

In the name of GOD

Antimicrobial Resistance

(ESBL, MDR, XDR & PDR)

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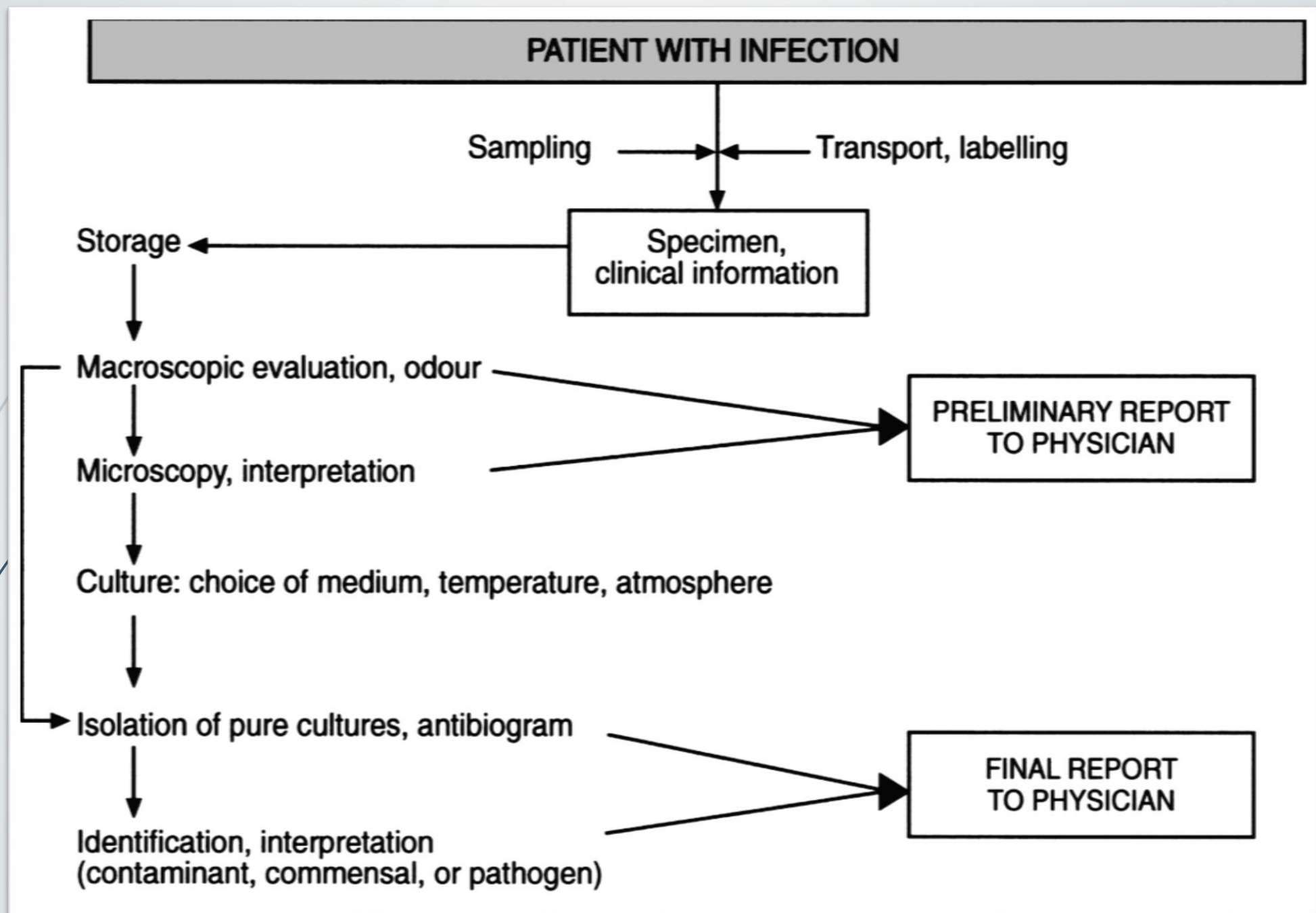
Microbiology Lab Director





Outline:

- ✓ **Introduction**
- ✓ **Methods of antimicrobial susceptibility test (AST)**
- ✓ **CLSI and important definitions & determinants**
- ✓ **Criteria of correct and appropriate antibiotic selection**
- ✓ **Correct analysis and interpretation of AST results**
- ✓ **Identification and report of ESBL, MDR, XDR & PDR strains**
- ✓ **Quality assurance and quality control (QA&QC) in AST**
- ✓ **WHONET 2019 software for correct and reliable analysis of AST results**



AST is essential for the selection
of the appropriate antibiotic

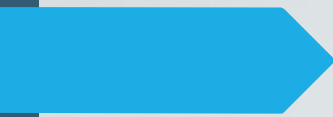


Antibiotic
Sensitivity
Testing

• Right Drug

• Right Microbe

• Right Cure



Purposes

- To guide the clinician in selecting the **best antibiotic** agent for an individual patient.
- To control the use of **inappropriate antibiotics** in clinical practice.
- To accumulate epidemiological information on the resistance of microorganisms of public health importance within the **community**.
- To reveal the **changing trends** in the local isolates.

Types

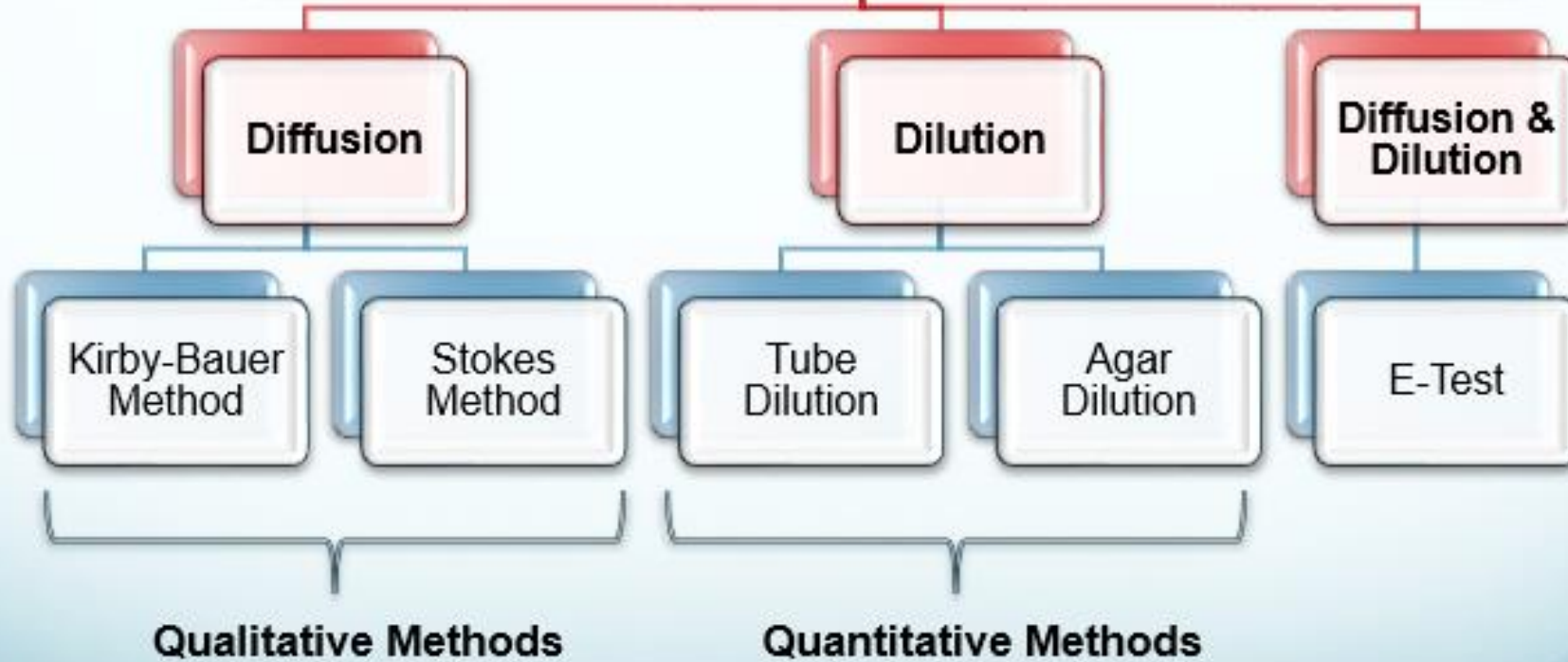
• Qualitative

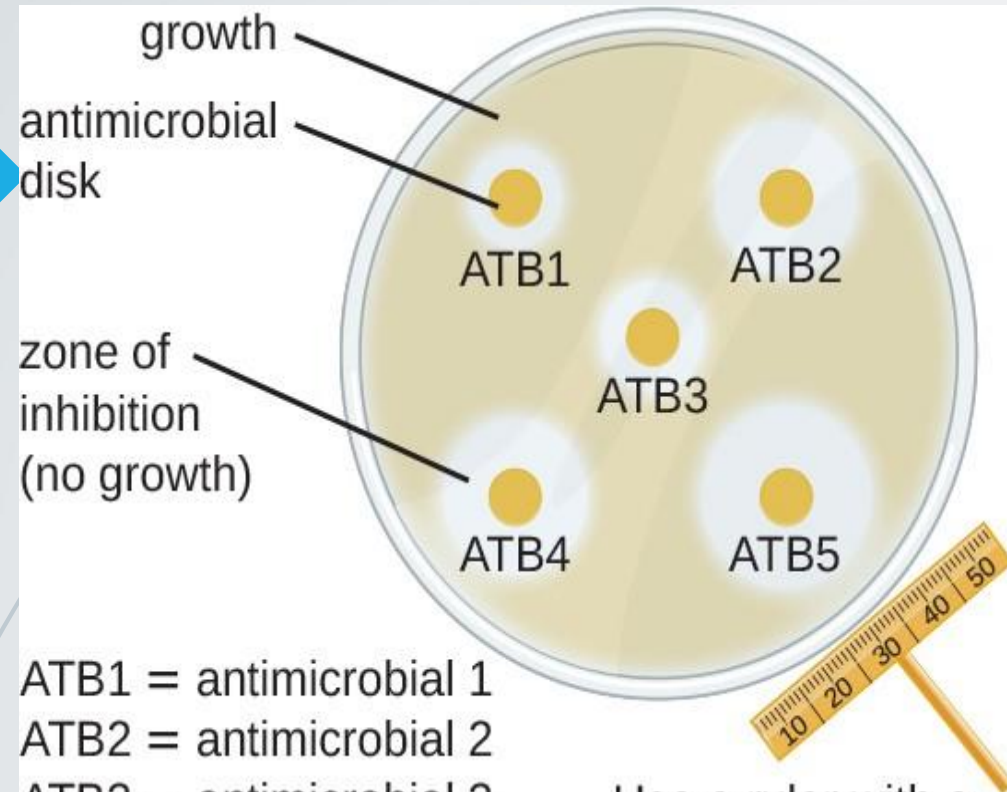
- For the testing of isolates from “healthy” patients with intact immune defenses.
- For **less serious infections** such as uncomplicated urinary tract infections.

