EXTENDED-SPECTRUM β-LACTAMASES

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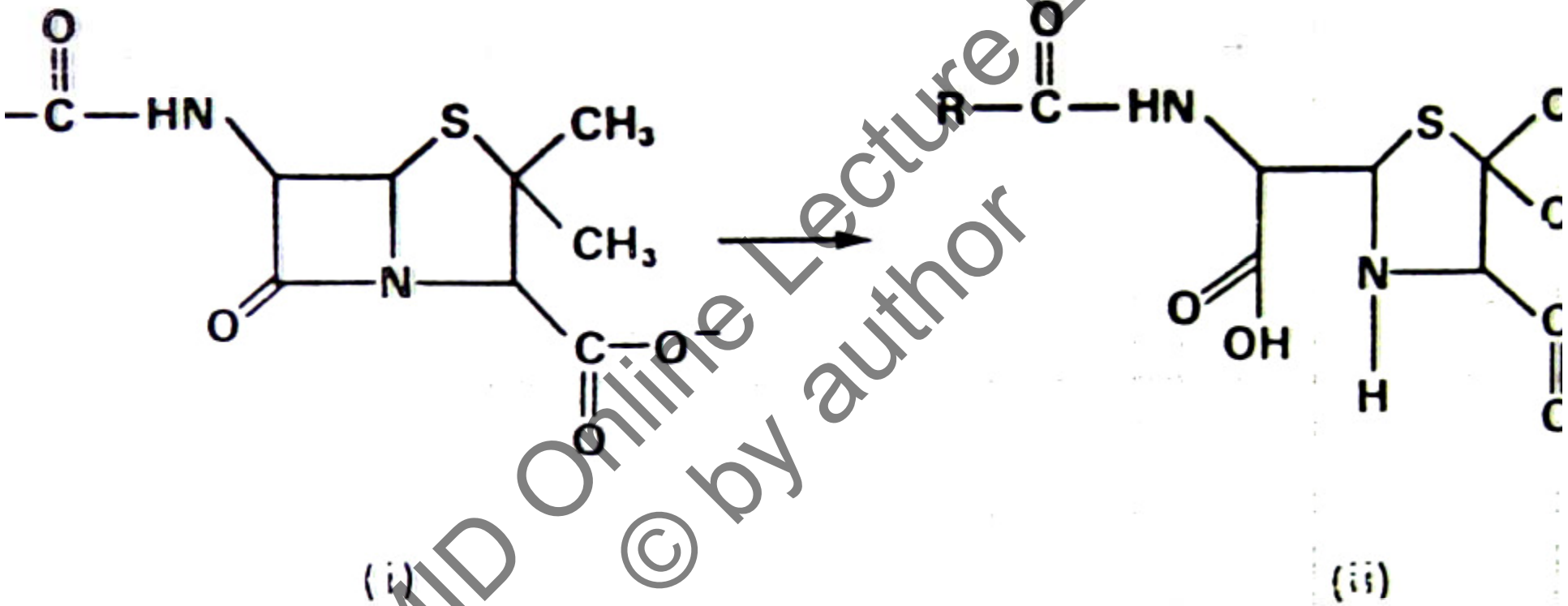
HYSTORY

- Emergence of resistance to β-lactam antibiotics began before the first β-lactam (penicillin) was developed.
- The first β-lactamase was identified in *Escherichia coli* prior to the release of penicillin for use in medical practice.
- *Staphylococcus aureus* developed rapid emergence of resistance due to a plasmid encoded penicillinase.

HYSTORY

- Most Gram-negative bacteria possess a naturally ocuring, chromosomally mediated β-lactamase.
- These enzymes have evolved from PBP molecules and show sequence homology.
- This development was due to the selective pressure exerted by soil organisms producing β-lactams.
- The first plasmid mediated β-lactamase was TEM-1 in *E. coli*.



neralized reaction catalysed by β-lactamases with penicillins as su
ıre; (ii) basic penicilloic acid structure.

ESBLs

- ESBLs are enzymes that hydrolyze oxyimino-cephalosporins and monobactams and render them inactive.
- Oxymino-cephalosporins were introduced in early 1980s to treat serious infections caused by gram-negative bacilli (*K. pneumoniae*, *E. coli*, *P. mirabilis* etc).
- These bacteria were in high percentage resistant to older generation cephalosporins due to production of plasmid mediated β-lactamases (TEM-1, TEM-2, SHV-1).

