

Extended-Spectrum β -Lactamases - An Emerging Worldwide Threat

Youri Glupczynski

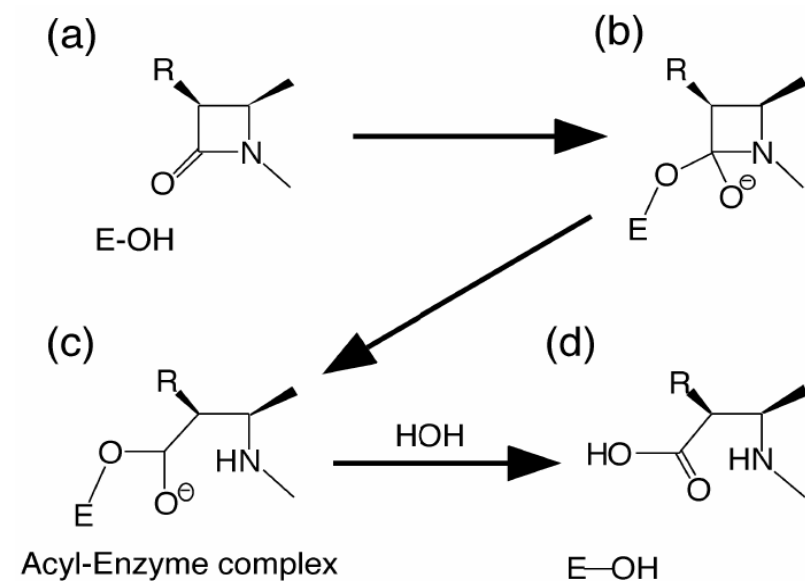
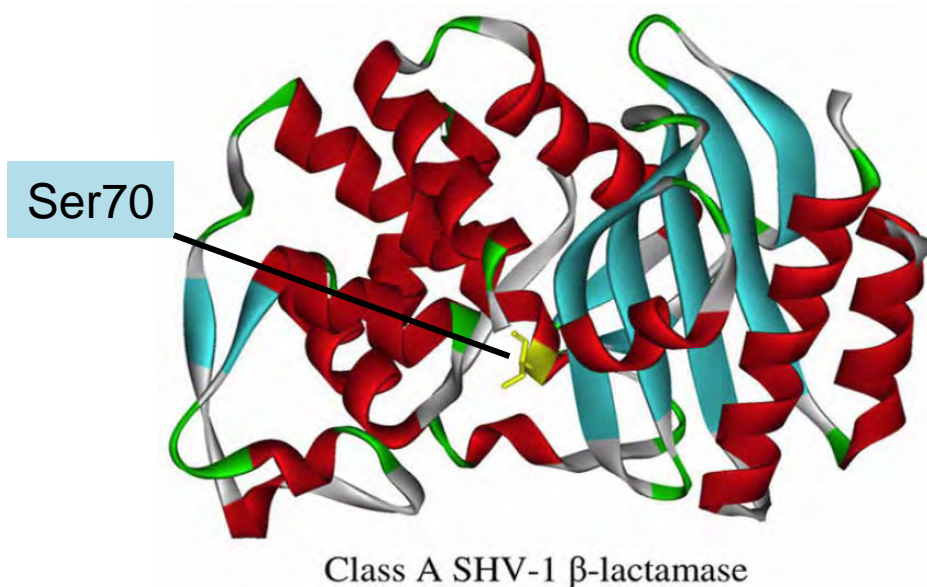
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β -lactamases

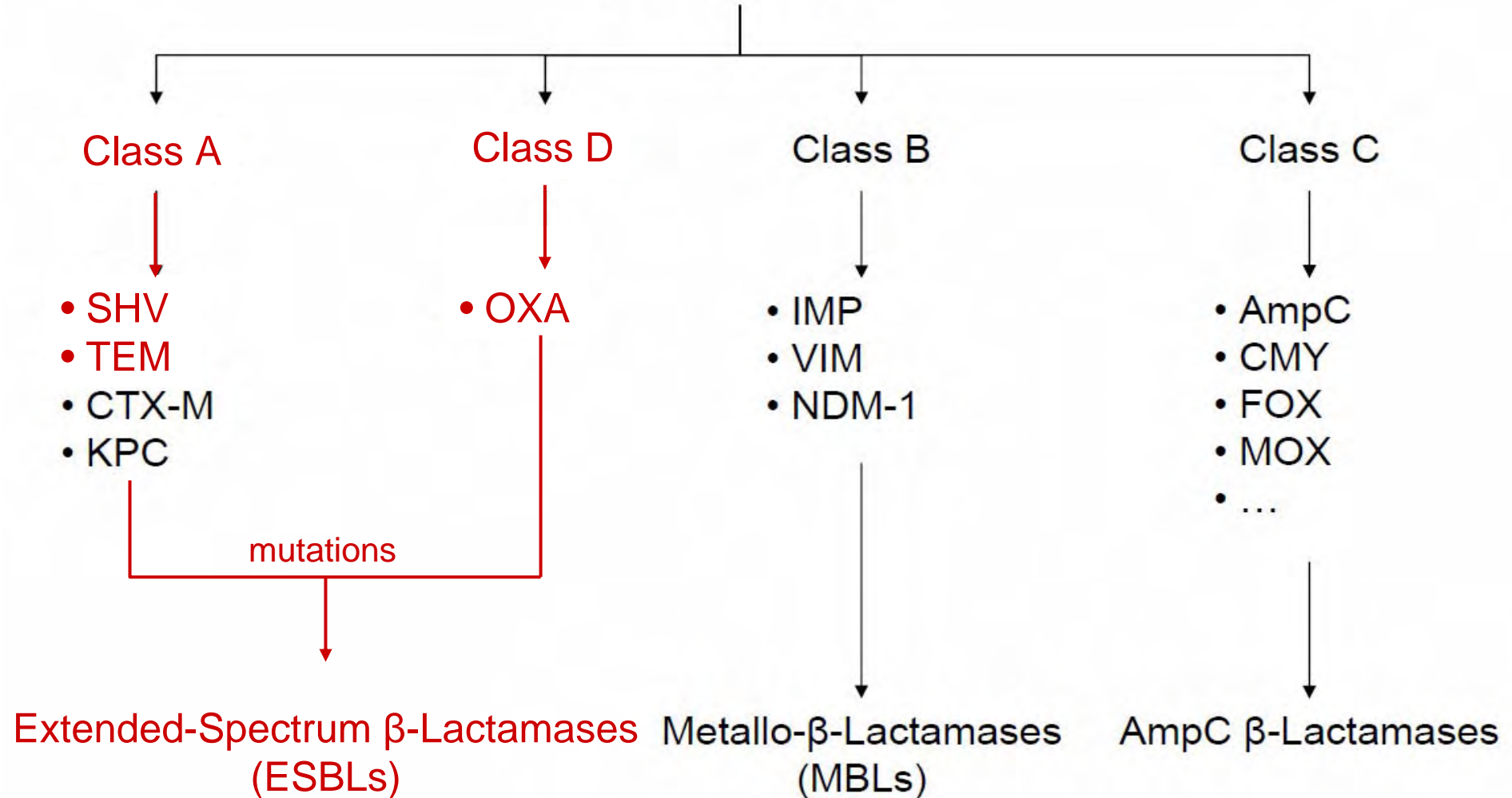
- Bacterial enzymes
- Inactivate β -lactam antibiotics
- Bind, acylate, and hydrolyze β -lactam ring
- H_2O molecule involved







Classification

β -Lactamases



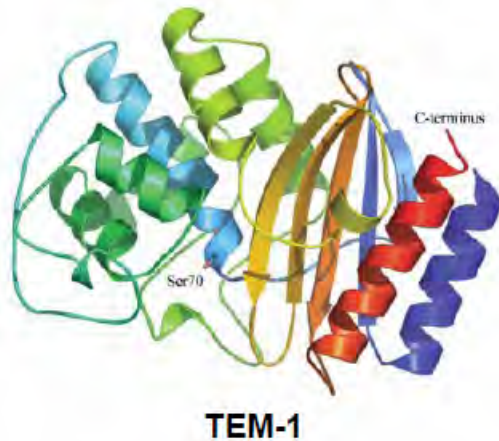


Extended-Spectrum β -Lactamases (ESBLs)

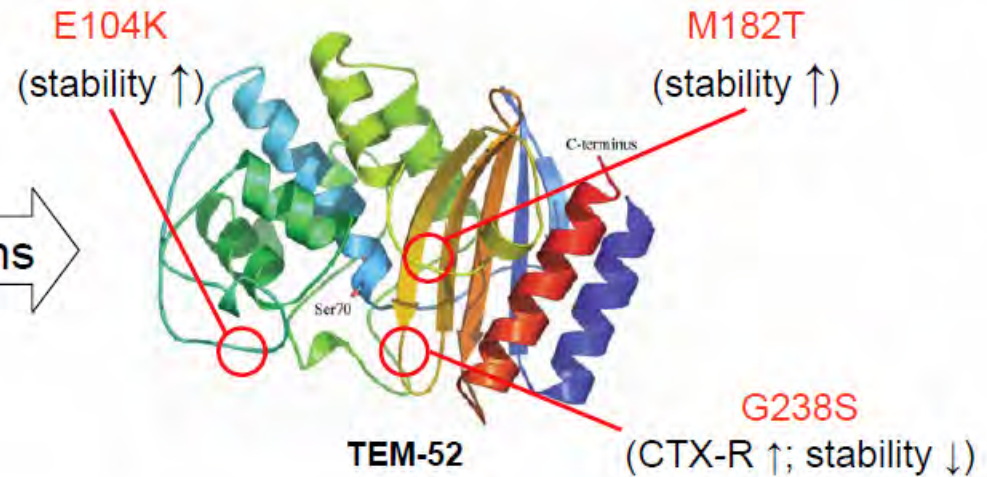


Spectrum

β-Lactamases



ESBLs



mutations

Spectrum:

- penicillins
- 1st generation cephalosporins
- 2nd generation cephalosporins

Extended Spectrum:

- penicillins
- 1st generation cephalosporins
- 2nd generation cephalosporins
- **3rd generation cephalosporins (e.g. cefotaxime)**
- **monobactams (e.g. aztreonam)**

Susceptible to cephamycins (cefotetan, cefoxitin), carbapenems (e.g. imipenem), and inhibitors

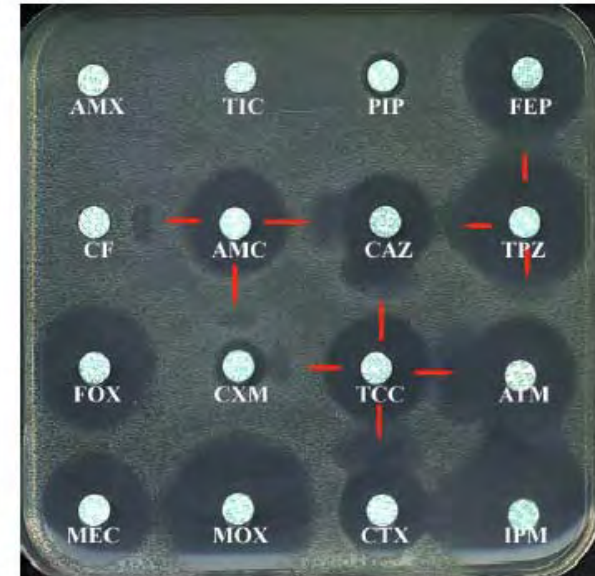
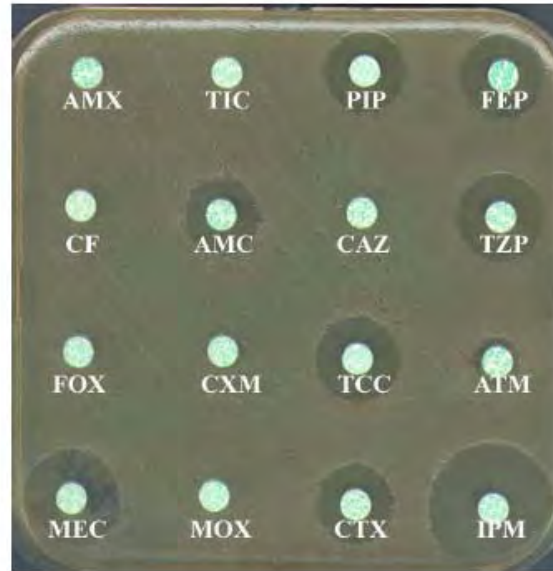
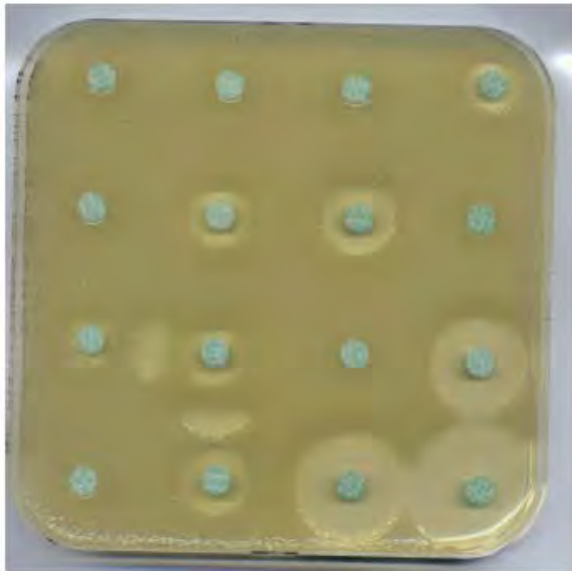
ESBL types and organisms

