Détection des BLSE chez les entérobactéries

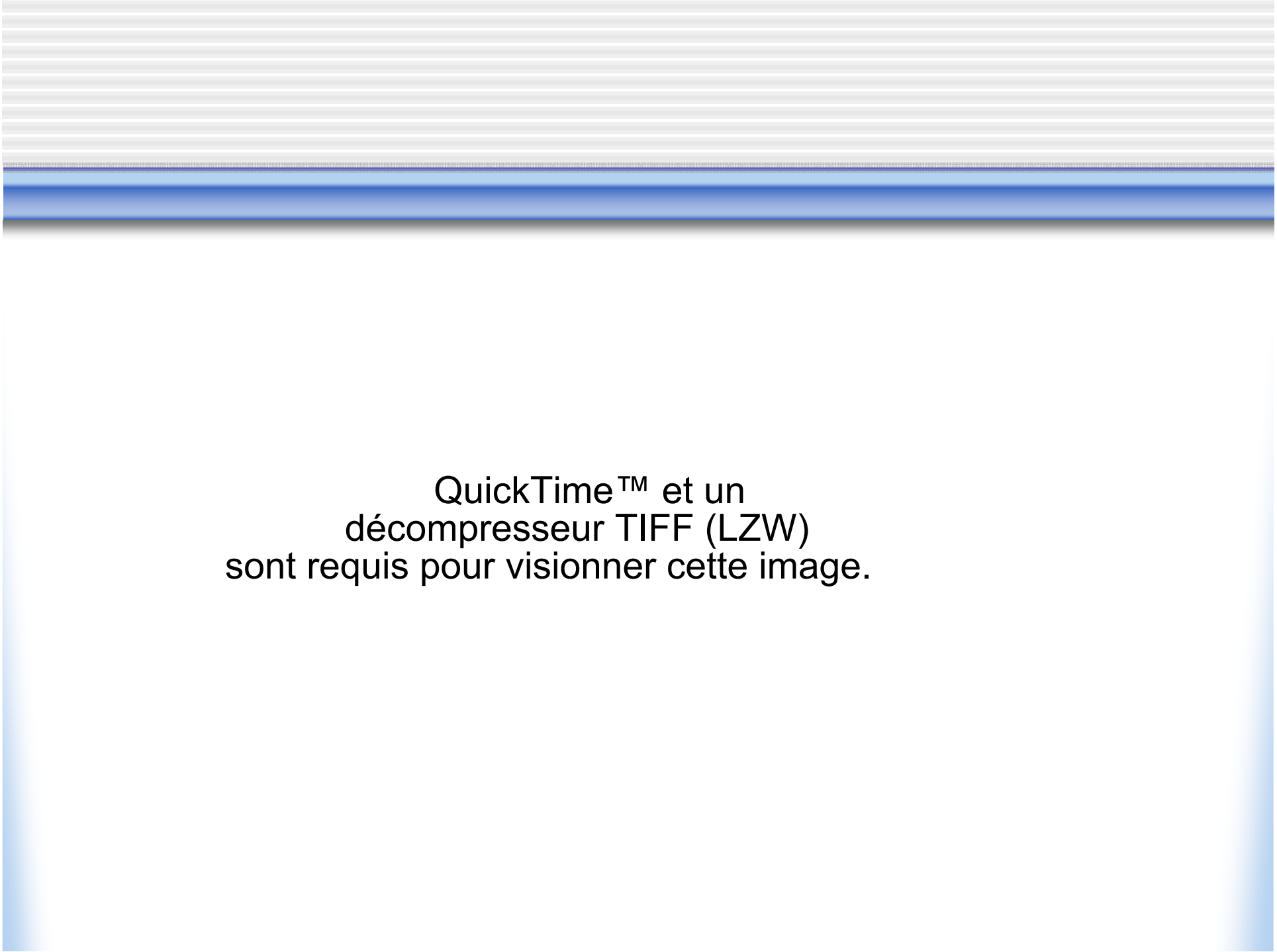
UNIVERSITE PARIS-SUD XI

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

Inserm U914
Résistances Emergentes aux Antibiotiques

P. Nordmann

Hôpital de Bicêtre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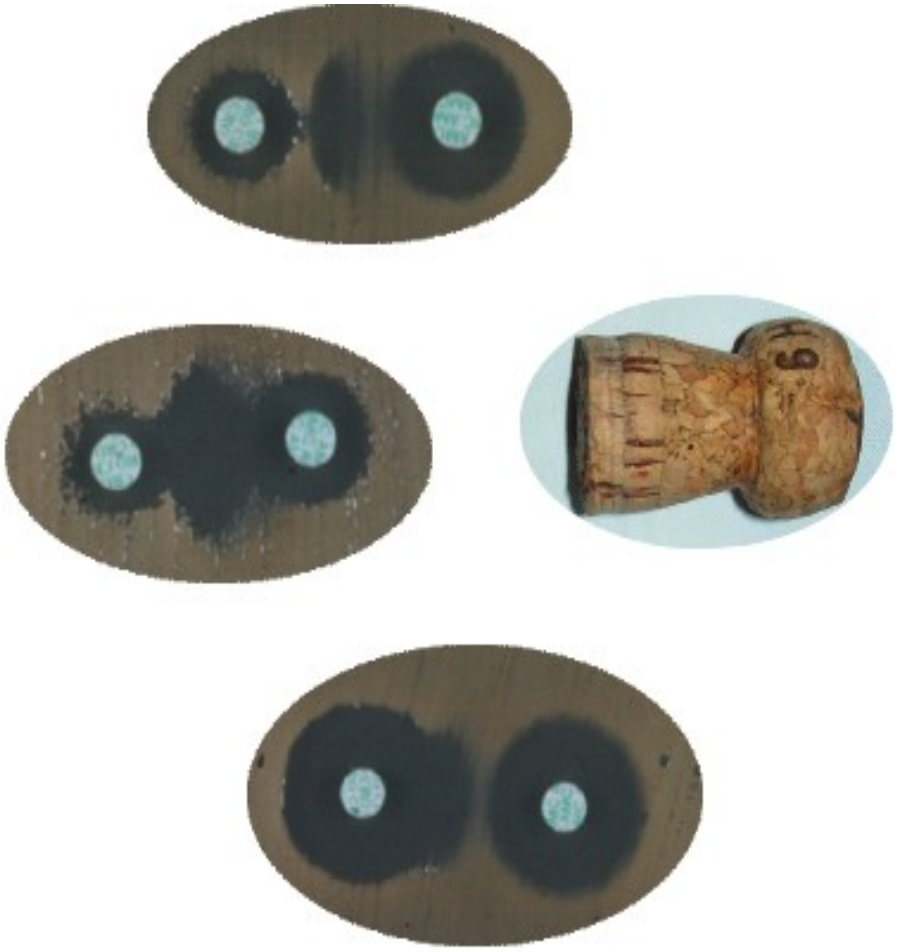
décompresseur TIFF (LZW)
sont requis pour visionner cette image.

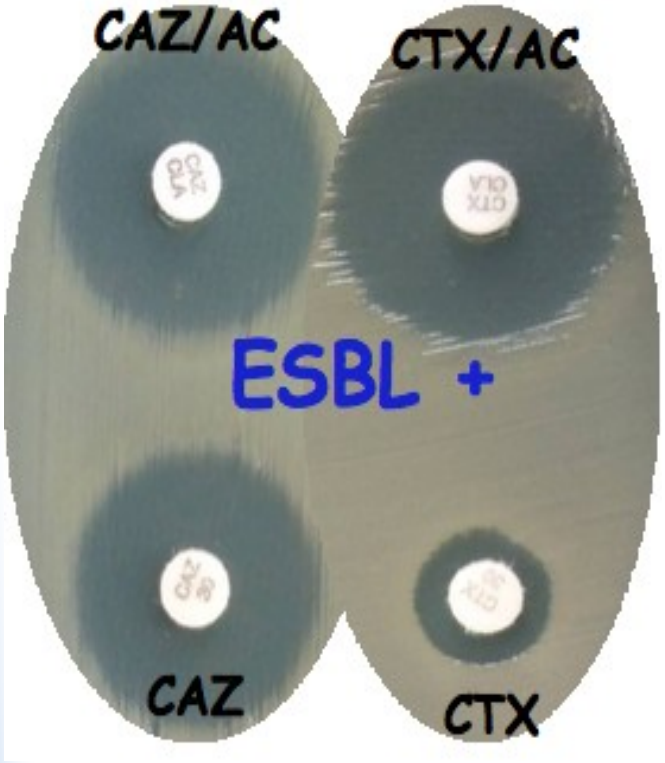
ESBL Detection on disc diffusion antibiogram

E. Coli (VEB-1)



ESBL Confirmatory Tests

Double-disk synergy (DDS) test

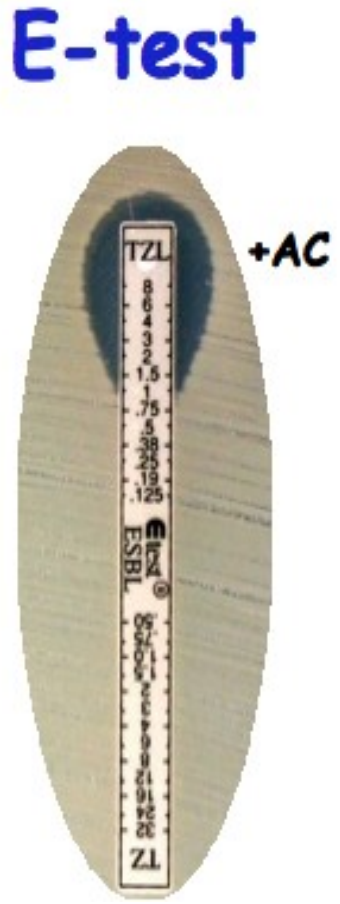


5 mm enhancement of the inhibition zone of antibiotic/CA combination vs antibiotic tested alone = ESBL

ESBL Confirmatory Tests
E-test

- CAZ and CAZ/CA E-strips
- CTX and CTX/CA E-strips

- A reduction in the MIC of antibiotic/CA of 3 dilutions vs antibiotic alone = ESBL




QuickTime™ et un
décompresseur TIFF (LZW)
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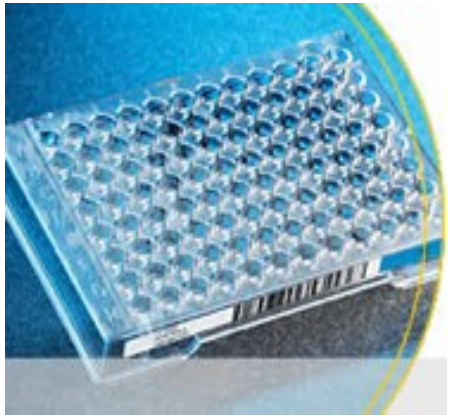
ESBL Confirmatory Tests

- Broth microdilution
  - CAZ and CAZ/CA
  - CTX and CTX/CA



- A 3 twofold concentration decrease in an MIC for either antibiotic tested in combination with CA vs its MIC when tested alone => ESBL

ESBL Detection: Automated Systems

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

Wiegand I, J Clin Microbiol. 2007, 45:1167-74.

ESBL Detection: Automated Systems

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2009, p. 358–361 Vol. 47, No. 2
0095-1137/09/$08.00+0 doi:10.1128/JCM.01687-08

Unreliable Extended-Spectrum β-Lactamase Detection in the Presence of Plasmid-Mediated AmpC in *Escherichia coli* Clinical Isolates[▽]

F. J. L. Robberts,[1] P. C. Kohner,[1] and R. Patel[1,2]*

TABLE 3. Correlation between phenotypic methods and gene amplification for detection of ESBLs

ESBL PCR result[a]	No. of samples							
	Total	Phenotypic ESBL method						
		Etest			Phoenix		Disk augmentation	
		+	−	ND[b]	+	−	+	−
+	7	2	0	5	4	3	6	1
−	19	0	1	18	5	14	9	10
Total	26	2	1	23	9	17	15	11

[a] Excluding TEM-1.
[b] ND, nondeterminable.

