Extended-Spectrum Beta-Lactamases (ESBLs) and the detection methods

by

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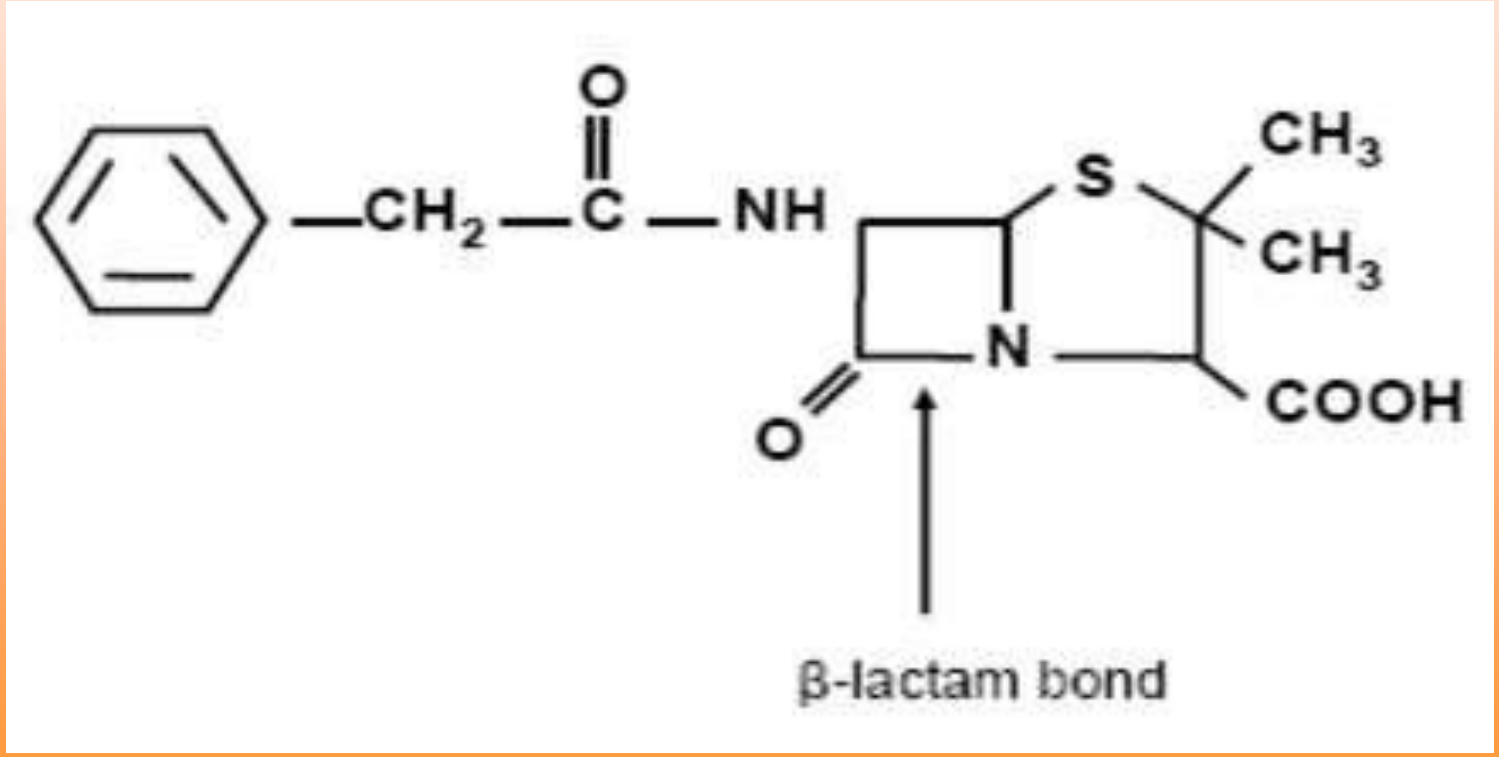
Why ESBL detection is important?

- **ESBL-producing Enterobacteriaceae have been responsible for numerous outbreaks of infection throughout the world.**
- **ESBL pose challenging infection control issues.**
- **ESBLs are clinically significant and indicate the appropriate antibacterial agents.**
- **Improve clinical outcome**
- **Inappropriate treatment leads to poor outcome**
- **Each 1 hour delay increases mortality by 7.6%.**
- **Identify emerging resistance problems**

- **β-lactam antibiotics**
- **– Penicillin**
- **– Cephalosporin**
- **– Monobactam**
- **– Carbapenem**



Penicillins

- **ß Lactamase-sensitive Penicillins**
- • Penicillin G/V
- • Ampicillin
- • Amoxicillin
- **Anti-staphylococcal Penicillins**
- • Oxacillin
- • Nafcillin
- • Dicloxacillin
- **Anti-pseudomonal Penicillins**
- • Ticarcillin
- • Piperacillin

Cephalosporins

- First generation (Moderate spectrum)
- Cefazolin · Cephalexin

- Second generation (Moderate spectrum)
- Cefaclor · Cefuroxime

- Third generation (Broad spectrum)
- Cefixime · Cefotaxime · Ceftriaxone Ceftazidime
- Fourth generation (Broad spectrum)
- Cefipime

Carbapenem
(Imipenem & Meropenem)

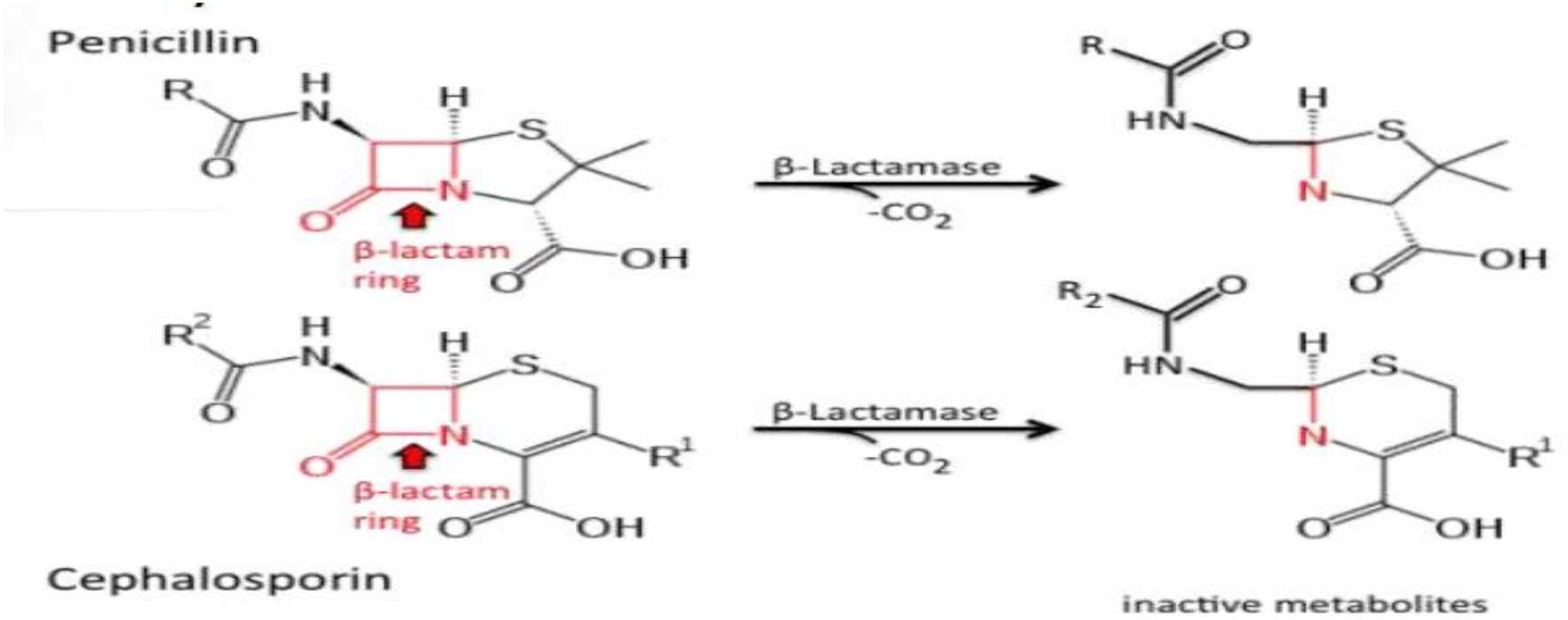
❖Mechanism of action:

- Same as penicillin and cephalosporin.
- Broad spectrum, resistant to B lactamase.
- Imipenem is quickly degraded by renal dehydropeptidase . So it's always administered with cilastatin (inhibitor of renal dehydropeptidase) to prevent the degradation of imipenem.

Monobactam (Aztreonam)

❖Mechanism of action:

- Prevents peptidoglycan cross linking like penicillins & cephalosporins.
- Resistant to B-lactamase.
- Synergistic with aminoglycosides.
- NO cross-allergenicity with penicillins.