Class	Type	Nr of subtypes	Most common subtypes	Predominant organisms
A	TEM	183	TEM-3;-4;-24;-26;-52	<i>Enterobacteriaceae</i>
	SHV	134	SHV-2;-4;-5;-12	
	CTX-M	102	CTX-M-3;-9;-14;-15	
D	OXA	194 (18 ESBLs)	OXA-2;-10	<i>Pseudomonas aeruginosa</i>

ESBL-producing *Enterobacteriaceae*

Period	Most prevalent types	Predominant species
20 th century	SHV + TEM	<i>Klebsiella</i> spp. (<i>Enterobacter</i> spp)
21 th century	CTX-M	

Changing pattern of predominant β -lactamases over time



Années 90s

Années 2000

Klebsiella pneumoniae
Productrice de BLSE

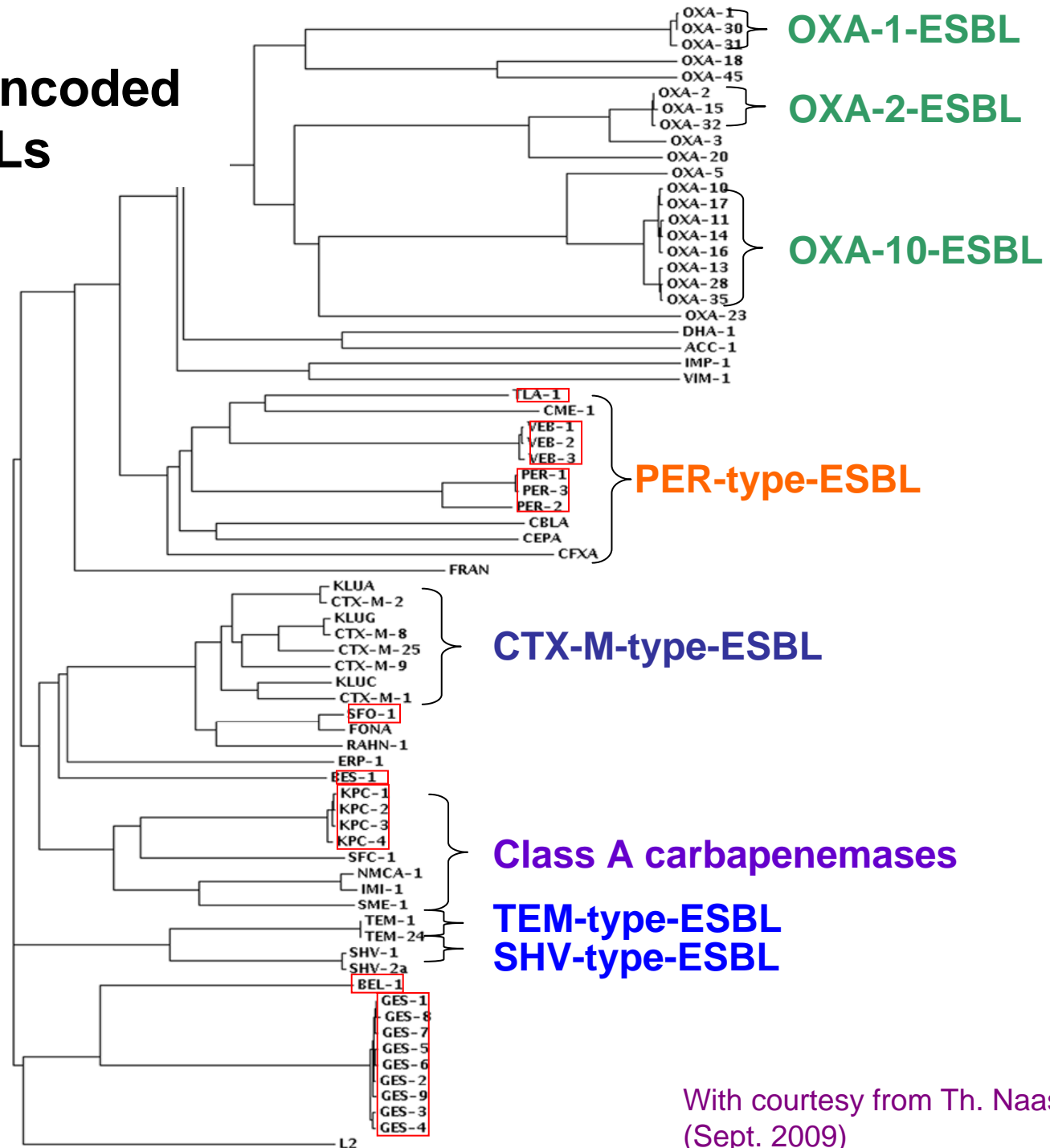
Enterobacter aerogenes
Hyper Producteur
de céphalosporinase
et de BLSE

Escherichia coli
producteur de BLSE

With courtesy from ML JOLY-GUILLOU
CHU d'Angers

Plasmid-encoded ESBLs

Different ESBL families



With courtesy from Th. Naas (Sept. 2009)

β -lactamases (>500) > 50% ESBLs



Plasmid encoded

- 1983 SHV-type (>130)
 - 1985 TEM-type (> 180)
 - 1989 CTX-M-type (> 100)
- } **“The oldies”**
- } **“ESBLs of growing importance”**

- 1988 SFO-1 **S**erratia **F**Onticola
 - 1991 TLA-1 **T**LAhuicas (indian tribe)
 - 1991 PER (7) **P**seudomonas **E**xtended **R**esistance
 - 1996 VEB (7) **V**ietnam **E**xtended-spectrum β -lactamase
 - 1996 BES-1 **B**razilian **E**xtended-**S**pectrum β -lactamase
 - 1998 GES (16) **G**uyana **E**xtended-**S**pectrum β -lactamases
 - 2005 BEL (2) **B**elgium **E**xtended-spectrum β -**L**actamase
 - 2005 TLA-2 ??? (Plasmid, waste water)
 - 1998 KPC (10) **K**lebsiella **p**neumoniae **C**arbapenemase
 - 1991 OXA-ESBL (18) (OXA-2, -10, -13 derivatives, OXA-18, OXA-45)
- } **“infrequent ESBLs”**
- } **“peculiar ESBLs”**

Chromosomally-encoded

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

Detection of ESBL production (1)

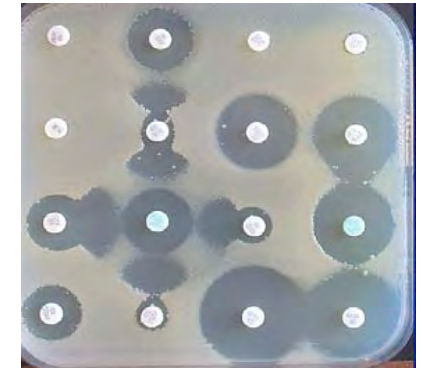
- Phenotypic:

- Direct screening of clinical samples:

- β -Lactamase Screening Agar (BLSE agar bi-plate)
- Chromogenic media (e.g. chromID ESBL)

- Screening:

- Disk diffusion
- Ceftazidime, cefotaxime, ceftriaxone, aztreonam



Detection of ESBL production (2)

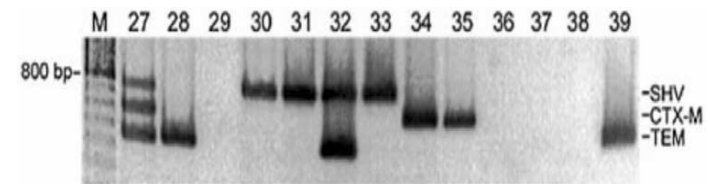
- Phenotypic:

- Confirmation :

- Cephalosporin (CTAX, CAZ)/clavulanate combination discs
 - Broth microdilution (MIC determination)
 - Cefepime/Clavulanate combination discs or CTAX/CAZ combination discs on cloxacillin (250 mg/L) containing media

- Genotypic:

- PCR detection of *bla* genes (+ sequencing)
 - Multiplex PCR
 - ESBL DNA Micro-array (Checkpoints)



Multiplex PCR

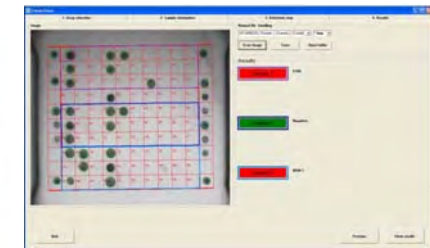


Cephalosporin-clavulanate combination discs
Cephalosporin-clavulanate combination discs



Carbapenemase	NDM-1 and KPC
ESBL	CTX-M, TEM, SHV
AmpC	CMY, DHA, FOX, MOX, ACC, MIR, ACT

ESBL DNA Micro-array (Check-Points)



Typing of ESBL producers

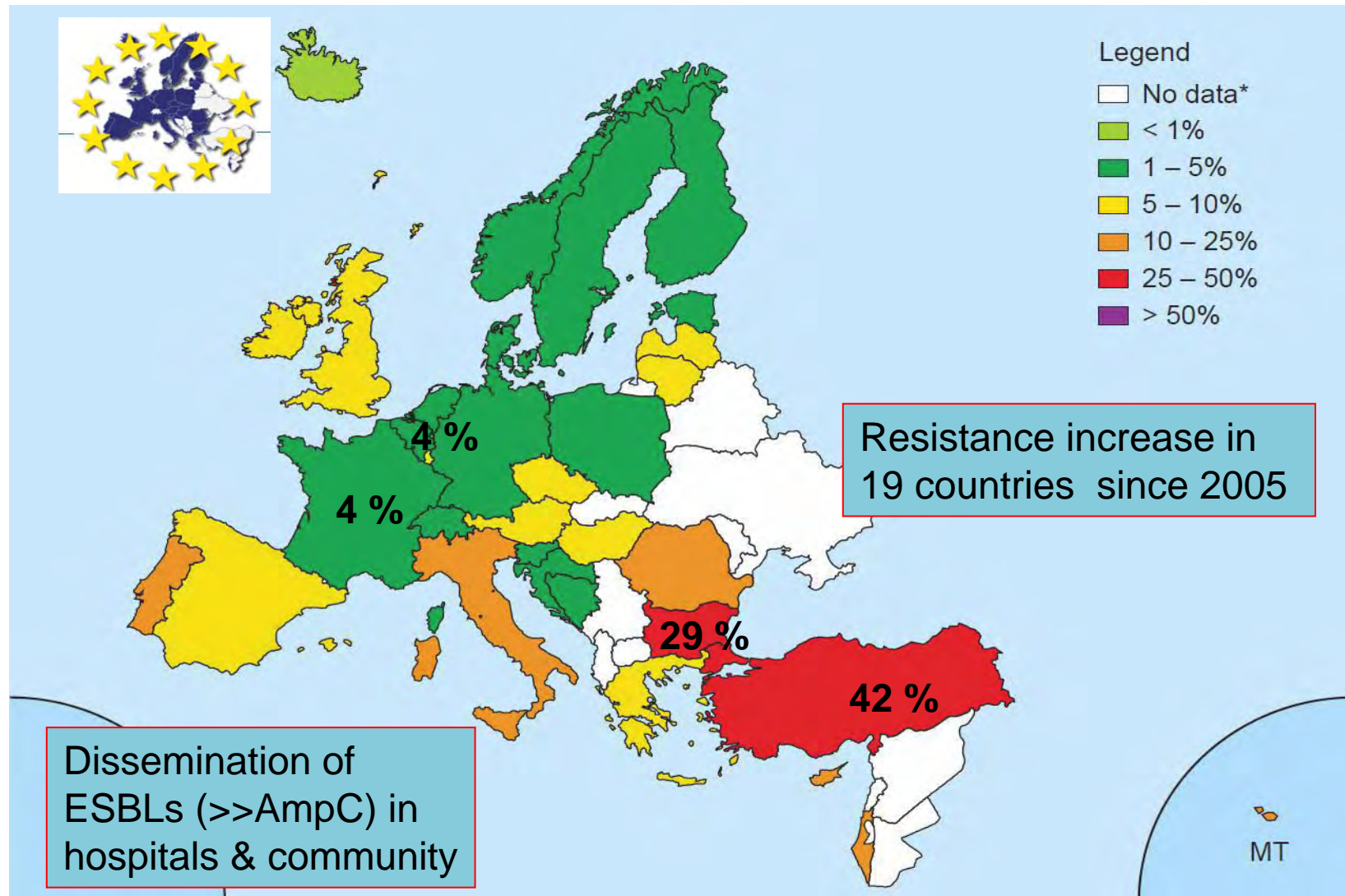
- **PFGE:**
 - Pulsed field gel electrophoresis
 - Restriction of genome with rare-cutters (e.g. *XbaI*)
 - Gold standard to study genetic background
- **MLST:**
 - Multi locus sequence typing
 - Sequence 7 housekeeping genes
 - *adhA, fumC, gyrB, icd, mdh, purA, recA*
 - Widely used, unambiguous databases
- **mMLST (mini-MLST):**
 - Sequence more variable regions in only 3 genes
 - Allows quick detection during outbreak
- **Serotyping**