ESBLs

- 1982 –first description of extended-spectrum β-lactamase in *K. oxytoca* from Germany.
- They are derived from parenthal TEM-1, TEM-2 and SHV-1 β-lactamases by point mutations in bla_{TEM} or bla_{SHV-1} genes that expand their spectrum of activity.
- ESBLs are inhibited by suicide inhibitors such as clavulanic acid and sulbactam.
- Cephamycins are not hydrolyzed by ESBLs.

ESBLs

- ESBLs spread throught Europe first and then in USA and the rest of the world.
- At the beginning they were confined only to *K. pneumoniae* and *E. coli* but later they were reported from other enteric bacteria and even non-fermentative bacteria.
- Belong to Ambler's molecular class A β-lactamases and have serine in active site

TEM β-lactamases

- There are over 90 TEM type ESBLs .
- They are derived from TEM-1 or TEM-2 β-lactamases by point mutations in bla_{TEM} gene.
- 90% of ampicillin resistance in *E. coli* is due to TEM-1 β-lactamase.
- TEM-1 β-lactamase is also responsible for ampicillin and penicillin resistance in *N. gonorrhoeae* and *H. influenzae*.
- TEM-1 is able to hydrolyze ampicillin and older cephalosporins (cephalotin and cephaloridine)
- It is strongly inhibited by clavulanic acid and sulbactam.

B-lactamase	Species	County of origin	Year of report	pI	CTX	CAZ	AMT
TEM-3	*K. pneumoniae*	France	1984	6.3	32	64	16
TEM-4	*E. coli*	France	1986	5.9	32	32	16
TEM-5 (CAZ-1)	*K. pneumoniae*	France	1987	5.5	4	128	8
TEM-6	*E. coli*	Germany	1987	5.9	1	128	64
TEM-7	*C. freundii*	France	1988	5.4	0.5	64	2
TEM-9	*K. pneumoniae*	UK	1987	5.5	2	128	128
TEM-10	*K. pneumoniae*	USA	1989	5.5	1	64	32
TEM-11 (CAZ-10)	*K. pneumoniae*	Belgium	1989	5.7	0.06	4	0.25
TEM-12	*E. coli*	USA	1987	5.2	0.06	4	0.25
TEM-14	*K. pneumoniae*	USA	1990	6.3			

SHV β-LACTAMASE

- SHV-1 β-lactamase is most commonly found in *K. pneumoniae* and is integrated into bacterial chromosome.
- It is responsible for ampicillin resistance in this species.
- In other species (*E. coli*) SHV-1 is plasmid-mediated.
- Unlike TEM type β-lactamase there are only few derivatives (>25)
- SHV means sulphydryl variable.

					MIC (mg/L)		
B-lactamase	Species	County of origin	Year of report	pI	CTX	CAZ	AMT
SHV-1	E. coli	Switzerland	1974	7.6	0.12	1	0.5
SHV-2	K. ozaenae	Germany	1983	7.6	64	32	32
SHV-3	K. pneumoniae	France	1986	7.0	64	32	32
SHV-4 (CAZ-5)	K. pneumoniae	France	1987	7.7	128	128	256
SHV-5	K. pneumoniae	France	1987	8.2	64	128	256

SHV β-lactamases

	8	20	35	43	54	116	130	140	179	192	193	205	238	240	pI
SHV-1	I	L	L	R	G	G	S	A	D	K	L	R	G	E	7.6
SHV-2								T					S		7.6
SHV-2a			Q										S		7.6
SHV-3												L	S		7.0
SHV-4												L	S	K	7.8
SHV-5													S	K	8.2
SHV-7	F			S									S	K	7.6
SHV-12			Q										S	K	8.2

CTX-M β-LACTAMASES

- Novel class A ESBLs that do not belong to TEM or SHV family.
- They were inicially reported in the second half on 1980 ties but become widespread after 1995 (today over 40 enzymes).
- According to the substrate profile they are cefotaximases.
- The first one was FEC-1 reported in 1986. from Japan, isolated from the feacal flora of the laboratory dog used for pharmacokinetic studies.