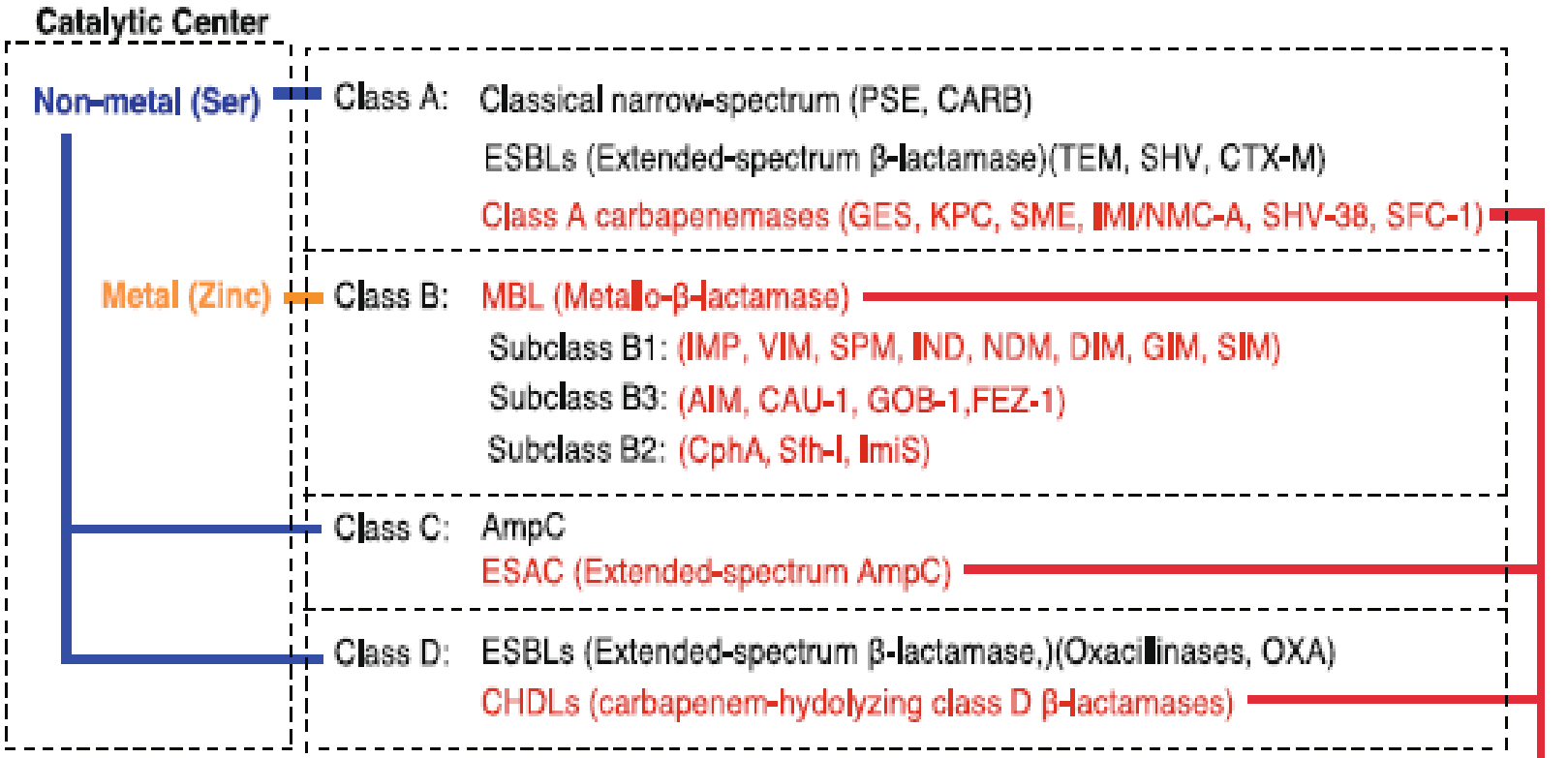


- **β lactamases**
- **Beta lactamases are enzymes produced by some bacteria that hydrolyze beta lactam antibiotics.**
- **Penicillinases, Cephalosporinases**
- **Extended spectrum β-lactamases (ESBL)**
- **Metallo β lactamases**
- **Amp C**
- **Carbapenemase**

TYPES OF B-LACTAMASES

- **β-lactamases**
  - Penicillinase: gene *blaZ* , inducible, on transposon (can move between chromosome and plasmid).
- **Broad spectrum β-lactamases**
  - (plasmid encoded)
  - TEM
  - SHV
  - OXA (mainly in pseudomonas)
- **ESBLs**
  - TEM related
  - SHV related
  - OXA related
  - CTX-M
  - Other
- ***ampC* β-lactamases**
  - Resistant to β-lactamase inhibitors
  - chromosomal
- **Carbapenemases**
  - Metallo- β-lactamases
  - Serine carbapenemases

Ambler molecular classification



Bush-Jacoby-Medeiros functional classification

Group 1: cephalosporinases (Ambler Class C)

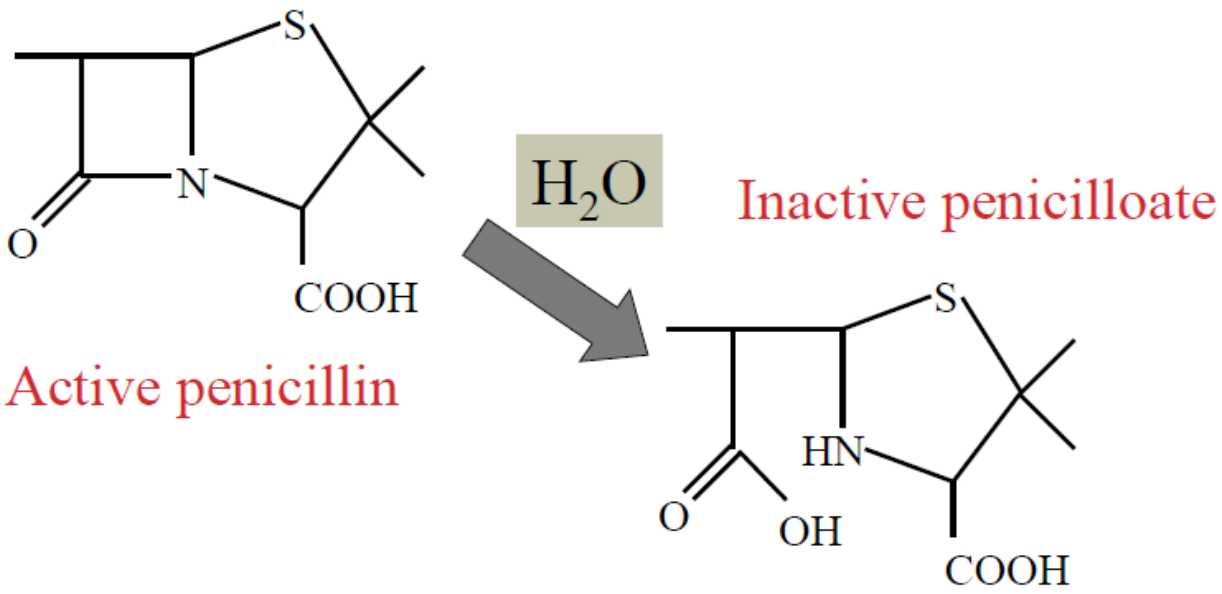
Group 2: serine-β-lactamase (Ambler Class A and D)

Group 3: metallo-β-lactamase (Ambler Class B)

- **Definition**
- **ESBLs are enzymes**
- – hydrolyzing most penicillins and cephalosporins,
- and monobactam (aztreonam).
- – but not cephamycins and carbapenems
- – Susciptable to β-lactamase inhibitors
- (clavulanate, sulbactam and tazobactam)

Genes

bla-SHV
*bla*TEM
*bla*CTX-M
*bla*OXA
*bla*PER
*bla*VEB



ESBL

- Confer resistance to 1st , 2nd, 3rd cephalosporins.
  - Most are susceptible to β-lactamase inhibitors
  - Most are susceptible to 4th cephalosporins
  - All are susceptible to carbapenems
- Diversity of ESBL
  - SHV (widespread)
  - TEM (>100 types)
  - OXA
    - Predominantly in Pseudomonas
    - less susceptible to β-lactamase inhibitors
  - CTX-M
    - Probably independent evolution
    - Highly resistant to 3rd generation cephalosporins
    - initially in South America, Far East & Eastern Europe
    - Probably most frequent worldwide
    - Clonal spread has been documented

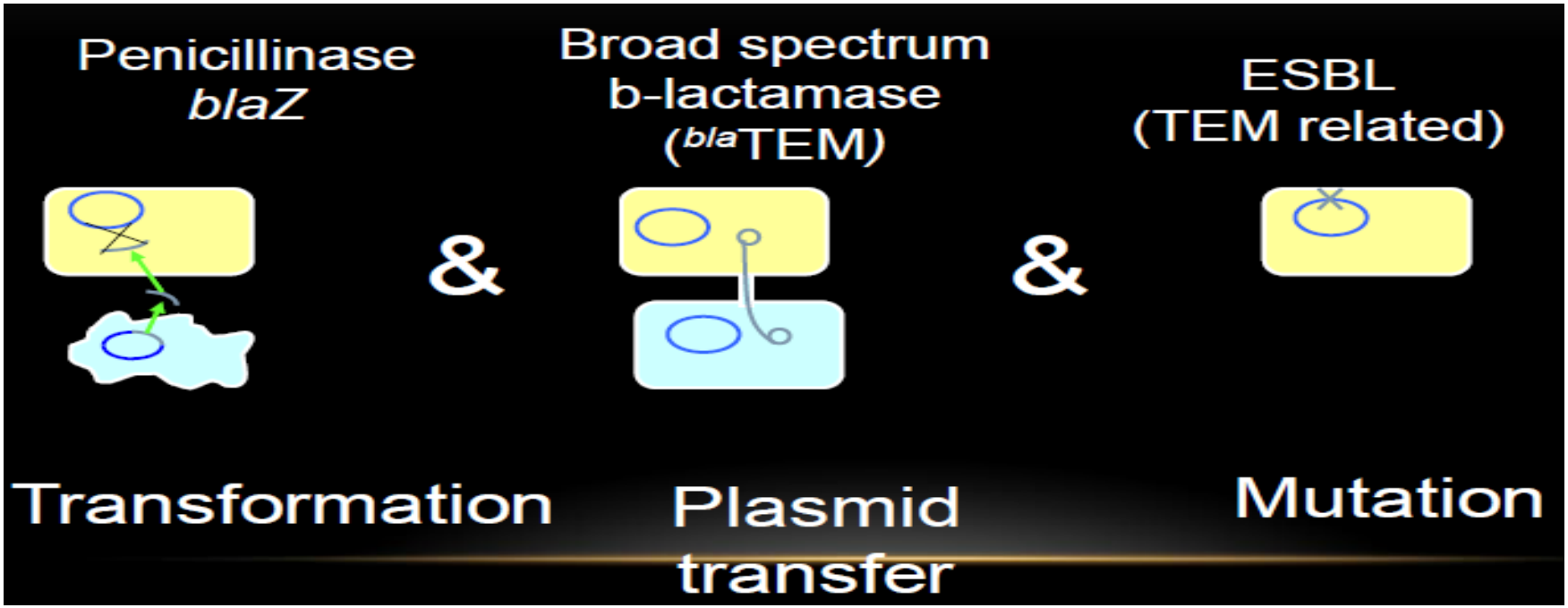
BETA-LACTAMASE INHIBITORS

- **Resemble β-lactam antibiotic structure Bind to β-lactamase and protect the antibiotic from destruction**
- **Most successful when they bind the β-lactamase irreversibly**
- **<u>Three important in medicine</u>**
- **Clavulanic acid**
- **Sulbactam**
- **Tazobactam**

