

Antibiotic Technology Laboratory

Lab 5: Bacterial Resistance tests

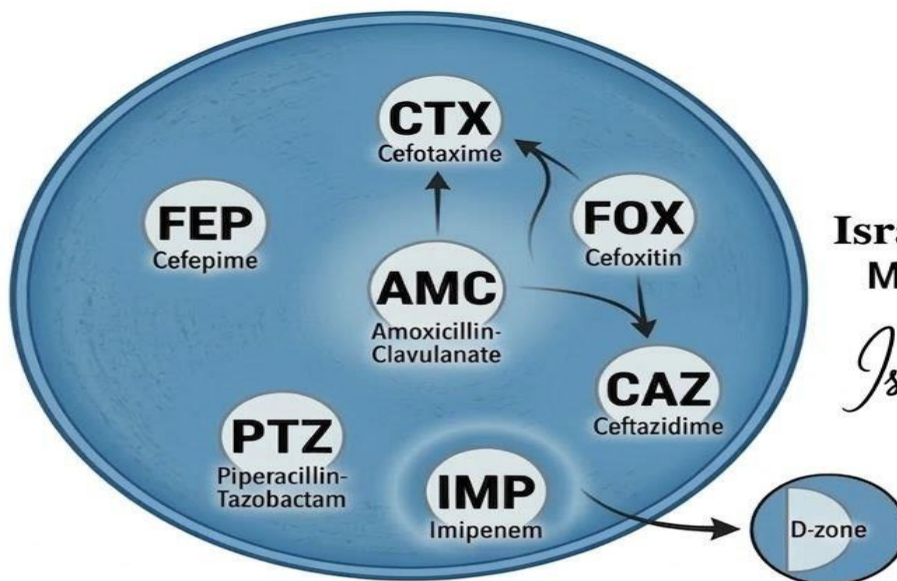
Subtitle: A Clinical & Microbiological Guide to *ESBL*, *AmpC*, *MBL* & *MRSA*

In the war against superbugs, the laboratory dish is our primary intelligence report. However, a "Sensitive" result on paper doesn't always translate to a "Cure" in the patient. We must learn to read between the lines of the Antibigram.

The *ESBL* Family: Optical Illusions in the Lab

- * Definition: Enzymes that hydrolyze Penicillins and Cephalosporins up to the 3rd generation.
- * The IDSA Golden Rule: For severe systemic infections (Sepsis, Pneumonia), Carbapenems (Meropenem/Imipenem) are the "Kings" .
- * The Sensitivity Trap: Even if Augmentin or Tazocin appear sensitive in vitro, clinical failure is common in critically ill patients due to the "Inoculum Effect" and biofilm.
- * Lab Note: Any ESBL producer must be reported as Resistant (R) to all Cephalosporins, regardless of the zone size.

Beta-lactam Resistance



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- **Keyhole** synergy between AMC and other Cephalosporins indicates **ESBL**
- **D-Zone** (induction) between IMP and Ceftazidime or Pr/ piptaz indicates **ampC**
Beta-Lactamase
- Response of **Fox** and **AMC**

→ Sensitive to FOX and AMC	→ ESBL
→ Resistant to FOX and AMC	→ ampC
- **IMP Resistant** Resistance extending to Penicillins and Cephalosporins indicates **Carbapenemase resistance** (e.g. Carbapenem-Resistant Enterobacteriaceae or CRE)

The *AmpC* Beast: Hidden (Inducible) Resistance

- * Organisms (SCEMoP): Serratia, Citrobacter, Enterobacter, Morganella, Providencia.
- * The Danger: They may look sensitive to 3rd gen cephalosporins initially, but the drug induces the gene during treatment, leading to failure.
- * The Survivor: Cefepime (4th gen) remains stable and is the preferred choice.
- * Differentiation: AmpC is resistant to Cefoxitin and is NOT inhibited by Clavulanic acid.

Carbapenemases: Breaking the Last Line

- * KPC (Serine-based): Destroys almost all beta-lactams.
- * MBL (Zinc-dependent): Uses Zinc ions to break the carbapenem ring. Zinc ions snap carbapenem ring; EDTA chelates zinc for synergy test. Often Aztreonam-sensitive

The Key: EDTA inhibits the enzyme by binding Zinc, restoring sensitivity (Synergy Test).