BD Phoenix NMIC/ID-132 panel (BDDiagnostics), which employs ceftazidime, TZL, ceftriaxone-clavulanate, CTL, cefpodoxime, and cefepime for ESBL detection

Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-009-0713-9

BRIEF REPORT

Evaluation of the capability of the VITEK 2 system to detect extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates, in particular with the coproduction of AmpC enzymes

H. M. Chen · J. J. Wu · P. F. Tsai · J. Y. Wann · J. J. Yan

VITEK 2 ESBL test, including ceftazidime, cefotaxime, and cefepime in the presence and absence of clavulanic acid,

- 317 K. pneumoniae and 291 E. coli
β-lactamases were characterised by PCR.
- The sensitivity and specificity for ESBLs were 98.9% and 98.5%, respectively.
- Ninety of the isolates were AmpC and ESBL positive
- 74 isolates (82.2%) were flagged ESBL producers

ESBL Detection: Automated Systems

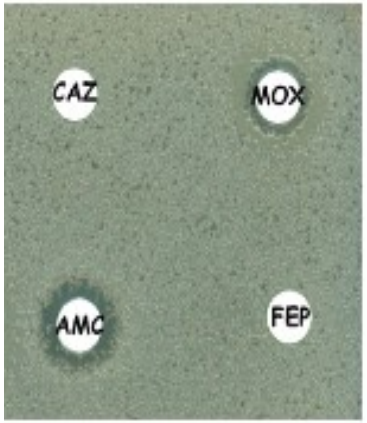
Detection Method	Sensitivity %	Specificity %	PPV %	NPV %
MicroScan	83.5	72.9	81.6	75.4
Phoenix	98.8	52.2	75	96.6
Vitek2	85.9	78	84.9	79.3
DDS	92.9	96.6	97.5	90.5
Etest	94.1	84.7	89.9	90.9

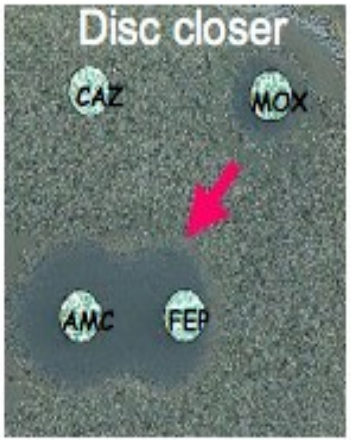
Wiegand I, JCM 2007, 45:1167-74.

Problems associated with ESBL detection

- High level expression of AmpC (chromosome- or plasmid borne may mask the phenotypic expression of ESBL)
- Clavulanic acid may act as inducer ofAmpC and masks the ESBL phenotype
- Low level resistance
- Complex ESBL
- Impermeability associated with ESBL
- Multidrug resistance due to association of plasmid-mediated AmpCs and/or carbapenemases
- Des exceptions…

ESBL + AmpC





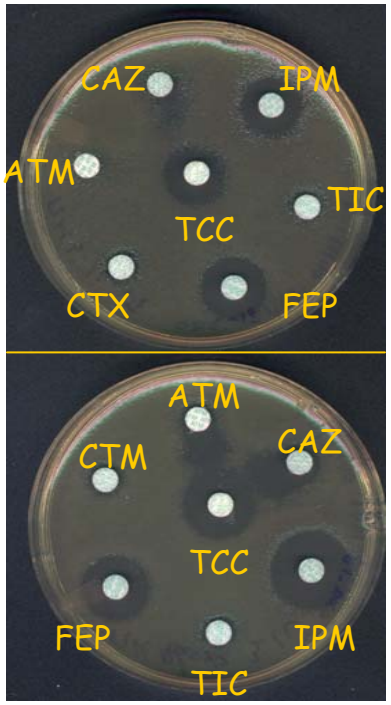
ESBL + MBL

Poirel, Pathology. 2004, 36:366-7.

ESBL + porin mutation

= > Disc closer
= > Conjugation
= > PCR

AmpC and ESBLs



+ CLOXA (250 mg/L)


QuickTime™ et un
décompresseur TIFF (LZW)
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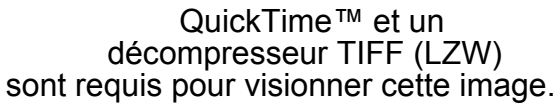
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décompresseur TIFF (LZW)
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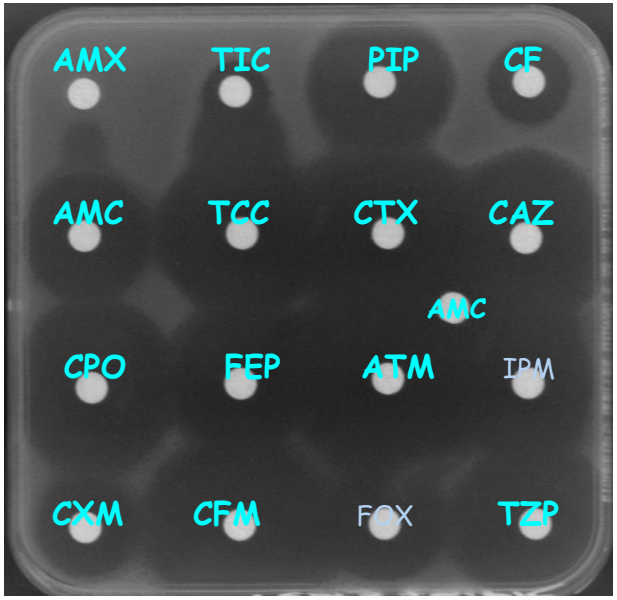
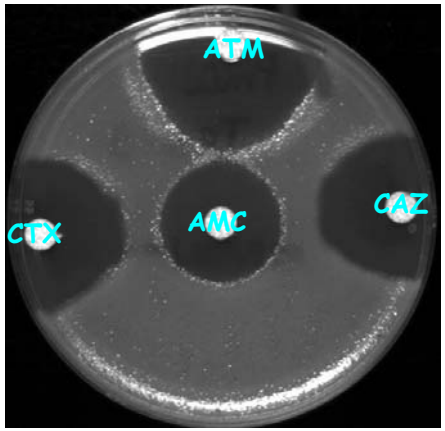
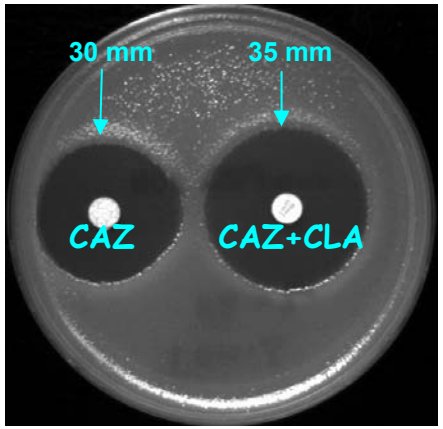
P. mirabilis BLSE (TEM-3)

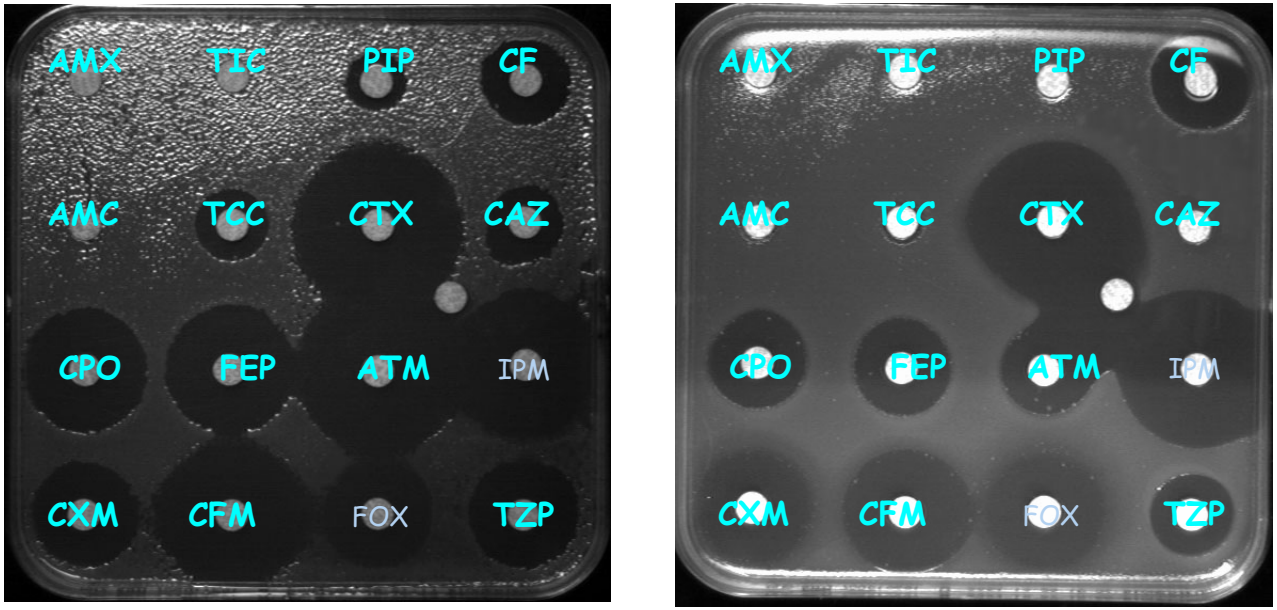






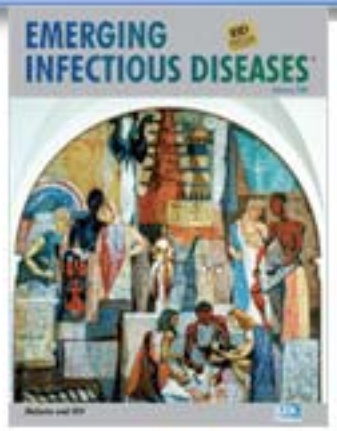
Courtesy R. Bonnet

E. Coli CMT (Complex Mutant TEM)



Courtesy R. Bonnet

ESBL + impermeability

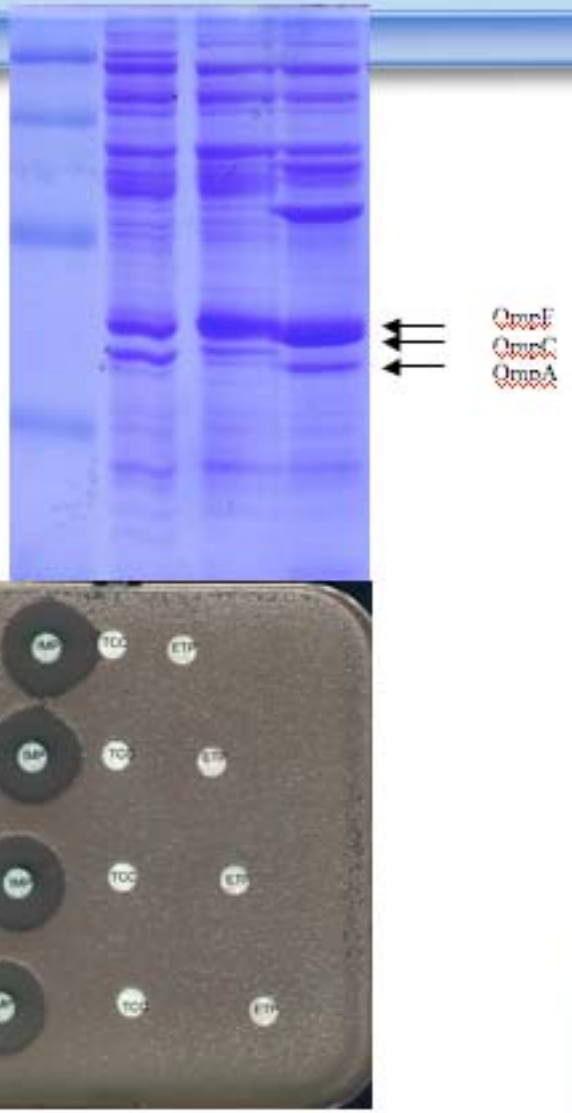


Ertapenem Resistance of *Escherichia coli*

Marie-Frédérique Lartigue,* Laurent Poirel,*
Claire Poyart,† Hélène Réglier-Poupet,†
and Patrice Nordmann*

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 13, No. 2, February 2007

An ertapenem-resistant *Escherichia coli* isolate was recovered from peritoneal fluid in a patient who had been treated with imipenem/cilastatin for 10 days. Ertapenem resistance may be explained by a defect in the outer membrane protein and production of extended-spectrum β-lactamase CTX-M-2.

