries.

Today, β -lactam antibiotics are most often prescribed for bacterial infections, among which penicillins (ampicillin, amoxicillin), cephalosporins (ceftriaxone, cefixime) and carbapenems (imipenem, meropenem) are distinguished.

They have a long history of use and are safe and effective against a wide range of bacterial pathogens. However, the extensive and long-term use of this class of antimicrobials has led to the emergence and spread of microorganisms that realize antibiotic resistance through the production of various variants of β -lactamases – enzymes that destroy β -lactam antibiotics, or through the modification of penicillin-binding proteins, which are the target of β -lactam antibiotics.



The results of scientific studies point to the need to determine sensitivity before prescribing antimicrobial therapy. Knowledge of bacterial resistance to antibiotics is essential for successful disease control.

Premature and insufficient treatment of nosocomial and serious infections increases the probability of death. Therefore, it is very important to first identify the type of antibiotic resistance in the initial stages of infection in order to determine the most effective antimicrobial therapy. In case of severe infections, this should be done as soon as possible.

Negative effects of resistance:

- ✓ **Complication of the course of the disease and increase in mortality**
- ✓ **Spread of infection throughout the body**
- ✓ **The probability of spreading the infection between relatives**
- ✓ **Failure of drug therapy**
- ✓ **Difficulty choosing antibiotics, limited choice of drugs**
- ✓ **Complex and expensive treatment**
- ✓ **An increase in the frequency of relapses and the transition of the disease to a chronic form**
- ✓ **Risk of complications after trauma, surgery or therapeutically induced immunosuppression**
- ✓ **Increased risk of opportunistic infections**
- ✓ **Increased risk of superinfection**

Unlike **traditional microbiological methods**, the **PCR method** makes it possible to identify genetic determinants of resistance of microorganisms, including difficult-to-cultivate bacteria, within 4 hours. It is characterized by high accuracy and smaller requirements for material sampling, does not require nutrient media, antibiotic disks and other reagents. Determining antibiotic resistance using PCR makes it possible to predict the emergence of resistance to different groups of antimicrobial drugs, as well as to assess the spread of resistant strains at the local and regional level.

Microbiological methods

- ✓ Lack of standardized methods
- ✓ Time cost (24-72 hours to get the result)
- ✓ Difficulty with cultivation and identification of some microorganisms
- ✓ The probability of not detecting the main source of infection
- ✓ Difficulties in assessing susceptibility to new antibiotics
- ✓ The subjectivity of sensitivity assessment
- ✓ Use of expensive media, reagents, etc.

vs.

PCR methods

- ✓ Identification of genetic determinants of resistance of microorganisms, including difficult-to-cultivate ones, within 2-4 hours
- ✓ High accuracy of analysis
- ✓ Predicting the emergence of resistance to different groups of antibiotics
- ✓ Evaluation of the spread of resistant strains

Antibiotic resistance detection by PCR is an excellent addition to traditional microbiological testing.

Main groups of antibiotics:

- ✓ **β -lactam**
- ✓ **Macrolides (erythromycin, azithromycin)**
- ✓ **Aminoglycosides (streptomycin, kanamycin, amikacin)**
- ✓ **Glycopeptides (vancomycin, teicoplanin)**
- ✓ **Fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin)**
- **Penicillins (ampicillin, oxacillin)**
- **Cephalosporins (cefotaxime, ceftriaxone, cefepime)**
- **Carbapenems (imipenem, meropenem, doripenem)**

In modern clinical practice, several variants of antibacterial resistance can be distinguished, which leads to extremely serious socio-economic consequences. These options include:

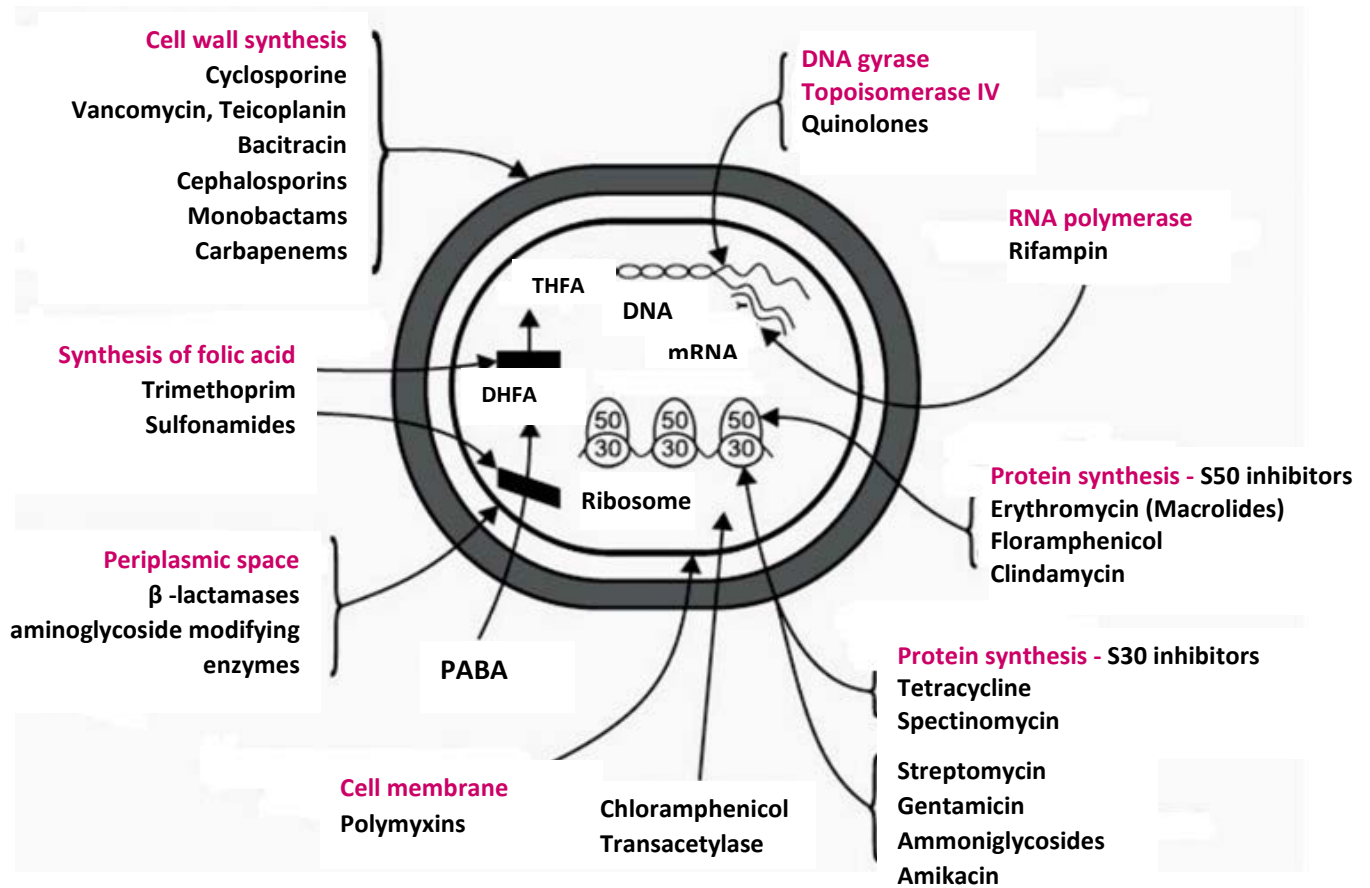
- ✓ resistance of gram-negative bacteria to **β -lactam antibiotics**, especially *Enterobacteriaceae* and *Pseudomonas aeruginosa*, the resistance mechanism is associated with **β -lactamase** production;
- ✓ resistance to **glycopeptides** in *Enterococcus spp.* bacteria;
- ✓ resistance to **β -lactam antibiotics** in *Staphylococcus aureus*;
- ✓ **fluoroquinolone** resistance among gram-positive and gram-negative bacteria;
- ✓ resistance to **macrolides** in bacteria *Streptococcus spp.*

Most of the listed resistances are caused by the bacteria of this group acquiring a genetic determinant of resistance (Table 1), which can be detected by PCR.