Treatment option for ESBL-producing *Enterobacteriaceae*

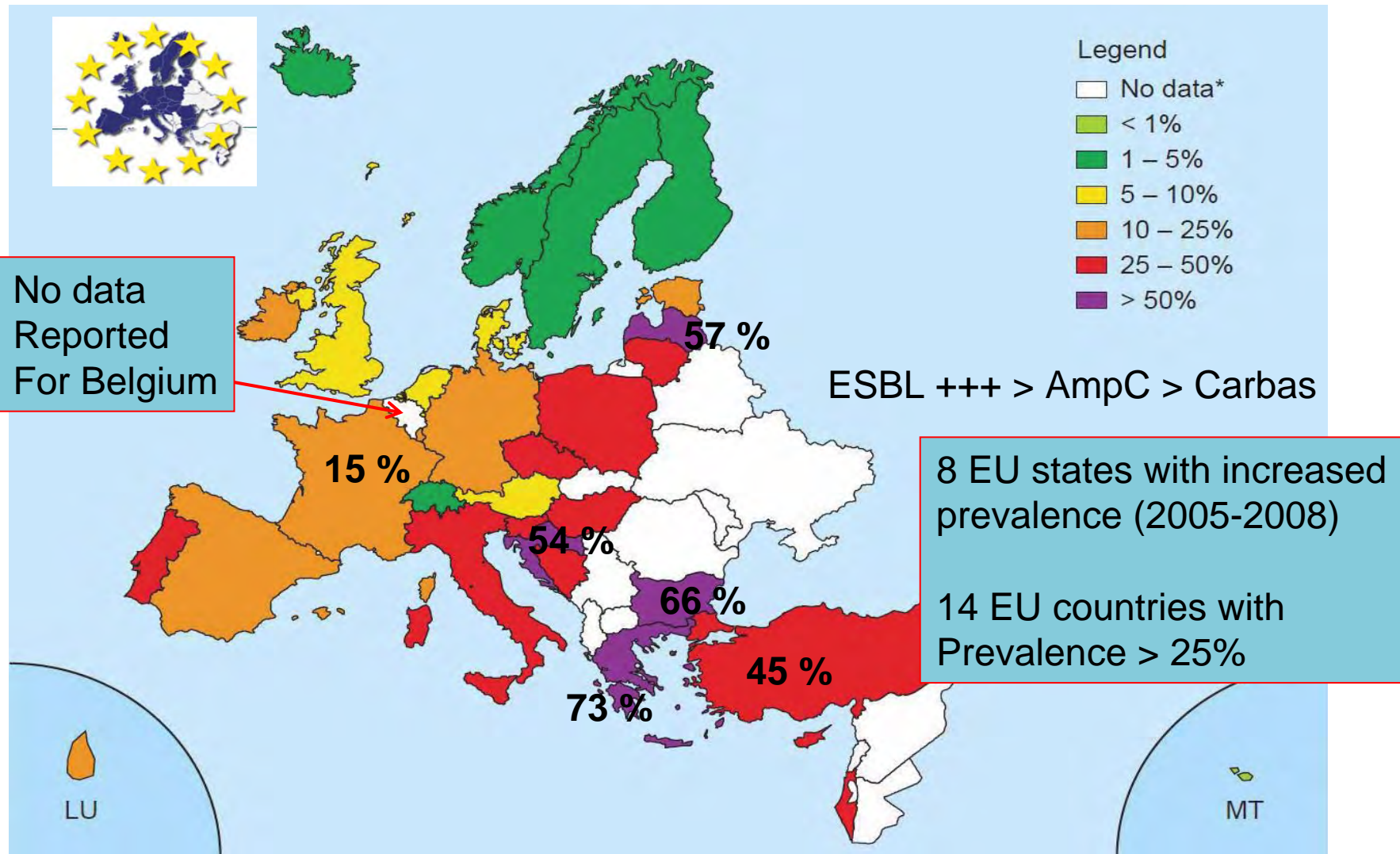
- **Carbapenems** widely regarded as the drugs of choice for the treatment of severe infections due to ESBL-producing *Enterobacteriaceae*
- **Nitrofurantoin and fosfomycin** are alternative options for the treatment of uncomplicated UTIs

Type of infection	Origin	First-line therapy	Alternative therapy
Systemic (primary bacteraemia, pneumonia, intra-abdominal sepsis, complicated UTI)	Community-onset	Ertapenem	Amikacin
Systemic (primary bacteraemia, pneumonia, intra-abdominal sepsis, complicated UTI)	Nosocomial	Imipenem/cilastatin or meropenem	Amikacin
Uncomplicated UTI	Community-onset	Fosfomycin	Nitrofurantoin, amoxicillin/clavulanate
Uncomplicated UTI	Nosocomial	Fosfomycin	Nitrofurantoin, amoxicillin/clavulanate

Proportion of Invasive *E. coli* Isolates Resistant to 3rd Generation Cephalosporins in 2008



Proportion of Invasive *K. pneumoniae* Isolates Resistant to 3rd Generation Cephalosporins in 2008



Massive emergence of ESBLs in countries with low socioeconomic income

- Multidrug-resistant *Enterobacteriaceae* in blood culture isolates of children in Ghana:

Organisms	N° of Isolates	Resistance percentages:					
		Ampicillin	Cotrimoxazole	Chloramphenicol	Tetracycline	Ciprofloxacin	Ceftriaxone
non-typhoid <i>Salmonella enterica</i>	113	80.5	82.3%	83.2%	10.6%	0%	0%
<i>Salmonella</i> Typhi	39	69.2%	60.0%	66.7%	60.0%	0%	0%
<i>Escherichia coli</i>	15	100%	93.3%	53.3%	86.7%	26.7%	-
<i>Klebsiella spp.</i>	9	100%	11.1%	77.8%	100%	33.3%	-
<i>Shigella boydii</i>	1	100%	100%	100%	100%	0%	0%
<i>Enterobacter spp.</i>	1	100%	100%	100%	100%	0%	100%

- *Escherichia coli*: 20.0% ESBL-producers
- *Klebsiella spp.*: 77.8% ESBL-producers

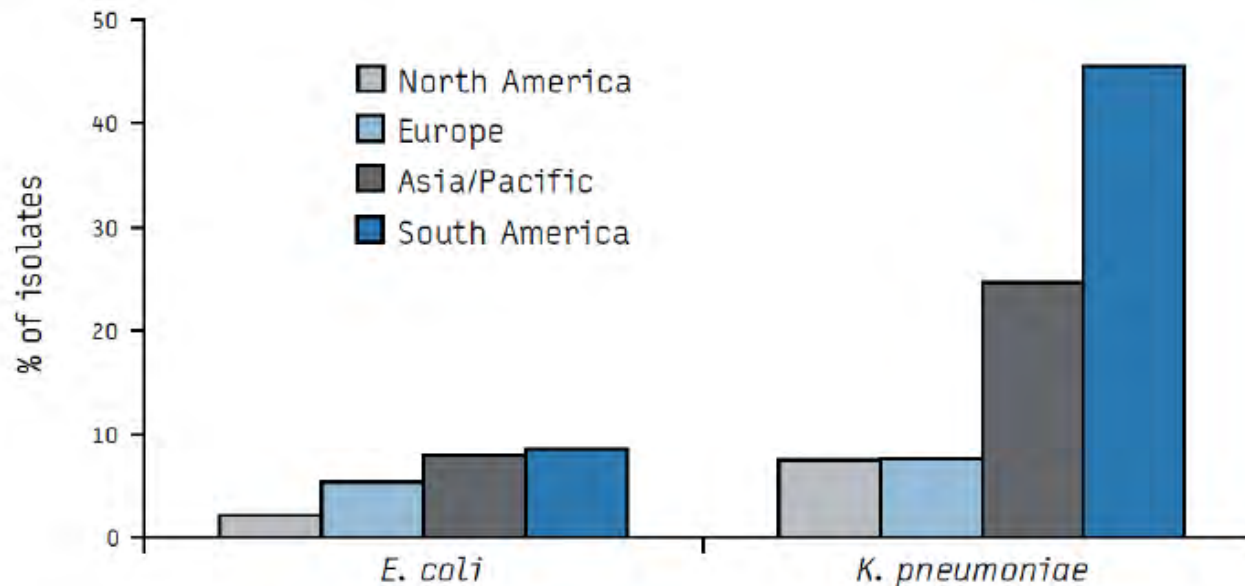
→ Massive emergence of ESBL producers indicates a serious medical and economic burden for African healthcare systems

Epidemiology: Global

INCREASING PREVALENCE OF ESBL-PRODUCING ENTEROBACTERIACEAE IN EUROPE

T M Coque (mcoque.hrc@salud.madrid.org)^{1,2}, F Baquero^{1,2}, R Canton^{1,2}

Frequency of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates reported in the TEST surveillance study (2004-2006) in different geographic areas [27]



ESBL: extended-spectrum beta-lactamases; TEST: Tigecycline Evaluation and Surveillance Trial.