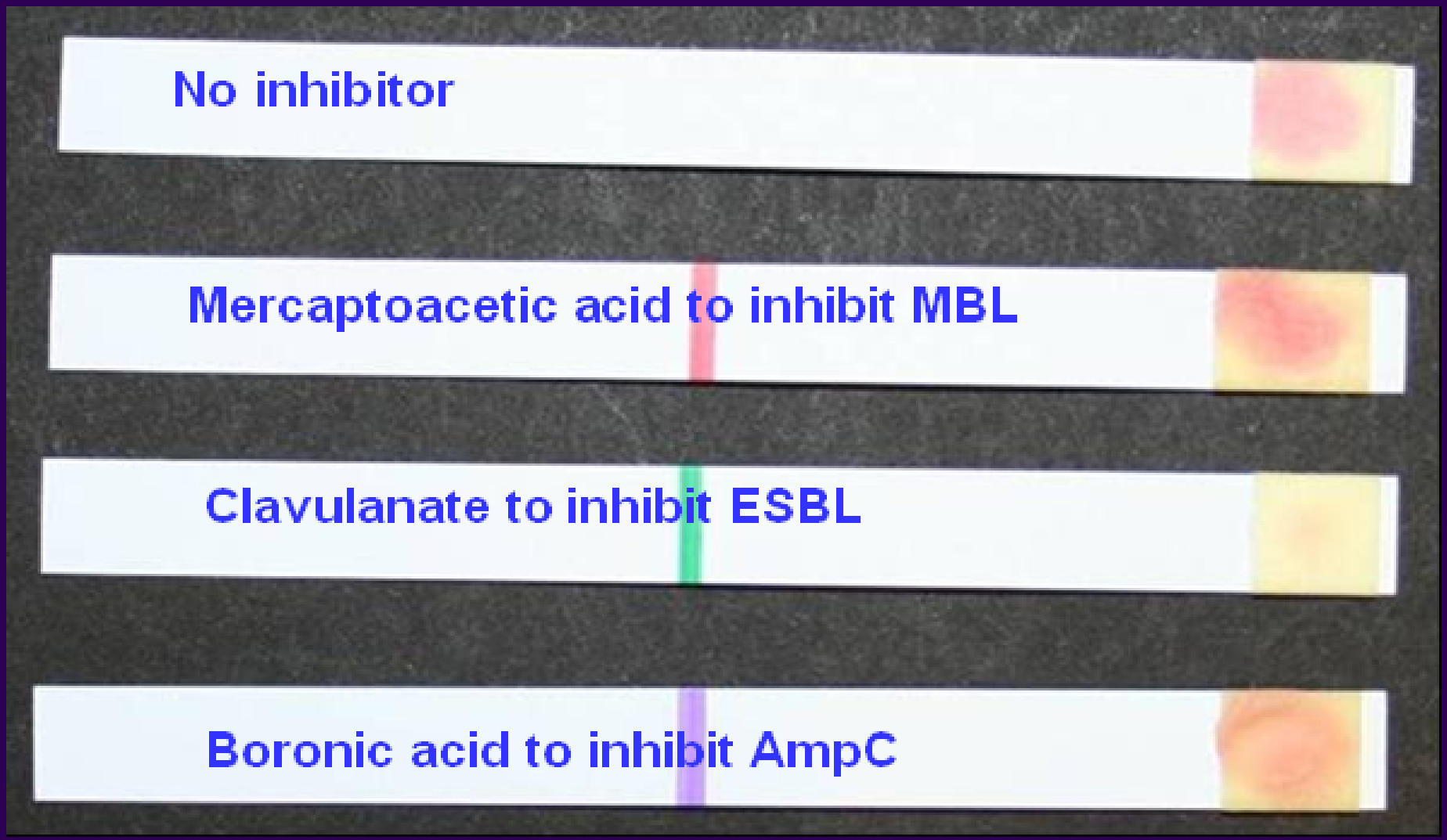


K. pneumoniae NDM-1

CTX-M-15, NDM-1

P. Nordmann and L. Poirel

Cica β-Test (Mast)



Molecular detection tools

ß-lactamases (>500) > 50% ESBLs

SHV-type (> 120), TEM-type (> 160), CTX-M-type (> 80)

SFO-1, TLA-1, PER (5) , VEB (7), BES-1, GES (12), BEL (2), TLA-2,

PCR and sequencing

- The gold standard
- Can detect all variants
- Easy to perform but labor intensive and costly

- **PCR,** Multiplex-PCR, Real-time PCR (Sybr green, Taqman, Hybridization probes), microarray

Only seeked genes can be found

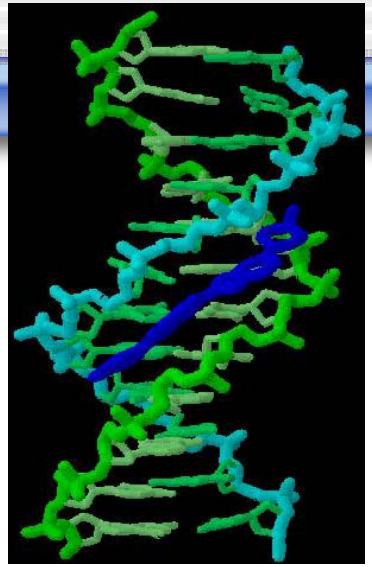
⇒ **Only** detection of DNA and not of expressed genes

⇒ **Requires isolation of the strain for identification and susceptibility testing**

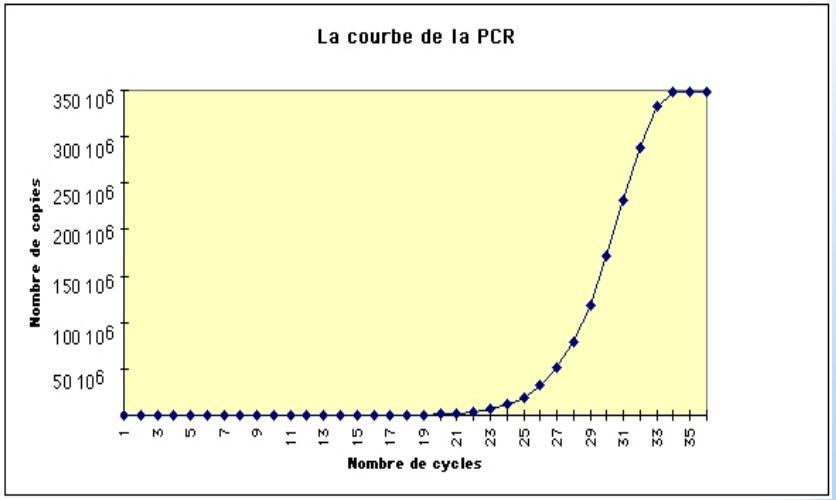
⇒ **Most of these assays are confirmative tests (on cultures)**

Real time PCR

LightCycler®
(Roche Diagnostics)



- Fast thermalcycler with microfluorimeter
- 32 samples in one run
- 40 cycles per run: at each cycle Syber green fluorescence reading
  (3 sec for the 32 capillaries)
- Run time : 40 mn
- **20 µl sample size**



Journal of Medical Microbiology (2005), **54**, 1183–1187 DOI 10.1099/jmm.0.46160-0

Rapid and simple detection of *bla*$_{CTX-M}$ genes by multiplex PCR assay

Li Xu,[1,2] Vicki Ensor,[2] Savita Gossain,[1] Kathy Nye[1] and Peter Hawkey[1,2]

[1]Health Protection Agency West Midlands Public Health Laboratory, Birmingham Heartlands & Solihull NHS Trust, Birmingham B9 5SS, UK

[2]Division of Immunity and Infection, University of Birmingham, UK

Correspondence
Li Xu
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A novel multiplex PCR assay is described (CTX-Mplex PCR) that allows rapid detection of *bla*$_{CTX-M}$ genes and discrimination between groups 1, 2, 9 and 25/26. The specificity and sensitivity of the assay were evaluated with 10 control strains and then applied to 62 clinical isolates. The multiplex PCR detected and classified *bla*$_{CTX-M}$ genes with 100 % accuracy. The utilization of a denaturing HPLC WAVE system to size the PCR products automatically from the multiplex PCR enhances the assay by saving time and costs.

Received 18 May 2005
Accepted 5 August 2005

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2005, p. 4486–4491 Vol. 43, No. 9
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.9.4486–4491.2005

Development of a Multiplex PCR and SHV Melting-Curve Mutation Detection System for Detection of Some SHV and CTX-M β-Lactamases of *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* in Taiwan

Ju-Hsin Chia,[1] Chishih Chu,[3] Lin-Hui Su,[1] Cheng-Hsun Chiu,[2] An-Jing Kuo,[1] Chien-Feng Sun,[1] and Tsu-Lan Wu[1]*

Department of Clinical Pathology, Chang Gung Memorial Hospital,[1] and Department of Pediatrics, Chang Gung Children's Hospital,[2] Taoyuan, Taiwan, and Department of Applied Microbiology, National Chiayi University, Chiayi, Taiwan[3]

Journal of Antimicrobial Chemotherapy (2009) **64**, 986–989
doi:10.1093/jac/dkp336
Advance Access publication 10 September 2009

JAC

Rapid detection of CTX-M-producing Enterobacteriaceae in urine samples

Cynthia Oxacelay†, Ayla Ergani†, Thierry Naas* and Patrice Nordmann

Objectives: **CTX-M extended-spectrum β-lactamases (ESBLs) are emerging worldwide. Fast and reliable detection techniques may become mandatory for implementing proper treatment and infection control measures. Here, a *bla*$_{CTX-M}$-specific LightCycler real-time PCR (LC-PCR) assay based on hybridization probes was developed.**

Methods: **Urine samples positive for Gram-negative bacilli as revealed by Gram staining were collected over a 3 month period at Bicêtre hospital, France. Aliquots of these urine samples were frozen for subsequent molecular analysis, and the bacteria were cultured and identified by standard bacteriological techniques (biochemical tests, disc diffusion antibiogram and synergy testing). LC-PCR and standard PCR followed by sequencing was performed on all ESBL-positive and on 70 randomly chosen ESBL-negative urine samples.**

