

# New Resistance in Gram Negative Rods (GNRs)

Baha Abdalhamid, Ph. D.  
Clinical Microbiology Fellow  
Pathology-Microbiology Department  
University of Nebraska Medical Center

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# Disclosure

- No financial interest

# Objectives

- Understand the complexity of treatment of infections caused by multidrug resistant GNRs
- Understand the mechanisms of  $\beta$ -lactam resistance in GNRs
- Know the characteristics of each class of  $\beta$ -lactamases
- Be aware of unresolved issues in  $\beta$ -lactamases

# Infections caused by GNRs

- UTIs
- Bloodstream infections
- Intra-abdominal infections
- Pneumonia
- Peritonitis
- CNS infections

# Therapeutic Issues

- Treatment of infections caused by GNRs is difficult because of emergence of antibiotic resistance
  - $\beta$ -lactam resistance
  - Aminoglycoside resistance
  - Fluoroquinolone resistance
  - Resistance for other antibiotic groups

# Mechanism of $\beta$ -Lactam Action

- Bactericidal
- $\beta$ -lactams bind and inhibit penicillin binding proteins (PBPs)
- PBPs are responsible for assembly, maintenance, and regulation of peptidoglycan (cell wall) metabolism.
- Disruption of peptidoglycan synthesis

# Mechanisms of GNR Resistance to $\beta$ -Lactams

- Outer-membrane permeability
  - Porin mutation
- PBP alterations:
  - PBP down regulation (*Acinetobacter baumannii*)
- $\beta$ -lactamase production: the most common mechanism

# Common $\beta$ -Lactamases in GNRs

$\beta$ -Lactamase	Examples	Substrates	Inhibition by Clavulanic Acid*	Molecular Class
Broad-spectrum	TEM-1, TEM-2, SHV-1	Benzylpenicillin (penicillin G), aminopenicillins (amoxicillin and ampicillin), carboxypenicillins (carbenicillin and ticarcillin), ureidopenicillin (piperacillin), narrow-spectrum cephalosporins (cefazolin, cephalothin, cefamandole, cefuroxime, and others)	+++	A
	OXA family	Substrates of the broad-spectrum group plus cloxacillin, methicillin, and oxacillin	+	D
Expanded-spectrum	TEM family and SHV family	Substrates of the broad-spectrum group plus oxymino-cephalosporins (cefotaxime, cefpodoxime, ceftazidime, and ceftriaxone) and monobactam (aztreonam)	++++	A
	Others (BES-1, GES/IBC family, PER-1, PER-2, SFO-1, TLA-1, VEB-1, and VEB-2)	Same as for TEM family and SHV family	++++	A
	CTX-M family	Substrates of the expanded-spectrum group plus, for some enzymes, cefepime	++++	A
AmpC	OXA family	Same as for CTX-M family	+	D
	ACC-1, ACT-1, CFE-1, CMY family, DHA-1, DHA-2, FOX family, LAT family, MIR-1, MOX-1, and MOX-2	Substrates of expanded-spectrum group plus cephamycins (cefotetan, cefoxitin, and others)	0	C
Carbapenemase	IMP family, VIM family, GIM-1, and SPM-1	Substrates of the expanded-spectrum group plus cephamycins and carbapenems (ertapenem, imipenem, and meropenem)	0	B
	KPC-1, KPC-2, and KPC-3	Same as for IMP family, VIM family, GIM-1, and SPM-1	+++	A
	OXA-23, OXA-24, OXA-25, OXA-26, OXA-27, OXA-40, and OXA-48	Same as for IMP family, VIM family, GIM-1, and SPM-1	+	D

# Mechanisms of Carbapenem Resistance

- Carbapenemase hydrolyzing enzymes
- Porin loss “OprD”
- ESBL or AmpC + porin loss