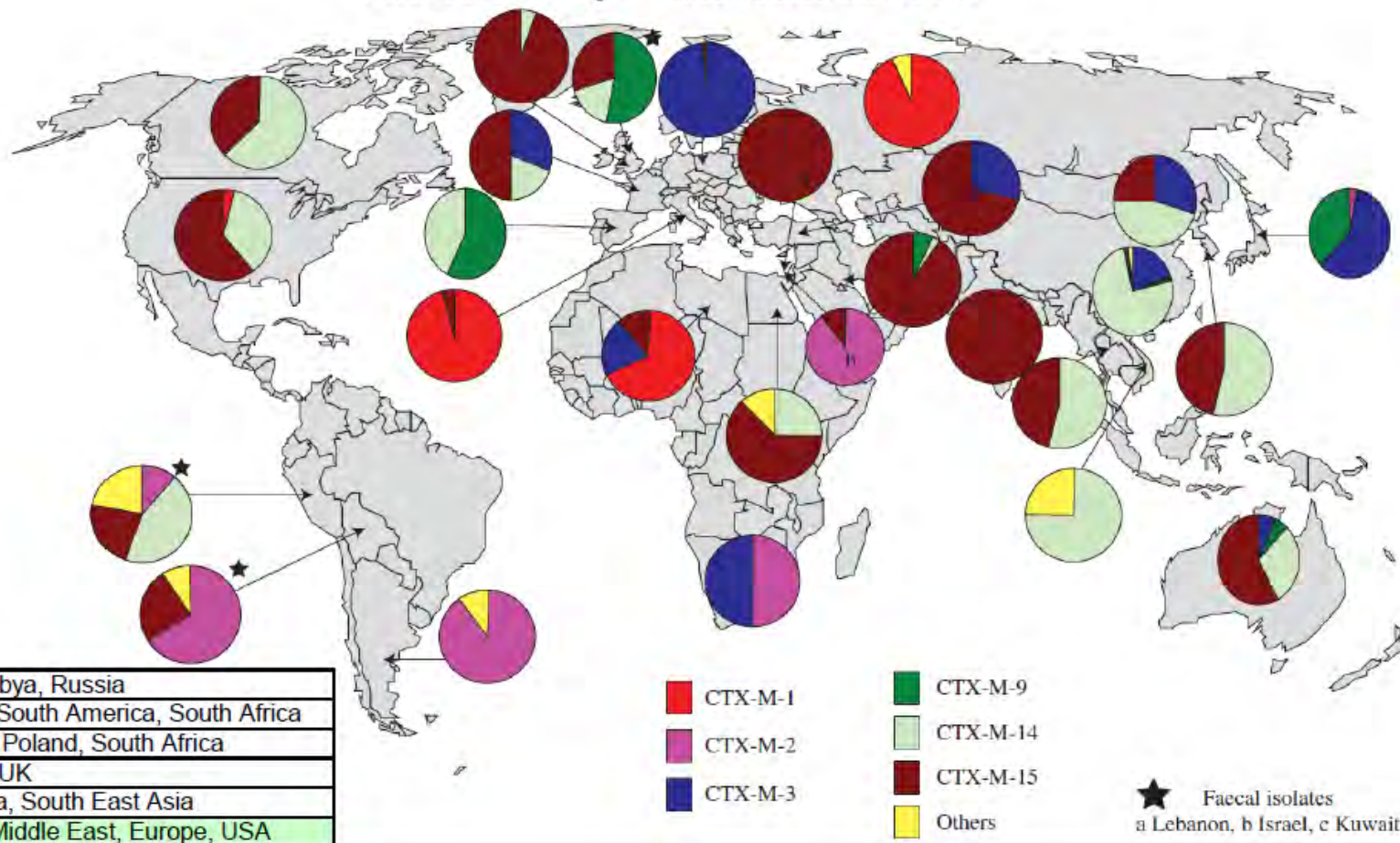
Epidemiology of CTX-M β -Lactamases

Journal of Antimicrobial Chemotherapy (2009) **64**, Suppl. 1, i3–i10

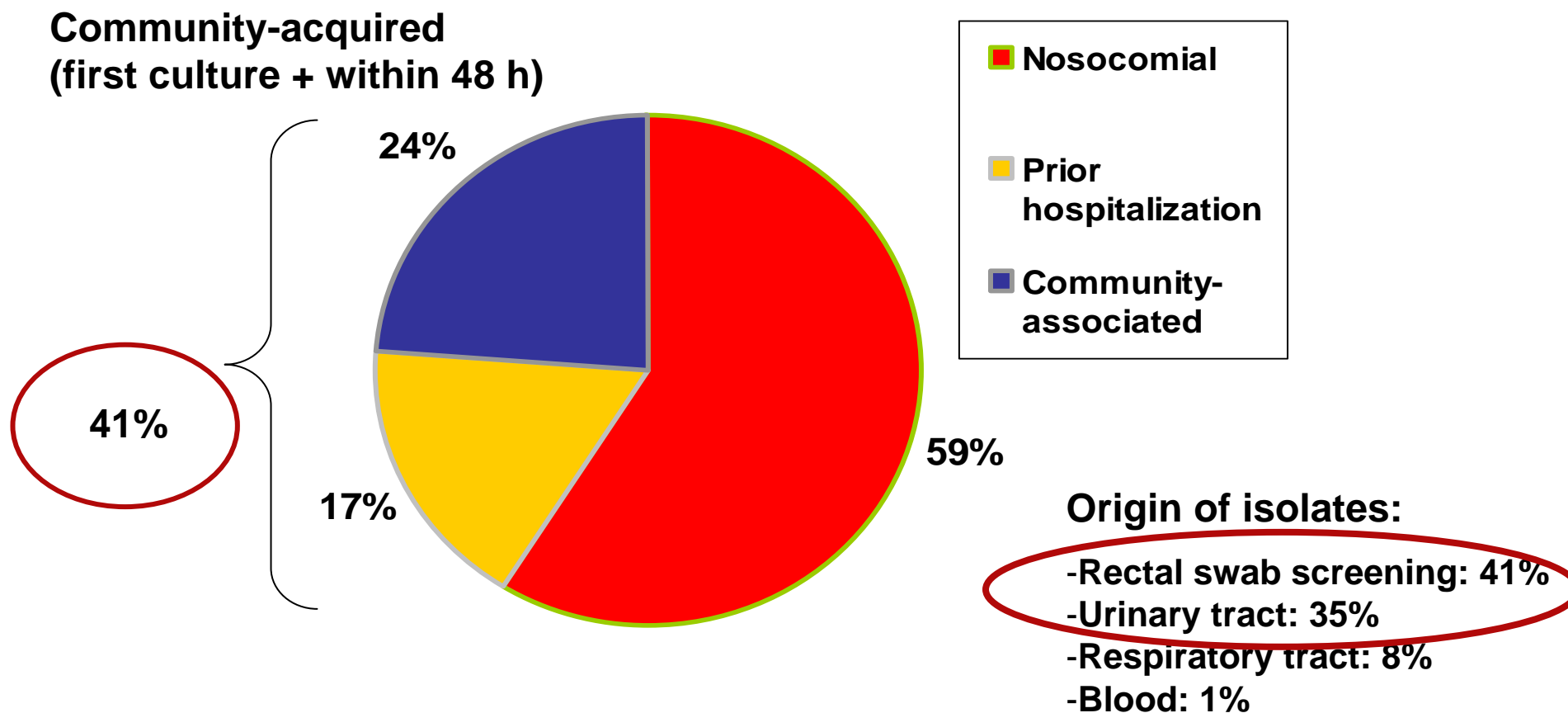
doi:10.1093/jac/dkp256

The changing epidemiology of resistance

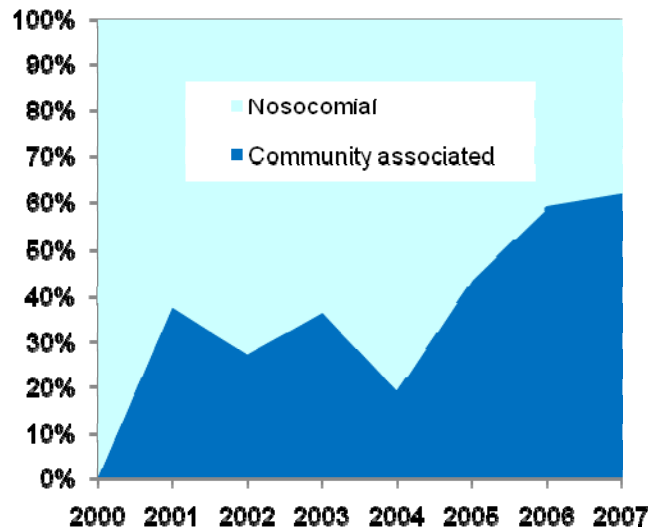
Peter M. Hawkey^{1,2*} and Annie M. Jones³



Community-associated emergence of CTX-M ESBL-producing *E. coli*



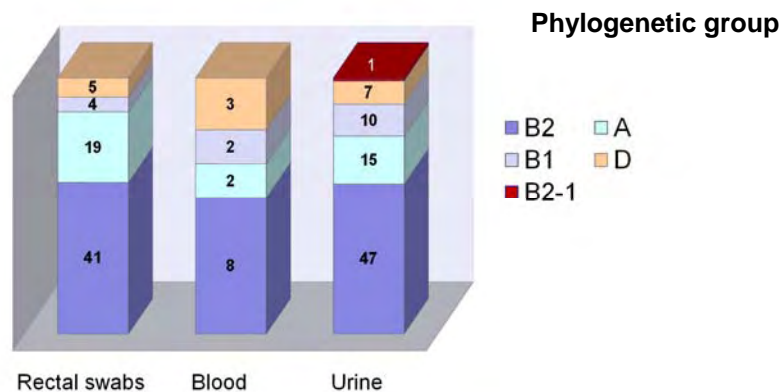
Increase of CTX-M enzymes among ESBL producing *E.coli*. Erasme Hospital - ULB 2000-2007



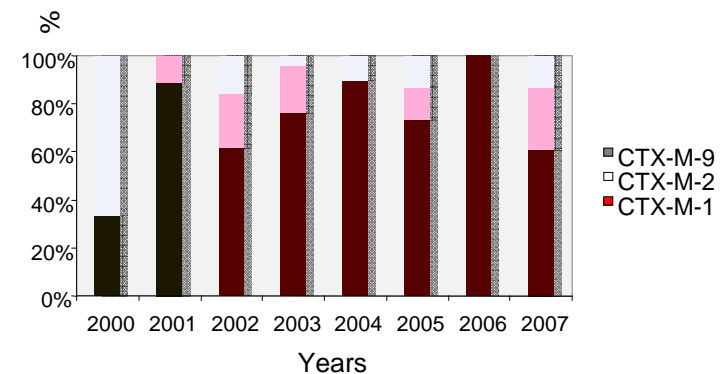
Annual trend in proportion of CTX-M in ESBL-producing *E. coli* strains , Erasme Hospital, 2000-2007

- Increase:
- proportion of *E. coli*-BLSE isolates
 - 0,92 % in 2000 → 8% in 2007
- proportion of *E. coli*-BLSE in Blood cultures.
 - 1,2 % 2000 → 7,5% 2007
- proportion of CTX-M.

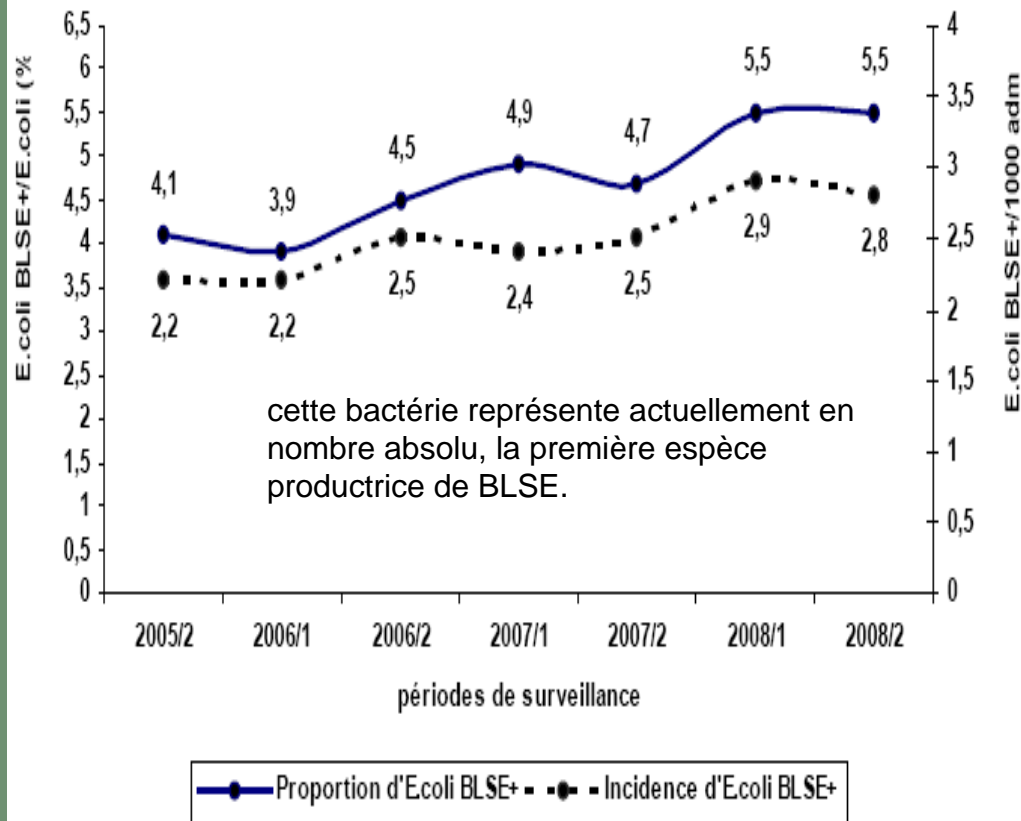
Phylogenetic group distribution in CTX-M producing *E. coli* strains by anatomic source of specimen



Distribution of CTX-M groups in *E. coli* strains by year



evolution of mean proportion and incidence rates of ESBL+ *E. coli*



Source: NSIH Survey Belgium National surveillance, B. Jans et al.

PROPORTION OF ESBL-PRODUCING ISOLATES BY SPECIES IN BELGIAN ICU'S

5% in 2003

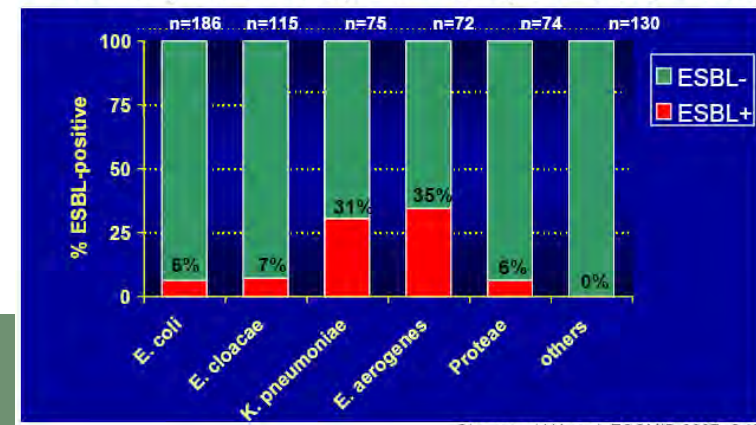
Glupczynski et al ICAAC 2003

Enterobacteriaceae (total: 561 isolates; 20 labs)	% of ESBL in screened isolates*	% of ESBL among total isolates	% of all ESBL
<i>E. aerogenes</i> (140)	76 (54.3)	76/180 (42.2)	84.4
<i>K. pneumoniae</i> (52)	7 (13.5)	7/112 (6.3)	7.8
<i>E. coli</i> (177)	3 (1.7)	3/367 (0.8)	3.3
<i>K. oxytoca</i> (35)	1 (2.9)	1/91 (1.1)	1.1
<i>E. cloacae</i> (75)	1 (1.3)	1/146 (0.7)	1.1
<i>Citrobacter</i> spp. (11)	1 (9.1)	1/22 (4.5)	1.1
<i>Providencia</i> spp. (7)	1 (14.2)	1/11	1.1
<i>S. marcescens</i> (36)	0	0/71	-
<i>M. morgani</i> (27)	0	0/56	-
Total	90/561 (16.0)	90/1703 (5.3)	