Table 1:











Group	Typical drugs	Gene-marker of resistance	Bacteria – carriers of genetic resistance
β-lactam antibiotics			
penicillins	ampicillin amoxicillin benzylpenicillin piperacillin	<i>blaTEM-1,2</i> <i>blaSHV-1.11</i>	<i>Enterobacteriaceae</i> (<i>Escherichia coli</i> , <i>Klebsiella spp.</i> , <i>Proteus spp.</i> , <i>Enterobacter spp.</i> , <i>Serratia spp.</i> , <i>Citrobacter spp.</i> , <i>Shigella spp.</i> , <i>Salmonella spp.</i>), <i>Pseudomonas aeruginosa</i> , <i>Acinetobacter spp.</i>
cephalosporins	I. generation: cefazolin cephalothin cephalexin	<i>blaCTX-M</i> <i>blaSHV-5.12</i> <i>blaOXA-10</i>	
	II. generation: cefuroxime cefaclor		
	III. generation: cefotaxime ceftriaxone ceftazidime cefixime		
	IV. generation: cefepime	<i>AmpC</i> (<i>blaDHA, blaMOX,</i> <i>blaCMY, blaFOX,</i> <i>blalat, blaact,</i> <i>blaMIR</i>)	
carbapenems	meropenem imipenem doripenem	<i>blaVIM</i> <i>blaIMP</i> <i>blaNDM</i> <i>blaKPC</i> <i>blages</i> <i>blaOXA23-like</i> <i>blaOXA40-like</i> <i>blaOXA48-like</i> <i>blaOXA58-like</i>	
β-lactam antibiotics	oxacillin	<i>MecA</i>	<i>Staphylococcus aureus</i>
glycopeptides	vancomycin	<i>VanA</i> <i>VanB</i>	<i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i>
	teicoplanin	<i>VanA</i>	
macrolides	erythromycin azithromycin clarithromycin	<i>Mef</i> <i>ErmB</i>	<i>Streptococcus spp.</i>
fluoroquinolones (II., III. and IV. generation of quinolones)	II. generation: ciprofloxacin ofloxacin	GyrA ParC QnrA	<i>Streptococcus spp.</i> , <i>Pseudomonas aeruginosa</i> , <i>Enterobacteriaceae</i>
	III. generation: levofloxacin		
	IV. generation: moxifloxacin		











The main mechanisms of the effect of antibiotics on the cell






RESISTOM diagnostic kits

Cat.№	Kit name and description	Number of tests
Extraction kits for isolation of nucleic acids		
E0225	DNA-EXPRESS Kit for rapid isolation of DNA from various clinical material	100
E0232	NA Sorbent Base	100
E0232-4	NA Sorbent Blood Isolation of nucleic acids from whole blood	100
E02104	Hemoculture DNA Rapid Extraction Kit	100