Common ESBL producers

Type	Major sources
TEM, SHV	*E. coli, K. pneumoniae*
Cefotaxime hydrolyzing (CTX-M)	*S.* Typhimurium, *E. coli, K. pneumoniae*
Oxacillin hydrolyzing (OXA)	*P. aeruginosa*
PER-1	*P. aeruginosa, A. baumanii, S.* Typhimurium
PER-2	*S.* Typhimurium
VEB-1	*E. coli, P. aeruginosa*

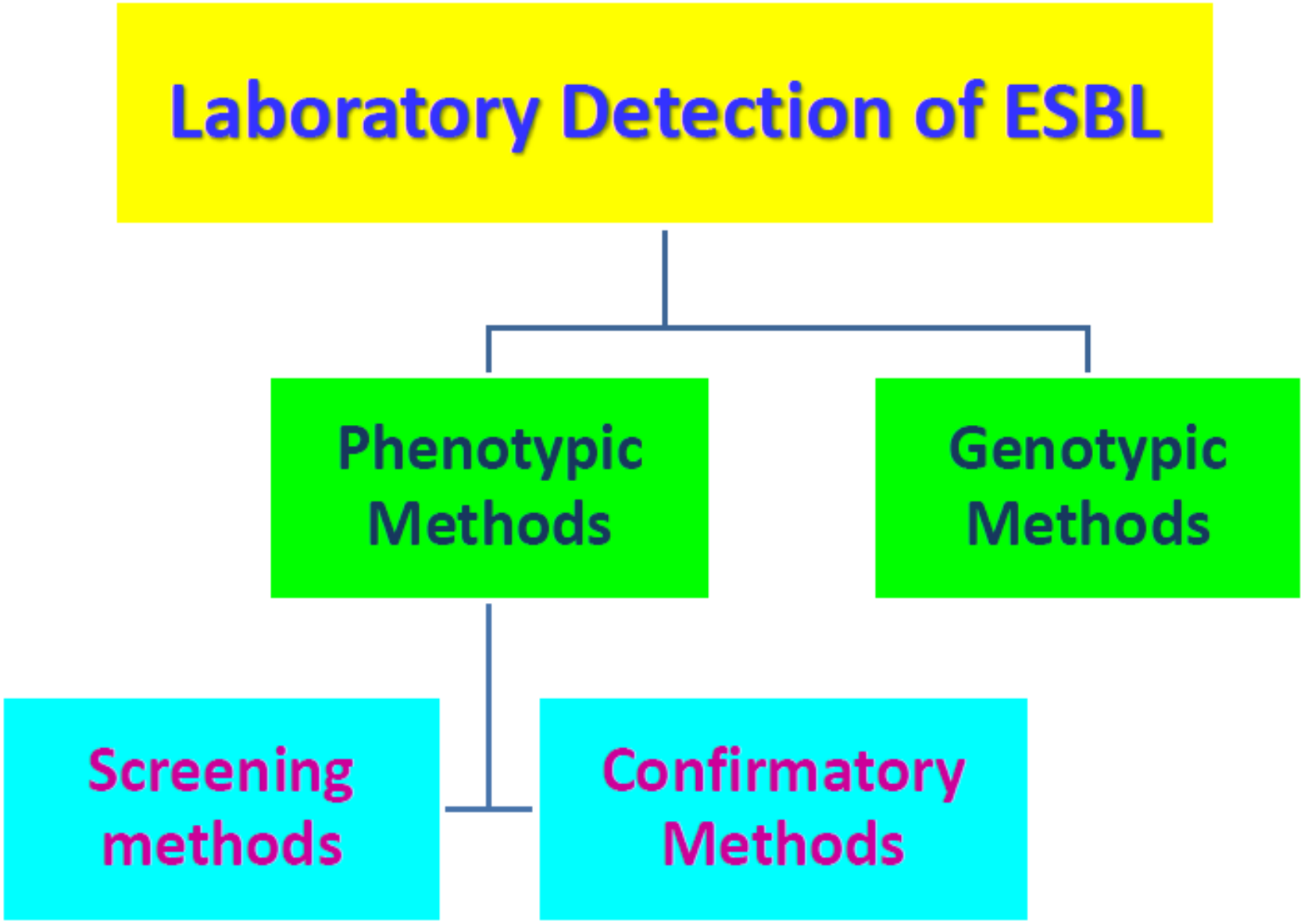
- **Mechanisms of resistance**
- The majority of ESBLs are acquired enzymes (Plasmids).
- **Different resistance phenotypes to:**
- Different expression levels
- Different biochemical characteristics such as
- Activity against specific β-lactams
- Co-presence of other resistance mechanisms (other β-lactamases, efflux, altered permeability).



CHOICE OF INDICATOR CEPHALOSPORIN

- **TEM & SHV – obvious resistance to ceftazidime, variable to cefotaxime**
- **CTX-M – obvious resistance to cefotaxime, variable to ceftazidime**
- **All ESBLs – obvious resistance to**
- **Cefpodoxime.**
- **Cefuroxime, cephalexin and cephradine are unreliable indicators**

- **Risk factors**
- Many ESBL producers are multi-resistant to non-β- lactam antibiotics such as quinolones, aminoglycosides and trimethoprim, narrowing treatment options.
- ESBLs destroy cephalosporins, main hospital antibiotics, given as first-line agents to many severely-ill patients, including community-acquired pneumonias and bacteraemias.
- Critically ill patients, Immunosuppression and ICU patients.
- Invasive procedures: intubation, mechanical ventillation, catheter
- Family member with multidrug-resistant pathogens
- High frequency of antibiotic resistance in the community or in the specific hospital unit.

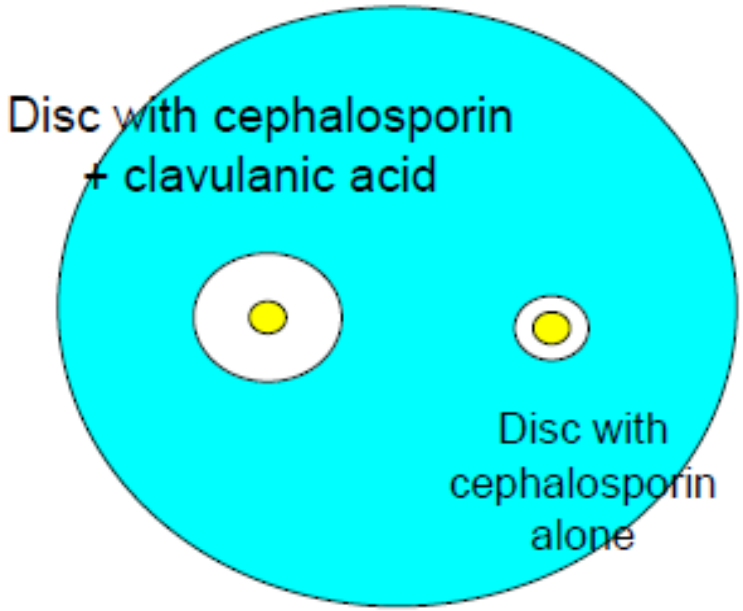

Laboratory Detection of ESBL
Phenotypic Methods
Genotypic Methods
Screening methods
Confirmatory Methods