• Quantitative

- In the treatment of **serious infections** such as endocarditis or osteomyelitis.
- For infections in high-risk patient groups such as **immunocompromised** patients (e.g., transplant patients).
- Those who are **critically ill**.

Antibiotic Sensitivity Tests





ATB1 = antimicrobial 1
 ATB2 = antimicrobial 2
 ATB3 = antimicrobial 3
 ATB4 = antimicrobial 4
 ATB5 = antimicrobial 5


Use a ruler with a handle if calipers are unavailable.

(a)



(b)




$$(1) \text{Weight (mg)} = \frac{\text{Volume (mL)} \cdot \text{Concentration } (\mu\text{g/mL})}{\text{Potency } (\mu\text{g/mg})}$$

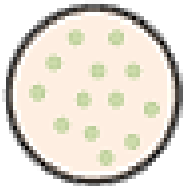
or

$$(2) \text{Volume (mL)} = \frac{\text{Weight (mg)} \cdot \text{Potency } (\mu\text{g/mg})}{\text{Concentration } (\mu\text{g/mL})}$$

1. Obtain required colonies of bacterial strain to test.

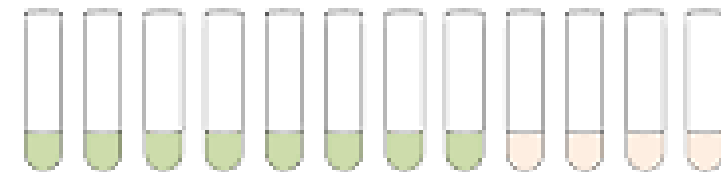
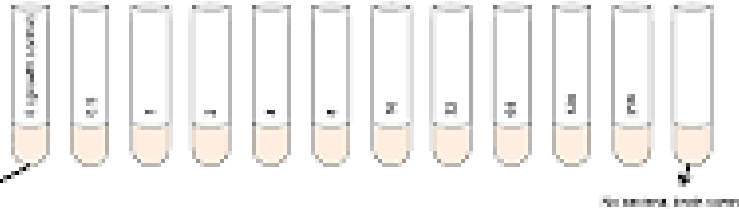


4. Plate aliquot of growth control (i.e., no antibiotic added) to verify viable counts, incubate overnight and count colonies.

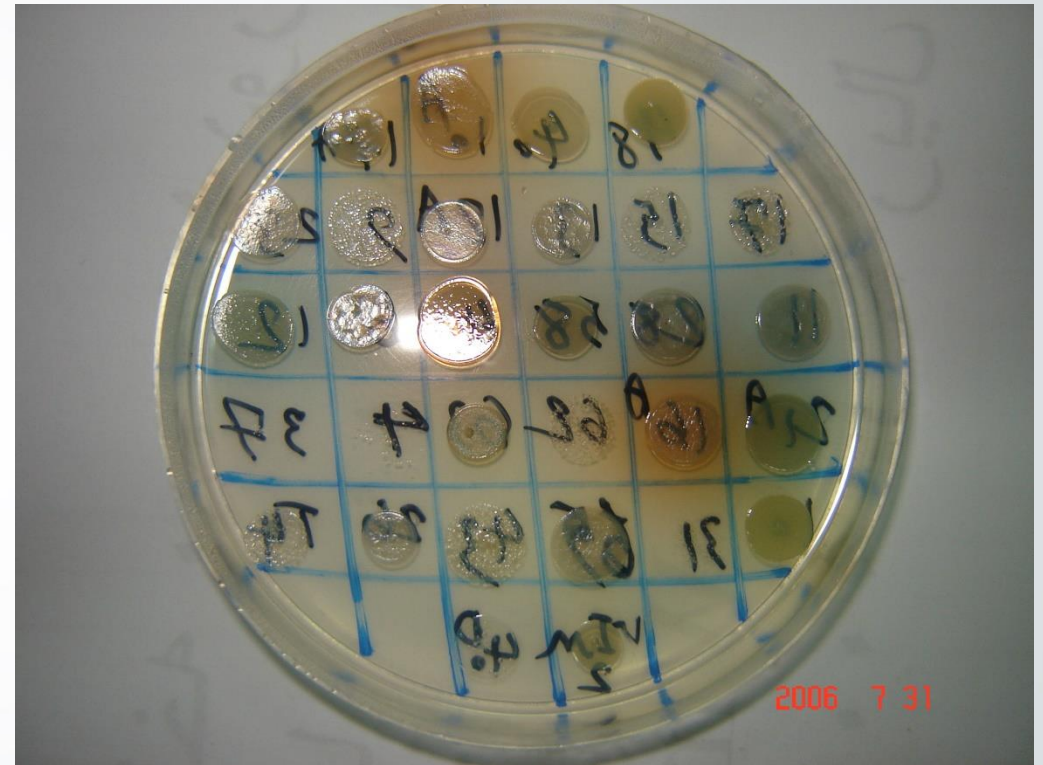


Broth dilution method for measuring minimum inhibitory concentration of antibiotics

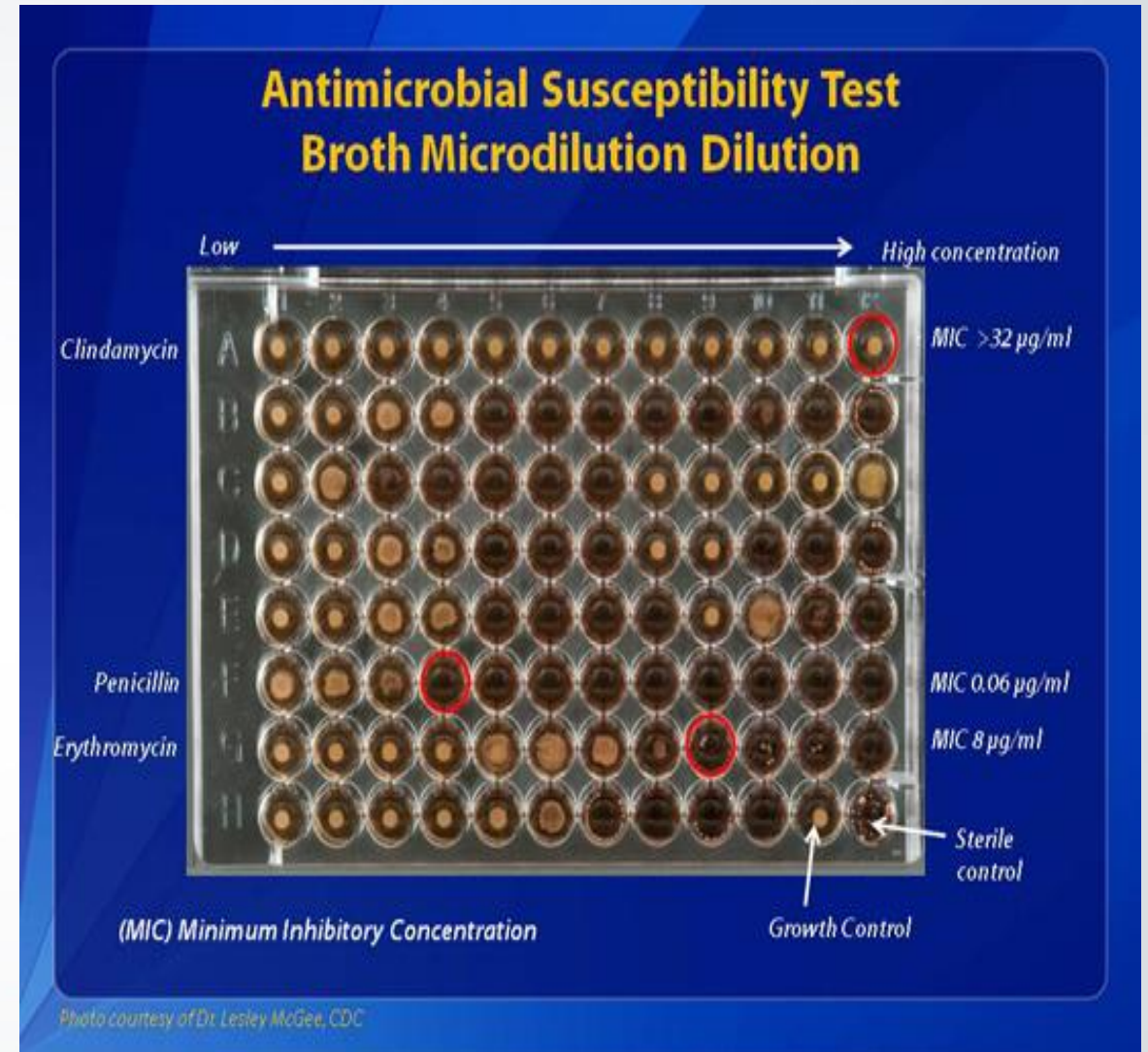
3. After overnight incubation shown at left, add rich broth with appropriate dilution series of test antibiotic to test tubes. Example concentrations (mg/L) are shown below. Inoculate bacteria to a final density of 5×10^7 c.f.u./ml.