# Carbapenemases

- The most versatile family of  $\beta$ -lactamases
- Two major groups based on the hydrolytic mechanism at the active site
  - Serine at the active site: class A and D
  - Zinc at the active site: class B
- All carbapenemases hydrolyze penicillins, extended spectrum cephalosporins, and carbapenems

# Carbapenemase Classification

Molecular Class	<b>A</b>	<b>B</b>	<b>D</b>
Functional Group	<b>2f</b>	<b>3</b>	<b>2d</b>
Aztreonam Hydrolysis	<b>+</b>	<b>-</b>	<b>-</b>
EDTA Inhibition	<b>-</b>	<b>+</b>	<b>-</b>
Clavulanate Inhibition	<b>+</b>	<b>-</b>	<b>±</b>

# Carbapenemases Class A

- First identified 1982 in UK
- Four major families
- Chromosomally encoded
  - *Serratia marcescens* enzyme (SME)
  - Not metalloenzyme carbapenemases (NMC)
  - Imipenem-hydrolyzing  $\beta$ -lactamases (IMI)
- Plasmid encoded
  - *Klebsiella pneumoniae* carbapenemases (KPC)
  - Guiana Extended-Spectrum (GES)

# Carbapenemases Class A

- Hydrolysis of penicillins, cephalosporins, carbapenems, and aztreonam
- GES enzymes do not hydrolyze aztreonam
- Most common in Enterobacteriaceae

# SME, NMC, and IMI carbapenemases

- Chromosomally encoded
- Rare: no association with mobile DNA elements
- Induced by imipenem and ceftazidime
  - Two component signal transduction system
- Only, IMI-2 is plasmid encoded in *Enterobacter cloacae*.

# KPC

- Molecular class A and functional group 2f
- Inhibited by clavulanic acid but not by EDTA
- Confers resistance to ALL  $\beta$ -LACTAMASES
- Plasmid-encoded
  - Associated with other resistant genes (aminoglycosides, fluoroquinolones)
  - Transferable

# KPC Epidemiology

- Predominantly in *K. pneumoniae* (KP)
- Reported in *Enterobacter* spp., *Salmonella* spp., *E. coli*, *P. aeruginosa*, and *Citrobacter* spp.
- First identified in KP clinical isolate from North Carolina in 1996 (KPC-1)
- KPC-2, -3, and -4 have been reported.
- Mostly identified at the East coast

# KPC Epidemiology

- KPC producers have been identified outside USA
  - France
  - Brazil
  - Columbia
  - China

# When to Suspect a KPC Producer

- *Enterobacteriaceae*
- Resistance to extended spectrum cephalosporins (cefotaxime, ceftazidime, and ceftriaxone)
- Variable susceptibility to cephamycins (cefoxitin, cefotetan)
- Carbapenem MICs  $\geq 2 \mu\text{g/ml}$