11% in 2007

Glupczynski et al ECCMID 2007

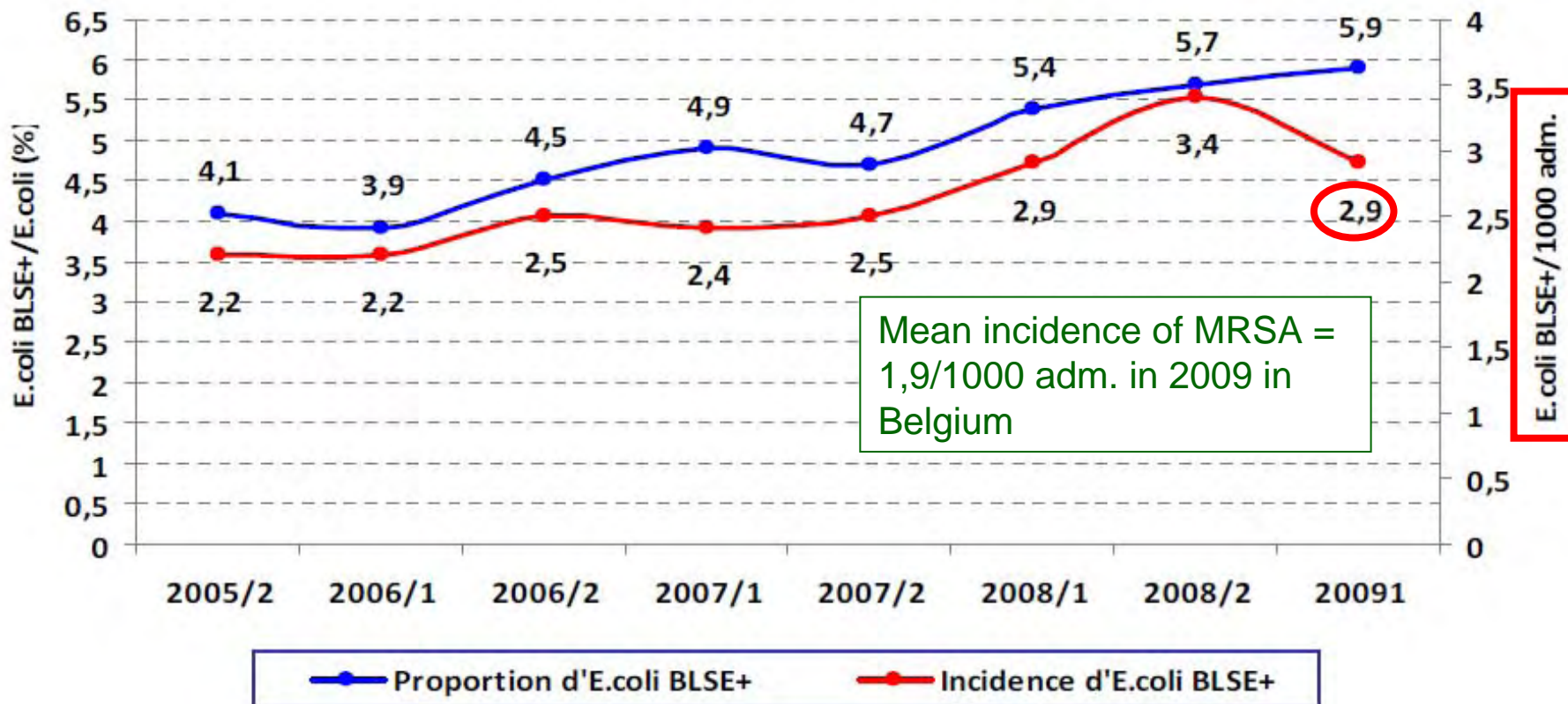
N=75 ESBL-positive isolates (Mean: 11.5% by centre; range: 3-29%)



Glupczynski Y et al ECCMID 2007, 0439

Increase of ESBL in *E. coli*, decrease in *E. aerogenes*

Surveillance of ESBL-producing *Escherichia coli* in Belgian hospitals



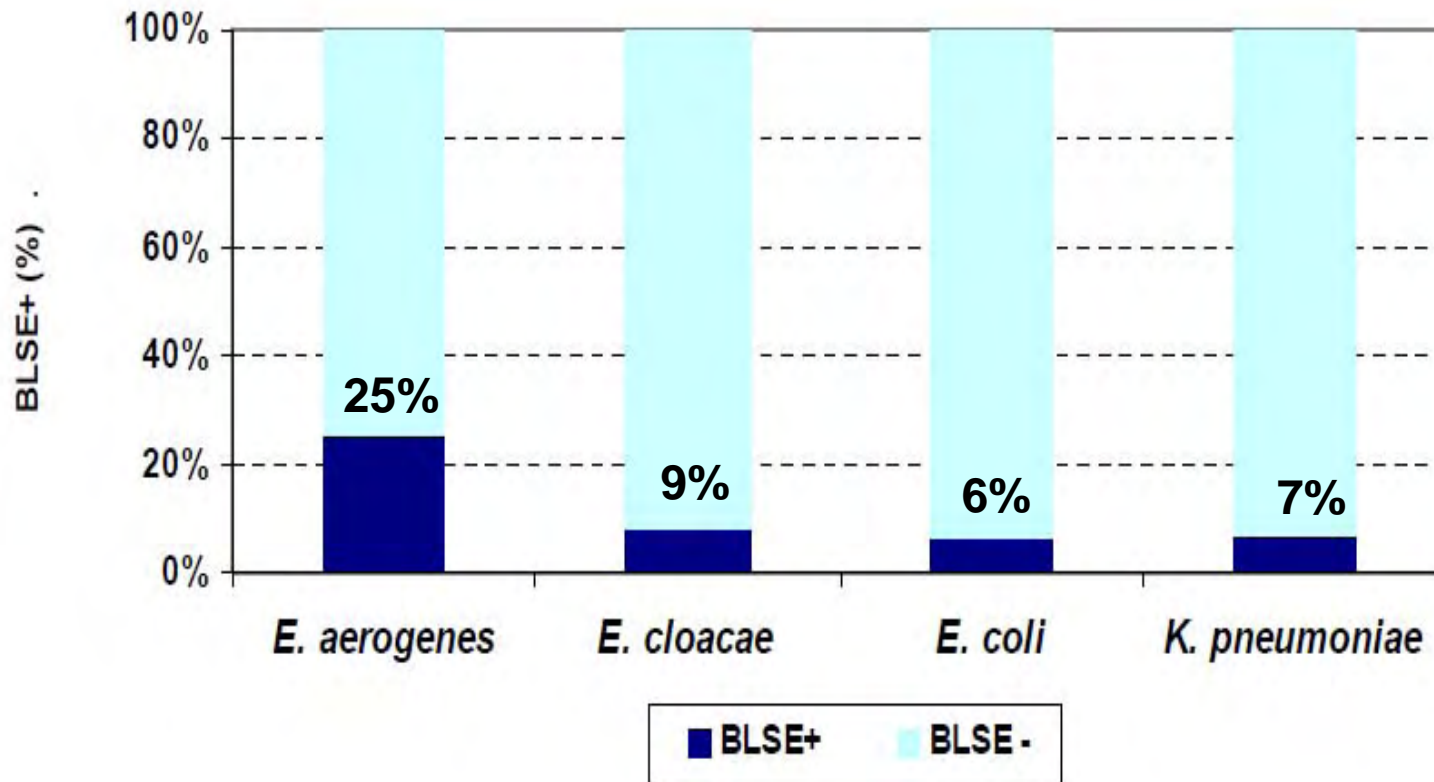
70% of ESBL+ *E. coli* isolates from urine

6-7 % from blood culture

No difference according to hospital size (nr bed), duration of stay, region

www.nshih.be
report 2009/1

Surveillance of ESBL-producing *Escherichia coli* in Belgian hospitals



E. coli (58% of all Enterobacteriaceae) -> first ESBL-producing Enterobacteriaceae in absolute number

Analysis of ESBL-producing *E.coli* in Belgium: a multicentre surveillance study

■ Regional distribution:

- 46% from Flanders, 28% from Wallonia and 26% from Brussels

■ Patients admission wards:

- Medicine 37% Surgery, 20%
- ICU, 13% Geriatric, 10%
- Oncology, 3%; Other hospital wards 3%
- Outpatients, 14%

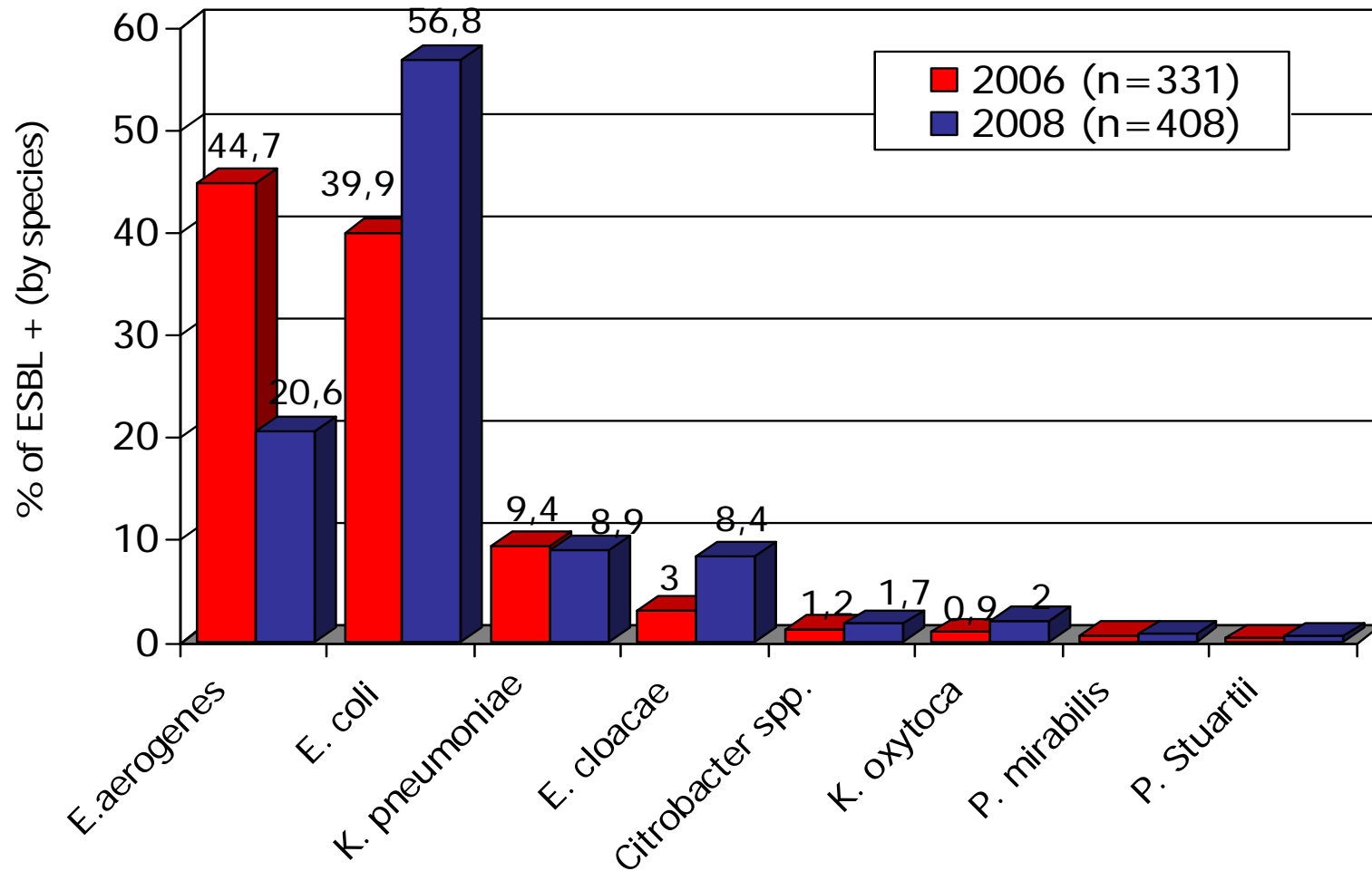
■ Anatomic source of specimen:

- **Urine, 79%**; Wounds, 12%; Respiratory tract, 6%; Blood, 3%.

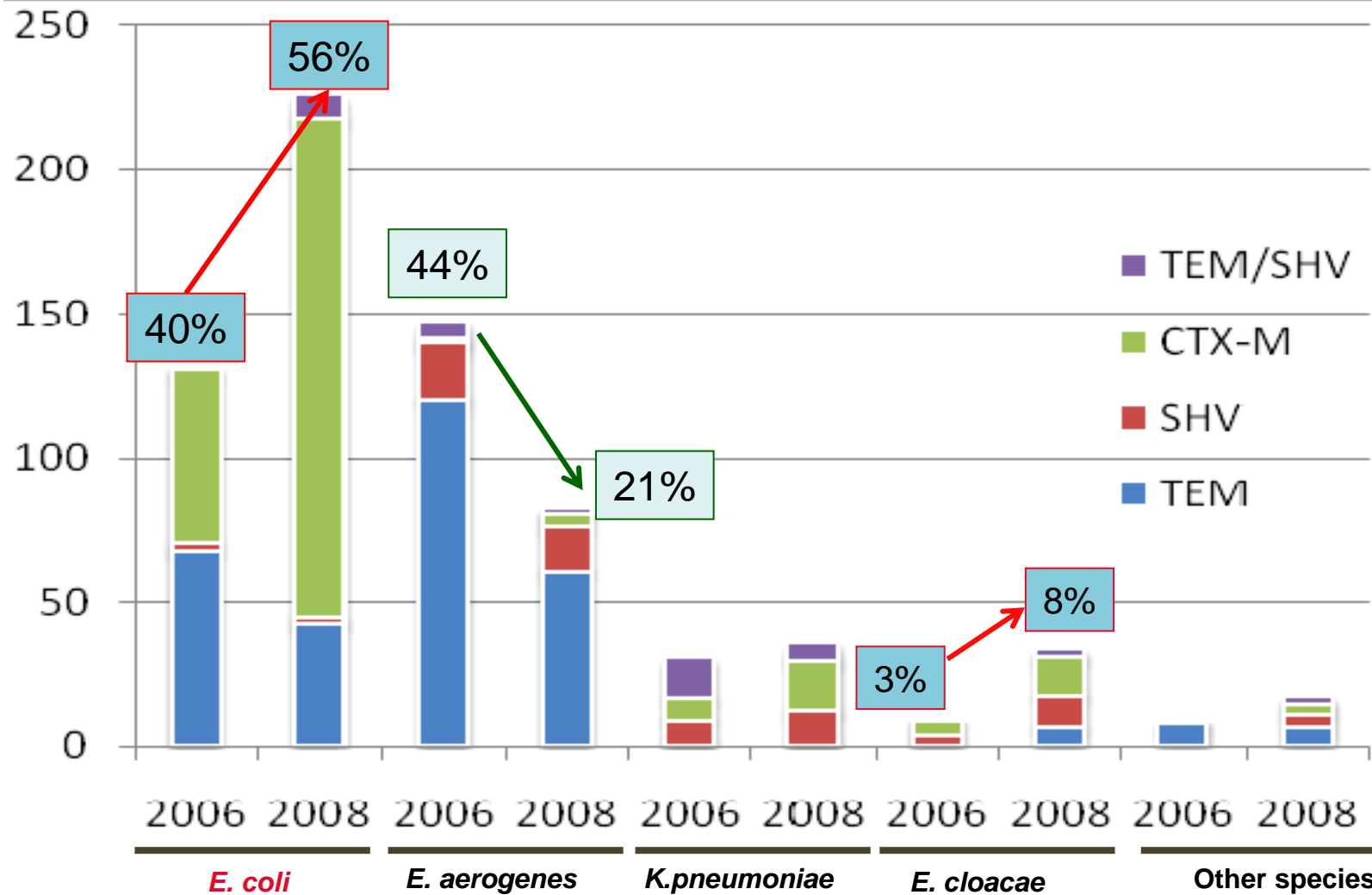
■ Probable origin of acquisition of the isolates:

- nosocomial, 45%; **community-associated, 39%**; ND 15%

Distribution of ESBL-producing *Enterobacteriaceae* in Belgian hospitals (2006-2008)

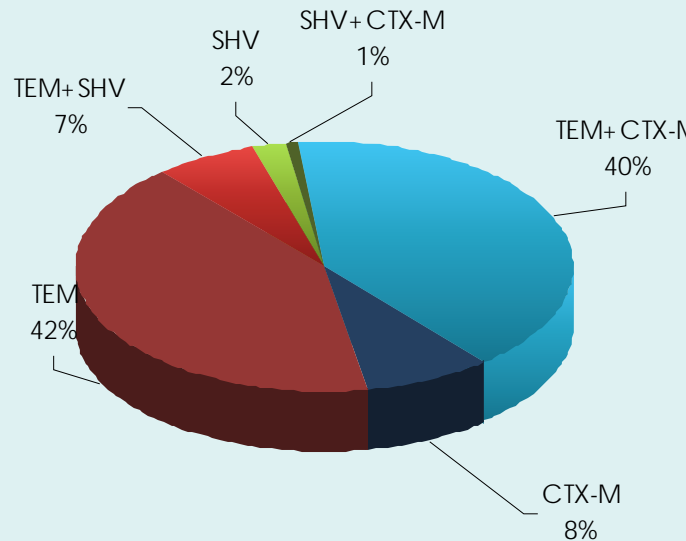


Evolution of species distribution and ESBL gene family by species (n=732 isolates) Belgium 2000-2008

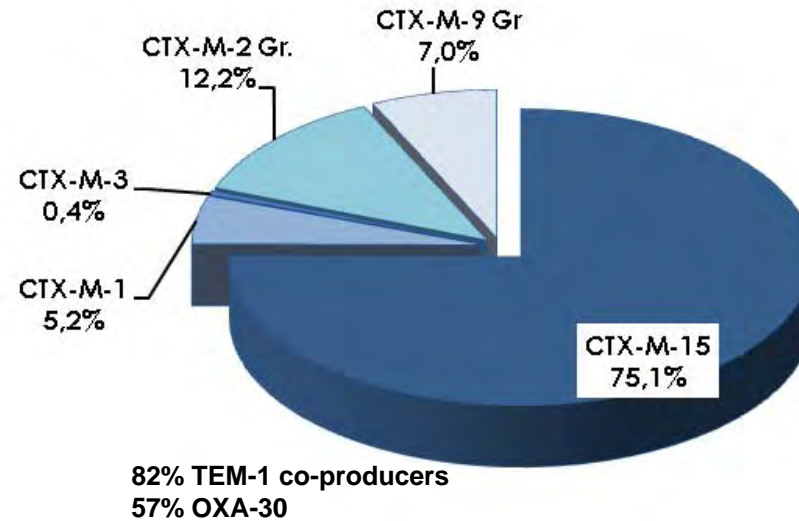


Analysis of ESBL-producing *E.coli* in Belgium: a multicentre surveillance study (2006-8)

Distribution of *E. coli* isolates by ESBL family



Distribution of CTX-M genes



Espèce (Nr isolats)	TEM	SHV	CTX-M	CTX-M/SHV ou CTX-M/TEM
<i>E. coli</i> (283)	TEM-52 (39), TEM-24 (2) TEM-19 (1)	SHV-4/-5/-12 (6) SHV-2a (3)	CTX-M-1 (12) CTX-M-15 (167) CTX-M-2 (26) CTX-M-3 (1) CTX-M-9 (15) CTX-M-16 (1)	CTX-M-1/15 + TEM-11 (3) CTX-M-15 + TEM-52 (2) CTX-M-2 + TEM-11 (1) CTX-M-2- + TEM-24 (1) CTX-M-9 + SHV-12 (1)

In Belgium like in other European countries, *bla*_{CTX-M} are present in half of ESBL-producing *E. coli* isolates and appeared present on admission in a third of cases.

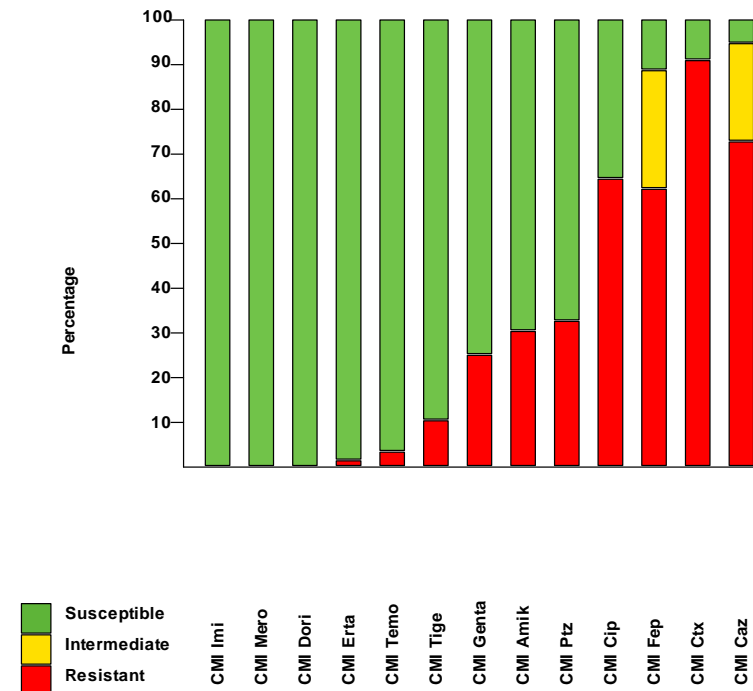
Distribution of ESBL types found in *Enterobacteriaceae* in Belgium 2008 (n=402 isolates, 91 hospitals)

Species (Nr isolates)	Classes of ESBL enzymes ^a			
	TEM	SHV	CTX-M	CTX-M/SHV or CTX-M/TEM
<i>E. coli</i> (226)	TEM-52 (39), TEM-24 (2) TEM-19 (1)	SHV-4/-5/-12 (6) SHV-2a (3)	CTX-M-1/15 (134) CTX-M-2 (18) CTX-M-9 (13)	CTX-M-1/15 + TEM-11 (3) CTX-M-15 + TEM-52 (2) CTX-M-2 + TEM-11 (1) CTX-M-2 + TEM-24 (1) CTX-M-9 + SHV-12 (1)
<i>E. aerogenes</i> (84)	TEM-24 (61)	SHV-4 (20) SHV-5/-12 (1)	CTX-M-1/-15 (2)	
<i>K. pneumoniae</i> (36)	TEM-5 (1) TEM-24 (1)	SHV-4 (13) SHV-2a (2) SHV-31 (1)	CTX-M-1/15 (13) CTX-M-9 (3)	CTX-M-1/-15 + SHV-2a (1) CTX-M-1/-15 + TEM-5 / SHV-2a (1)
<i>E. cloacae</i> (34)	TEM-24 (6)	SHV-5/-12 (8)	CTX-M-9 (3) CTX-M-1/15 (1)	CTX-M-9 + SHV-12 (15)
<i>K. oxytoca</i> (8)	TEM-3 (2) TEM-5/-7 (1) TEM-24 (1)	SHV-5/-12 (4)		
<i>Citrobacter</i> spp. (7)	TEM-24 (2)	SHV-5-12 (3)	CTX-M-15 (1)	CTX-M-9 + SHV-12 (1)
<i>P. mirabilis</i> (3)	TEM-24 (1)		CTX-M-15 (1) CTX-M-2 (1)	
<i>P. stuartii</i> (2)	TEM-24 (2)			
<i>Salmonella</i> spp. (1)	TEM-52 (1°)			

CTX-M > 50% (CTX-M15 >> CTX-M-2, CTX-M-9);
variation of prevalent ESBL types by species,
combination of several ESBLs

Percentage of CTX-M *E.coli* isolates inhibited by 13 antimicrobials agents S/I/R categorization by EUCAST breakpoints

Compound	<i>E. coli</i> (n=132)		
	Range of MICs	MIC ₅₀ /MIC ₉₀	% susceptible
Piperacillin/tazobactam	0.25->256	2/32	67.4
Cefotaxime	0.06->256	256/>256	9.1
Ceftazidime	0.5->256	16/>256	5.3
Cefepime	0.25->256	16/256	11.4
Temocillin	0.5-64	8/16	96.6
Imipenem	0.125-0.5	0.25/0.25	100
Meropenem	0.008-0.25	0.03/0.06	100
Ertapenem	0.008-2	0.03/0.25	98.6
Doripenem	0.008-0.125	0.03/0.06	100
Ciprofloxacin	0.004->32	>32/>32	35.6 (22.8)
Tigecycline	0.125-2	0.5/2	89.6
Amikacin	1->256	4/16	69.7
Gentamicin	0.5->256	1/64	75



70-80 % of isolates are co-resistant to ciprofloxacin and to cotrimoxazole

Co-resistance rates of ESBL-producing *Enterobacteriaceae* by species (Belgium 2009)

E-test MICs (EUCAST breakpoints)

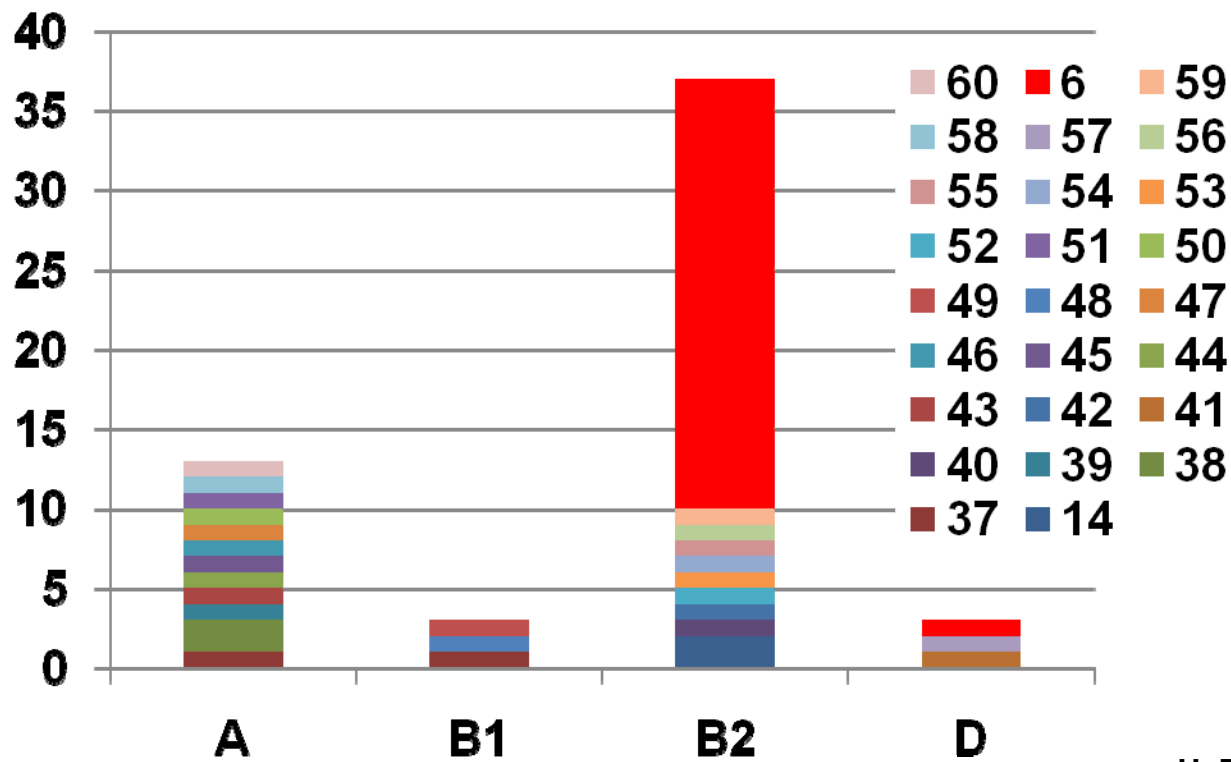
Agent	Co-resistance rates (I+R%)			
	<i>E. coli</i> (n=226)	<i>E. aerogenes</i> (n=84)	<i>E. cloacae</i> (n=34)	<i>K. pneumoniae</i> (n=36)
Amikacin	15.9	81.0	55.8	41.7
Gentamicin	6.5	2.4	52.9	38.9
Ciprofloxacin	73.5	97.6	67.6	75.0
Cotrimoxazole	60.2	94.1	67.7	80.5
Pipera/tazo	13.3	39.3	29.4	31.0
Cefepime	42.0	4.8*	8.8	36.1
Meropenem	0.0	2.4	0.0	0.0
Temocillin	3.5	13.1	17.6	8.3
Tigecycline	14.6	89.3	70.6	66.7