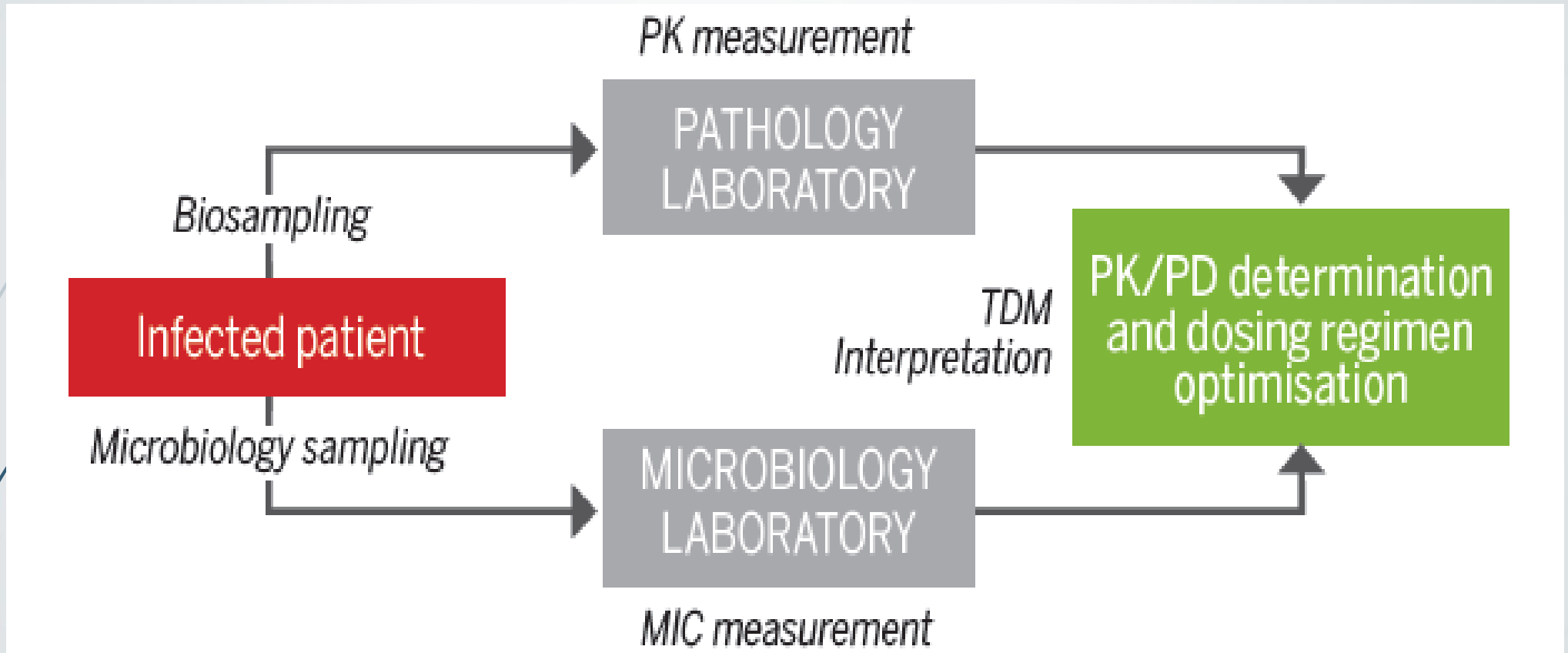


5. After overnight incubation, check culture for growth. The MIC is the lowest concentration of antibiotic that prevents visible growth. In this example, the MIC is 64 mg/L.

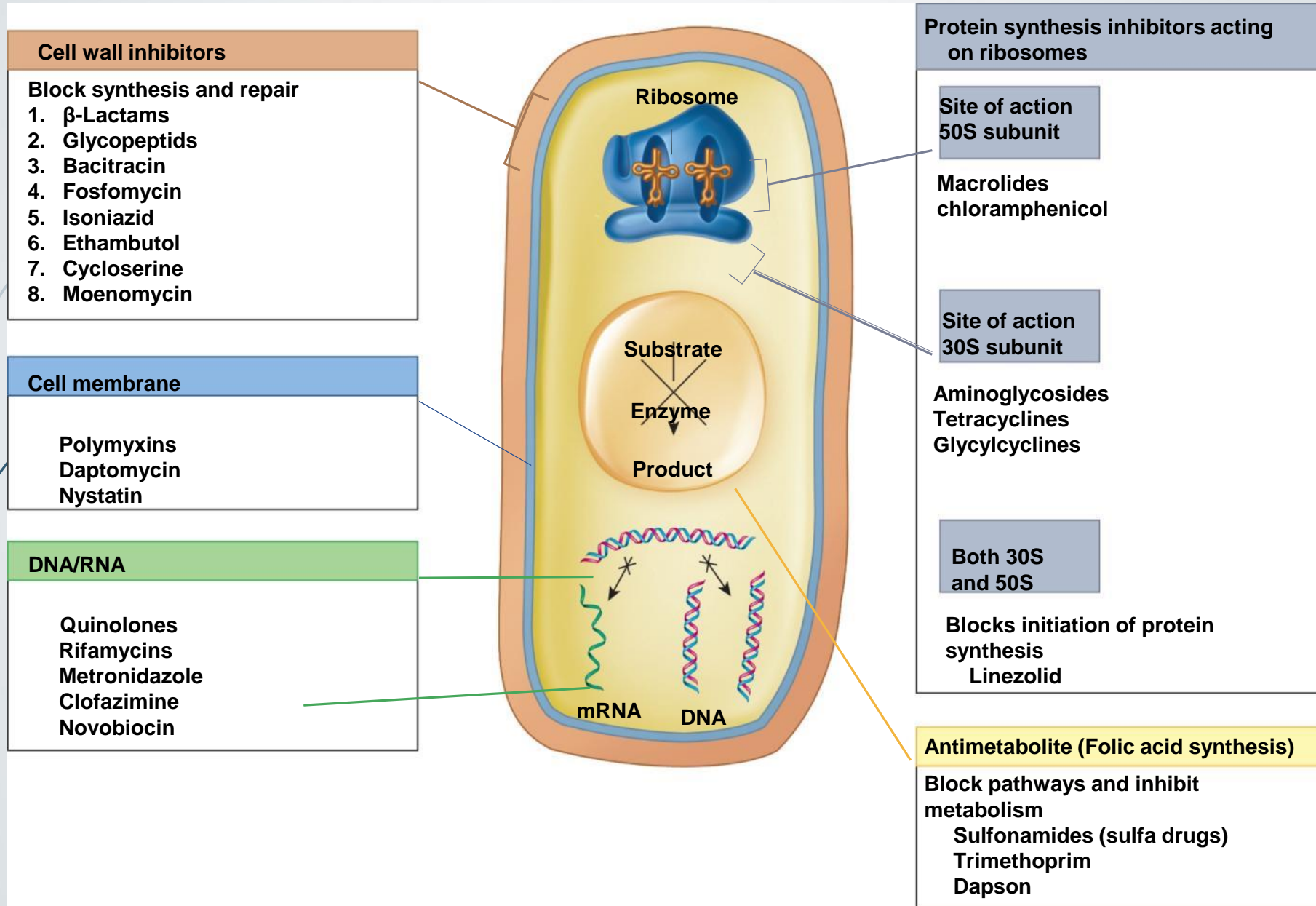


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Targets of Antimicrobials



Antimicrobial Class	Antimicrobial Subclass(es)		Agent(s) Included; Generic Name(s)
Penicillins	Penicillinase-labile penicillins ^a	Penicillin	Penicillin
		Aminopenicillins	Amoxicillin Ampicillin
		Carboxypenicillins	Carbenicillin Ticarcillin
		Ureidopenicillins	Azlocillin Mezlocillin Piperacillin
	Penicillinase-stable penicillins ^b		Cloxacillin Dicloxacillin Methicillin Nafcillin Oxacillin
	Aminopenicillin		Mecillinam
β -lactam combination agents			Amoxicillin-clavulanate Ampicillin-sulbactam Aztreonam-avibactam Cefepime-tazobactam (1:1) Cefepime-zidebactam Ceftaroline-avibactam Ceftazidime-avibactam Ceftolozane-tazobactam Imipenem-relebactam Meropenem-vaborbactam Piperacillin-tazobactam Ticarcillin-clavulanate
Cephems (parenteral)	Cephalosporins I ^c		Cefazolin Cephalothin Cephapirin Cephradine
	Cephalosporins II ^c		Cefamandole Cefonicid Cefuroxime (parenteral)
	Cephalosporins III ^c		Cefoperazone Cefotaxime Ceftazidime Ceftizoxime Ceftriaxone
	Cephalosporins IV ^c		Cefepime Cefpirome