Cat.№	Gene-marker	Kit name and description	Format	Number of tests
Monoplex kits				
E01681-RT-120 E01781-RT-TS-96 E01781-RT-TS-60	<i>blaCTX-M</i>	RESISTOM.CTX-M Diagnostic kit for the detection of <i>blaCTX-M</i> cephalosporin resistance genes in Gram-negative bacteria	 •• 12x8 Strip •• P02	130 96 60
E01682-RT-120 E01782-RT-TS-96 E01782-RT-TS-60	<i>MecA</i>	RESISTOM.MecA Diagnostic kit for detection of <i>MecA</i> β -lactam resistance gene in <i>Staphylococcus spp.</i>	 •• 12x8 Strip •• P02	130 96 60
E01865-RT-120 E01965-RT-TS-96 E01965-RT-TS-60	<i>blaOXA10</i>	RESISTOM.OXA10 Diagnostic kit for the detection of <i>blaOXA10</i> cephalosporin resistance genes in Gram-negative bacteria	 •• 12x8 Strip •• P02	130 96 60
E01868-RT-120 E01968-RT-TS-96 E01968-RT-TS-60	<i>blaDHA</i>	RESISTOM.DHA Diagnostic kit for the detection of <i>blaDHA</i> resistance genes to protected penicillins and cephalosporins in Gram-negative bacteria	 •• 12x8 Strip •• P02	130 96 60
E01684-RT-120 E01784-RT-TS-96 E01784-RT-TS-60	<i>blaVIM</i>	RESISTOM.VIM Diagnostic kit for the detection of carbapenem resistance <i>blaVIM</i> genes in Gram-negative bacteria	 •• 12x8 Strip •• P02	130 96 60
E01685-RT-120 E01785-RT-TS-96 E01785-RT-TS-60	<i>blaNDM</i>	RESISTOM.NDM Diagnostic kit for the detection of <i>blaNDM</i> carbapenem resistance genes in Gram-negative bacteria	 •• 12x8 Strip •• P02	130 96 60
E01686-RT-120 E01786-RT-TS-96 E01786-RT-TS-60	<i>blaOXA48-like</i>	RESISTOM.OXA48-like Diagnostic kit for the detection of <i>blaOXA48-like</i> carbapenem resistance genes in Gram-negative bacteria	 •• 12x8 Strip •• P02	130 96 60
E01646-RT-120 E01746-RT-TS-96 E01746-RT-TS-60	<i>blaKPC</i>	RESISTOM.KPC Diagnostic kit for the detection of <i>blaKPC</i> carbapenem resistance genes in Gram-negative bacteria	 •• 12x8 Strip •• P02	130 96 60
E01860-RT-120 E01960-RT-TS-96 E01960-RT-TS-60	<i>blaGES</i>	RESISTOM.GES Diagnostic kit for the detection of <i>blaGES</i> carbapenem resistance genes in Gram-negative bacteria	 •• 12x8 Strip •• P02	130 96 60
E01860-RT-120 E01960-RT-TS-96 E01960-RT-TS-60	<i>blaOXA23-like</i>	RESISTOM.OXA23-like Diagnostic kit for the detection of <i>blaOXA23-like</i> carbapenem resistance genes in Gram-negative bacteria	 •• 12x8 Strip •• P02	130 96 60