B-lactamase	pI	Country	Species	Year
FEC-1	8.2	Japan	*E. coli*	1986
CTX-M-1	8.4	Germany	*E. coli*	1989
CTX-M-2	7.9	Argentina	*Nontyphoid Salmonella, V. cholerae, E. coli*	1992
CTX-M-3	8.4	Poland, Greece, France	*C. freundii, E. coli, K. pneumoniae*	1996
CTX-M-4	8.4	Russia, Hungary	*S. enterica*	1996
CTX-M-5	8.8	Latvia	*S. enterica*	1996
CTX-M-6	8.4	Greece	*S. enterica*	1997
CTX-M-7	8.4	Greece	*S. Enterica*	1996
CTX-M-8	7.6	Brazil	*E. cloacae, E. aerogenes*	1996

B-lactamase	pI	Country	Species	Year
CTX-M-9	8.1	France	*E. coli*	1994
CTX-M-10	8.1	Spain	*E. coli, K. pneumoniae*	1990
CTX-M-12	9.0	Kenya	*K. pneumoniae*	1999
CTX-M-13	8.2	China	*K. Pneumoniae*	1998
CTX-M-14	8.3	France	*E. Coli*	1994
CTX-M-15	8.6	India	*E. coli, K. pneumoniae*	1999
CTX-M-16	8.2	Brazil	*E. Coli*	1996
CTX-M-17		Vietnam	*K. Pneumoniae*	1996
CTX-M-19	8.0	France	*K. Pneumoniae*	1999

EPIDEMIOLOGY OF CTX-M β-LACTAMASES

1. South America: Argentina and Brazil

   Outbreak of nontyphoidal Salmonella in Argentina in La Plata hospital in1989. The strain spread to neonatology units in Buenos Aires and from there to neighboring countries. The dissemination of $bla_{\text{CTX-M-2}}$ gene was demonstrated in different members of the family Enterobacteriaceae.

EPIDEMIOLOGY OF CTX-M β-LACTAMASES

- Far East: Japan, China, Korea, Taiwan, Vietnam and India.
- Predominant types: CTX-M-2, CTX-M-3, CTX-M-14 and CTX-M-15.
- Eastern Europe: Poland, Latvia, Russia, Greece, Hungary, Bulgaria, Romania and Turkey
- Predominant types: CTX-M-3 and CTX-M-15

EPIDEMIOLOGY OF CTX-M β-LACTAMASES

- Western Europe: France, UK, Spain, Portugal.
- Dominant types: CTX-M-3 and CTX-M-15.

- CTX-M β-lactamases are typical β-lactamases in community acquired isolates in contrast to SHV and TEM β-lactamases which are usually associated with nosocomial infections.

PHENOTYPIC CHARACTERISTICS

- CTX-M confer resistance to:

1. Aminopenicillins (ampicillin and amoxycillin),
2. Carboxypenicillins (carbenicillin and ticarcillin),
3. Ureidopenicillins (piperacillin)
4. Narrow spectrum cephalosporins (cephalotin, cephaloridine, cefuroxime)

The susceptibility to cephamycins (cefoxitin) and carbapenems is usually unchanged.

PHENOTYPIC CHARACTERISTICS

- Most CTX-M enzymes provide:
- high level resistance to cefotaxime and ceftriaxone,
- Variable levels of resistance to cefepime and cefpirome,
- MICs of ceftazidime are elevated slightly and often are within susceptibility range,
- MICs of aztreonam are also high,
- CTX-M β-lactamases are inhibited by clavulanic acid, the susceptibility to β-lactam-inhibitor combinations depends on the amount of enzyme.

OXA β-LACTAMASES

- Belong to molecular class D and funcional group 2d.
- Confer resistance to ampicillin and cephalotin and are characterized by high hydrolytic activity against oxacillin and cloxacillin.
- Are poorly inhibited by clavulanate.
- Have little sequence homology with other groups of ESBLs.

OXA β-LACTAMASES

- In contrast to other ESBLs, OXA type ESBLs have been predominantly found in *P. aeruginosa*.
- Most of them are derived from OXA-10 by point mutation in the blaOXA gene.
- OXA type ESBLs provide weak resistance to oxymino-cephalosporins when cloned in E. coli but high-level resistance in P. aeruginosa.
- They are widesprad only in Turkey, a few were found in France.

OXA Extended-spectrum β-lactamases

B-lactamase	124	126	143	150	157	pI	Species
OXA-10 (PSE-2)	A	E	N		G	6.1	P. aeruginosa
OXA-11			S		D	6.4	P. aeruginosa
OXA-14 (OXA-13)					D	6.2	P. aeruginosa
OXA-16	T				D	6.2	P. aeruginosa
OXA-2				D		7.7	P. aeruginosa
OXA-15				G		8.0	P. aeruginosa

Amp C β-lactamases

- Confer resistance to third generation cephalosporins and cephamycins (cefoxitin, cefotetan, moxalactam).
- Usually are not inhibited by clavulanate
- Susceptibility to fourth generation cephalosporins (cefepime) is maintained.
- They arose due to the escape of chromosomal ampC gene of the genus Enterobacter, Serratia and Pseudomonas on the plasmid.