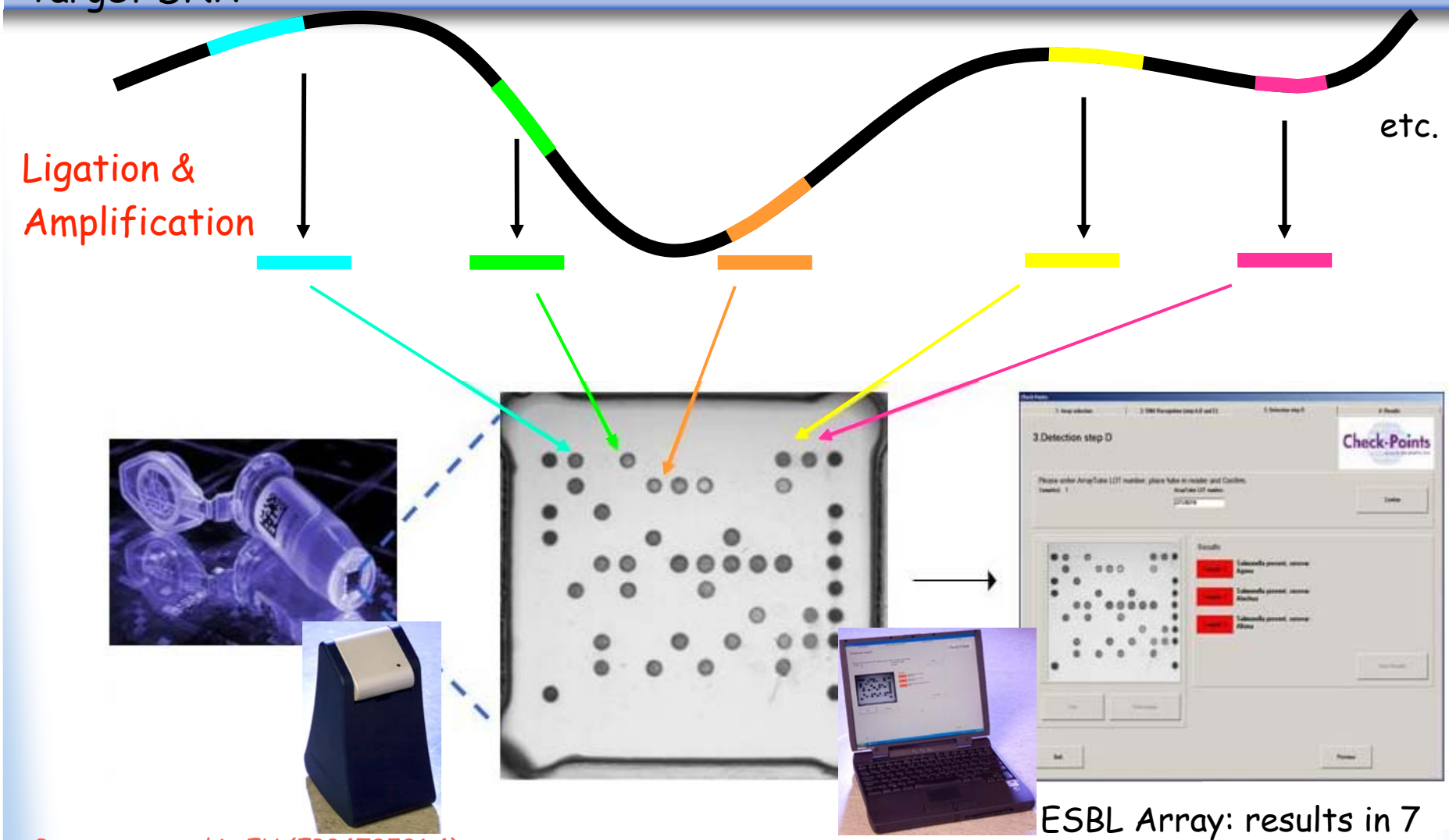
Results: **Over the study period, 810 urine samples were collected from 655 patients. Thirty-six ESBL-producing Enterobacteriaceae, mostly *Escherichia coli* (77%), were identified from 29 patients, of which half were outpatients. Twenty-five urine samples (19 patients) were found to be positive for *bla*$_{CTX-M}$ genes using the LC-PCR assay. The *bla*$_{CTX-M}$ genes belonged to the *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-9}$ and *bla*$_{CTX-M-2}$ groups (68%, 24% and 8%, respectively). Standard PCR and sequencing of the entire *bla*$_{CTX-M}$ genes confirmed the LC-PCR results; 17 CTX-M-15, 6 CTX-M-9 and 2 CTX-M-2. Among the remaining ESBLs, eight were of the TEM type and three of the SHV type.**

Conclusions: **The LC-PCR assay represents a powerful tool for rapid identification of CTX-M producers in urine samples.**

Rapid Molecular ESBL Detection: micro-array

- Accurate molecular ESBL assay, with results in < 7 h
- Tracking tool for Outbreak management
  - includes molecular typing of ESBL
- Identifies ESBL from non-ESBL:
  - for CTX-M, TEM & SHV
  - for all ESBL producing organisms

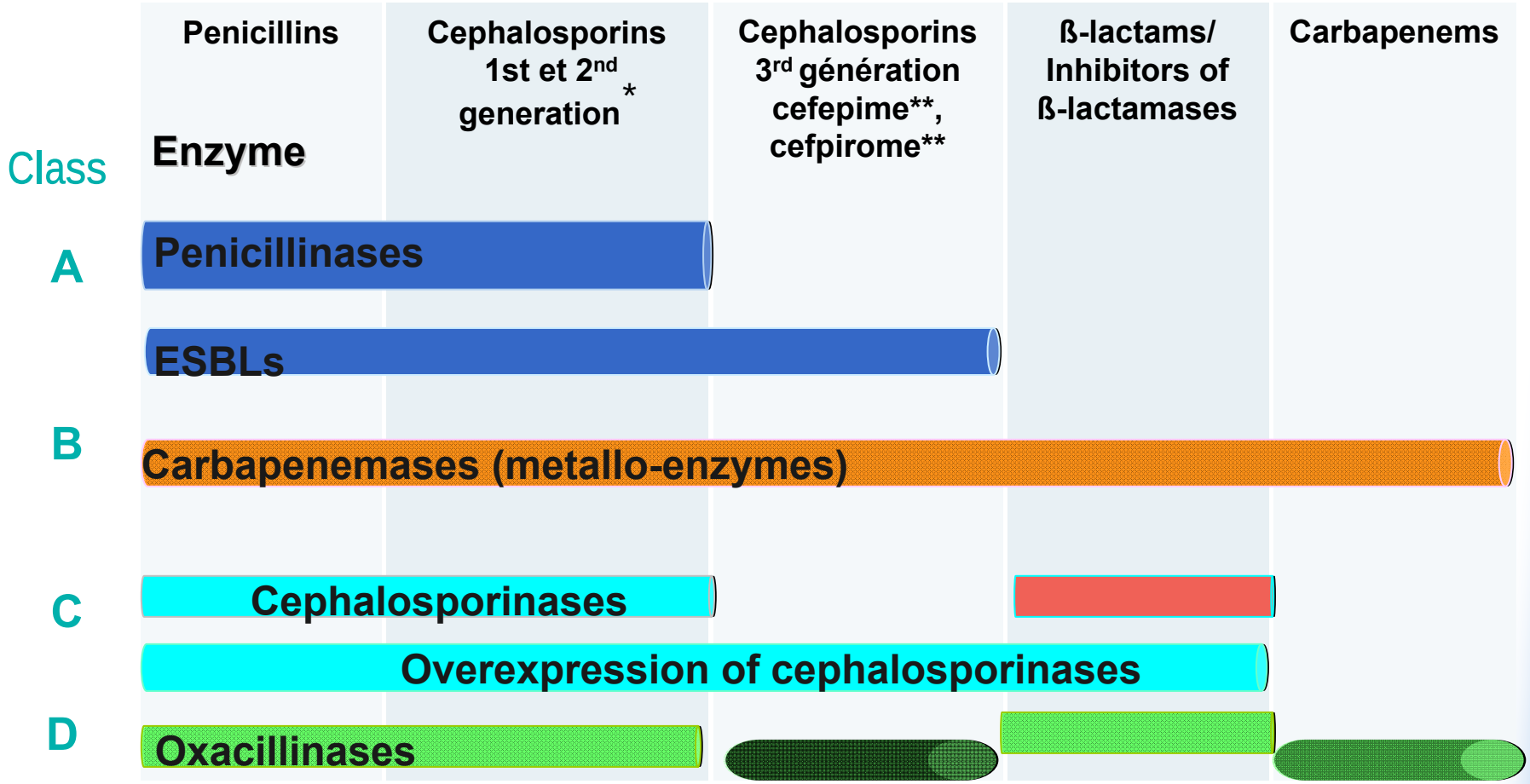
System ESBL Array:



Patent granted in EU (EP0473531.4)

ESBL Array: results in 7 h

In-vitro spectrum of activity of ß-lactamases



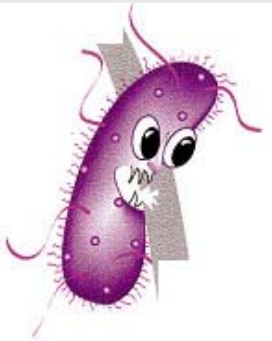
* Cephamycins excluded for ESBLs

** Cefepime, cefpirome excluded for overexpressed cephalosporinase

Definition

- Hydrolysis of penicillins + expanded-spectrum ß-lactams including most cephalosporins
- Inhibited by clavulanic acid, tazobactam and sulbactam
- Do hydrolyze neither cephamycins nor carbapenems (usually !)
- Ambler class A ß-lactamases
- Plasmid-encoded genes

ß-lactamases (>500)
> 50% ESBLs



Plasmid encoded

- 1983 SHV-type (>120)
- 1985 TEM-type (> 160)

"The oldies"

- 1989 CTX-M-type (> 80)

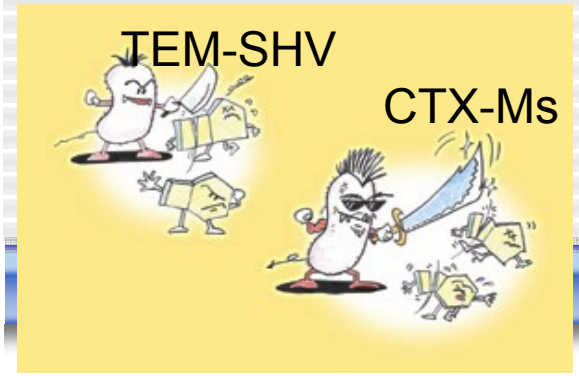
"ESBLs of growing importance"

- 1988 SFO-1 Serratia FOnticola
- 1991 TLA-1 TLAhuicas (indian tribe)
- 1991 PER (5) Pseudomonas Extended Resistance
- 1996 VEB (7) Vietnam Extended-spectrum ß-lactamase
- 1996 BES-1 Brazilian Extended-Spectrum ß-lactamase
- 1998 GES (12) Guyana Extended-Spectrum ß-lactamases
- 2005 BEL (2) Belgium Extended-spectrum ß-Lactamase
- 2005 TLA-2 ???? (Plasmid, waste water)

"infrequent ESBLs"

Chromosomally-encoded

- http://www.lahey.org/studies/webt.asp
- Naas et al., 2008, CMI, Suppl 1:42-52)



Why detect ESBls?