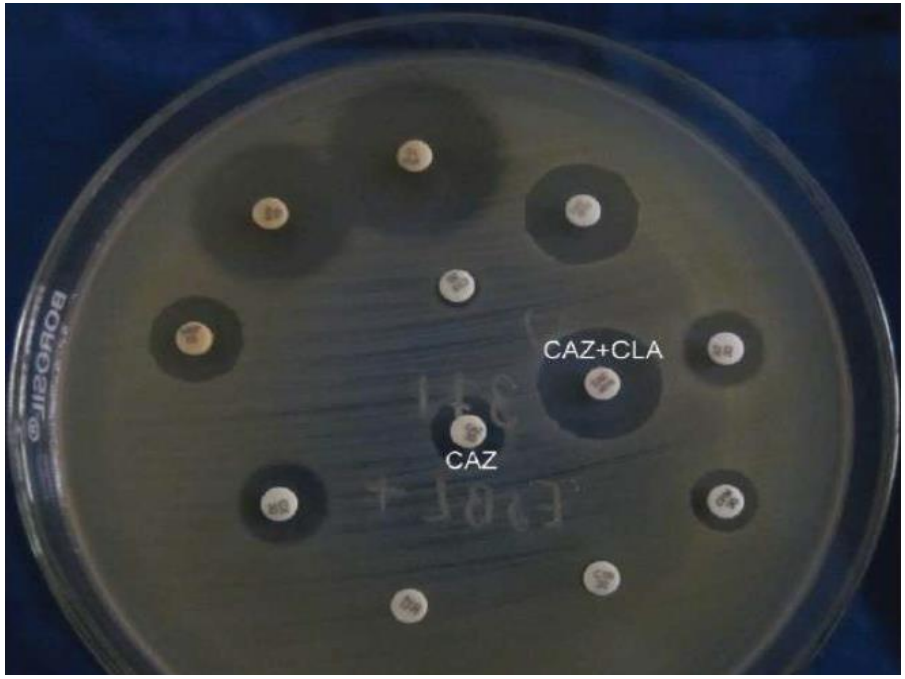
Screening methods

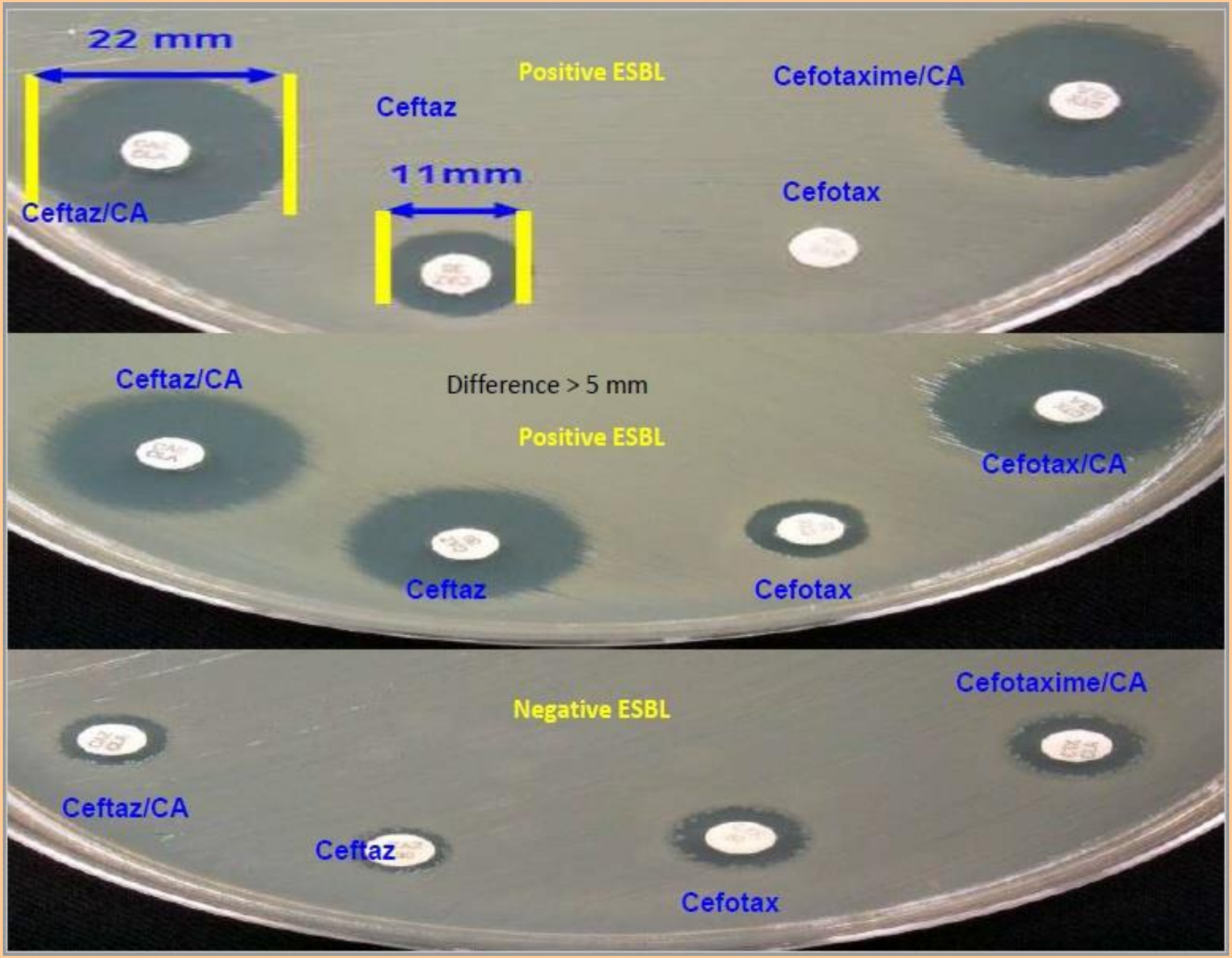
Screening for ESBL producers.

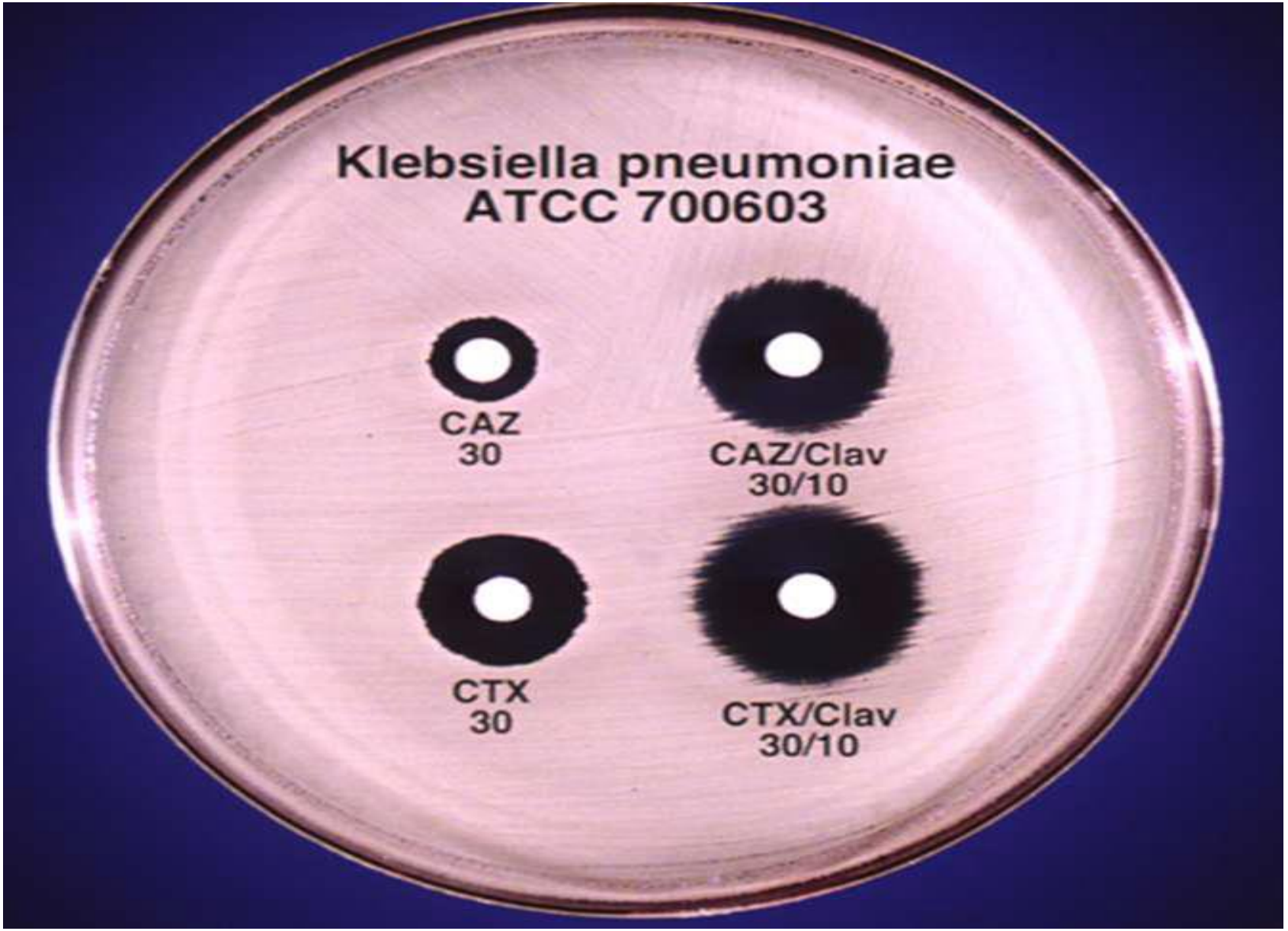
- (i) Disk diffusion methods.
- Isolates with a cefpodoxime (10-µg) zone diameter of ≤17 mm should undergo phenotypic confirmatory tests for ESBL production.
- (ii) Screening by dilution antimicrobial susceptibility tests.
- Useful screening test for cefpodoxime is to use a cefpodoxime MIC of ≥8 µg/ml, as a trigger to perform phenotypic confirmatory tests for ESBL production

- **Confirmatory methods**
- **1- Combination disk**
- – Uses 2 disks of 3rd cephalosporin alone and
- combined with clavulanic acid
- – An increase of ≥5 mm in zone inhibition with use
- of the combination disk.