Plasmid mediated AmpC β-lactamases

B-lactamase	Species	Country	Year	pI	CTX	CAZ	AMT	FOX	IMI	Inhibition by cla
CMY-1	K. Pneumoniae	South Korea	1989	8.0	64	4	16	256	0.25	+/-
CMX-2	K. Pneumoniae	Greece	1990	8.1	32	128	64	256	0.25	+/-
MIR-1	K. Pneumoniae	USA	1988	8.4	64	128	128	256	1	-

AMP C beta-lactamases

Examples	Substrates	Inhibition by clavulanate	Molecular class
ACC, ACT, CMY, DHA, FOX, LAT, MOR, MOX	Expanded-spectrum cephalosporins, narrow spectrum cephalosporins, aminopenicillins, ureidopenicillins and carboxypenicillins, cephamycins	0	C

ESBLs in the community settng

- First ESBL producing isolate in the community setting was reported in 1998 in Ireland.
- *E. coli* resistant to nalidixic acid producing ESBL was isolated from urine of an eldery patient.
- The patient did not have a recent history of hospitalization but had received multiple coursed of antibiotics.
- The type of ESBL was not determined

ESBLs in the community settng

- A laboratory study on the prevalence of ESBLs in the community was published in 2001 in Israel.
- The study found the prevalence of ESBL of 1% in community acquired urinary tract infections (CAUTI).
- Risk factors were: previous hospitalization, previous antibiotic treatment, male gender age over 60 years and diabetes mellitus.

Distribution of ESBLs in Gram-negative bacteria

Klebsiella pneumoniae
Escherichia coli
Proteus mirabilis
Enterobacter cloacae
Enterobacter aerogenes
Serratia marcescens
Citrobacter spp
Salmonella
Shigella
Morganella

Geographic distribution of ESBLs

Europa	America	Asia	Australia	Africa
Belgium	USA	India		Algeria
France	Argentina	Indonesia		South Africa
Germany	Chile	Japan		Tunisia
Portugal	zsil	Saudi Arabia		Cameroon
Spain	Mexico	Singapur		
The Netherlands	Canada	South Korea		
Italy		Turkey		
Greece				
Sweden				
UK				
Croatia				

Risk factors for colonization and infection with ESBL producing bacteria

1. Stay in intensive care units
2. Recent operative procedure
3. Intravenous or urinary catheters
4. Prolonged stay in the hospital
5. Previous therapy with expanded-spectrum cephalosporins