Distribution of PFGE types among phylogenetic group of CTX-M

26 PFGE types: major PFGE type 6

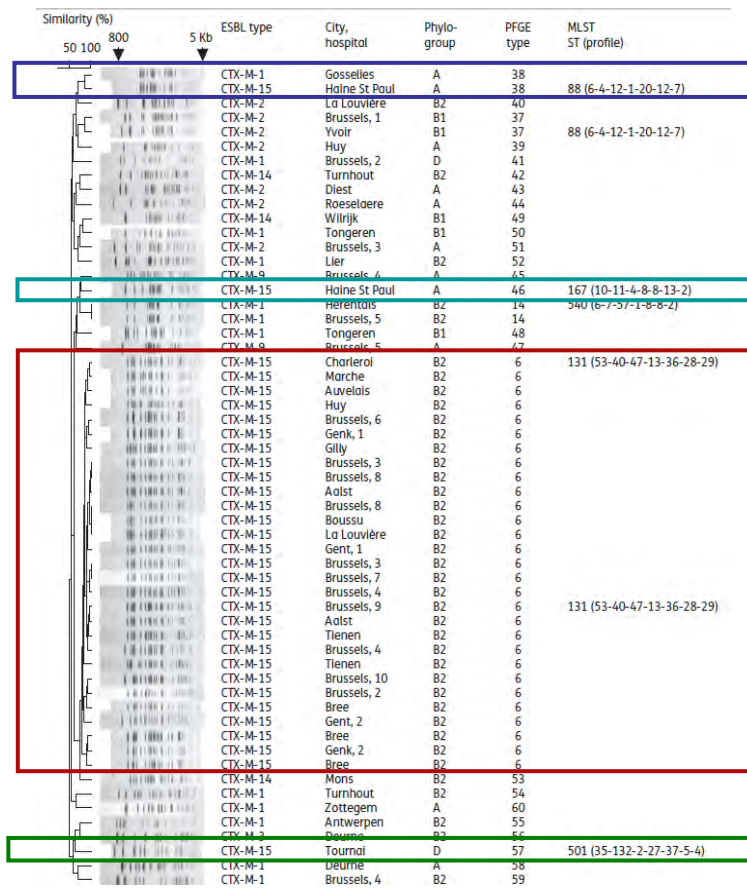
Phylogroup: 21% A, 5% B1, 67% B2, 7% D

Number of strains



H. Rodriguez et al. JAC 2010

Geographical distribution of CTX-M-15 *E.coli* clones in Belgium, 2006-2008



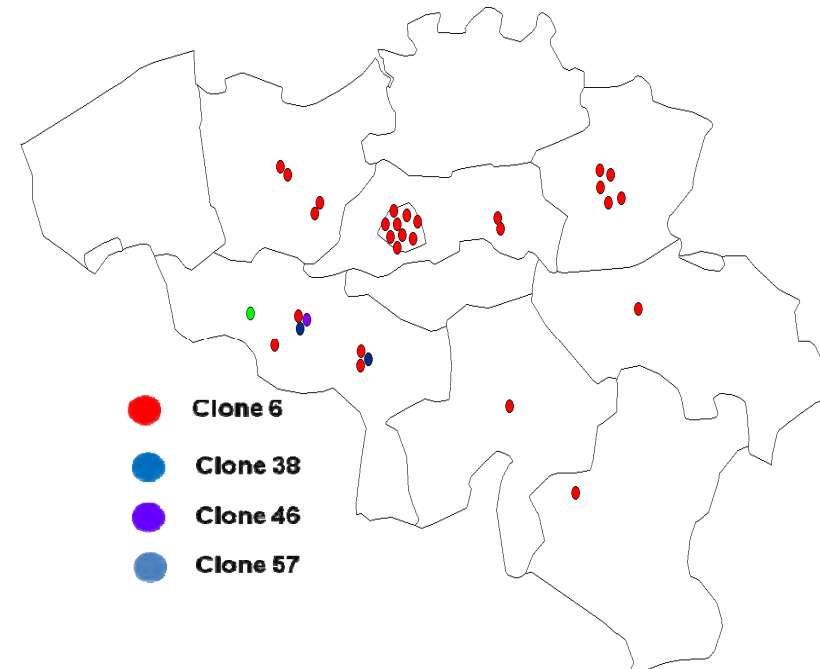
Phylogroup A ST188

Majority (27/33) of CTX-M-15 isolates belonged to a major PFGE type (PFGE type 6) found in 18 centers (1 to 4 isolates/center)

Phylogroup A ST167

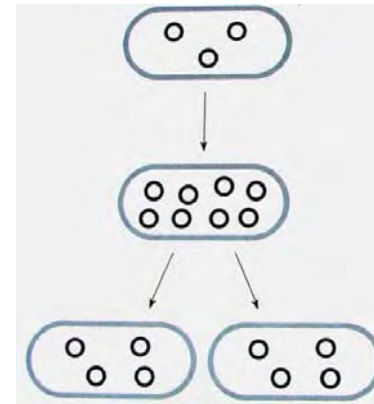
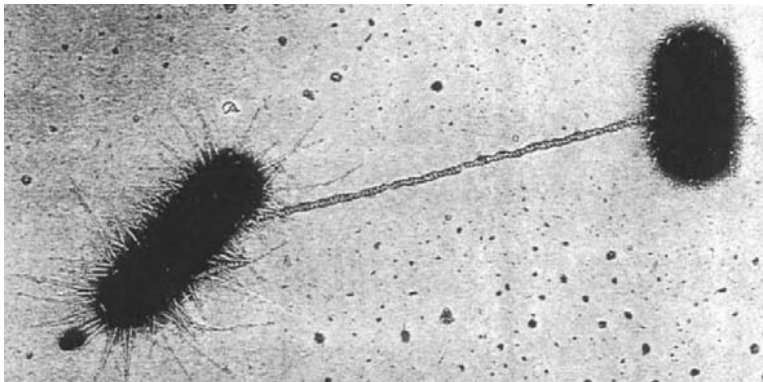
Phylogroup B2 ST131, O25b:H4

Phylogroup D ST501

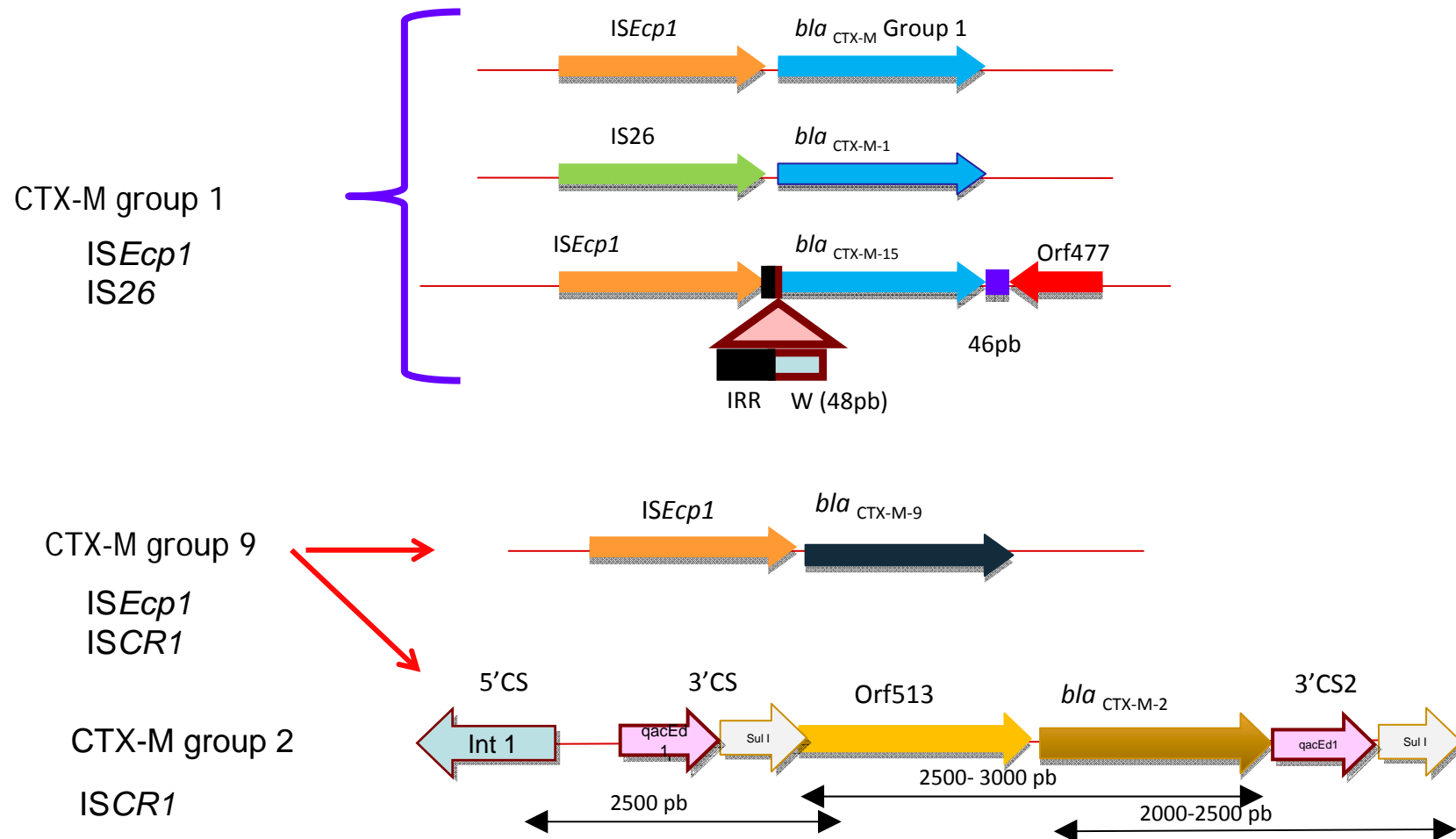


Transmission

- ***bla* genes mobilized from chromosomes to plasmids:**
 - Integrases, transposases, and insertion sequences involved
 - *bla*CTX-M mobilizes 10x more frequently than *bla*SHV & *bla*TEM
- **Horizontal & vertical transmission of plasmids:**
 - Horizontal transmission: plasmid-mediated conjugation
 - Vertical transmission: clonal transmission by normal cell division



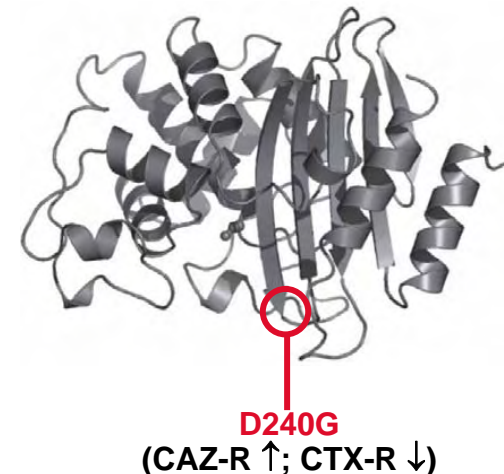
Schematic representation of the genetic environment of *bla* CTX-M genes from *E.coli* isolates in Belgium



Pandemic Spread of *bla*CTX-M-15-ST131

- ***bla*CTX-M-15**

- Located on **multiple plasmids** belonging to the incompatibility group IncFII
- *bla*CTX-M-15 mostly downstream *ISEcpI*
 - Multidrug co-resistance: *bla*OXA-1
 - *bla*TEM-1
 - *aac6'-Ib-cr* (aminoglycoside acetyltransferase)
 - *tet(A)* (tetracycline efflux protein), ...