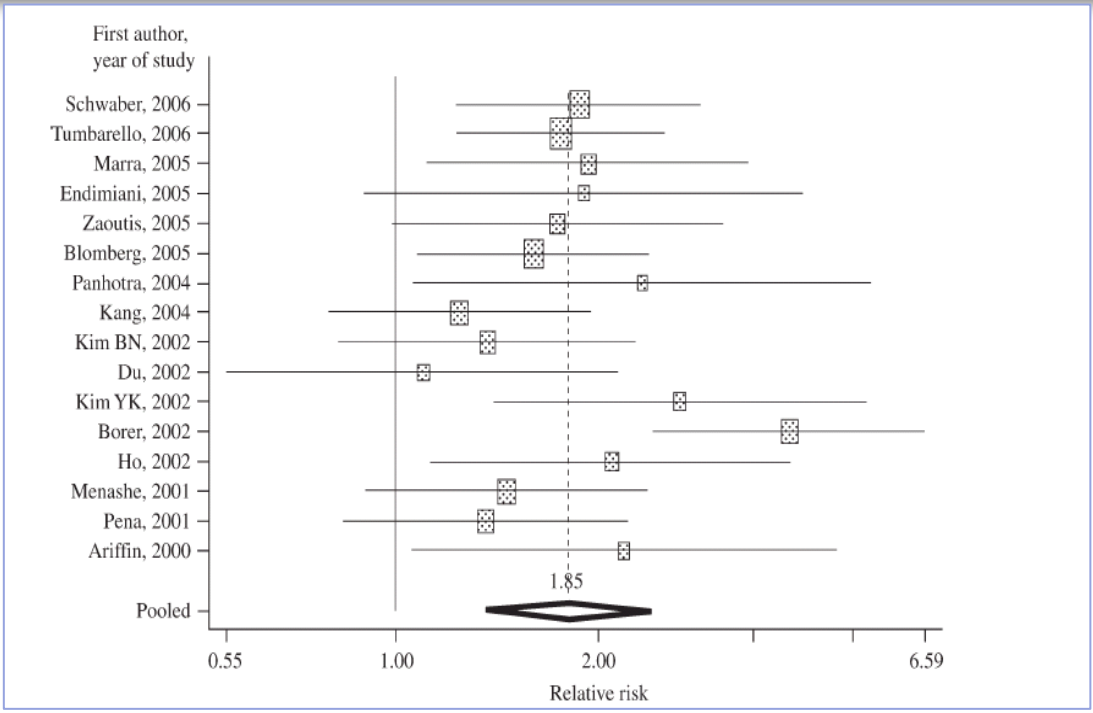
- ESBLs destroy activity of penicillins, cephalosporins, aztreonam
- ESBLs are plasmid encoded that facilitates their spread
- ESBL producing organisms represent significant infection control problems
- ESBLs are associated to multi-resistance to non- ß-lactam antibiotics such as fluoroquinolones, aminoglycosides and trimethoprim

=> **Carbapenems** (imipenem, ertapenem) are the drugs of choice

=> Emergence of carbapenem-producing isolates

=> Delayed recognition and inappropriate treatment of severe infection

Meta-analysis: ESBL bloodstream infections and mortality; *Mortality ESBL: (34%) vs. Non-ESBL: (20%)*



(Schwaber and Carmeli, JAC, 2007)

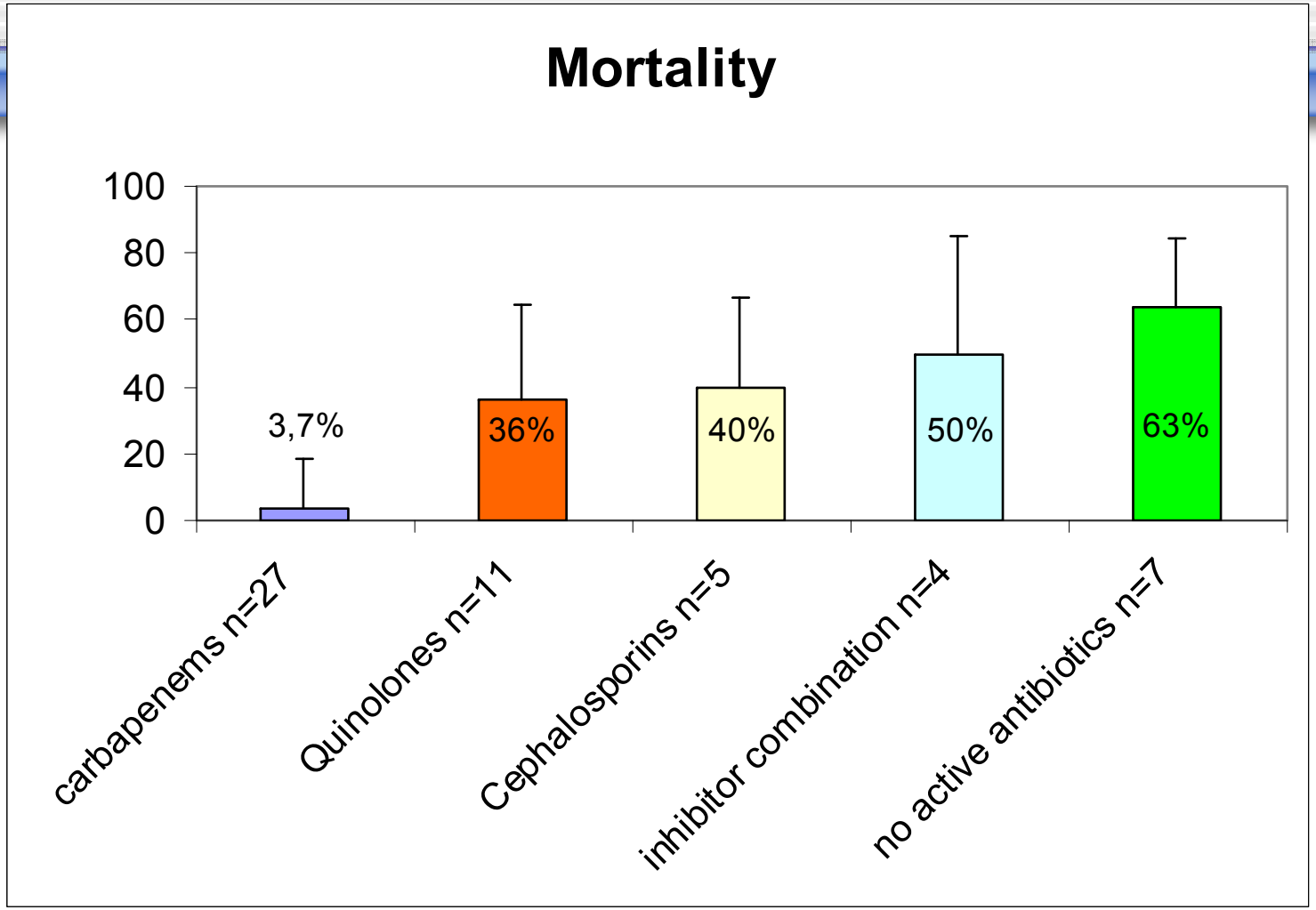
ESBL: n= 519
Non-ESBL: n=1091

Relative Risk: 1,85 (95% CI 1.39-2,47)

Potential causes of excess mortality in ESBL infections:

- Selection bias (i.e. risk-factors for ESBL are also risk-factors for mortality)
- ESBL is associated with virulence genes
- Delay in effective therapy

Day 14 mortality per antibiotic class

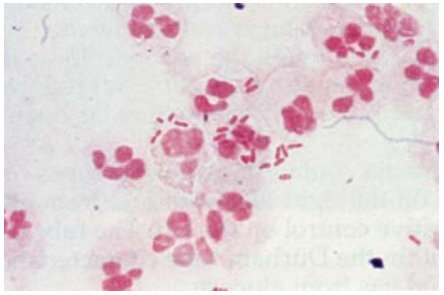


Paterson et al., CID, 2004

Where and How Detect ESBLs?

Enterobacteriaceae

(E.coli, K. pneumoniae,Enterobacter …)



- Initial screen by disk diffusion or broth microdilution for resistance to:
  - Cefpodoxime, ceftriaxone, ceftazidime, cefotaxime, and aztreonam
  - The use of several antibiotics improves the test sensitivity

⇒ Growth at or above the screening MICs suggests ESBL production

⇒ Zones of inhibition smaller than that of the standard breakpoints suggests ESBL production

⇒ **Confirmatory tests are required**

Société Française de Microbiologie

COMITE DE L'ANTIBIOGRAMME DE LA SOCIETE FRANCAISE DE MICROBIOLOGIE

Recommandations 2010

(Edition de Janvier 2010)

Concentrations, diamètres critiques et règles de lecture interprétative pour *Enterobacteriaceae:* céphalosporines

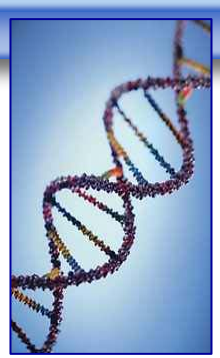
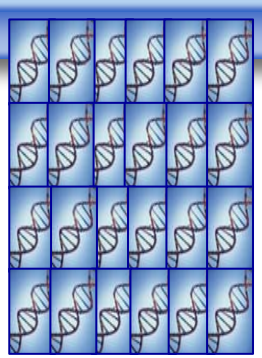
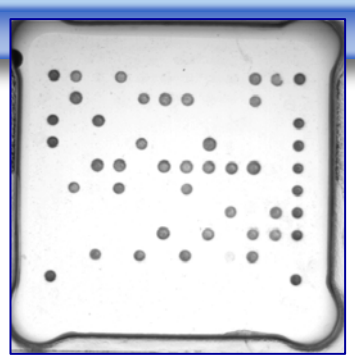
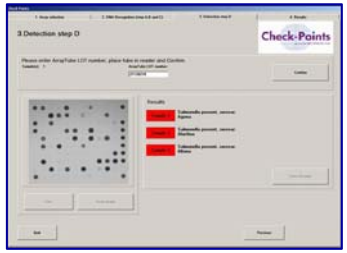
Antibiotiques	Concentrations critiques (mg/L)	
	S	R
Céfalotine	Š 8	> 32
Céfuroxime	Š 8	> 8
Céfamandole	Š 8	> 32
Céfoxitine	Š 8	> 32
Céfotétatn	Š 4	> 32
Latamoxef	Š 4	> 32
Cefotaxime	Š 1	> 2
Céftriazone	Š 1	> 2
Ceftazidime	Š 1	> 8
Céfépime	Š 1	> 8
Cefpirome	Š 1	> 8
Céfixime	Š 1	> 2

• Interpreter I un résultat S à toutes les céphalosporines sauf les céphamycines (céfoxitine et céfotétan) et à l'aztréonam en présence d'une synergie significative entre au moins l'une des céphalosporines de 3ème génération (C3G) ou l'aztréonam et AMC. Ce phénotype est évocateur d'une β-lactamamase à spectre élargi (BLSE). Dans ce cas, il n'y pas lieu d'interpréter I les souches caractérisées à S à l'Augmentin lorsqu'elles sont responsables d'infections urinaires basses.

• Cette synergie significative d'une BLSE (typiquement « en bouchon de champagne ») est habituellement visible sur l'antibiogramme standard où les disques sont distants de 30mm (centre à centre). Toutefois, chez les souches résistantes à haut niveau aux β-lactamines (souches cumulant plusieurs mécanismes de résistance, dont l'hyperproduction de céphalosporinase), la détection d'une BLSE est facilitée par la recherche d'une synergie entre les disques de céfépime ou cefpirome et d'AMC, que l'on peut rapprocher, et/ou la réalisation d'un antibiogramme standard (comprenant éventuellement des disques de C3G + AMC) sur gélose Mueller-Hinton additionnée de 250 mg/L de cloxacilline. Chez certaines espèces intrinséquement très sensibles aux β-lactamines (*P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. stuartii* et *P. rettgeri*), les BLSE s'expriment à bas niveau, leur détection est facilitée par la recherche d'une synergie entre les C3G ou l'aztréonam et AMC avec des disques placés de 40-45mm.