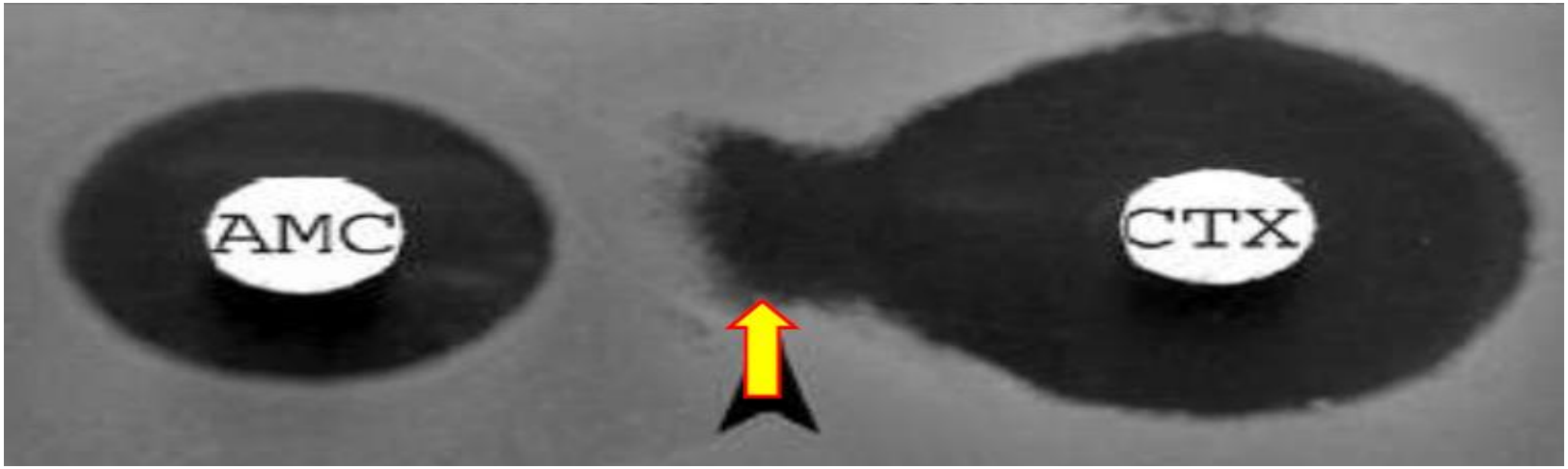


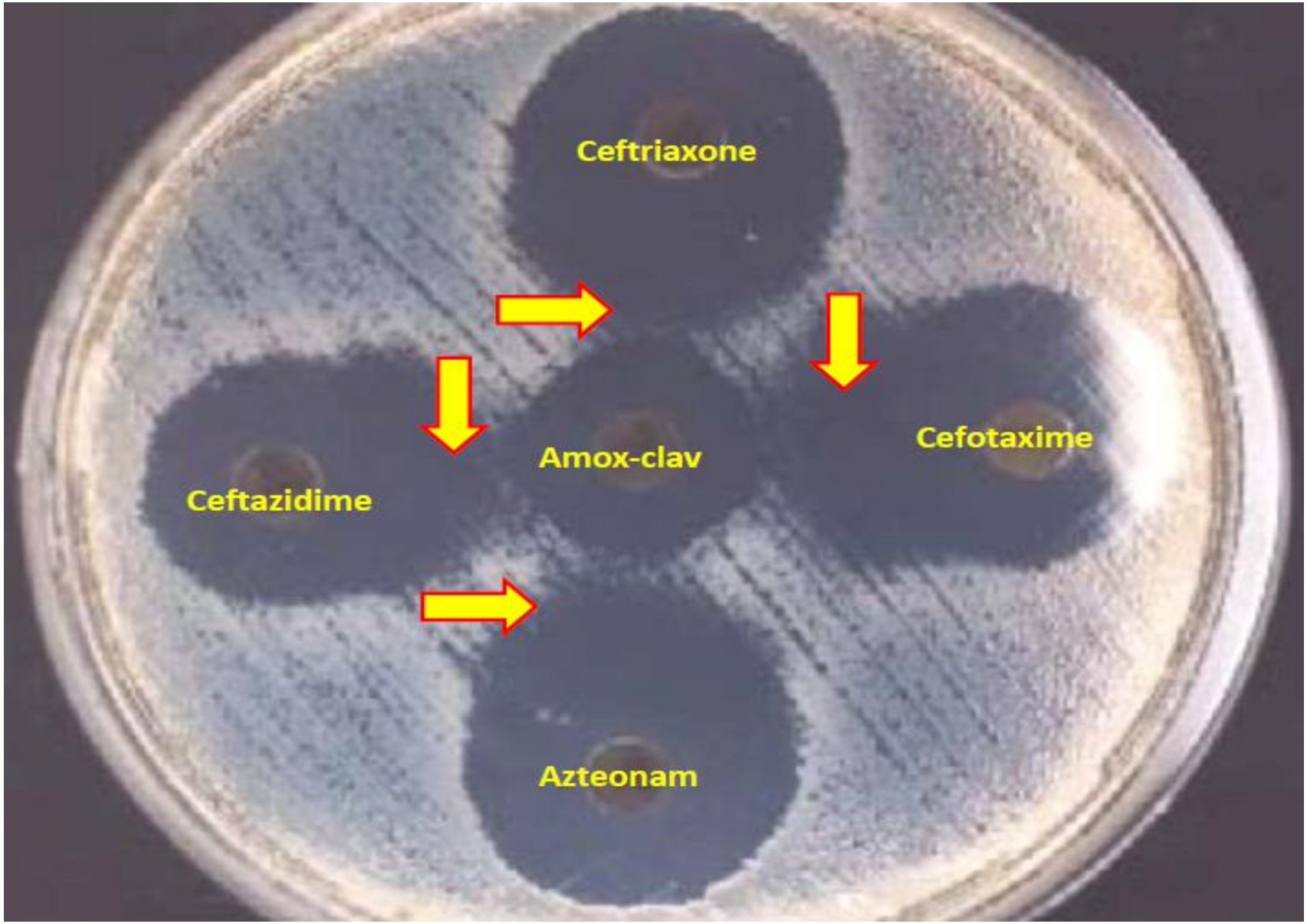

22 mm
Positive ESBL
Cefotaxime/CA
Ceftaz
11mm
Cefotax
Ceftaz/CA
Ceftaz/CA
Difference > 5 mm
Positive ESBL
Cefotax/CA
Ceftaz
Cefotax
Negative ESBL
Cefotaxime/CA
Ceftaz/CA
Ceftaz
Cefotax

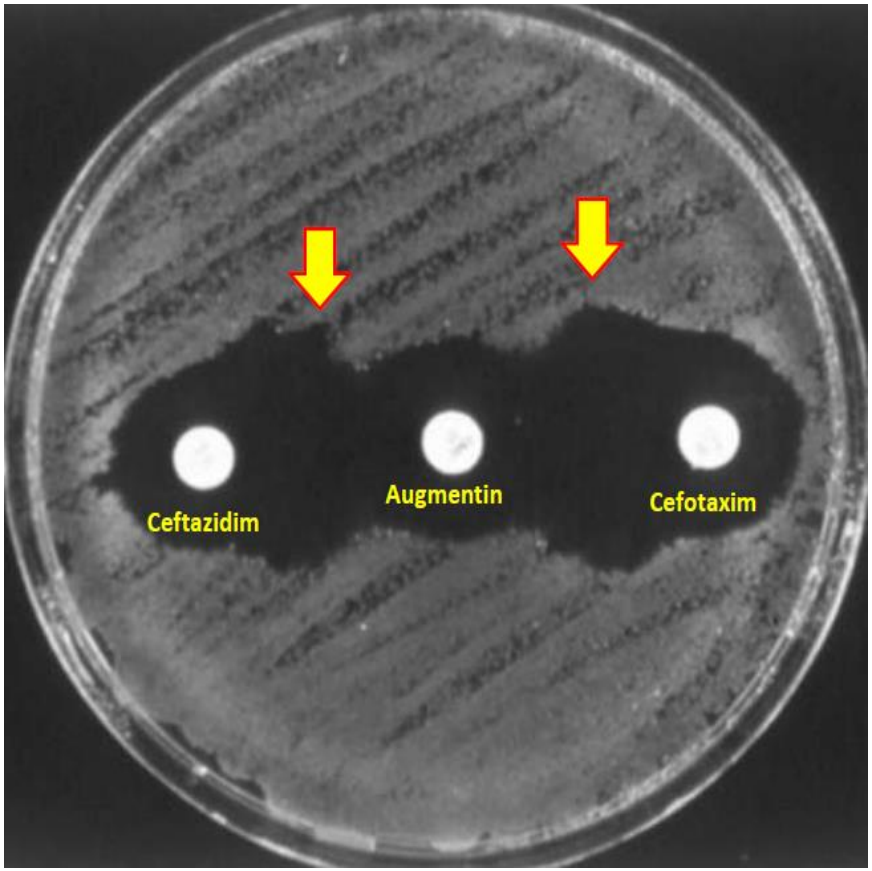

Klebsiella pneumoniae
ATCC 700603
CAZ
30
CAZ/Clav
30/10
CTX
30
CTX/Clav
30/10

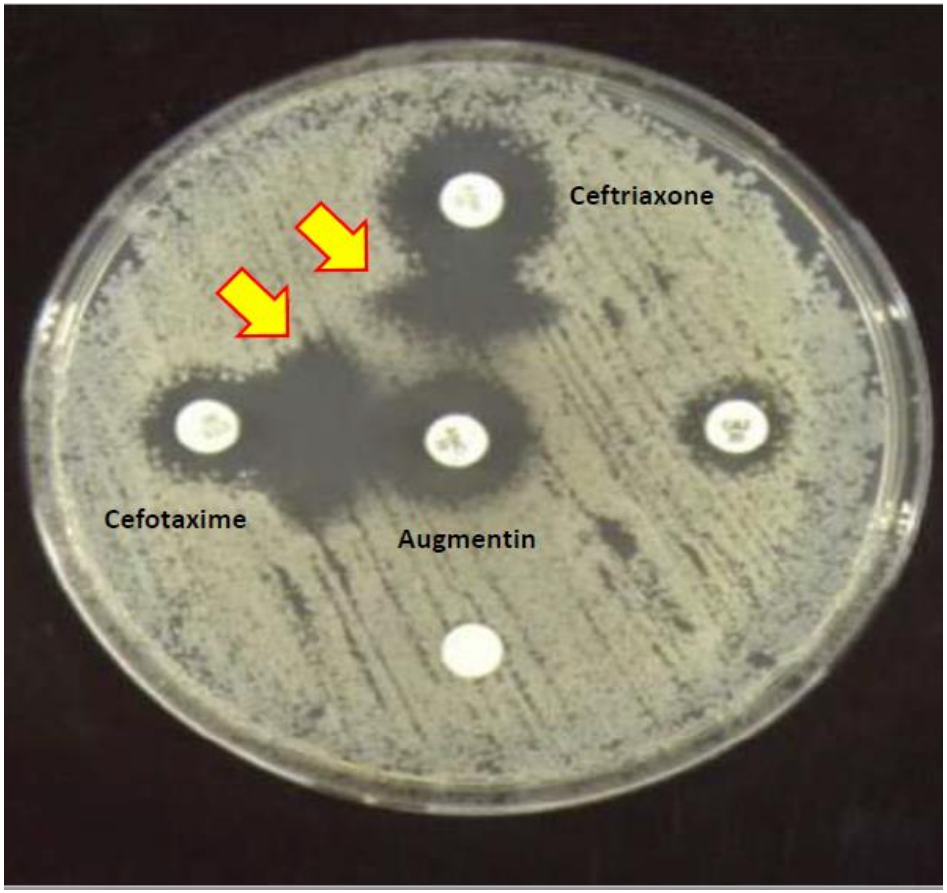
Phenotypic conformation

- **2- Double disk approximation or double disk synergy**
- – Disk of 3rd cephalosporin placed 30 mm from amoxicillin clavulanic acid
- – Result: Enhanced inhibition (A keyhole or ghost zone).