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Cephems (parenteral (Continued))	Cephalosporins with anti-MRSA activity	Ceftaroline Ceftobiprole
	Cephamycins	Cefmetazole Cefotetan Cefoxitin
	Oxacephem	Moxalactam
	Siderophore cephalosporin	Cefiderocol
Cephems (oral)	Cephalosporins	Cefaclor Cefadroxil Cefdinir Cefditoren Cefetamet Cefixime Cefpodoxime Cefprozil Ceftibuten Cefuroxime (oral) Cephalexin Cephradine
	Carbacephem	Loracarbef
Monobactams		Aztreonam
Penems	Carbapenems	Biapenem Doripenem Ertapenem Imipenem Meropenem Razupenem
	Penems	Faropenem Sulopenem

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Aminocyclitols		Spectinomycin
Aminoglycosides		Amikacin Gentamicin Kanamycin Netilmicin Plazomicin Streptomycin Tobramycin
Aminoglycoside-fosfomycin		Amikacin-fosfomycin
Ansamycins	Rifamycins	Rifabutin Rifapentine Rifampin Rifaximin
Folate pathway antagonists	Dihydrofolate reductase inhibitors	Iclaprim Sulfonamides Trimethoprim Trimethoprim-sulfamethoxazole
	Sulfonamides	Sulfamethoxazole Sulfisoxazole
	Combination	Trimethoprim-sulfamethoxazole
Fosfomycins		Fosfomycin
Glycopeptides	Glycopeptide	Vancomycin
	Lipoglycopeptides	Dalbavancin Oritavancin Teicoplanin Telavancin Ramoplanin
Lincosamides		Clindamycin Lincomycin
Lipopeptides		Daptomycin Surotomycin
	Polymyxins	Colistin Polymyxin B
Macrocyclic lactone		Fidaxomicin

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Macrolides		Azithromycin Clarithromycin Dirithromycin Erythromycin
	Fluoroketolide	Solithromycin
	Ketolides	Nafithromycin Telithromycin
Nitroheterocyclics	Nitrofurantoin	Nitrofurantoin
	Nitroimidazoles	Metronidazole Secnidazole Tinidazole
	Thiazolides	Nitazoxanide Tizoxanide
Oxazolidinones		Linezolid Tedizolid
Peptide	Magainin	Pexiganan
Phenicol		Chloramphenicol Thiamphenicol
Pleuromutilins		Lefamulin Retapamulin
Pseudomonic acid		Mupirocin
Quinolones		Cinoxacin Garenoxacin Nalidixic acid
	Benzoquinolizine	Levonadifloxacin
	Fluoroquinolones	Besifloxacin Ciprofloxacin Clinafloxacin Delafoxacin Enoxacin Finafloxacin Fleroxacin Gatifloxacin Gemifloxacin Grepafloxacin Levofloxacin Lomefloxacin Moxifloxacin Norfloxacin Ofloxacin Pefloxacin Sparfloxacin Trovafoxacin Ulifloxacin (prulifloxacin)

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Quinolonyl oxazolidinone		Cadazolid
Steroid	Fusidane	Fusidic acid
Streptogramins		Quinupristin-dalfopristin
Tetracyclines		Doxycycline Minocycline Tetracycline
	Fluorocycline	Eravacycline
	Glycylcycline	Tigecycline
	Aminomethylcycline	Omadacycline
Triazaacenaphthylene		Gepotidacin



What are factors promote antimicrobial resistance?

- ✓ Exposure to sub-optimal levels of antimicrobial
- ✓ Inappropriate use
- ✓ Exposure to microbes carrying resistance genes

CAUSES OF ANTIBIOTIC RESISTANCE



Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause.



Over-prescribing
of antibiotics



Patients not finishing
their treatment



Over-use of antibiotics in
livestock and fish farming



Poor infection control
in hospitals and clinics



Lack of hygiene and poor
sanitation



Lack of new antibiotics
being developed

www.who.int/drugresistance

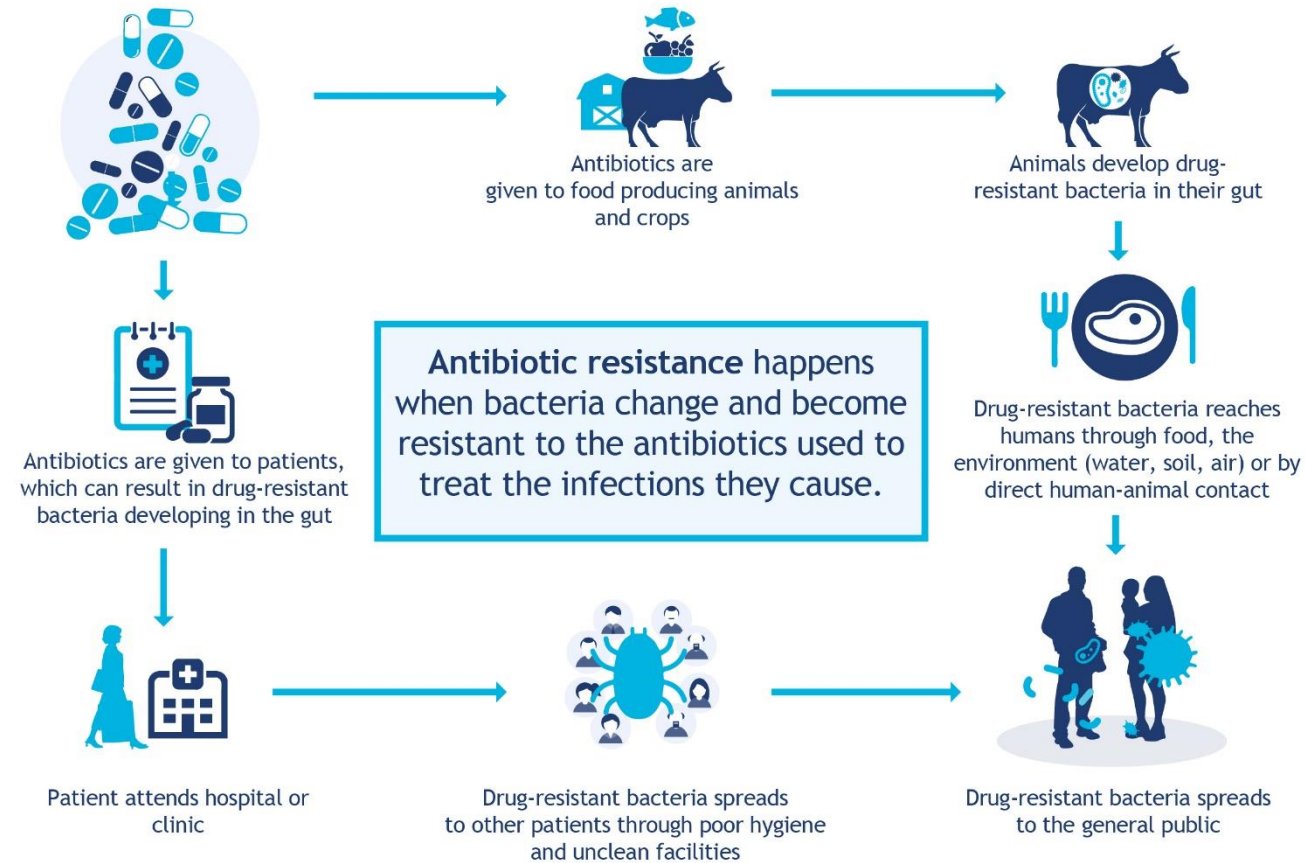
#AntibioticResistance



**World Health
Organization**

ANTIBIOTIC RESISTANCE

HOW IT SPREADS



www.who.int/drugresistance

#AntibioticResistance

ANTIBIOTIC RESISTANCE

WHAT YOU CAN DO



Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause.



- 1 Only use antibiotics when **prescribed** by a certified health professional
- 2 Always take the **full prescription**, even if you feel better
- 3 **Never use left over** antibiotics
- 4 **Never share** antibiotics with others
- 5 **Prevent infections** by regularly washing your hands, avoiding contact with sick people and keeping your vaccinations up to date

www.who.int/drugresistance

#AntibioticResistance

ANTIBIOTIC RESISTANCE

WHAT POLICY MAKERS CAN DO



Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause.



- 1 Ensure you have a robust **national action plan** to tackle antibiotic resistance
- 2 Improve **surveillance** of antibiotic-resistant infections
- 3 Strengthen **infection prevention** and control measures
- 4 **Regulate and promote** the appropriate use of quality medicines
- 5 Make information on the **impact** of antibiotic resistance available

www.who.int/drugresistance

#AntibioticResistance

ANTIBIOTIC RESISTANCE

WHAT HEALTH WORKERS CAN DO



Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause.



- 1 Prevent infections by ensuring your hands, instruments and environment are clean
- 2 Keep your patients' vaccinations up to date
- 3 If you think a patient might need antibiotics, where possible, **test to confirm** and find out which one
- 4 Only prescribe and dispense antibiotics when they are **truly needed**
- 5 Prescribe and dispense the **right antibiotic at the right dose for the right duration**

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#AntibioticResistance

ANTIBIOTIC RESISTANCE

WHAT THE AGRICULTURE SECTOR CAN DO



Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause.



- 1 Ensure that antibiotics given to animals—including food-producing and companion animals—are **only used to control or treat** infectious diseases and under veterinary supervision
- 2 **Vaccinate** animals to reduce the need for antibiotics and **develop alternatives** to the use of antibiotics in plants
- 3 Promote and apply **good practices** at all steps of production and processing of foods from animal and plant sources
- 4 Adopt **sustainable systems** with improved hygiene, biosecurity and stress-free handling of animals
- 5 Implement **international standards** for the responsible use of antibiotics and guidelines, set out by OIE, FAO and WHO

www.who.int/drugresistance
www.oie.int/antimicrobial-resistance
www.fao.org/antimicrobial-resistance

#AntibioticResistance



Food and Agriculture
Organization of the
United Nations



World Health
Organization

Clinical & Diagnostic Microbiology Lab

- ✓ Isolation, purification and identification of microbes from clinical specimens
- ✓ AST on standard protocols with QA & QC
- ✓ Selection of type of AST (qualitative or quantitative or both)
- ✓ Selection of best and suitable antibiotics
- ✓ Accurate analysis and interpretation of AST results
- ✓ Identification and detection of important resistance strains:
 - MRSA, VISA, VRSA, VRE, ESBL, MDR, XDR, PDR , etc.
- ✓ Rapid and timely reporting of AST results

CLSI: Definition of antimicrobial agent groups;

A,B,C,O,Inv,U

➡ Group A:

- Appropriate for routine, primary testing panel.
- Routine reporting of results for the specific organism groups.