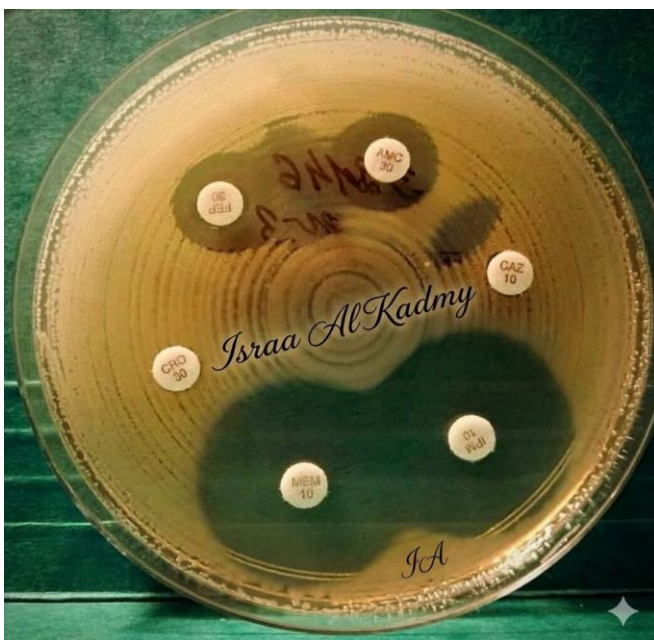
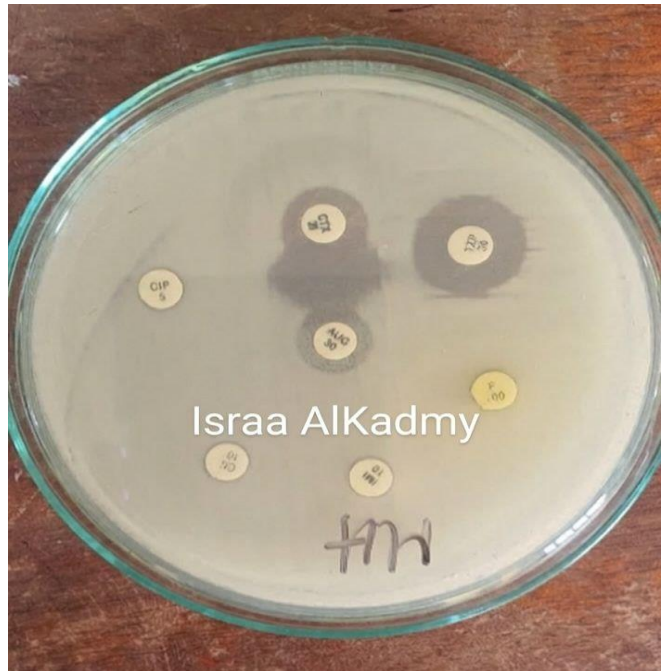
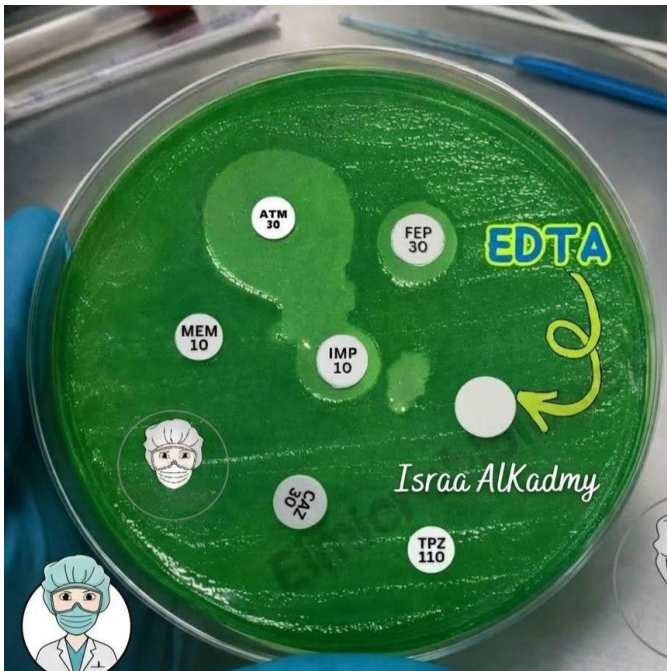
Weakness: Often sensitive to Aztreonam.

OXA-48: The "Silent" killer. Often shows false sensitivity to cephalosporins.