# How to Detect a KPC Producer

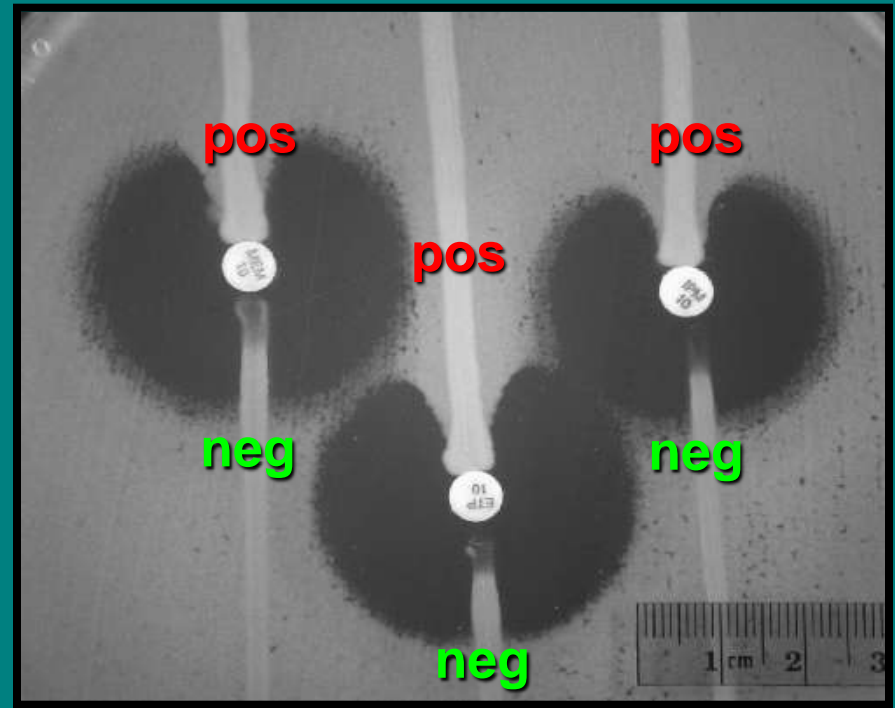
- Antimicrobial susceptibility tests (ASTs)
  - MIC
    - Carbapenem MIC  $\geq 2 \mu\text{g/ml}$
  - Disk diffusion
    - Carbapenem: “I” or “R”
  - Among carbapenems, ertapenem:
    - Most sensitive
    - less specific

# How to Detect a KPC Producer

- Commercial systems
  - Inconsistent detection of KPC-producing isolates
    - » Tenover et al. 2006. EID. 12:1209-1213
  - Breakpoints do not match CLSI recommendations

# Definitive ID of a KPC Producer

- Modified Hodge test
  - 100% sensitivity to detect KPC
- 1. Swab *E. coli* ATCC 25922 onto plate to create lawn  
Place imipenem disk in center.
- 2. Streak **test isolates** from edge of disk to end of plate.
- 3. Incubate overnight.
- 4. Look for growth of *E. coli* around **test isolate** streak - indicates carbapenem-hydrolyzing enzyme.



meropenem ertapenem imipenem

# Definitive ID of a KPC Producer

- PCR
  - The method of choice to confirm KPC
  - Fast
  - Detection of which enzyme is present

# Alternative Treatment for a KPC Producer

- Tigecycline (100.0% effective)
- Colistin (88.1% effective)
  - » SENTRY report. AAC. 2008. Feb;52(2):570-3
- No CLSI interpretive criteria for those drugs in Enterobacteriaceae
- A strategy for susceptibility testing is needed

# Oxacillin (OXA) Hydrolyzing $\beta$ -Lactamases

- Class D and functional group 2d
- Poorly inhibited by CA
- A large amount of variability in amino acid sequences
- Penicillinase capable of hydrolyzing oxacillin
- Extended-spectrum OXAs: carbapenem hydrolyzing ability

# OXA $\beta$ -Lactamases

- Most common in Enterobacteriaceae and Pseudomonas
- Carbapenem-hydrolyzing OXAs are most common in multidrug resistant *A. baumannii*.
- Main cause of wound infections
- Major problem for American soldiers returning from Iraq and Afghanistan

# OXA Carbapenemases

- More than 30 enzymes
- Identified at different geographical locations: Europe, Asia, South America
- OXA-40 was first OXA identified in USA in *A. baumannii*
- Mostly chromosomally encoded

# OXA Carbapenemases

- Hydrolysis spectrum: penicillins and early cephalosporins
- No aztreonam hydrolysis
- Variable hydrolysis of extended spectrum cephalosporins
- Confer only reduced susceptibility to the carbapenems

# Metallo- $\beta$ -Lactamases (MBL)

- First identified in Japan (*P. aeruginosa*), 1988
- Class B, functional group 3  $\beta$ -lactamases
- Requires  $Zn^{2+}$  for activity
- Inhibited by EDTA but not by CA
- Chromosomally or plasmid mediated
- Broad substrate spectrum including penicillins, cephalosporins, and carbapenemases