➡ Group B:

- Antimicrobial agents that may warrant primary testing
- Organism is resistant to same antimicrobial class, as in group A
 - ✓ A third-generation cephalosporin for enteric bacilli from CSF
 - ✓ Trimethoprim-sulfamethoxazole for urinary tract isolates.
- A polymicrobial infection.
- Infections involving multiple sites.
- Cases of patient allergy, intolerance, or failure to respond to an antimicrobial agent in group A.

Continued...

➡ Group C:

- Agents that may necessitate testing for **endemic or epidemic strains resistant to several of the primary drugs**
- For treatment of **unusual organisms** (e.g., chloramphenicol for extraintestinal isolates of *Salmonella* spp.)
- For reporting to infection control as an **epidemiological aid**.



Continued...

➡ Group O (“other”):

- Includes antimicrobial agents that have a clinical indication for the organism group but are generally **not candidates for routine testing** and reporting in the United States.

➡ Group Inv. (“investigational”):

- Includes antimicrobial agents that are investigational for the organism group and **have not yet been approved by the FDA** for use in the United States.



Continued...

➡ Group U (“urine”):

- Includes certain antimicrobial agents (e.g., nitrofurantoin and certain quinolones) that are used only or **primarily for treating UTIs**.
- These agents **should not be routinely reported against pathogens recovered from other infection sites**. An exception to this rule is cefazolin is agent for oral cephalosporins.
- Other antimicrobial agents with broader indications may be included in group U for specific urinary pathogens (e.g., *Enterococcus* and ciprofloxacin).

Breakpoint and Interpretive Category Definitions

- ☐ Susceptible(S)
- ☐ Susceptible-dose dependent (SDD)
- ☐ Intermediate(I)
- ☐ Resistant(R)
- ☐ Nonsusceptible(NS)

Table 2B-1. *Pseudomonas aeruginosa* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
O	Piperacillin	100 µg	≥21	15–20	≤14	≤16	32–64	≥128	(5) Breakpoints for piperacillin (alone or with tazobactam) are based on a piperacillin dosage regimen of at least 3 g every 6 h.
β-LACTAM COMBINATION AGENTS									
A	Piperacillin-tazobactam	100/10 µg	≥21	15–20	≤14	≤16/4	32/4–64/4	≥128/4	(6) Breakpoints for piperacillin (alone or with tazobactam) are based on a piperacillin dosage regimen of at least 3 g every 6 h.
B	Ceftazidime-avibactam	30/20 µg	≥21	–	≤20	≤8/4	–	≥16/4	(7) Breakpoints are based on a dosage regimen of 2.5 g (2 g ceftazidime + 0.5 g avibactam) every 8 h over 2 days
B	Ceftolozane-tazobactam	30/10 µg	≥21	17–20	≤16	≤4/4	8/4	≥16/4	(8) Breakpoints are based on a dosage regimen of 1.5 g every 8 h.
O	Ticarcillin-clavulanate	75/10 µg	≥24	16–23	≤15	≤16/2	32/2–64/2	≥128/2	(9) Breakpoints for ticarcillin (alone or with clavulanate) are based on a ticarcillin dosage regimen of at least 3 g every 6 h.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
A	Ceftazidime	30 µg	≥18	15–17	≤14	≤8	16	≥32	(10) Breakpoints are based on a dosage regimen of 1 g every 6 h or 2 g every 8 h.
B	Cefepime	30 µg	≥18	15–17	≤14	≤8	16	≥32	(11) Breakpoints are based on a dosage regimen of 1 g every 8 h or 2 g every 12 h.
MONOBACTAMS									
B	Aztreonam	30 µg	≥22	16–21	≤15	≤8	16	≥32	(12) Breakpoints are based on a dosage regimen of 1 g every 6 h or 2 g every 8 h.

Table 2A. Enterobacteriaceae (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)											
U	Cefazolin	30 µg	≥15	–	–	≤14	≤16	–	–	≥32	(12) Breakpoints when cefazolin is used for therapy of uncomplicated UTIs due to <i>E. coli</i> , <i>K. pneumoniae</i> , and <i>P. mirabilis</i> . Breakpoints are based on a dosage regimen of 1 g every 12 h. See additional information in CEPHEMS (ORAL).
C	Ceftaroline	30 µg	≥23	–	20–22	≤19	≤0.5	–	1	≥2	(13) Breakpoints are based on a dosage regimen of 600 mg every 12 h.
B	Cefepime	30 µg	≥25	19–24	–	≤18	≤2	4–8	–	≥16	(14) The breakpoint for susceptible is based on a dosage regimen of 1 g every 12 h. The breakpoint for SDD is based on dosage regimens that result in higher cefepime exposure, either higher doses or more frequent doses or both, up to approved maximum dosage regimens. See Appendix E for more information about breakpoints and dosage regimens. Also see the definition of SDD in the Instructions for Use of Tables section.

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2A. <i>Enterobacteriaceae</i>				
Azithromycin (<i>Salmonella Typhi</i>)	≤16 µg/mL	500 mg daily	N/A	
Aztreonam	≤4 µg/mL	1 g every 8 h	N/A	
Cefazolin	≤2 µg/mL	2 g every 8 h	N/A	
Ceftaroline	≤0.5 µg/mL	600 mg every 12 h	N/A	
Cefepime	≤2 µg/mL	1 g every 12 h	4 µg/mL	1 g every 8 h or 2 g every 12 h
			8 µg/mL or zone diameter: 19–24 mm	2 g every 8 h (Because it is not possible to correlate specific zone diameters with specific MICs, an isolate with a zone diameter in the SDD range should be treated as if it might be an MIC of 8 µg/mL.)

Table 2H-1. *Streptococcus* spp. β -Hemolytic Group (Continued)

(5) Breakpoints for *Streptococcus* spp. β -hemolytic group are proposed based on population distributions of various species, pharmacokinetics of the antimicrobial agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available for review with many of the antimicrobial agents in this table.

NOTE: Information in boldface type is new or modified since the previous edition.

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
(6) An organism that is susceptible to penicillin can be considered susceptible to antimicrobial agents listed here when used for approved indications and does not need to be tested against those agents. For groups A, B, C, and G β-hemolytic streptococci, penicillin is a surrogate for ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefepime, ceftaroline, cephadrine, cephalothin, cefotaxime, ceftriaxone, ceftizoxime, imipenem, ertapenem, and meropenem. For group A β-hemolytic streptococci, penicillin is also a surrogate for cefaclor, cefdinir, cefprozil, ceftibuten, cefuroxime, and cefpodoxime.									
A	Penicillin or	10 units	≥24	—	—	≤0.12	—	—	See general comment (4).
A	ampicillin	10 µg	≥24	—	—	≤0.25	—	—	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
See comment (6).									
B	Cefepime or	30 µg	≥24	—	—	≤0.5	—	—	
B	cefotaxime or	30 µg	≥24	—	—	≤0.5	—	—	
B	ceftriaxone	30 µg	≥24	—	—	≤0.5	—	—	



Criteria for Antibiotic Selection

- ☐ Type of Microbe
- ☐ Type of specimen and infection site
- ☐ Hospitalized or community patients
- ☐ History of disease and etc.
- ☐ Antibiotic therapy and types of antibiotics
- ☐ Hospitalized unit (ICU, NICU,)
- ☐ Nosocomial infections ,

AST Results

(Analysis, Interpretation & Report)



Example 1: Warning

Warning

Some of the comments in the tables relate to dangerously misleading results that can occur when certain antimicrobial agents are tested and reported as susceptible against specific organisms. These are denoted with the word “Warning.”

Location	Organism	Antimicrobial Agents
“Warning”: The following antimicrobial agent/organism combinations may appear active <i>in vitro</i> , but are not effective clinically and must not be reported as susceptible.		
Table 2A	<i>Salmonella</i> spp., <i>Shigella</i> spp.	1st- and 2nd-generation cephalosporins, cephamycins, and aminoglycosides
Table 2D	<i>Enterococcus</i> spp.	Aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole
“Warning”: The following antimicrobial agents that are included in this document should not be routinely reported for bacteria isolated from CSF. These antimicrobial agents are not the drugs of choice and may not be effective for treating CSF infections caused by these organisms (ie, the bacteria included in Tables 2A through 2J):		
Tables 2A through 2J	Bacteria isolated from CSF	Agents administered by oral route only, 1st- and 2nd-generation cephalosporins and cephamycins, clindamycin, macrolides, tetracyclines, and fluoroquinolones

Abbreviation: CSF, cerebrospinal fluid.

Example 2: Fosfomycin

Table 2A. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
FOSFOMYCINS											
U	Fosfomycin	200 µg	≥16	–	13–15	≤12	≤64	–	128	≥256	(44) For testing and reporting of <i>E. coli</i> urinary tract isolates only. (45) The 200-µg fosfomycin disk contains 50 µg of glucose-6-phosphate. (46) The only approved MIC method for testing is agar dilution using agar media supplemented with 25 µg/mL of glucose-6-phosphate. Broth dilution MIC testing should not be performed.