Laboratory Patterns: Reading the Plates (**Types of inhibition zone**)

Phenomenon / Test	Visual Shape	Laboratory Interpretation
Clear Circle zone	Full Sensitivity	No major resistance
Keyhole zone	Drug + Inhibitor Synergy	ESBL (e.g., CAZ + Clavulanate)
D-Shape Zone	Inducible Resistance	erm (Staph) or AmpC (G-)
Flattened Edge zone	Zinc Chelation/Antagonism	MBL + EDTA
Cloverleaf zone	Multi-directional synergy	Carbapenemase producers (KPC/NDM/OXA48)
Eagle Eye (Double Zone)	A clear inner circle with a secondary hazy ring of growth	Bacteria are susceptible to highly concentrated antibiotic (outer zone > MIC) but develop resistance to lower concentrations (inner zone < MIC) after prolonged incubation. Report as Resistant or recommend MIC testing.
Elliptical Inhibition (E-test)	A clear, Symmetrical Oval/Egg shape (Tear-drop) that intersects the plastic strip	Gold Standard for MIC. The point where the "Ellipse" (the growth) touches the strip is the exact MIC value
DDST or "Ghost" or "Balloon" Zone	Synergy	Confirms Enzyme Production (like ESBL)

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The "Magic" Combination: CAZ-AVI + ATM

- * Avibactam: Neutralizes Serine enzymes (KPC, OXA-48).
- * Aztreonam: Resists the MBL (NDM, VIM) enzymes.
- * Synergy: Together, they cover almost all known Carbapenem-Resistant Enterobacteriaceae (CRE).

MRSA: The Oxacillin Giant

Marker: Cefoxitin disk—small zone = MRSA (*mecA* gene → PBP2a mutation) .

Consequence: All β -lactams fail (except Ceftaroline). Even untested ones reported R .

Visual: Bacteria "pummels" Cefoxitin; others sit out a losing fight .

Why the Combination ?

Resistance Type	Issue	Combo Fix
Serine (KPC/OXA/ESBL/AmpC)	Breaks Aztreonam	Avibactam blocks them, protects Aztreonam
MBL (Zinc)	Unaffected by Avibactam	Aztreonam resists MBL

Efflux Pumps: The Ejection Door

Bacteria don't negotiate—they boot antibiotics out via Efflux Pumps, like a bouncer at a club .

-Mechanism: Drug enters cell → pump detects threat → ejects it instantly (e.g., Ciprofloxacin, Meropenem, Ceftazidime) .

-Multi-Drug Power: One pump expels multiple classes simultaneously .

-Upgrade: Mutations amp up pump strength, causing "weird" multi-resistance .

-Lab Clue: Not enzyme-based—pure mechanical resistance

The "Different Doors" Mystery: Imipenem vs. Meropenem

In the lab, you might see a strange result: Imipenem is Resistant, but Meropenem is Sensitive. Does this mean the bacteria produces Carbapenemases? Not necessarily!

Think of the bacterial cell as a fortress. For an antibiotic to work, it must first enter the fortress through specific doors (Porin Channels).

Why does this matter in the Lab?

If you only test Imipenem, you might report the bacteria as "Carbapenem Resistant" (suggesting a scary enzyme), when in reality, it just has a locked door.

The Rule:

* Isolated Imipenem Resistance: Likely a Porin (*OprD*) issue.

* Dual Resistance (Imi + Mero): Now you should suspect a Carbapenemases or a powerful Efflux Pump.

Don't let the bacteria trick you—check both doors!

Resistance Mechanism	Imipenem	Meropenem	What happened?
OprD Mutation	Resistant	Sensitive	The Imipenem "Door" is locked
MPM Mutation	Sensitive	Resistant	The Meropenem "Door" is locked
Efflux Pump	Resistant	Resistant	Both are "Booted Out" after entry
Carbapenemases	Resistant	Resistant	Both are "Shredded" by enzymes

Comparison of ESBLs & AmpC β -lactamases

Key differences between the two types of β -lactamases in inhibition, induction, and detection

ESBLs	VS	AmpC
β -Lactamase Inhibitors		β -Lactamase Inhibitors
✓ Susceptible to traditional inhibitors (Clavulanic acid, Sulbactam, Tazobactam)	VS	✓ Susceptible only to newer inhibitors (like Avibactam)
Cefoxitin / Cefotetan	✓	Resistant (R) ✗
Variable (depend on (R/S))	⚖	Susceptible (S) ✓
Monobactam (Aztreonam)	✗	Usually susceptible (S) ✓
💡 Induction	💡	Inducible ✓
Keyhole effect between disk inhibitor & 3rd gen cephalosporin		D-zone Formed toward strong inducer like: Imipenem, AUG, Cefoxitin

Quick Summary

- ✓ **ESBLs:** Inhibited by traditional inhibitors, not inducible, detected by **Keyhole effect**
- ✓ **AmpC:** Resistant to most traditional inhibitors, inducible, detected by **D-zone**



Homework:

What the type of resistance in picture?