# MBLs

- Do not hydrolyze aztreonam
- Most common in *P. aeruginosa*, *A. baumannii*, and then Enterobacteriaceae
- The most common MBL families are:
  - The largest group: Imipenemases (IMP)
  - The second largest group: Verona imipenemases (VIM)
  - German imipenemases (GIM)
  - Seoul imipenemases (SIM)

# MBL Epidemiology

- Most common in Europe
  - Italy, Greece, France, Germany, Spain
- Also spread in other countries
  - Korea, Brazil, Argentina
- Spread to USA
  - First identified in *P. aeruginosa* strains in Texas, 2001

# MBL Detection

- Etest:

A reduction in the MIC of imipenem of  $\geq 3$  dilution in the presence of EDTA is interpreted as positive

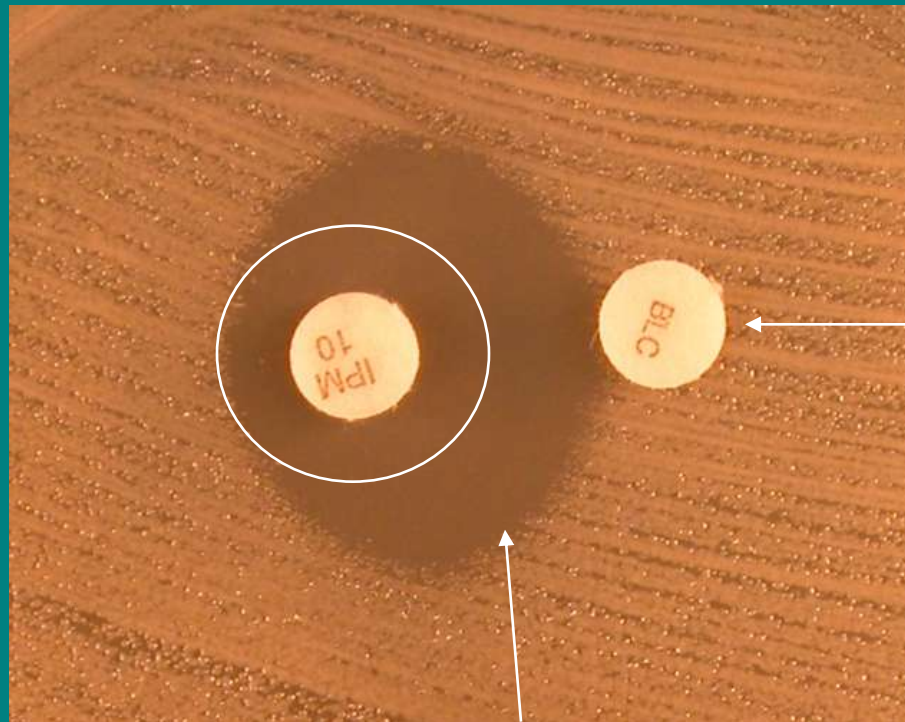


Imipenem + EDTA

Imipenem

# MBL Detection

## Disk Approximation Test



EDTA

7-mm increase of  
inhibition zone= MBL

# MBL Detection

- Different combinations of antibiotics and inhibitors to detect MBL producers with different sensitivity and specificity
  - Imipenem-EDTA: *P. aeruginosa* and *A. baumannii*
  - Ceftazidime-CA/EDTA: *K. pneumoniae*
  - Cefepime-CA/EDTA: *E. cloacae* and *C. freundii*

# MBL Detection

- PCR, cloning, and sequencing
  - Molecular gold standard method
  - Specific
  - Expensive
  - Labor intensive

# ESBLs

- Molecular class A, functional group 2be
- Inhibited by CA
- Hydrolyze penicillins, cephalosporins, and aztreonam
- Do not hydrolyze cephamycins (cefoxitin, cefotetan)
- Emerged in early eighties of last century
- Encoded on mobile DNA elements