Example 3: MIC for Colistin

LIPOPEPTIDES									
O	Colistin		-	-	-	≤2	-	≥4	<p>(5) Colistin (methanesulfonate) should generally be given with a loading dose and at maximum recommended doses and used in combination with other agents.</p> <p>(6) Applies to <i>A. baumannii</i> complex only.</p> <p>(7) The only approved MIC method for testing is broth microdilution. Disk diffusion and gradient diffusion methods should not be performed.</p>
O	Polymyxin B	-	-	-	-	≤2	-	≥4	
AMINOGLYCOSIDES									
A	Gentamicin	10 µg	≥15	13-14	≤12	≤4	8	≥16	
A	Tobramycin	10 µg	≥15	13-14	≤12	≤4	8	≥16	
B	Amikacin	30 µg	≥17	15-16	≤14	≤16	32	≥64	
O	Netilmicin	-	-	-	-	≤8	16	≥32	

Example 4: Tetracyclines

TETRACYCLINES

(8) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.

B	Doxycycline	30 µg	≥13	10-12	≤9	≤4	8	≥16	
B	Minocycline	30 µg	≥16	13-15	≤12	≤4	8	≥16	
U	Tetracycline	30 µg	≥15	12-14	≤11	≤4	8	≥16	

Example 5: MIC for VRSA, VISA

Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
GLYCOPEPTIDES									
(19) For <i>S. aureus</i> , vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy.									
B	Vancomycin (For <i>S. aureus</i>)	—	—	—	—	≤2	4–8	≥16	(20) MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of <i>S. aureus</i> from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and -resistant isolates of CoNS, all of which give similar size zones of inhibition.

Example 6: HLAR in *Enterococci*

Table 3I
Test for High-Level Aminoglycoside Resistance in *Enterococcus* spp.

Table 3I. Test for Detection of High-Level Aminoglycoside Resistance in *Enterococcus* spp.^a (Includes Disk Diffusion)

Test	Gentamicin HLAR			Streptomycin HLAR		
Test method	Disk diffusion	Broth microdilution	Agar dilution	Disk diffusion	Broth microdilution	Agar dilution
Medium	MHA	BHI ^b broth	BHI ^b agar	MHA	BHI ^b broth	BHI ^b agar
Antimicrobial concentration	120-µg gentamicin disk	Gentamicin, 500 µg/mL	Gentamicin, 500 µg/mL	300-µg streptomycin disk	Streptomycin, 1000 µg/mL	Streptomycin, 2000 µg/mL
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface
Incubation conditions	35°C±2°C; ambient air	35°C±2°C; ambient air	35°C±2°C; ambient air	35°C±2°C; ambient air	35°C±2°C; ambient air	35°C±2°C; ambient air
Incubation length	16–18 hours	24 hours	24 hours	16–18 hours	24–48 hours (if susceptible at 24 hours, reincubate)	24–48 hours (if susceptible at 24 hours, reincubate)
Results	6 mm = resistant 7–9 mm = inconclusive ≥ 10 mm = susceptible MIC correlates: R = > 500 µg/mL S = ≤ 500 µg/mL	Any growth = resistant	> 1 colony = resistant	6 mm = resistant 7–9 mm = inconclusive ≥ 10 mm = susceptible MIC correlates: R = > 1000 µg/mL (broth) and > 2000 µg/mL (agar) S = ≤ 1000 µg/mL (broth) and ≤ 2000 µg/mL (agar)	Any growth = resistant	> 1 colony = resistant
Additional testing and reporting	<p>Resistant: is not synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin).</p> <p>Susceptible: is synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin) that is also susceptible.</p> <p>If disk diffusion result is inconclusive: perform an agar dilution or broth dilution MIC test to confirm.</p>					

Example 7: Intrinsic or Innate Resistance

Each laboratory should decide which agents to test and report in consultation with institutional leaders representing infectious diseases practitioners, the pharmacy and therapeutics and infection control committees of the medical staff, and the antimicrobial stewardship team. If tested, the result for an antimicrobial agent/organism combination listed as having intrinsic resistance should be reported as resistant. Consideration may be given to adding comments regarding intrinsic resistance of agents not tested. See Appendix A, footnote "a."

B1. Enterobacteriaceae

Antimicrobial Agent Organism	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Piperacillin	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporin II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
<i>Citrobacter freundii</i>	R	R	R			R	R	R						
<i>Citrobacter koseri</i>	R			R	R									
<i>Enterobacter cloacae</i> complex ^a	R	R	R			R	R	R						

B1. Enterobacteriaceae (Continued)

Antimicrobial Agent Organism	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Piperacillin	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporin II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
<i>Salmonella</i> and <i>Shigella</i> spp.	There is no intrinsic resistance to β -lactams in these organisms; refer to WARNING below for reporting.													
<i>Serratia marcescens</i>	R	R	R			R	R	R				R	R	
<i>Yersinia enterocolitica</i>	R	R			R	R								

Microbial Resistant strains

- ☐ MRSA: Methicillin Resistant *Staphylococcus aureus*
- ☐ MR-CoNS: Methicillin Resistant Coagulase Negative *Staphylococci*
- ☐ VRSA: Vancomycin Resistant *Staphylococcus aureus*
- ☐ VRE: Vancomycin Resistant *Enterococci*
- ☐ **ESBL: Extended Spectrum Beta Lactamases-producing strains**
- ☐ Carbapenemase-producing strains
- ☐ **MDR: Multidrug-Resistant**
- ☐ **XDR: Extensively Drug-Resistant**
- ☐ **PDR: Pandrug-Resistant**



ESBL producing-Strains:

➤ Resistance to most beta-lactam antibiotics including:

- ✓ Penicillins
- ✓ Cephalosporins
- ✓ Monobactam aztreonam

Test	Criteria for Performance of ESBL Test	
Test method	Disk diffusion	Broth microdilution
Medium	MHA	CAMHB
Antimicrobial concentration	<p>For <i>K. pneumoniae</i>, <i>K. oxytoca</i>, and <i>E. coli</i>:</p> <p>Cefpodoxime 10 µg or Ceftazidime 30 µg or Aztreonam 30 µg or Cefotaxime 30 µg or Ceftriaxone 30 µg</p> <p>For <i>P. mirabilis</i>:</p> <p>Cefpodoxime 10 µg or Ceftazidime 30 µg or Cefotaxime 30 µg</p> <p>(Using more than one antimicrobial agent improves the sensitivity of ESBL detection.)</p>	<p>For <i>K. pneumoniae</i>, <i>K. oxytoca</i>, and <i>E. coli</i>:</p> <p>Cefpodoxime 4 µg/mL or Ceftazidime 1 µg/mL or Aztreonam 1 µg/mL or Cefotaxime 1 µg/mL or Ceftriaxone 1 µg/mL</p> <p>For <i>P. mirabilis</i>:</p> <p>Cefpodoxime 1 µg/mL or Ceftazidime 1 µg/mL or Cefotaxime 1 µg/mL</p> <p>(Using more than one antimicrobial agent improves the sensitivity of ESBL detection.)</p>
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	16–18 hours	16–20 hours

Test	Criteria for Performance of ESBL Test	
Test method	Disk diffusion	Broth microdilution
Results	<p>For <i>K. pneumoniae</i>, <i>K. oxytoca</i>, and <i>E. coli</i>:</p> <p>Cefpodoxime zone ≤ 17 mm Ceftazidime zone ≤ 22 mm Aztreonam zone ≤ 27 mm Cefotaxime zone ≤ 27 mm Ceftriaxone zone ≤ 25 mm</p> <p>For <i>P. mirabilis</i>:</p> <p>Cefpodoxime zone ≤ 22 mm Ceftazidime zone ≤ 22 mm Cefotaxime zone ≤ 27 mm</p> <p>Zones above may indicate ESBL production.</p>	<p>Growth at or above the concentrations listed may indicate ESBL production (ie, for <i>E. coli</i>, <i>K. pneumoniae</i>, and <i>K. oxytoca</i>, MIC ≥ 8 µg/mL for cefpodoxime or MIC ≥ 2 µg/mL for ceftazidime, aztreonam, cefotaxime, or ceftriaxone; and for <i>P. mirabilis</i>, MIC ≥ 2 µg/mL for cefpodoxime, ceftazidime, or cefotaxime).</p>
Reporting		

ESBL Test	
Disk diffusion	Broth microdilution
MHA	CAMHB
Ceftazidime 30 µg Ceftazidime-clavulanate ^a 30/10 µg and Cefotaxime 30 µg Cefotaxime-clavulanate 30/10 µg (Testing necessitates using both cefotaxime and ceftazidime, alone and in combination with clavulanate.)	Ceftazidime 0.25–128 µg/mL Ceftazidime-clavulanate 0.25/4–128/4 µg/mL and Cefotaxime 0.25–64 µg/mL Cefotaxime-clavulanate 0.25/4–64/4 µg/mL (Testing necessitates using both cefotaxime and ceftazidime, alone and in combination with clavulanate.)
Standard disk diffusion procedure	Standard broth dilution procedure
35°C±2°C; ambient air	35°C±2°C; ambient air
16–18 hours	16–20 hours

ESBL Test	
Disk diffusion	Broth microdilution
A ≥5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone = ESBL (eg, ceftazidime zone = 16; ceftazidime-clavulanate zone = 21).	A ≥3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone = ESBL (eg, ceftazidime MIC = 8 µg/mL; ceftazidime-clavulanate MIC = 1 µg/mL).
For all confirmed ESBL-producing strains:	
If laboratories do not use current cephalosporin and aztreonam breakpoints, the test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam.	
If laboratories use current cephalosporin and aztreonam breakpoints, then test interpretations for these agents do not need to be changed from susceptible to resistant.	

ESBL Test

Disk diffusion

When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be used for routine QC (eg, weekly or daily).