• La présence d'une BLSE peut être confirmée en quantifiant la synergie soit par un antibiogramme comportant des disques de céfotatime, ceftazidime simples ou combinés à l'acide clavulanique, soit par la mesure de la CMI de ces molécules testées seules ou associées à l'acide clavulanique. Une synergie significative est définie comme l'augmentation de la zone d'inhibition ou la diminution d'au moins 3 dilutions de CMI en présence d'acide clavulanique. Cette synergie significative témoigne de la présence de BLSE et permet de distinguer ces enzymes de certaines pénécillinases plasmidiques (OXA-1/30, SHI-1). Une synergie non significative exclut, *a priori,* la présence d'une BLSE et la lecture interprétative associée à ce mécanisme.

ESBL/KPC Array

DNA extraction 1 h	Identification Amplification 3 h	Detection 2 h	Results on line

- Hands on time:
  - 3 samples: 1 h
  - 15 samples: 1 ½ h
  - 72 samples: 2 ½ h

72 samples/day, cost effective run: minimum 3 samples

ESBL - KPC Array:

- ESBL - non ESBL
- KPC present /absent

- When ESBL:
  - TEM
  - SHV
  - CTX-M group
  - or combinations of ESBL…

Spread of ESBLs in clinically-significant Gram negatives

	Enterobacteriaceae	
	K. pneumoniae *Enterobacter sp.*	*E. coli*
TEM	++	+/-
SHV	++	+/-
CTX-M	+	+++
VEB	+/-	+/-
PER	+/-	-
GES	+/-	+/-

3rd & 4th gen. cephalosporin breakpoints in Enterobacteriaceae

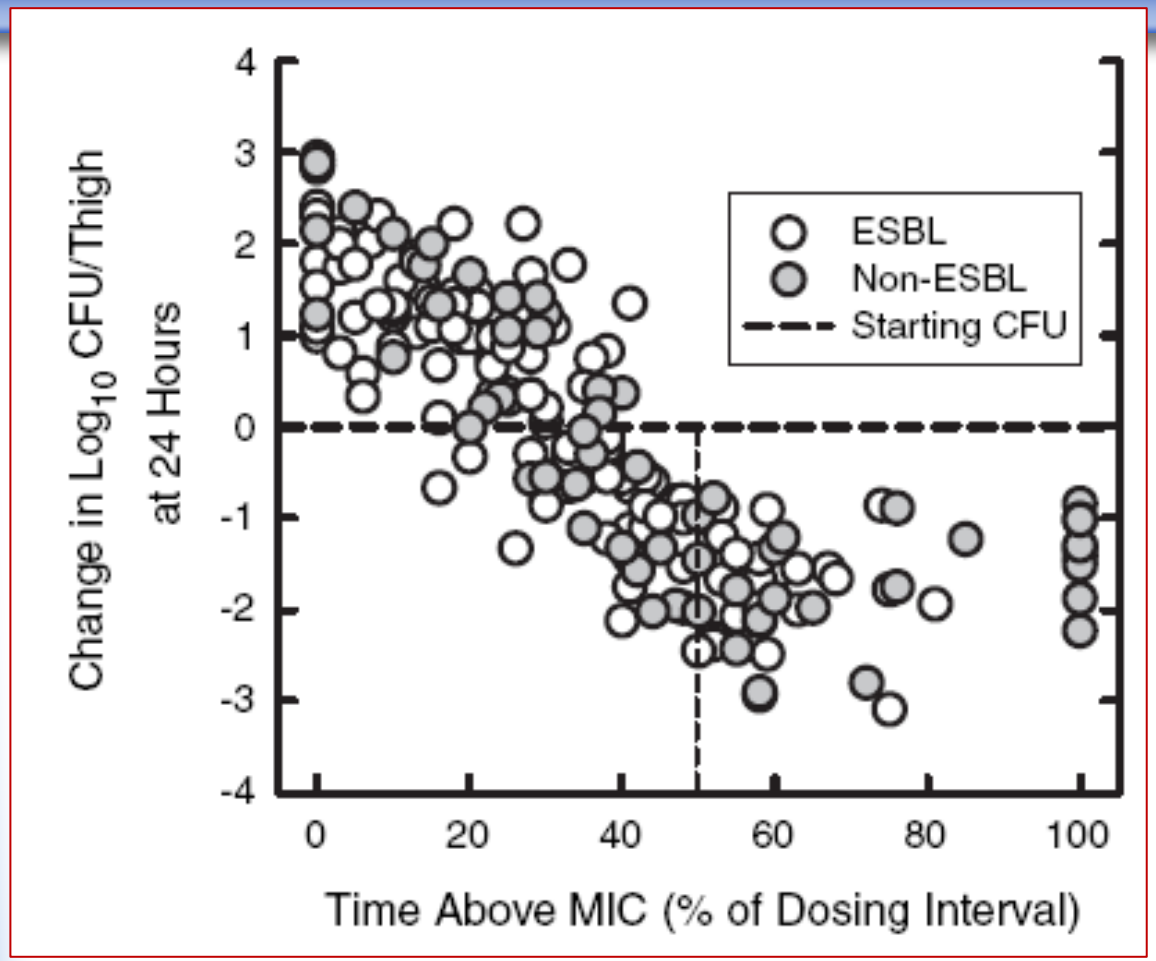
Cefalosporins	CLSI (2010)			EUCAST (2010)	
	S	R		S	R
Cefotaxime	**≤1** (8)*	**≥4** (64)	=	**≤1**	**>2**
Ceftriaxone	**≤1** (8)	**≥4** (64)	=	**≤1**	**>2**
Ceftazidime	**≤4** (8)	**≥16** (32)		**≤1**	**>4** (8)
Cefepime	**≤8**	**≥32**		**≤1**	**>4** (8)
Aztreonam	**≤4** (8)	**≥16** (32)		**≤1**	**>4** (8)

*2009

EUROPA, 2010

Detection in the lab: Does this really matter?

- The murine thigh infection model showed that the % T>CMI was similar in therapy (3rd/4th gen. cephalosporins) against ESBL and non-ESBL groups



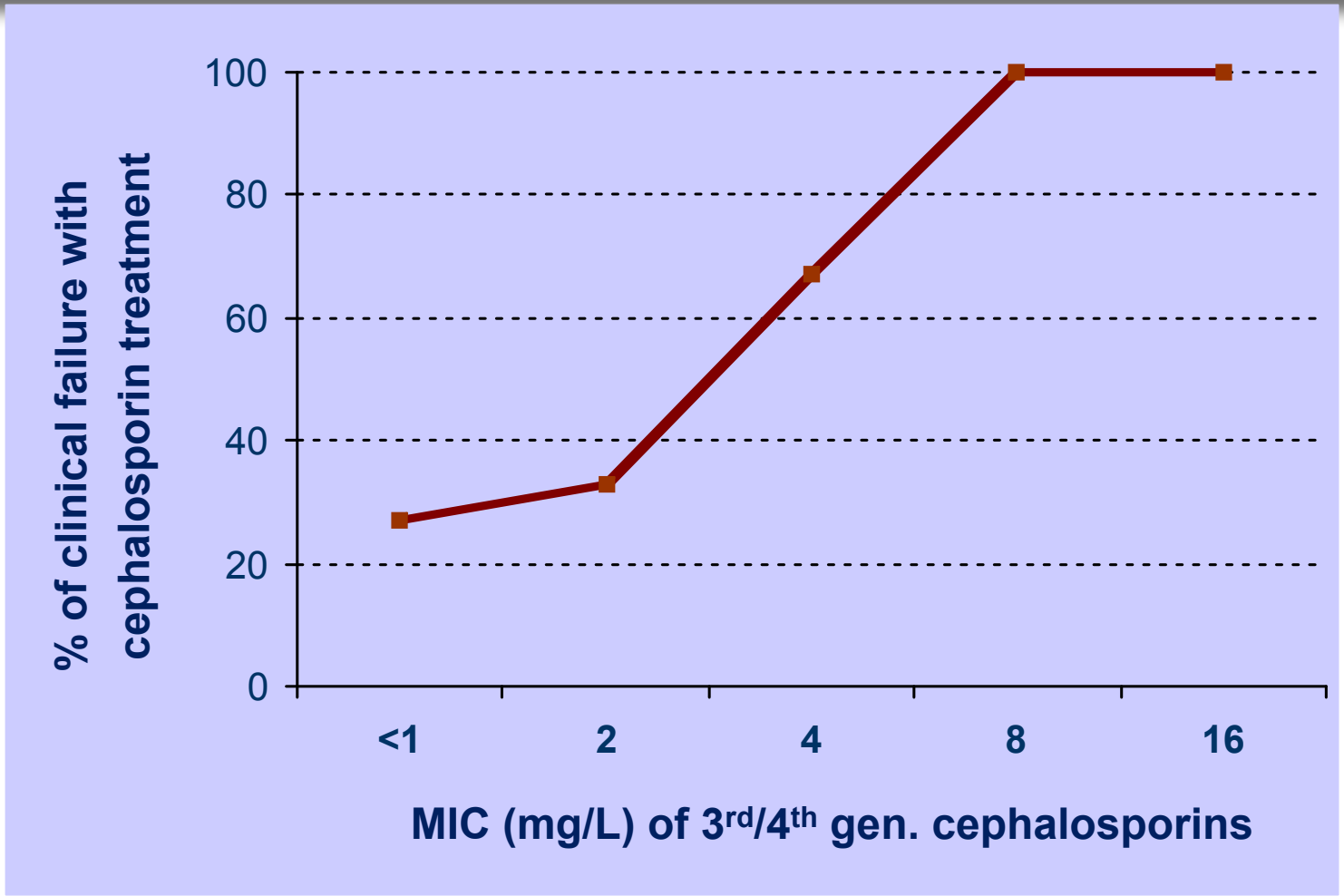
- PK/PD breakpoints should be independent of the resistance mechanisms

- The MIC of ß-lactam in an ESBL-producing isolate can be used to predict likely human outcomes from PK/PD models

Andes & Craig. Clin Microbiol Infect 2005; 11 (Supp. 6): 10-7

Detection in the lab: Does this really matter?

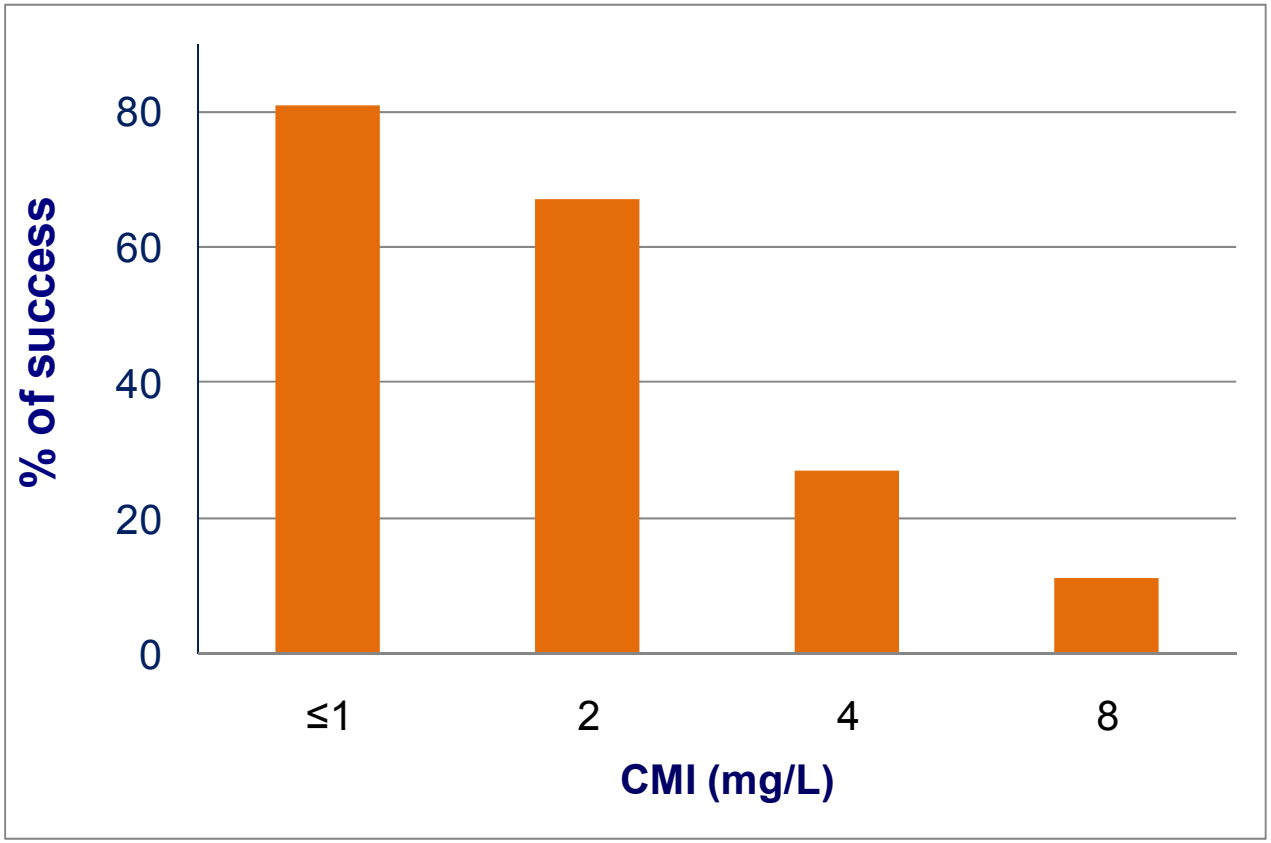
- Clinical outcome of 3er/4th gen. cephalosporins treatment in patients with serious infections due to ESBL-organisms