# ESBL Types

- Class A ESBLs:
  - TEM
  - SHV
  - CTX-M

} Predominant in Enterobacteriaceae especially *K. pneumoniae* and *E. coli*
- Class D ESBLs:
  - OXA: predominant in *P. aeruginosa*,  
Currently, the most prevalent ESBL worldwide

# ESBL Prevalence

- From 1997-1999: the percentage of ESBL producers:
    - 4700 *K. pneumoniae* strains
      - Latin America: 45.4%
      - Western Pacific: 24.6%
      - Europe: 22.6%
      - USA: 7.6%
      - Canada: 4.9%
- » CID. 2001. supplement 2:S94-S103

# ESBL Prevalence

- 13000 *E. coli* strains
  - Latin America: 8.5%
  - Western Pacific: 7.9%
  - Europe: 5.3%
  - USA: 3.3%
  - Canada: 4.2%

» CID. 2001. supplement 2:S94-S103

# ESBL Detection

- Initial screening by disk diffusion or broth microdilution for the following antibiotics
  - Cefpodoxime, ceftriaxone, ceftazidime, cefotaxime, and aztreonam
  - CLSI standards for the concentrations of antibiotics
- The use of several antibiotics improves the test sensitivity

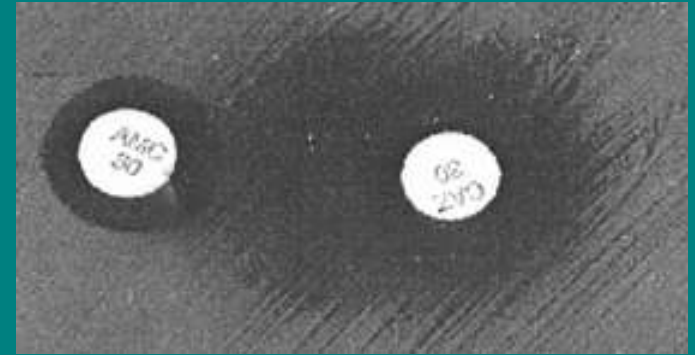
# ESBL Detection

- Initial screening
  - Growth at or above the screening MICs indicates ESBL production
  - Zones of inhibition smaller than that of the CLSI standard indicates ESBL production

# ESBL Confirmatory Tests

## Double-disk synergy (DDS) test

- CAZ and CAZ/CA disks
- CTX and CTX\CA disks
- Confirmatory testing requires using both CAZ and CTX alone and with CA
- 5 mm enhancement of the inhibition zone of antibiotic/CA combination vs antibiotic tested alone = ESBL

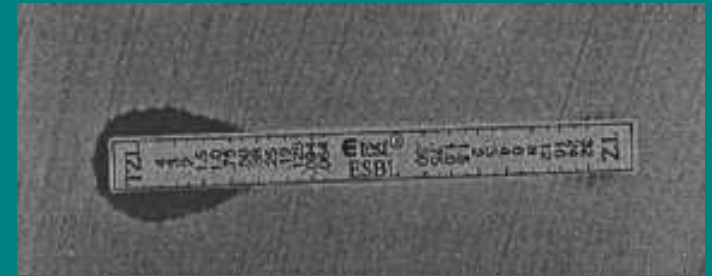


# ESBL Confirmatory Tests

- Broth microdilution
  - CAZ and CAZ/CA
  - CTX and CTX/CA
- A  $\geq 3$  twofold concentration decrease in an MIC for either antibiotic tested in combination with CA vs its MIC when tested alone = ESBL

# ESBL Detection by Etest

- CAZ and CAZ\CA Estrips
- CTX and CTX/CA Estrips
- A reduction in the MIC of antibiotic\CA of  $\geq 3$  dilutions vs antibiotic alone = ESBL



# Molecular Detection of ESBLs

- PCR and sequencing
  - The gold standard
  - Can detect all variants
  - Easy to perform
  - Labor intensive