Cat.№	Gene-marker	Kit name and description	Format	Number of tests
E01862-RT-120 E01962-RT-TS-96 E01962-RT-TS-60	<i>blaOXA40-like</i>	RESISTOM.OXA40-like Diagnostic kit for the detection of <i>blaOXA40-like</i> carbapenem resistance genes in Gram-negative bacteria	 •• 12x8 Strip •• P02	130 96 60
E01687-RT-120 E01787-RT-TS-96 E01787-RT-TS-60	<i>VanA</i> <i>VanB</i>	RESISTOM.Van Diagnostic kit for detection and differentiation of <i>VanA</i> and <i>VanB</i> glycopeptide resistance genes in <i>E. faecalis/E. faecium</i>	 •• 12x8 Strip •• P02	130 96 60
E01647-RT-120 E01747-RT-TS-96 E01747-RT-TS-60	<i>ErmB</i>	RESISTOM.ErmB Diagnostic kit for detection of <i>ErmB</i> resistance genes to macrolides, lincosamides and streptogramin B in <i>Streptococcus spp.</i> and <i>Staphylococcus spp.</i>	 •• 12x8 Strip •• P02	130 96 60
E01648-RT-120 E01748-RT-TS-96 E01748-RT-TS-60	<i>Mef</i>	RESISTOM.Mef Diagnostic kit for detection of <i>Mef</i> macrolide resistance genes in <i>Streptococcus spp.</i>	 •• 12x8 Strip •• P02	130 96 60
Multiplex kits				
E01873-RT-120 E01973-RT-TS-96	<i>Staphylococcus aureus</i> <i>Staphylococcus spp.</i> <i>MecA</i>	RESISTOM.MRSA Diagnostic kit for detection and differentiation of DNA of <i>Staphylococcus aureus</i> , <i>Staphylococcus spp.</i> and <i>MecA</i> β -lactam resistance gene in <i>Staphylococcus spp.</i>	 •• 12x8 Strip	130 96
E01874-RT-120 E01974-RT-TS-96	<i>Enterococcus faecalis/</i> <i>Enterococcus faecium</i> <i>VanA</i> <i>VanB</i>	RESISTOM.VRE type V Diagnostic kit for detection of <i>Enterococcus faecalis/Enterococcus faecium</i> DNA and differentiation of <i>VanA</i> and <i>VanB</i> glycopeptide resistance genes in <i>Enterococcus spp.</i>	 •• 12x8 Strip	130 96
E01875-RT-120 E01975-RT-TS-96	<i>Acinetobacter baumannii</i> <i>blaOXA23-like</i> <i>blaOXA40-like</i>	RESISTOM.CRAB Diagnostic kit for detection of DNA of <i>Acinetobacter baumannii</i> , <i>blaOXA23-like</i> and <i>blaOXA40-like</i> carbapenem resistance genes in Gram-negative bacteria	 •• 12x8 Strip	130 96
E01876-RT-120 E01976-RT-TS-96	<i>Pseudomonas aeruginosa</i> <i>blaVIM</i> <i>blaNDM</i>	RESISTOM.MDR-Pseudomonas Diagnostic kit for detection of DNA of <i>Acinetobacter baumannii</i> , <i>blaOXA23-like</i> and <i>blaOXA40-like</i> carbapenem resistance genes in Gram-negative bacteria	 •• 12x8 Strip	130 96
E01877-RT-120 E01977-RT-TS-96	<i>Klebsiella pneumoniae</i> <i>blaKPC</i> <i>blaOXA48-like</i>	RESISTOM.CRE-Klebsiella Diagnostic kit for detection of DNA of <i>Klebsiella pneumoniae</i> , <i>blaKPC</i> and <i>blaOXA48-like</i> carbapenem resistance genes in Gram-negative bacteria	 •• 12x8 Strip	130 96
E01878-RT-120 E01978-RT-TS-96	<i>Escherichia coli</i> <i>blaCTX-M</i> <i>blaOXA10</i>	RESISTOM.ESBL-E.coli Diagnostic kit for detection of DNA of <i>Escherichia coli</i> , <i>blaCTX-M</i> and <i>blaOXA10</i> cephalosporin resistance genes in Gram-negative bacteria	 •• 12x8 Strip	130 96

Cat.No	Gene-marker	Kit name and description	Format	Number of tests
E01879-RT-120 E01979-RT-TS-96	<i>Enterobacter species</i> <i>blaGES</i> <i>blaDHA</i>	RESISTOM.CRE-Enterobacter Diagnostic kit for detection of DNA of <i>Enterobacter species</i> , <i>blaGES</i> carbapenem resistance genes in Gram-negative bacteria and <i>blaDHA</i> resistance genes to protected penicillins and cephalosporins in Gram-negative bacteria	 •• 12x8 Strip	130 96
E01880-RT-120 E01980-RT-TS-96	<i>Streptococcus species</i> <i>Mef</i> <i>ErmB</i>	RESISTOM.MLSB- Streptococcus Diagnostic kit for detection of DNA of <i>Streptococcus species</i> , <i>Mef</i> and <i>ErmB</i> resistance genes to macrolides, lincosamides and streptogramin B in Gram-positive cocci	 •• 12x8 Strip	130 96
E01867-RT-120 E01967-RT-TS-96	<i>Enterococcus faecalis/</i> <i>Enterococcus faecium</i> <i>VanA</i> <i>VanB</i>	RESISTOM.VRE type E Diagnostic kit for detection and differentiation of DNA of <i>Enterococcus faecalis/ Enterococcus faecium</i> and <i>VanA/VanB</i> glycopeptide resistance genes in <i>Enterococcus spp.</i>	 •• 12x8 Strip	130 96

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