Acceptable QC:

E. coli ATCC® 25922: ≤ 2 -mm increase in zone diameter for antimicrobial agent tested in combination with clavulanate vs the zone diameter when tested alone.

K. pneumoniae ATCC® 700603: ≥ 5 -mm increase in zone diameter of ceftazidime-clavulanate vs ceftazidime alone; ≥ 3 -mm increase in zone diameter of cefotaxime-clavulanate vs cefotaxime alone.

Broth microdilution

When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be tested routinely (eg, weekly or daily).

Acceptable QC:

E. coli ATCC® 25922: < 3 twofold concentration decrease in MIC for antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.

K. pneumoniae ATCC® 700603: ≥ 3 twofold concentration decrease in MIC for an antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.

Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance

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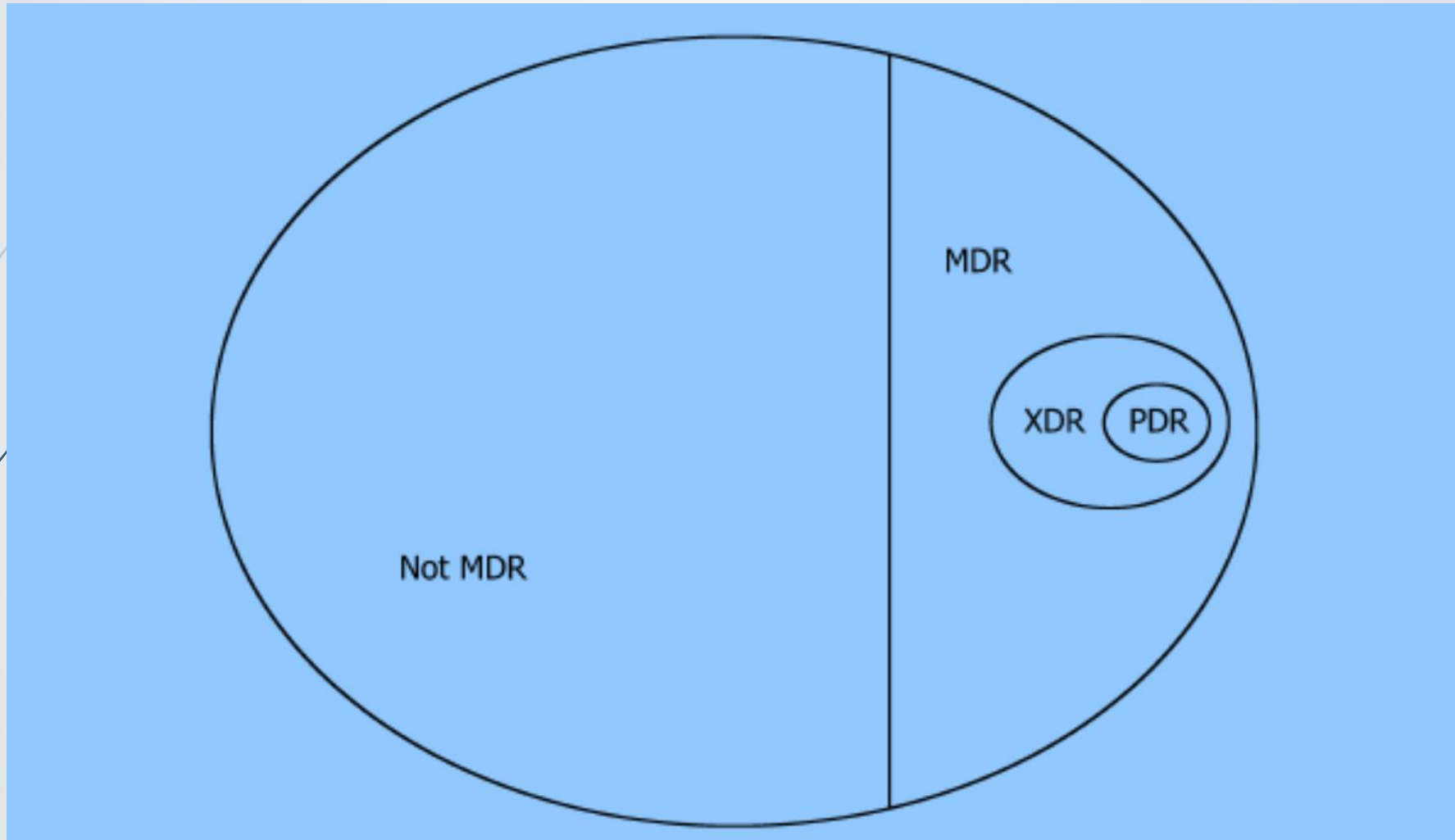


TABLE 6. Definitions for multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria

Bacterium	MDR	XDR	PDR
<i>Staphylococcus aureus</i>	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 1 ^a	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 1.	Non-susceptibility to all agents in all antimicrobial categories for each bacterium in Tables 1–5
<i>Enterococcus</i> spp.	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 2	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 2.	
<i>Enterobacteriaceae</i>	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 3	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 3.	
<i>Pseudomonas aeruginosa</i>	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 4	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 4.	
<i>Acinetobacter</i> spp.	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 5	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 5.	

^aAll MRSA isolates are defined as MDR because resistance to oxacillin or ceftiofex predicts non-susceptibility to all categories of β -lactam antimicrobials listed in this document, with the exception of the anti-MRSA cephalosporins (i.e. all categories of penicillins, cephalosporins, β -lactamase inhibitors and carbapenems currently approved up until 25 January 2011).

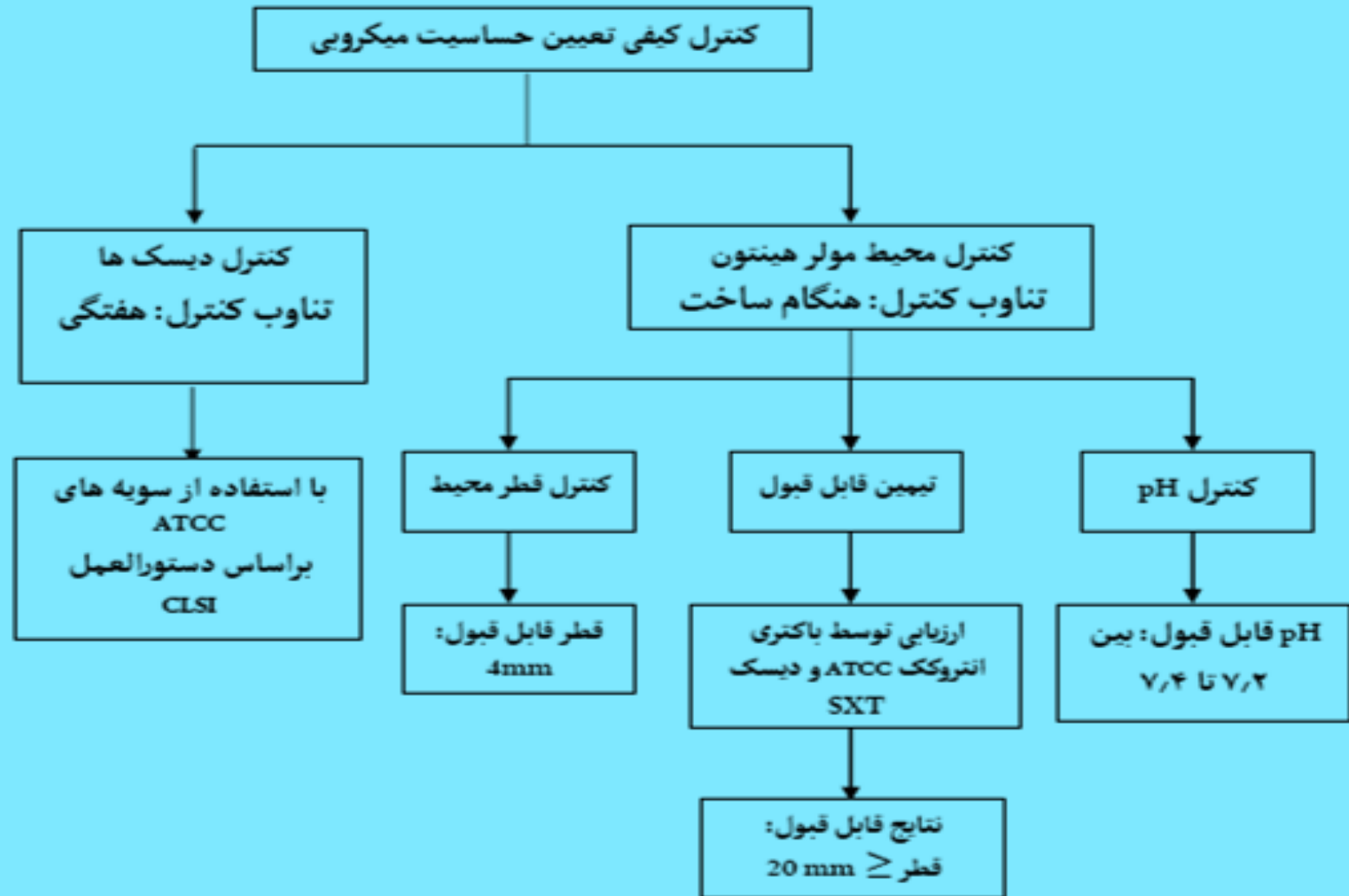
http://www.ecdc.europa.eu/en/activities/diseaseprogrammes/ARHAI/Pages/public_consultation_clinical_microbiology_infection_article.aspx.