# ESBL Detection: Automated Systems (AS)

- 144 putative of ESBL producers
- ESBL detection:
  - AS: Microscan, Vitek2, Phoenix
  - Phenotypic tests: Etest, DDS
  - Molecular tests: PCR, IsoElectric Focusing (IEF)
- Molecular identification: the reference method

» JCM. Apr. 2007, p.1167-1174

# ESBL Detection: Automated Systems

Detection Method	Sensitivity %	Specificity %	PPV %	NPV %
MicroScan	83.5	72.9	81.6	75.4
Phoenix	98.8	52.2	75	96.6
Vitek2	85.9	78	84.9	79.3
DDS	92.9	96.6	97.5	90.5
Etest	94.1	84.7	89.9	90.9

# Reporting of ESBL producers

- All confirmed ESBL-producing strains should be reported resistant to all penicillins, cephalosporins, and aztreonam

# AmpC $\beta$ -Lactamases

- Molecular class C, functional group 1
- Not inhibited by CA
- Confers resistance to penicillins, cephalosporins, monobactam, and cephamycin
- Chromosomally- or plasmid-mediated

# AmpC $\beta$ -Lactamases

- Many genera in Enterobacteriaceae encode chromosomal inducible AmpC
  - *Serratia marcescens*
  - *Enterobacter cloacae*
  - *Citrobacter freundii*
  - *Morganella morganii*
  - *Hafnia alvei*
  - *Yersenia enterocolitica*
- *Pseudomonas aeruginosa*

# AmpC $\beta$ -Lactamases

- Expression of the chromosomal *ampC* is generally low
- Inducible in response to certain  $\beta$ -lactams
- Factors involved in *ampC* induction:
  - $\beta$ -lactam interaction with PBPs
  - Byproducts of cell wall synthesis
  - Gene products
    - AmpR
    - AmpD
    - AmpG