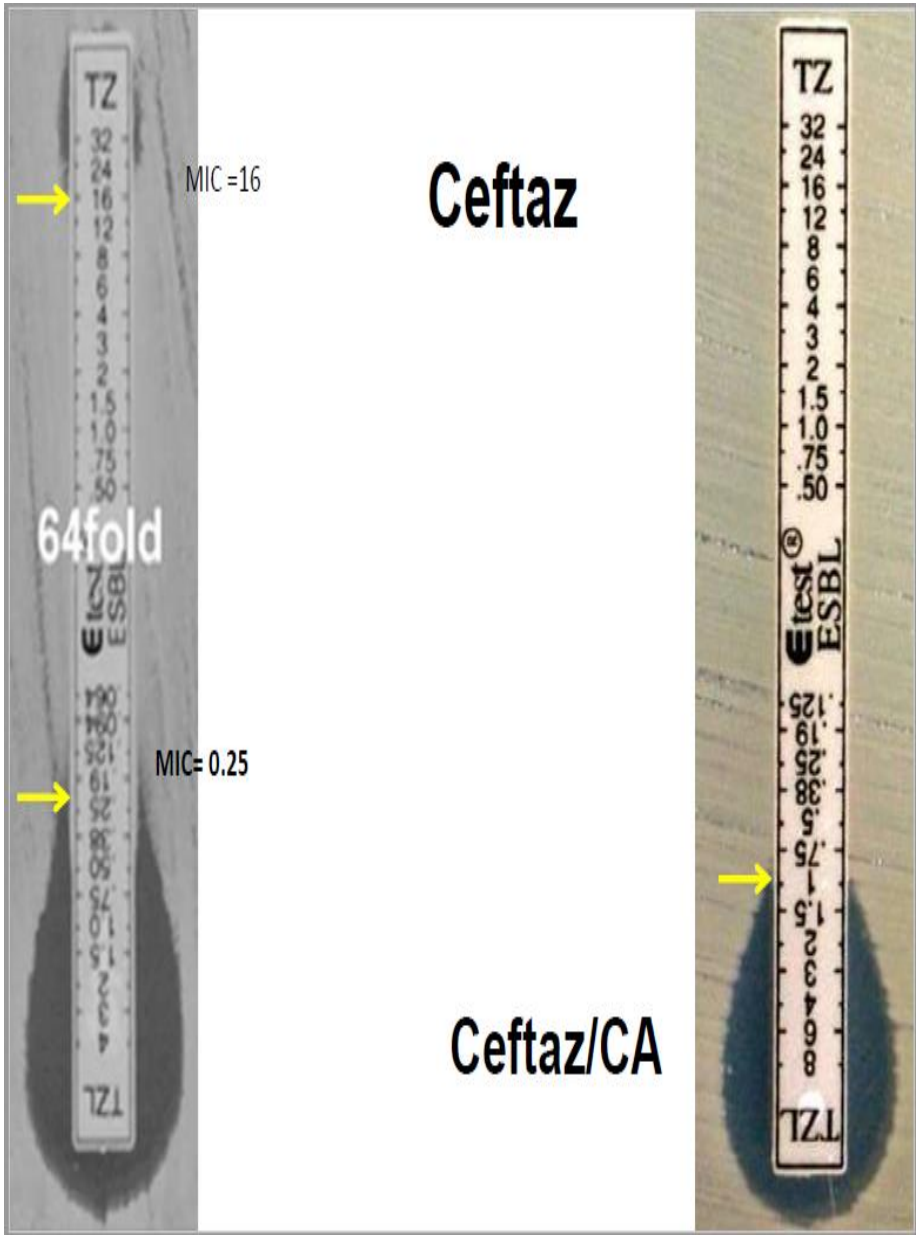


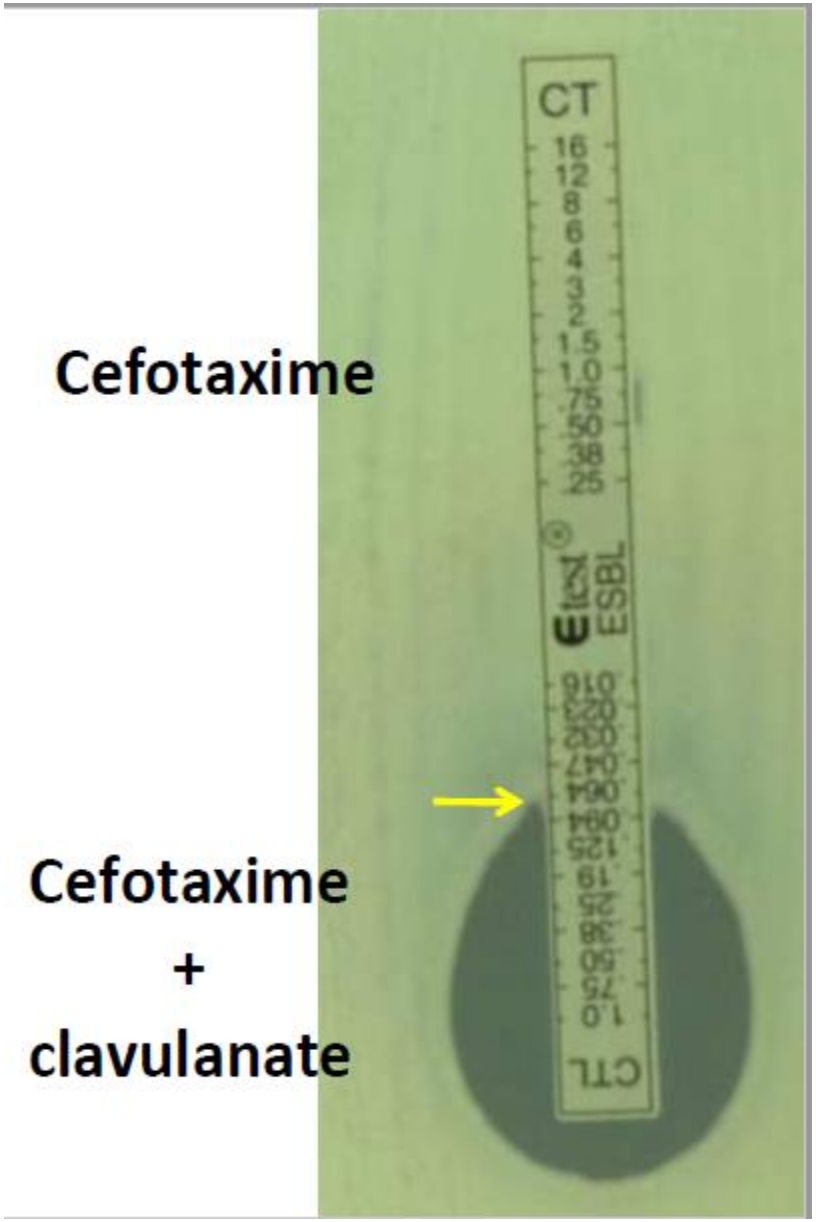

Ceftriaxone
Ceftazidime
Amox-clav
Cefotaxime
Azteonam


Ceftazidim
Augmentin
Cefotaxim

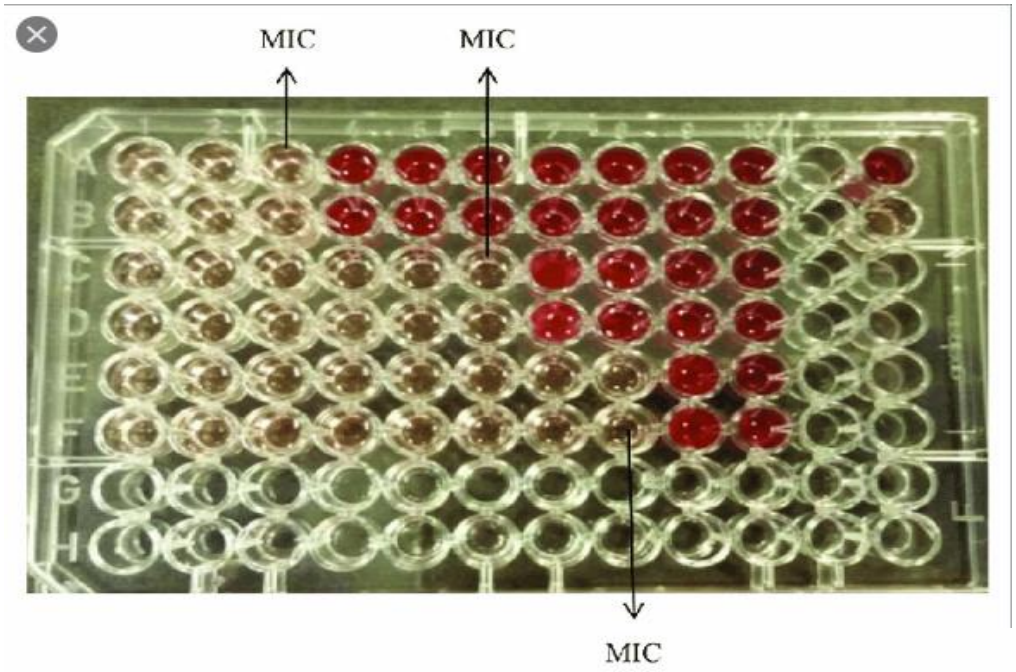

Ceftriaxone
Cefotaxime
Augmentin

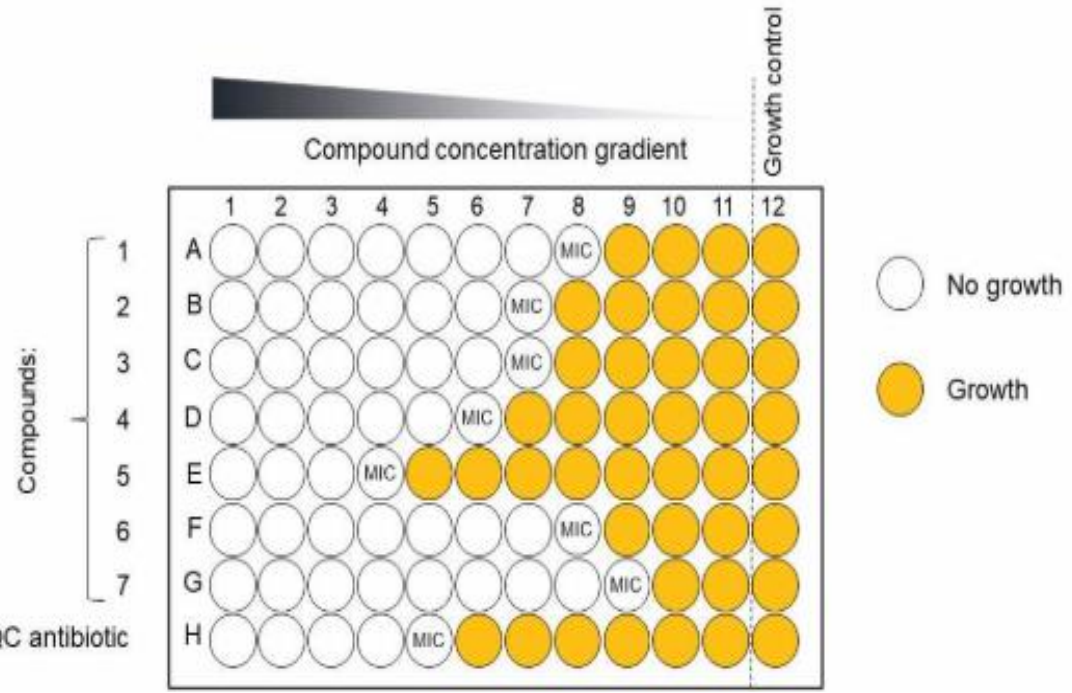
- **Phenotypic conformation**
- **3- E-test (MIC ESBL strips)**
- Two-sided strip containing cephalosporin on one side and cephalosporin -clavulanic acid on the other
- MIC ratio ≥8
- >8 fold reduction in MIC in presence of CA= ESBL
- or Phantom zone (deformed ellipse)