TABLE 7. *Pseudomonas aeruginosa*; examples of antimicrobial susceptibility profiles that fit MDR, XDR and PDR definitions; isolate no. 1 is PDR; isolate no. 2 is XDR and isolate no. 3 is MDR

Antimicrobial category	Antimicrobial agent	Isolate no. 1 (PDR)	Isolate no. 2 (XDR)	Isolate no. 3 (MDR)
Aminoglycosides	Gentamicin	X ^a	X	
	Tobramycin	X	^b	
	Amikacin	X		
	Netilmicin	X		
Antipseudomonal carbapenems	Imipenem	X	X	X
	Meropenem	X	X	
	Doripenem	X	X	
Antipseudomonal cephalosporins	Ceftazidime	X		X
	Cefepime	X	X	
Antipseudomonal fluoroquinolones	Ciprofloxacin	X	X	X
	Levofloxacin	X		
Antipseudomonal penicillins + β -lactamase inhibitors	Piperacillin-tazobactam	X		
	Ticarcillin-clavulanic acid	X	X	
Monobactams	Aztreonam	X	X	
Phosphonic acids	Fosfomycin	X		
Polymyxins	Colistin	X		
	Polymyxin B	X		

	• ادامه آزمایش تعیین حساسیت ضد میکروبی (آنتی بیوگرام)	سنجه	حداکثر امتیاز هر سنجه	حداکثر امتیاز هر سؤال	امتیاز کسب شده	کاربرد ندارد	توضیحات
۴۸	آیا در آزمایشگاه، عفونت های مقاوم ناشی از باکتری های MDR (Multidrug resistant)، XDR (Extensively drug resistant) و PDR (Pandrug resistant) به پزشک معالج و پرستار یا پزشک کنترل عفونت به عنوان نتیجه بحرانی و نیز به صورت دوره ای گزارش می شود؟ توجه: (اگر آزمایشگاه عفونت های مقاوم ناشی از هر کدام از باکتری های MDR، XDR، PDR را گزارش کند، امتیاز سنجه اول را به طور کامل می گیرد. ضمناً آزمایشگاه یا امتیاز سنجه گزارش توسط نرم افزار را می گیرد، یا امتیاز سنجه گزارش دستی را، بنابراین حداکثر امتیاز این سؤال، ۵ می باشد).	آگاهی کارکنان و گزارش موارد زیر: MDR: باکتری هایی که حداقل به سه آنتی بیوتیک از سه کلاس مختلف مقاوم باشند. XDR: باکتری هایی که تنها به یک یا دو آنتی بیوتیک از کلاس های مختلف حساس باشند. PDR: باکتری هایی که به تمامی آنتی بیوتیک های مورد آزمایش مقاوم باشند.	۱	۵			
		گزارش توسط نرم افزار WHONET 5.6	۲				
		گزارش دستی (غیر از نرم افزار WHONET)	۱				
		وجود سوابق گزارش فوری یا دوره ای و صحت سوابق	۲				

QA & QC in AST:



کنترل کیفی
نیم مک فارلند



کنترل کیفی ماهانه




تعیین جذب در ۶۲۵ نانومتر



جذب قابل قبول: بین ۰٫۱۳ - ۰٫۰۸



نگهداری در لوله های دریچ دار، در
دمای اتاق و در تاریکی



Test Modification	Required QC Frequency		
	1 Day	5 Days	15-Replicate Plan or 20- or 30-Day Plan
Disks			
Use new shipment or lot number.	X		
Use new manufacturer.	X		
Addition of new antimicrobial agent to existing system.			X

Table 4A-1. Disk Diffusion QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β -Lactam Combination Agents^a

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		<i>Escherichia coli</i> ATCC ^{®b} 25922	<i>Staphylococcus aureus</i> ATCC [®] 25923	<i>Pseudomonas aeruginosa</i> ATCC [®] 27853
Amikacin	30 μ g	19–26	20–26	18–26
Ampicillin	10 μ g	15–22	27–35	–
Azithromycin	15 μ g	–	21–26	–
Azlocillin	75 μ g	–	–	24–30
Aztreonam	30 μ g	28–36	–	23–29
Carbenicillin	100 μ g	23–29	–	18–24
Cefaclor	30 μ g	23–27	27–31	–
Cefamandole	30 μ g	26–32	26–34	–
Cefazolin	30 μ g	21–27	29–35	–
Cefdinir	5 μ g	24–28	25–32	–
Cefditoren	5 μ g	22–28	20–28	–
Cefepime	30 μ g	31–37	23–29	25–31
Cefetamet	10 μ g	24–29	–	–
Cefiderocol	30 μ g	25–31	–	22–31
Cefixime	5 μ g	20–26	–	–
Cefmetazole	30 μ g	26–32	25–34	–

Aminoglycosides Quinolones	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO ₂ incubation, which lowers pH.
	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Aminoglycosides	<i>P. aeruginosa</i> ATCC® 27853	Zone too small	Ca++ and/or Mg++ content too high	Use alternative lot of media.
Aminoglycosides	<i>P. aeruginosa</i> ATCC® 27853	Zone too large	Ca++ and/or Mg++ content too low	Use alternative lot of media.
Clindamycin Macrolides	<i>S. aureus</i> ATCC® 25923	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO ₂ incubation, which lowers pH.
	<i>S. aureus</i> ATCC® 25923	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Quinolones	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO ₂ incubation, which lowers pH.
Quinolones	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Tetracyclines	Any	Zone too large	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO ₂ incubation, which lowers pH.
Tetracyclines	Any	Zone too small	pH of media too high	Acceptable pH range = 7.2–7.4
Tetracyclines	Any	Zone too small	Ca++ and/or Mg++ content too high	Use alternative lot of media.
Tetracyclines	Any	Zone too large	Ca++ and/or Mg++ content too low	Use alternative lot of media.
Sulfonamides Trimethoprim Trimethoprim-sulfamethoxazole	<i>E. faecalis</i> ATCC® 29212	Zone ≤ 20 mm	Media too high in thymidine content	Use alternative lot of media.
ALL AGENTS				
Various	Various	Zone too small	Contamination Use of magnification to read zones	Measure zone edge with visible growth detected with unaided eye. Subculture to determine purity and repeat if necessary.
Various	Any	Inoculum too light Error in inoculum preparation Media depth too thin MHA nutritionally unacceptable	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Use agar with depth approximately 4 mm. Recheck alternate lots of MHA.	
Various	Any	Many zones too small	Inoculum too heavy Error in inoculum preparation Media depth too thick	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Use agar with depth approximately 4 mm. Recheck alternate lots of MHA.

ALL AGENTS (Continued)				
Various	Any	One or more zones too small or too large	Measurement error Transcription error Random defective disk Disk not pressed firmly against agar	Recheck readings for measurement or transcription errors. Retest. If retest results are out of range and no errors are detected, initiate corrective action.
Various	Various	Zone too large	Did not include lighter growth in zone measurement (eg, double zone, fuzzy zone edge)	Measure zone edge with visible growth detected with unaided eye.
Various	<i>S. pneumoniae</i> ATCC® 49619	Zones too large Lawn of growth scanty	Inoculum source plate too old and contains too many nonviable cells. Plate used to prepare inoculum should be 18–20 hours.	Subculture QC strain and repeat QC test, or retrieve new QC strain from stock.
Various	Any	One QC strain is out of range, but other QC organism(s) is in range with the same antimicrobial agent.	One QC organism may be a better indicator of a QC problem.	Retest this strain to confirm reproducibility of acceptable results. Evaluate with alternative strains with known MICs. Initiate corrective action with problem QC strain/antimicrobial agents.
Various	Any	Two QC strains are out of range with the same antimicrobial agent.	A problem with the disk	Use alternative lot of disks. Check storage conditions and package integrity.
Various	Any	Zones overlap.	Too many disks per plate	Place no more than 12 disks on a 150-mm plate and 5 disks on a 100-mm plate; for some fastidious bacteria that produce large zones, use fewer.

WHONET 2019 software for correct and reliable analysis of AST results

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The microbiology laboratory database software.

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This version of WHONET is still in development. In addition to the standard

WHONET 5.6

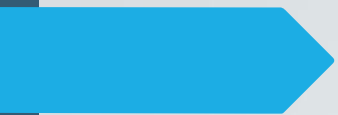


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Microbiology Critical Value

Microbiology Critical Values	
Test	Critical Value Notification
Blood Culture	Positive Smear Or Culture
Cerebrospinal Fluid(CSF) Exam	Positive Smear Or Culture
Acid Fast Bacilli – AFB	Positive Smear Or Culture
Herpes Culture	Positive Culture From Female Genital Specimen
Genital Culture	Positive Culture For Neisseria Gonorrhoeae And GroupB Strep From Inpatient /OB Genital , Placenta
Cryptococcal Antigens	Positive
Hepatitis Surface Antigen	Positive Postpartum Within 24 hrs
Positive Tissue Cultures (Liver,Biopsy Biopsies,etc.)	
Beta – Hemolytic Streptococci,Group A From Sterile Sites	
Neisseria Gonorrhoeae From Eyes	
Clostridium Botulinum, Clostridium Perfringenes, Clostridium Tetanus	
Corynebacterium Diphtheriae	
Legionella Species	
Listeria Species	
Positive Modified Acid Fast And Ziehl- Neelsen Stains	
Unusually Resistant Organisms Including:	
*Vancomycin Resistant Enterococci (VRE)	
*Methicillin Resistant Staphylococcus Aureus (MRSA)	
*Multiply Resistant Gram Negative Or Any Other Highly Resistant Organism Or Organism With Unusual Susceptibility Pattern.On Blood/Sterile Fluids/Wound/ANO2/Screening Swabs,BAL,Tracheal Aspirates	
*Extended Spectrum Beta Lactamases (ESBLs)	
*Glycopeptide Intermediate StaphylococcusAureus (GISA) Borderline Oxacilin Resistant	





Thanks for your attention