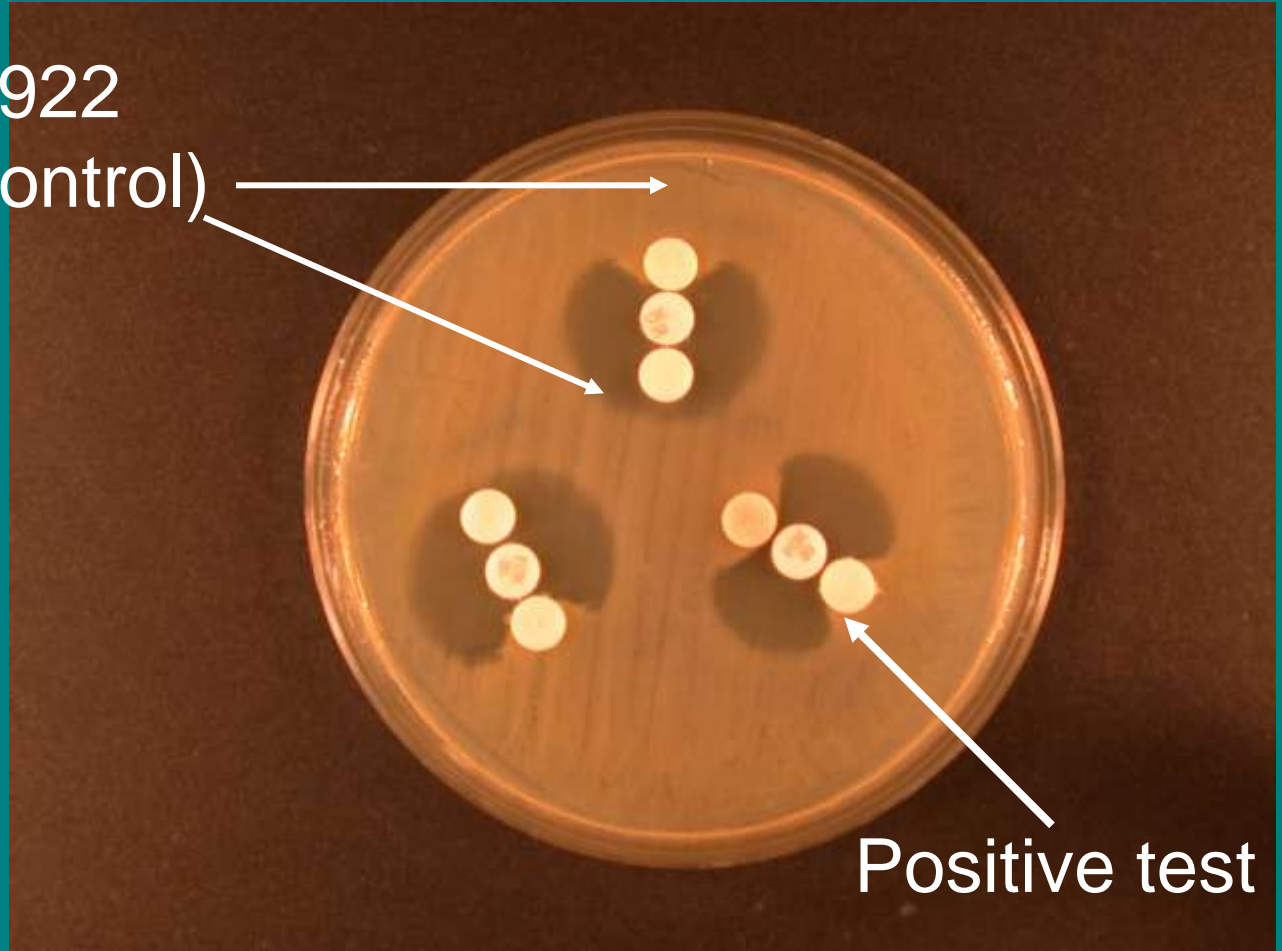
# AmpC $\beta$ -Lactamases

- Mutations in AmpD result in derepressed mutants and confer resistance to  $\beta$ -lactams
- 1980s, detection of plasmid-mediated AmpC (PmAmpC)(mostly noninducible)
- Mostly *K. pneumoniae*, *salmonella* spp, and *E. coli*