Paterson et al. J Clin Microbiol 2001; 39:2206-12

Detection in the lab: Does this really matter?

Clinical outcome in 42 patients with ESBL-producing *Klebsiella* spp. or *E. coli* bacteraemia and treated with cephalosporin monotherapy



Andes & Craig. Clin Microbiol Infect 2005; 11 (Supp. 6): 10-7

Screening

WHY ?

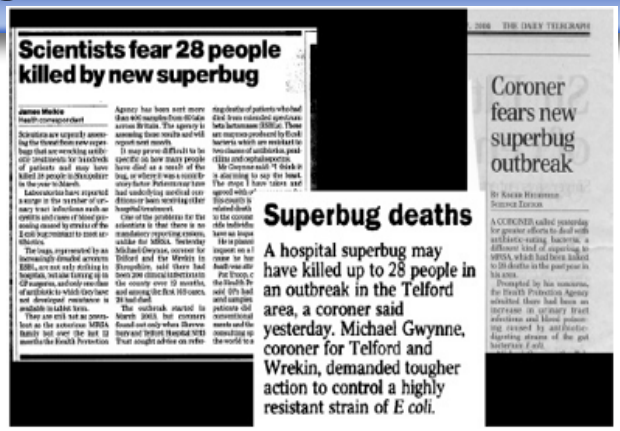
Prevent diffusion of ESBL-producing *enterobactericeae*

- **Individual Risks**

  - Inadequation and delay in treatment

    => increased morbidity and mortality

Melzer et al, *J Infect* 2007, 55:254;
Zahar et al, *Clin Microbiol Infect.* 2007 ;13:219
Schwaber et al, *J Antimicrob Chemother* 2007;60:913



Scientists fear 28 people killed by new superbug

James Meikle
Health correspondent

Coroner fears new superbug outbreak

Superbug deaths

A hospital superbug may have killed up to 28 people in an outbreak in the Telford area, a coroner said yesterday. Michael Gwynne, coroner for Telford and Wrekin, demanded tougher action to control a highly resistant strain of *E coli*.

- **General Risks: Dissemination community -> hospital**

  - Human reservoir

    - 13,7 % of enterobacterial blood-cultures isolated within the first 48 hours

    (Ben-Ami et al, *Clin Infect Dis* 2006, 42: 925)

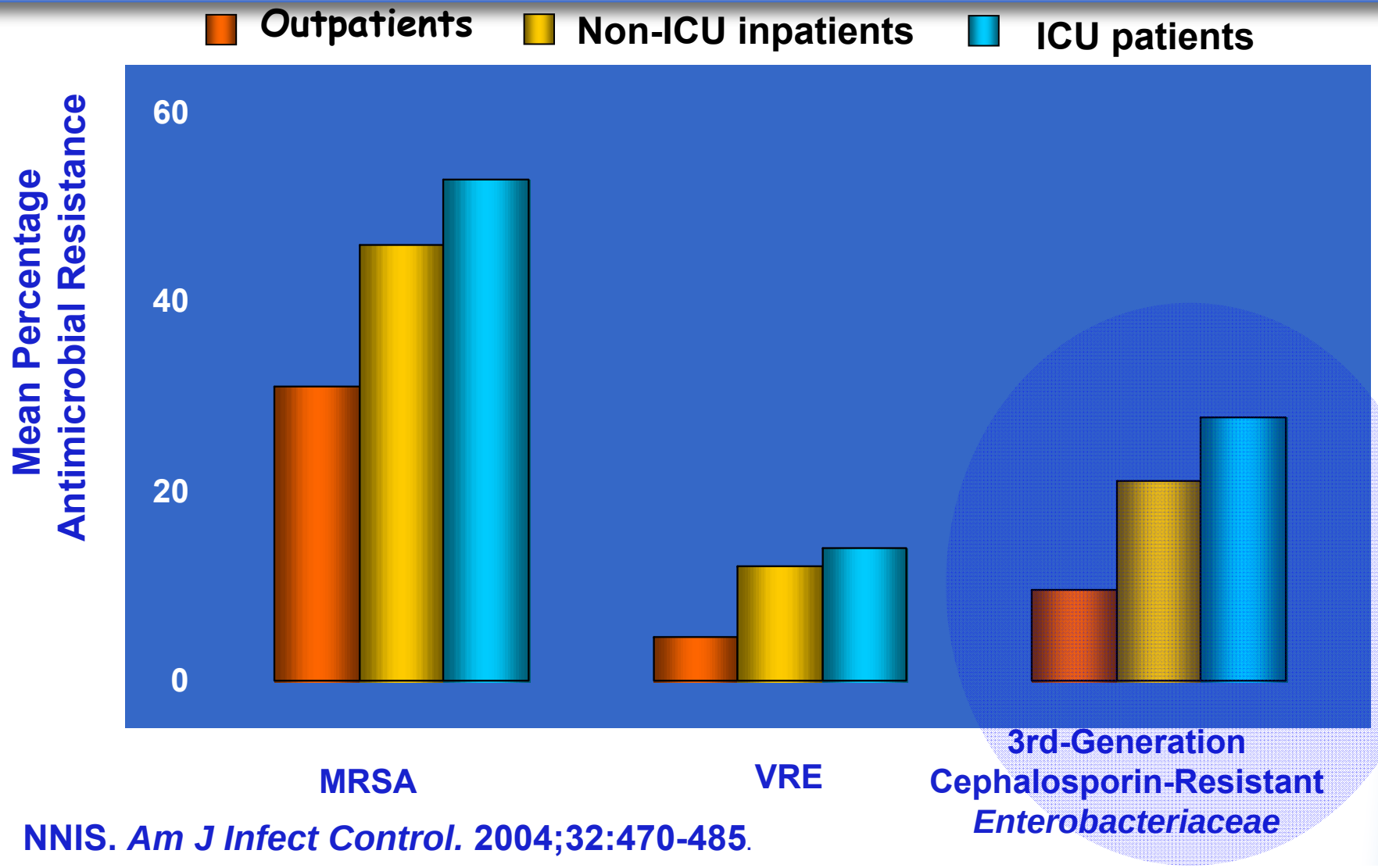
    - 51 % of bacteriemia with *E coli* are community-acquired (19% were true ones) (Rodriguez-Bano, *Clin Infect Dis* 2006; 43:1407)

  - Clonal diffusion

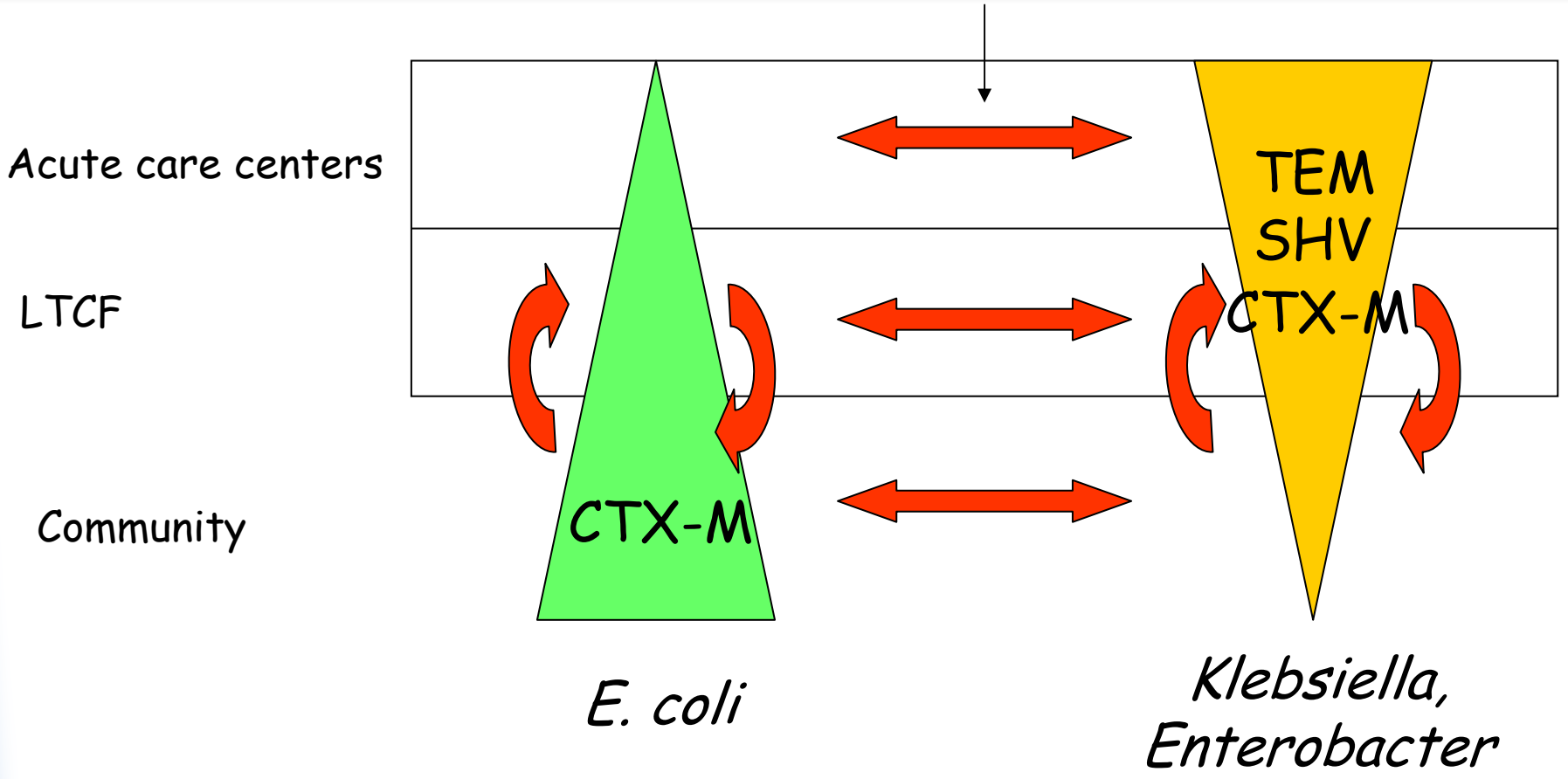
  - Transmission

Where:

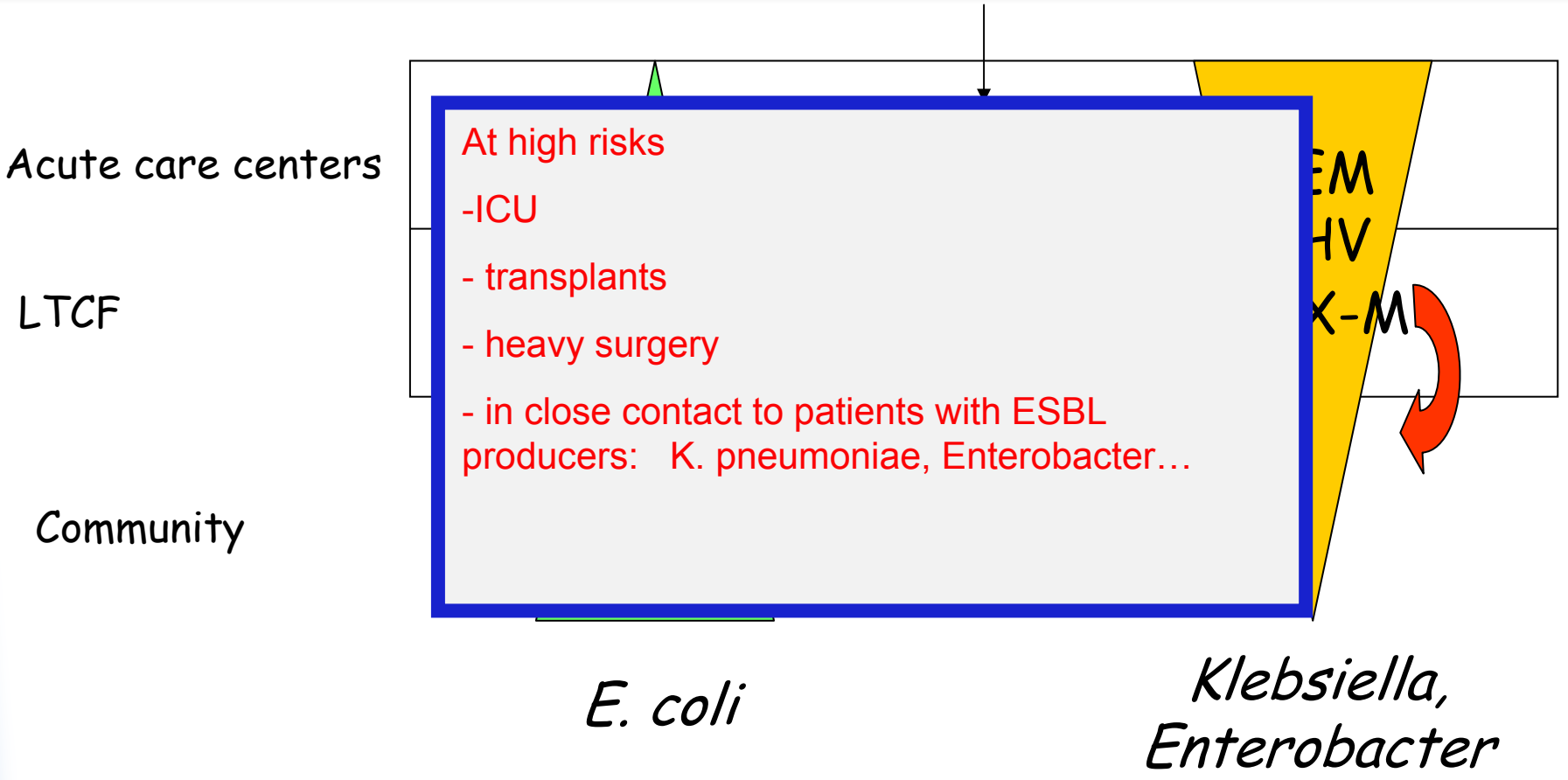
Resistant Bacteria Are Seen in a Variety of Clinical Settings



NNIS. *Am J Infect Control.* 2004;32:470-485.


Acute care centers
LTCF
Community
CTX-M
TEM
SHV
CTX-M
E. coli
Klebsiella,
Enterobacter

Which patients to be screened



Screening techniques

- **The percentage of clinical samples is low / carriage**
  - **Only 29 (25%) of the 117 carriers at admission had an ESBL-positve clinical sample**
    Harris *et al, Emerg Infect Dis* 2007;13:1144
  - **Only 35 (8.5%) of the 413 carriers had a blood culture infection**
    Reddy *et al, Clin infect Dis* 2007; 45:846

How to screen?:

=>Decreased susceptibility to extended-spectrum ß-lactams

- Home-made media containing either antibiotic: Cefpodoxime, Ceftazidime, Cefotaxime, Ceftriaxone, Aztreonam
- Three commercially available media
  - BLSE Agar (AES),
  - ChromID™ ESBL (bioMérieux),
  - CHROMagar ESBL, CTX (CHROMagar)

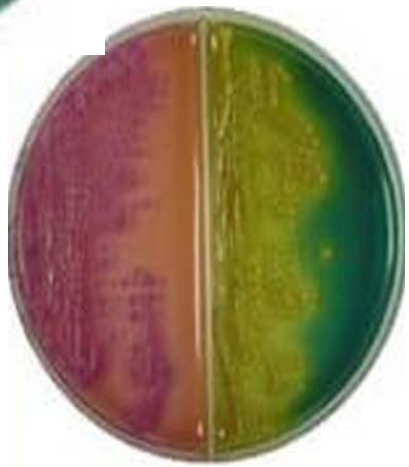
Evaluation of the two commercially available media

(**Glupczynski Y et al**. 2007. J Clin Microbiol; 45: 501; **Réglier-Poupet H et al**. 2008. J Med Microbiol. 57:310)

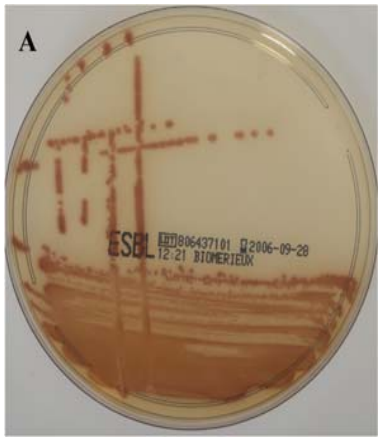
BLSE agar AES (France)

Drigalski
CTX 1.5 µg/ml

Mac Conkey
CAZ 2 µg/ml



chromID™ ESBL bioMérieux (France)

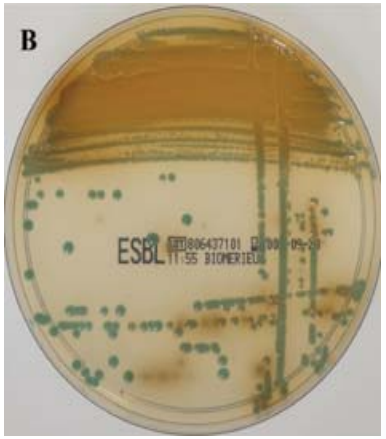


Cefpodoxime

E. coli: pink to burgundy

Klebsiella, Enterobacter, Serratia, Citrobacter (KESC): green/blue to browny-green

Proteeae (*Proteus, Providencia, Moraganella*): dark to light brown colouration



Screening specimens: rectal swab
(urine, respiratory secretions)*

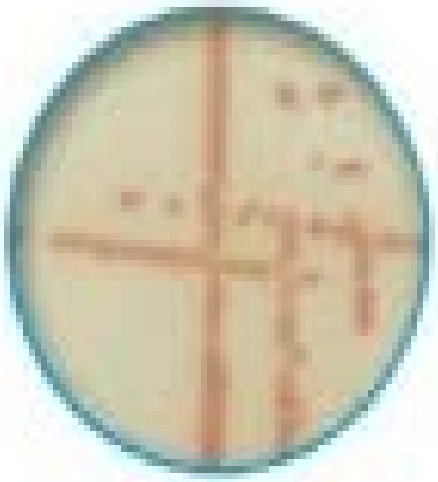
Incubation 18 - 24 h.

chromID™ ESBL

Direct identification of:

E. coli	pink to burgundy
KESC	green/blue to browny-green
Proteeae	dark to light brown

Confirmation of being an extended Spectrum ß-Lactamase-producer



Sensitivity and positive predictive value

Incubation		Number of isolates			Sensitivity[a]	PPV[b]
		True Positive (TP)	False Positive (FP)	False Negative (FN)		
24h	ChromID™ ESBL	29	46	4	**88%** ([72.3%; 95.3,%])	**38.7%** ([28.3% ; 50.2%]
	BLSE agar	28	156	5	85% ([68.7%; 93.5%])	15.4% ([10.1% ; 21.5%])
48h	ChromID™ ESBL	31	78	2	**94%** ([80.0%; 98.4%])	**28.4%** ([20.7% ; 37.7%])
	BLSE agar	28	220	5	85% ([68.7%; 93.5%])	11.3% ([7.9% ; 15.9%])

[a]: Sensitivity (TP/TP+FN); [b] PPV (TP/TP+FP)

(Réglier-Poupet H et al. 2008. J Med Microbiol. 57:310)

Evaluation of the two commercially available media

(**Glupczynski Y et al**. 2007. J Clin Microbiol; 45: 501; **Réglier-Poupet H et al**. 2008. J Med Microbiol. 57:310)

- 765 samples from 547 patients
  - rectal swabs (468),
  - urines (255),
  - pulmonary aspirations (42)

50 µl per plate

33

Species (no of isolates)	Classes of ESBL enzymes (N isolates)		
	CTX-M (17)	**TEM (10)**	**SHV (7)**
***E. coli* (16)**	CTX-M-1 (4), CTXM-15 (9)	TEM-3 (2)	SHV-2 (1)
K. pneumoniae* (8)	CTXM-15 (3)	TEM-24 (2)	SHV-5 (1), SHV-12 (3)
***C. koseri* (3)**		TEM-3 (3)	
***E. cloacae* (2)**			SHV-12 (2)
***C. freundii* (2)**		TEM-21 (2)	
***P. mirabilis* (1)**	CTX-M-1 (1)		
***E. aerogenes* (1)**		TEM-24 (1)	

* One *K. pneumoniae* isolate harboured two ESBLs

Various Enterobacterial species producing minor ESBLs (TLA-1, TLA-2, BES, VEB, PER, BEL, GES, OXA-18, KPC, …) grew well on both plates with the appropriate color on ChromID ESBL

(Réglier-Poupet H et al. 2008. J Med Microbiol. 57:310)

Strains requiring additional confirmations

	Bacterial species*	ChromID™ ESBL	BLSE agar
Overproduction of Chromosomal Enzymes	*Pseudomonas spp.*	13	73
	Enterobacter spp.	18	24
	Escherichia coli	8	13
	Morganella morganii		10
	Klebsiella spp	6	8
	Hafnia alvei	1	8
	Acinetobacter spp.		4
	Stenotrophomonas maltophilia		4
	Enterococcus faecalis		3
	Serratia marescens		2
	Other		5
	Total	46	154

(Réglier-Poupet H et al. 2008. J Med Microbiol. 57:310)

Take home message

- Detection of ESBLs is mandatory for patient treatment and outbreak prevention
- Limiting hospital-based outbreaks of ESBL producers will limit usage of carbapenems
- Phenotypic analysis mostly ok except for pandrug-resistant isolates
- Genotypic analysis remains the gold standard