TZ
MIC =16
Ceftaz
64fold
MIC= 0.25
Ceftaz/CA
TZL


Cefotaxime
Cefotaxime
+
clavulanate
CT
CTL

- **Phenotypic conformation**
- **4- Broth Microdilution**
- MIC of 3rd cephalosporin alone and combined with clavulanic acid
- >3-two fold serial dilution decrease in MIC of either cephalosporin in the presence of clavulanic acid compared to its MIC when tested alone.
- **Ceftazidim MIC =8 µg/mL**
- **Ceftazidime + Clavulanate= 1 µg/mL**
- **Or MIC ratio≥8**
- **5- MIC broth dilution**
- **MIC of 3rd cephalosporin** alone and combined with clavulanic acid
- A decrease in the MIC of the combination of > 3-two fold dilutions

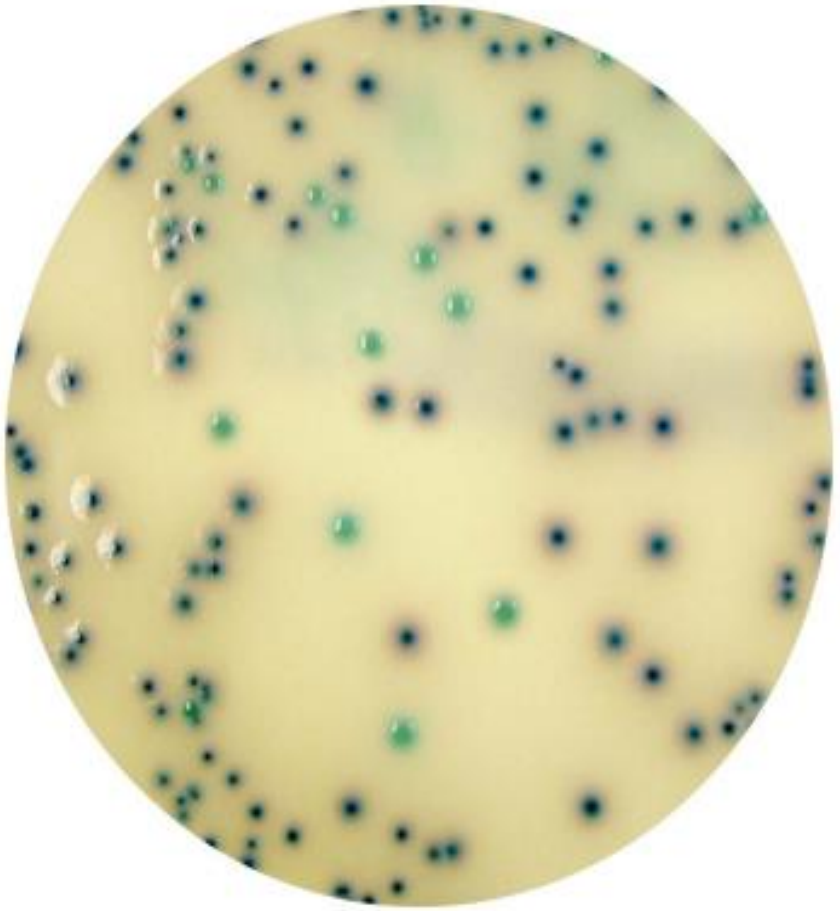

MIC
MIC
MIC


Compound concentration gradient
Growth control
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
MIC
Compounds:
1
2
3
4
5
6
7
QC antibiotic
No growth
Growth

Other confirmatory methods

Brilliance ESBL agar

- identification of ESBL-producing E. coli, Klebsiella, Enterobacter, Serratia and Citrobacter group, directly from clinical samples.
- two chromogens that specifically target enzymes→ green and blue colonies
- Negative →pink.
- Proteus, Morganella and Providencia → tan-coloured colonies with a brown halo



Selective agar with multiple antibiotics including cefpodoxime • Rapid detection of common ESBL+ enterobacteria within 18-24h of specimen.

- **Other confirmatory methods**
- **6- Automated instruments**
- Measure MICs and compare the growth of bacteria in presence of cephalosporin vs. cephalosporin - clavulanic acid

Vitek ESBL confirmatory test

Phoenix ESBL test (BD)

Microscan ESBL Panel

Evaluation of the automated detection

- The **BD Phoenix ESBL screening test**, included in both panels, utilizes the growth response to selected cephalosporins (cefotaxime, ceftazidime, cefpodoxime, and ceftriaxone), with or without clavulanic acid, to detect the production of ESBLs. The results are analyzed with the integrated BDXpert system.
- **For the evaluation of the Vitek 2 system**, the ESBL confirmatory test is incorporated on cards AST-N041 and AST-N062. This ESBL screening test utilizes the growth response to ceftazidime, cefepime, and cefotaxime in combination with or without clavulanic acid on both cards. All results were interpreted by using the Advanced Expert System (AES).

- **Genotypic confirmation**
- **Molecular detection (ESBL genes) in ESBL producing bacteria**
- – PCR
- – RFLP
- – Gene sequencing
- – DNA microarray-based method
- Targets specific nucleotide sequences to detect different variants of TEM and SHV genes.
- In the clinical diagnostic laboratory
- – Directly from specimens
- – Need to target key species
- – Format must be simple, rapid and cost-effective
- – Problems with genes in commensals.