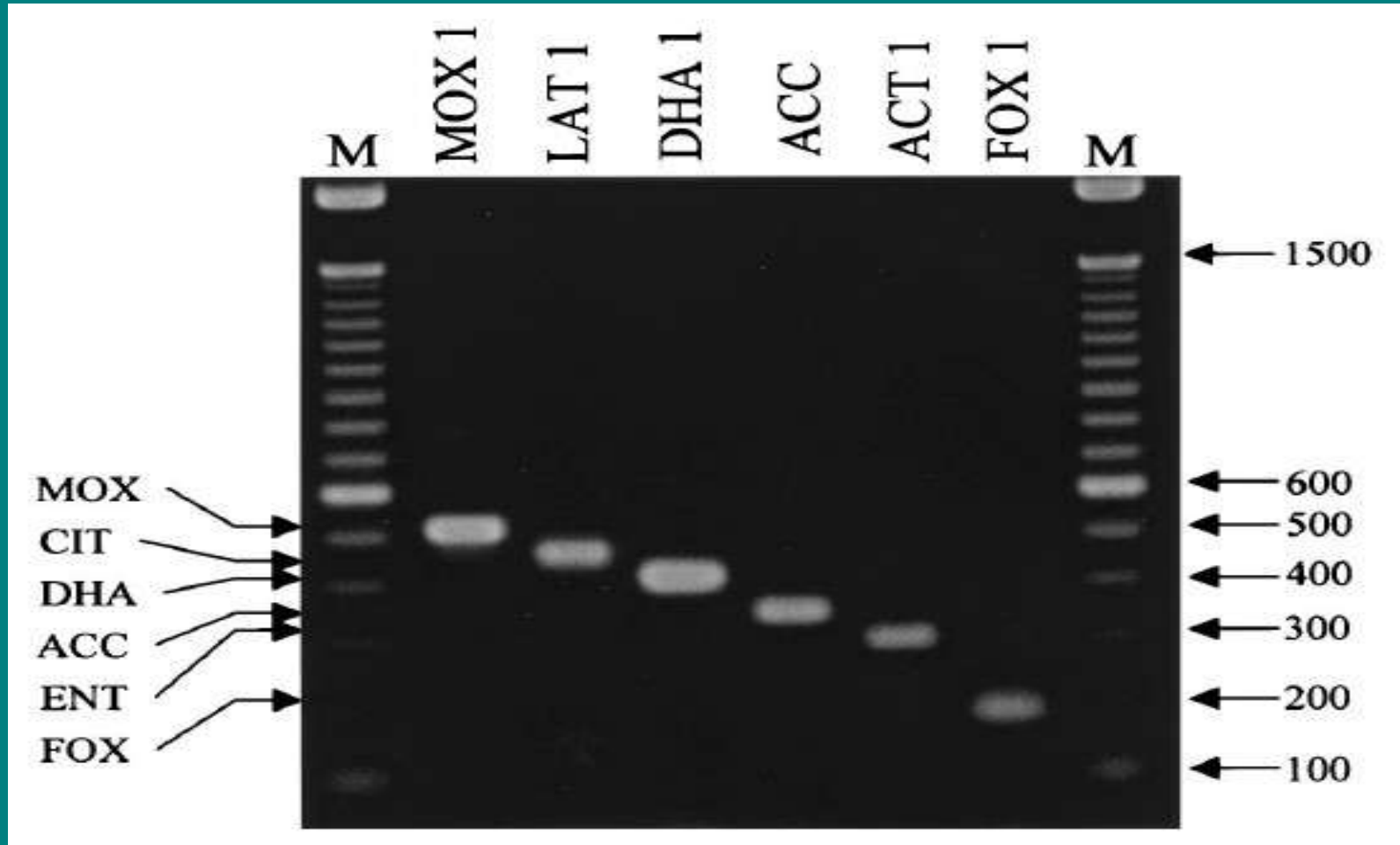
# PmAmpC Detection

- AmpC detection test

*E. coli* ATCC 25922  
(Lawn & Neg. Control)



# Detection of Plasmid-Mediated AmpC



# Issues with $\beta$ -Lactamases

- Reporting and ESBL-producing organisms other than *Klebsiella* and *E. coli*
- For the same third generation cephalosporin MICs
  - *E. coli* and *Klebsiella* will be considered ESBL producers and reported resistant to penicillins, cephalosporins, and aztreonam.
  - Other organisms would be reported as susceptible.

# Issues with $\beta$ -Lactamases

- ESBL detection in Enterobacteriaceae organisms other than *E. coli*, *K. pneumoniae*, and *K. oxytoca*
  - DDS: promising, BUT
  - AmpC: not inhibited by CA
  - Chromosomal inducible AmpC: can be induced by CA
  - High level expression of AmpC may render ESBL undetected

# Issues with $\beta$ -Lactamases

Isolate	Test agent	MIC $\mu\text{g/ml}$
SHV-2-producing <i>E. cloacae</i>	CAZ	2
	CAZ/CA	16

- CA induced chromosomal AmpC of *E. cloacae*
- Tazobactam and sulbactam are preferable inhibitors with these organisms (do not induce AmpC as much as CA does)

# Issues with $\beta$ -Lactamases

- Cefepime: minimally affected by AmpC
- Cefepime can be used as a screening agent for ESBL detection

# Summary

- Antibiotic resistance in GNRs is a serious issue
- MIC panels may need to be modified to reflect the new emerging resistance
- CLSI guidelines for ESBL-producers other than *E. coli* and *Klebsiella* are necessary
- CLSI guidelines for AmpC and carbapenemase producers are needed