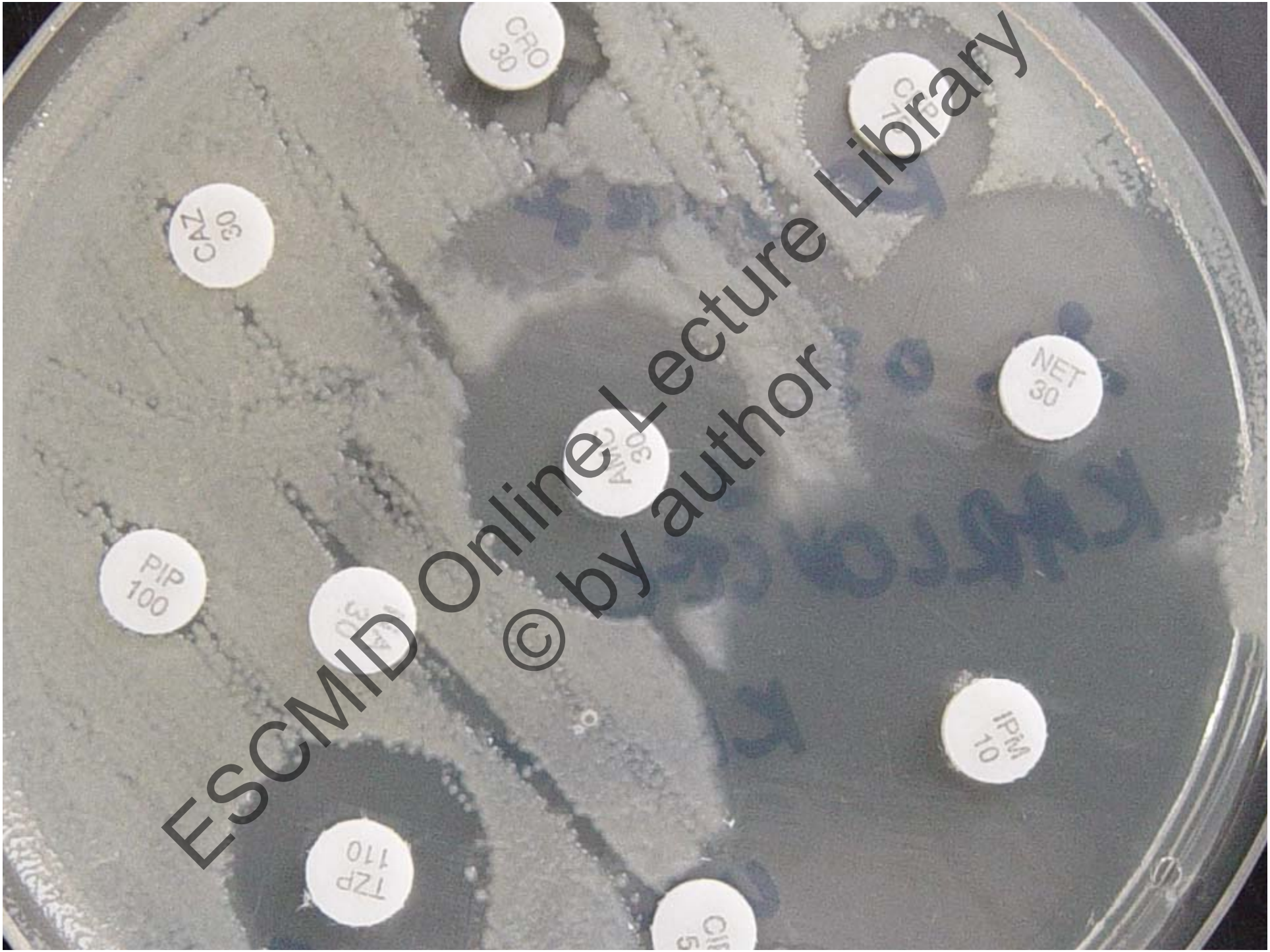
DETECTION OF ESBLs

Phenotypic methods

- Double-disk synergy test
- Disk on disk test
- CLSI combined disks test
- Three dimensional test
- E test
- Dilution method

MOLECULAR METHODS FOR DETECTION OF ESBLS

1. PCR with primers specific for TEM, SHV, CTX-M, PER and other β-lactamases.
2. Sequencing of PCR products to detect point mutations in *bla*$_{ESBL}$ genes.
3. DNA probes.



Antibiograms of *K. pneumoniae* and *E. coli* producing ESBLs and other types of β-lactamases

	Antimicrobial susceptibility							
Enzim	CAZ	CTX	CRO	CXM	CPD	FOX	ATM	IMI
ESBL (TEM ili SHV)	R	V	V	V	R	S	V	S
AmpC	R	R	R	R	R	R	R	S
K1	S	V	V/R	R	R	S	R	S
CTX-M	V	R	R	R	R	S	V	S

Effect of clavulanic acid on different types of β-lactamases

	Sinergism with clavulanate		
Enzim	CAZ	CTX	CPD
ESBL	+	+	+
AmpC	-	-	-
K1	-	+/-	-
CTX-M	+/-	+	+

Type of infection	First choice therapy	Alternative therapy
Bacteremia	Carbapenem (meropenem or imipenem)	Ciprofloxacin
Respiratory tract infection	Carbapenem (meropenem or imipenem)	Ciprofloxacin
Intra-abdominal infection	Carbapenem (meropenem or imipenem)	Ciprofloxacin or cephamycin
Urinary tract infection	Ciprofloxacin	Amoxycillin/clavulanate
Meningitis	Meropenem	Addition of polymixin B

Therapy of infections caused by ESBL producers

- Combination with inhibitors usually show in vitro susceptibility, but there are failures in vivo due to:

1. Pronounced inoculum effect
2. Development of hyperproducing mutants that are not inhibited by concentrations of inhibitors that are achieved during therapy.

Spectrum of activity of ESBLs

- Cephalosporins
- Monobactams
- Cephamycins
- Caboxypenicillins
- Ureidopenicillins
- Carbapenems