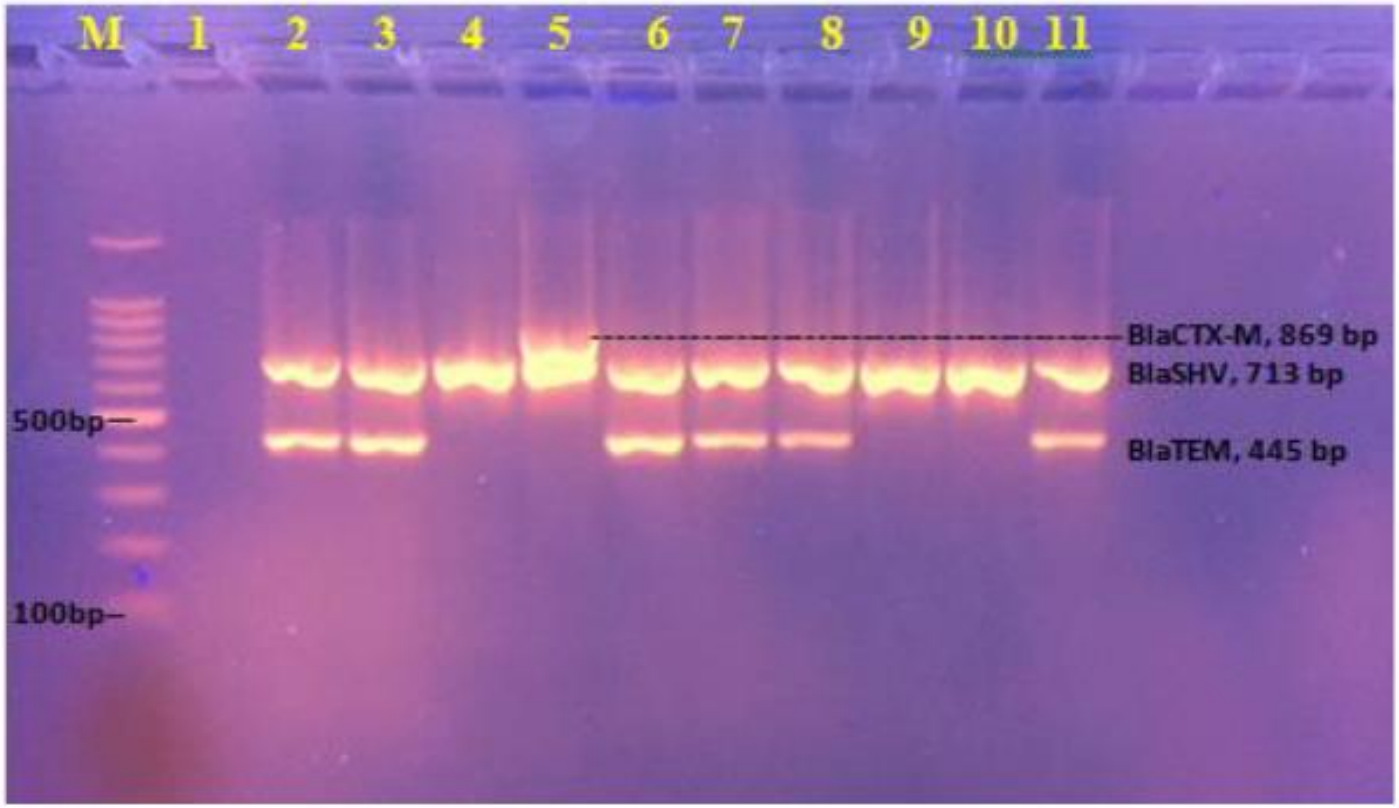
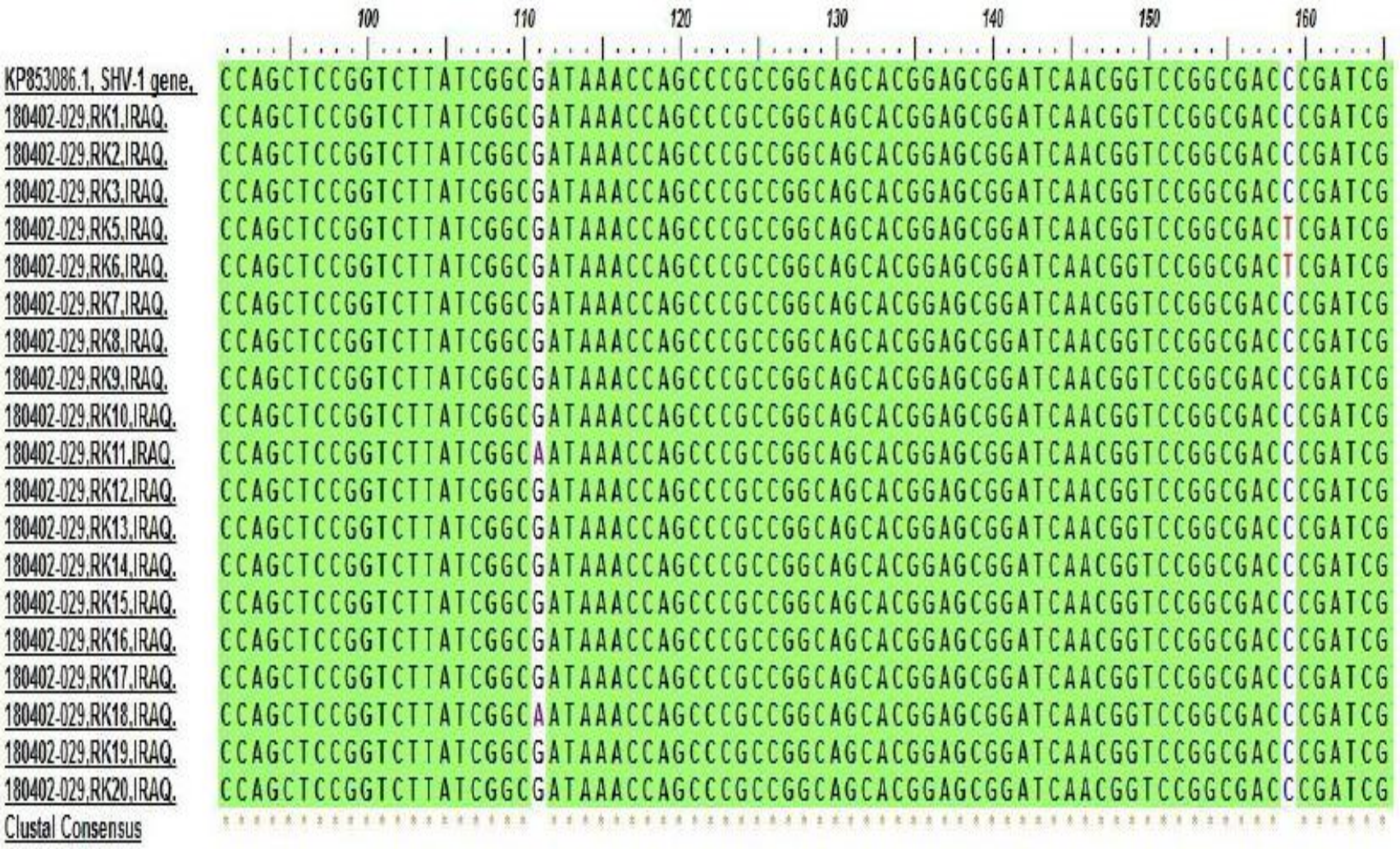
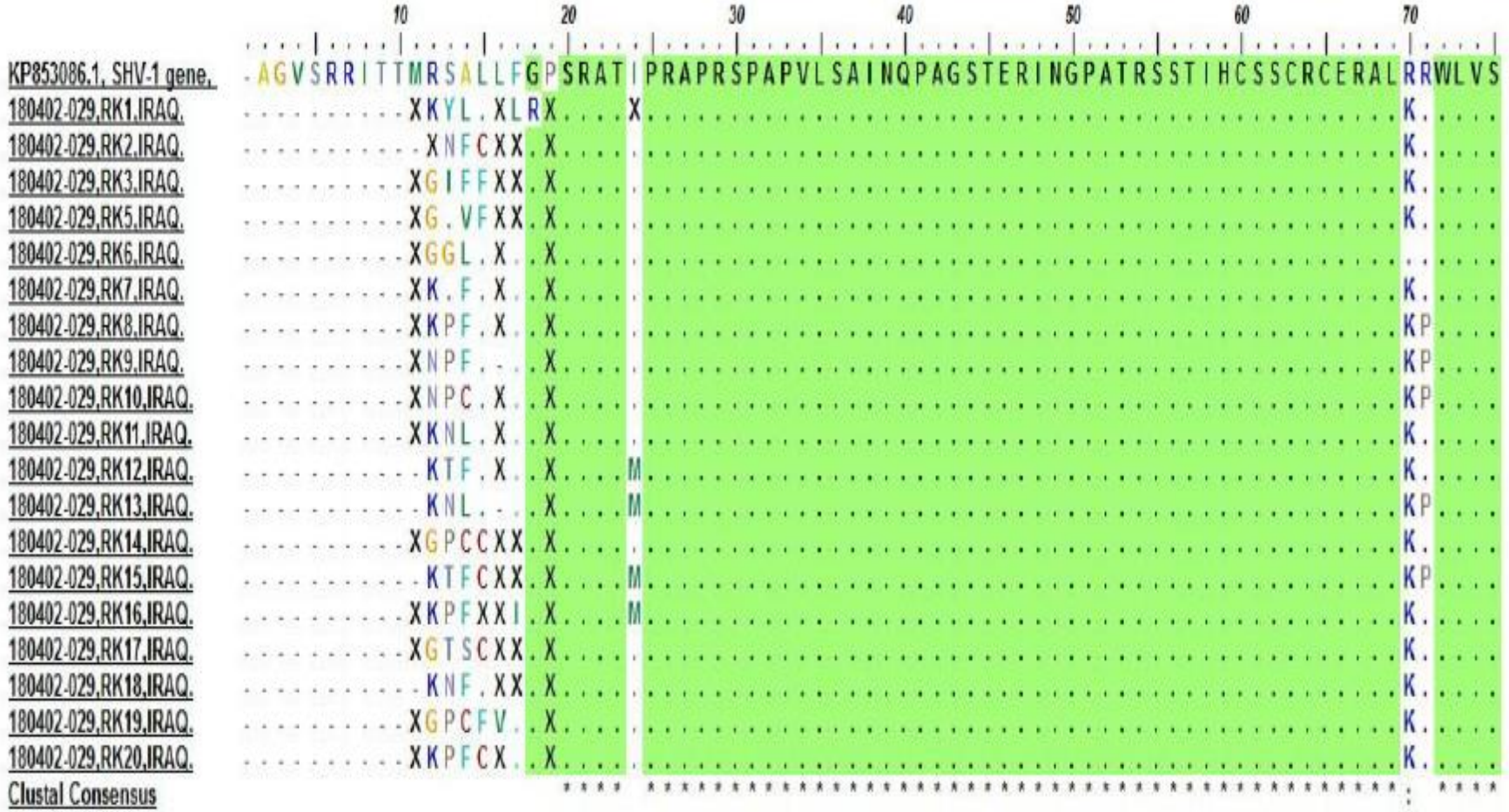


Figure (4-7): Agarose gel electrophoresis for detection the resistance genes *bla*SHV, *bla*TEM, and *bla*CTX-M- by multiplex PCR. Lane M: 100bp DNA ladder; lanes 2-13: *K. pneumoniae* 40-52 isolates; lane 1: Negative control. (70V for 2hr).

Alignment of *bla*SHV sequences of *Klebsiella pneumoniae* isolates

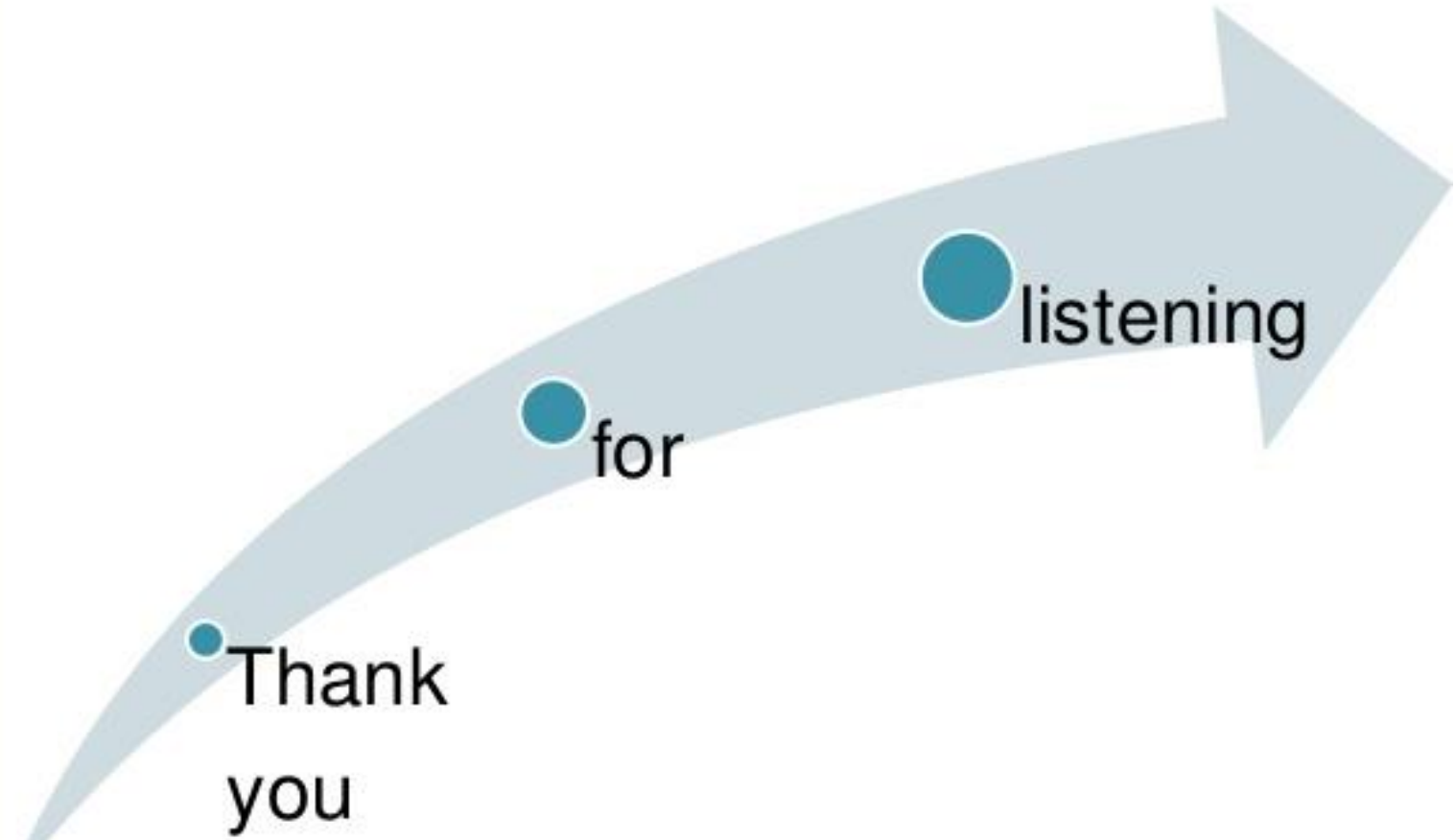


Appendix (6): Alteration of amino acids in *bla*SHV detected in *K. pneumoniae* local isolate by MEGA6 and Bio Edit softwares.



BACTERIA NOT TO TEST FOR ESBL'S

- *Acinetobacter*
  - Acinetobacter often S to clavulanate alone
- *S. maltophilia*
  - You get +ve results via inhibition of L-2 chromosomal β-lactamase, which is ubiquitous in the species


Thank
you
for
listening