- **MLST clone ST131**

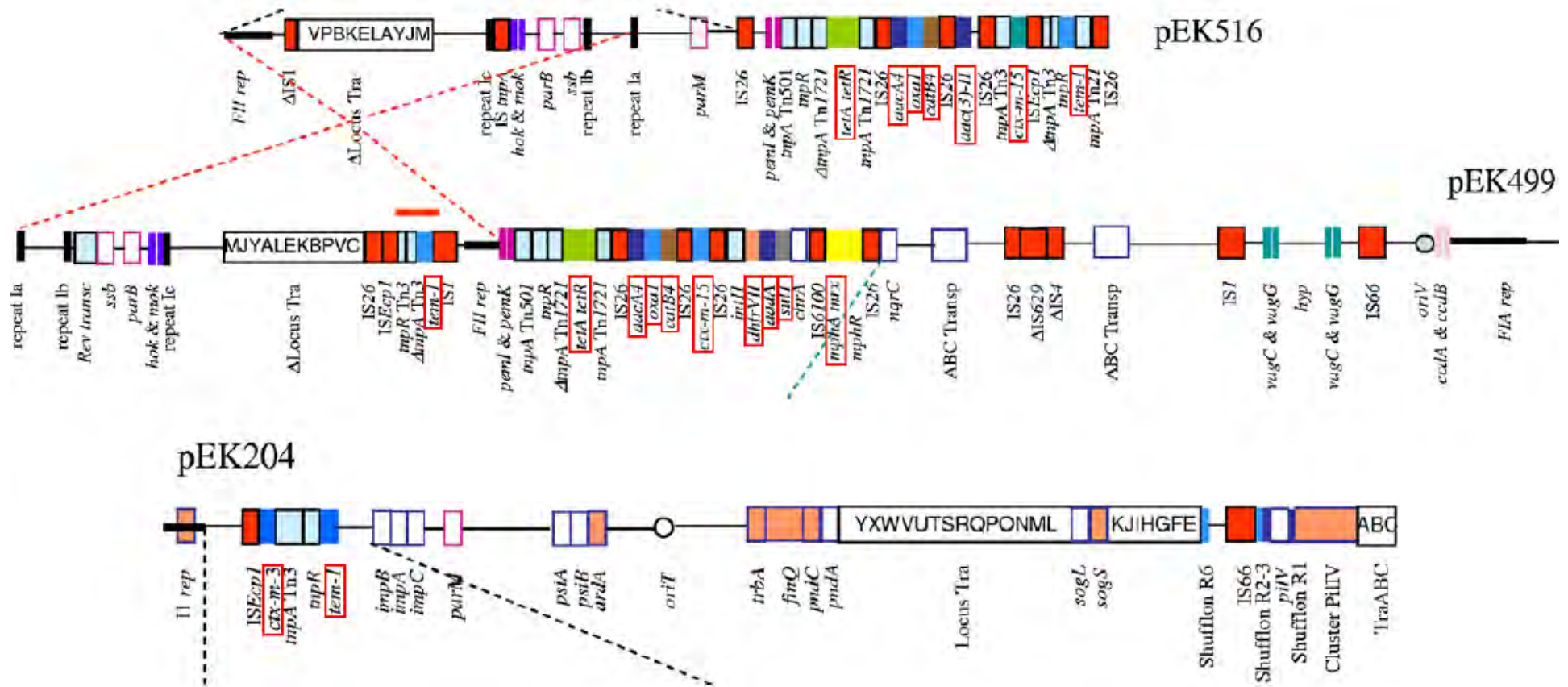
- Disseminated by a widespread, **successful clone**: Serotype O25:H4 & MLST profile ST131
- Causing urinary tract infections
- O25:H4-ST131 not exclusively associated with *bla*CTX-M-15 (same clone also found without CTX-M or any ESBL)

Plasmids Belonging to O25:H4-ST131

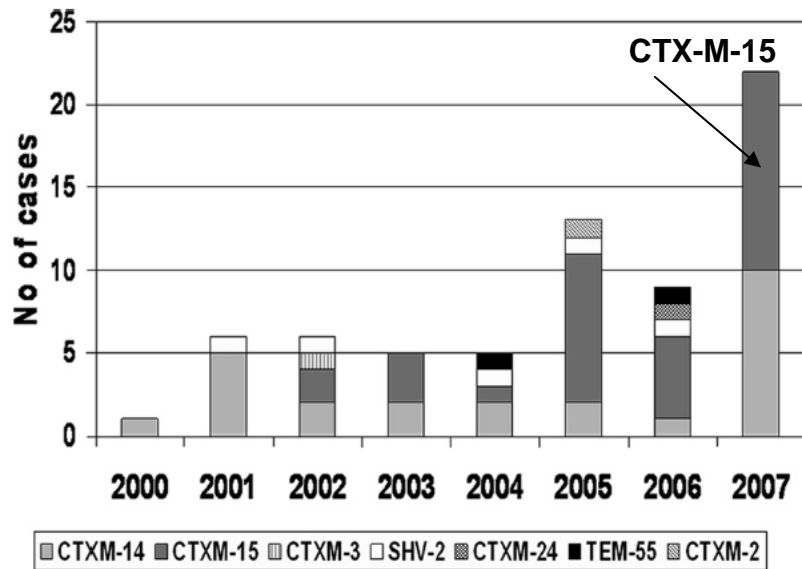
Complete Nucleotide Sequences of Plasmids pEK204, pEK499, and pEK516, Encoding CTX-M Enzymes in Three Major *Escherichia coli* Lineages from the United Kingdom, All Belonging to the International O25:H4-ST131 Clone

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2009, p. 4472-4482 Vol. 53, No. 10
0066-4804/09/508.00+0 doi:10.1128/AAC.00688-09
Copyright © 2009, American Society for Microbiology. All Rights Reserved.

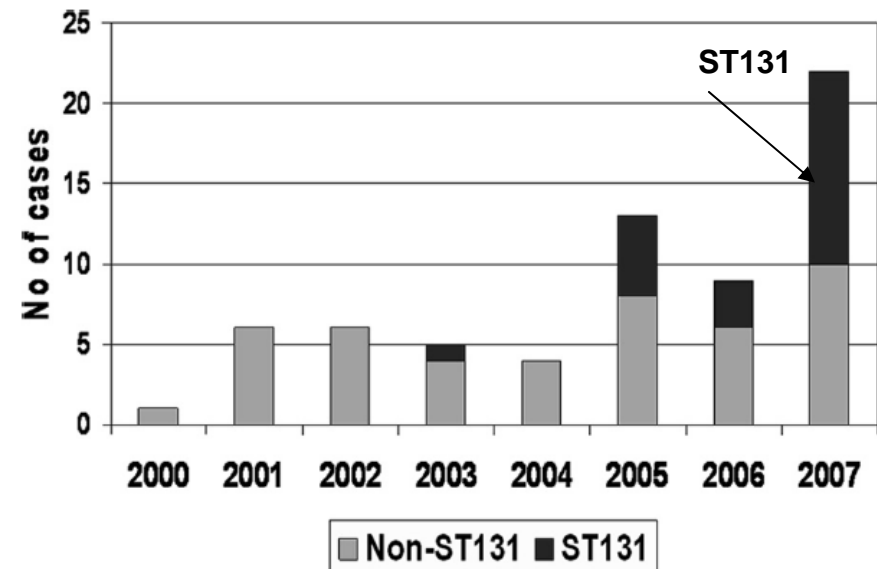
Neil Woodford,^{1*} Alessandra Carattoli,² Edi Karisik,¹ Anthony Underwood,¹ Matthew J. Ellington,¹ and David M. Livermore¹



Spread of MLST Clone ST131 in Canada



Distribution of the different ESBL-producing *Escherichia coli* isolates recovered from blood in the Calgary Health Region from 2000 to 2007



Distribution of *Escherichia coli* MLST clone ST131 isolated from blood in the Calgary Health Region from 2000 to 2007

Characteristics of MLST Clonal Complex ST131 Members Compared to Those of non-ST131 ESBL-Producing *E. Coli*

Characteristic	N° of isolates/total (%):		P value
	Clonal complex ST131	Non-ST131	
Antimicrobial susceptibilities (n = 209):			
GEN nonsusceptible	64/96 (67)	40/113 (35)	0.0001
TOB nonsusceptible	89/96 (93)	55/113 (49)	<0.0001
AMK nonsusceptible	43/96 (45)	39/113 (35)	0.2
TZP nonsusceptible	33/96 (34)	23/113 (20)	0.03
NIT nonsusceptible	4/96 (4)	13/113 (12)	0.07
PMQR determinants (n = 209):			
<i>aac(6')-Ib-cr</i> (FQ resistance)	66/96 (69)	46/113 (41)	0.0001
<i>aac(6')-Ib-cr</i> and <i>qnrB</i>	1/96 (1)	0/113	
Collection sites (n = 209):			
Community	55/96 (57)	75/113 (66)	0.2
Hospital	29/96 (30)	34/113 (30)	1.0
Nursing home	12/96 (13)	4/113 (4)	0.02
Specimens (n = 209):			
Urine	70/96 (73)	94/113 (83)	0.4
Blood	20/96 (21)	11/113 (10)	0.03
Other	6/96 (6)	8/113 (7)	1

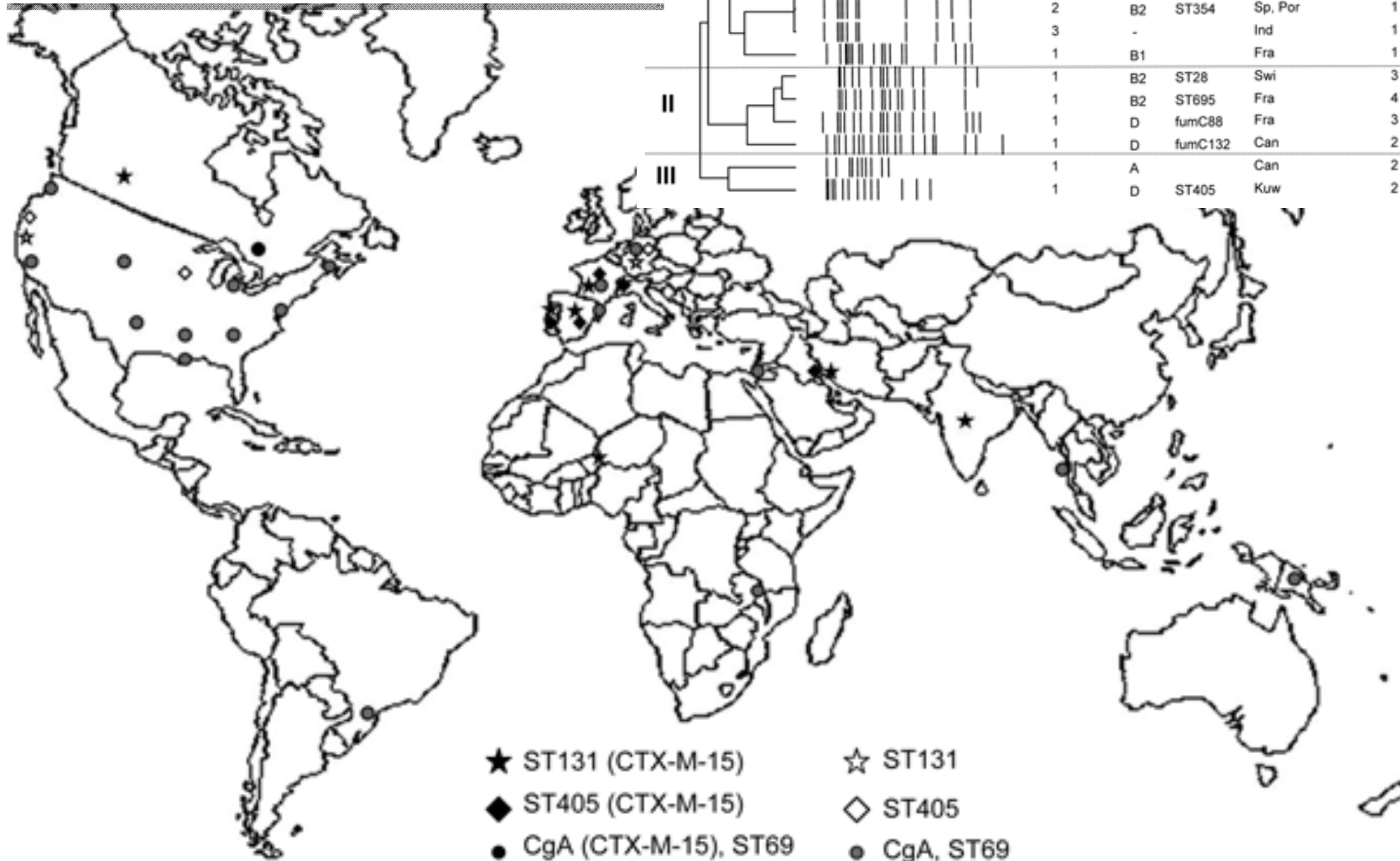
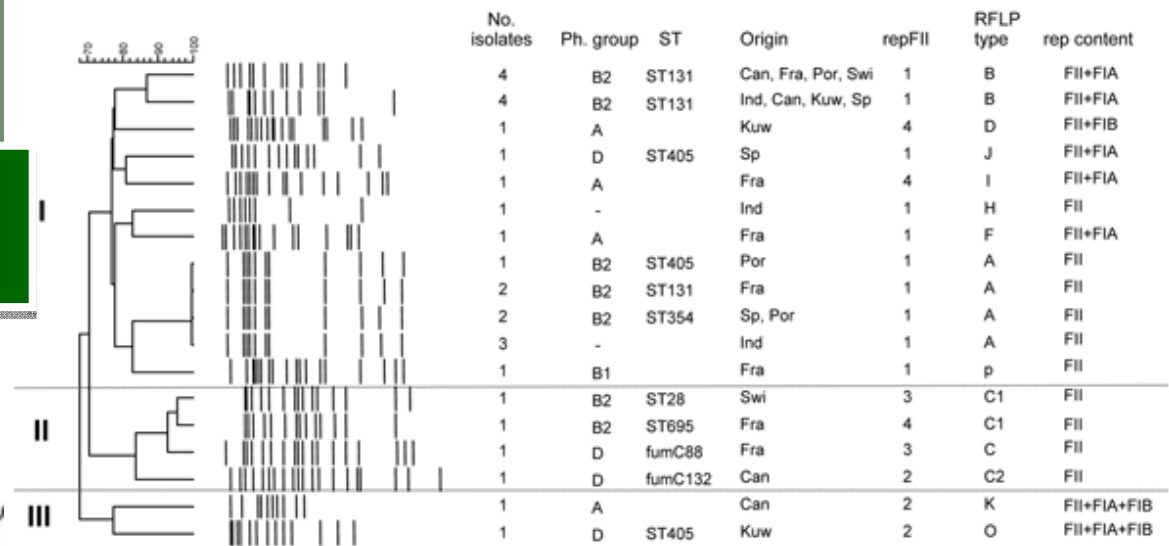
Characteristics of MLST clonal complex ST131 members (n = 96) compared to those of non-ST131 (n = 113) ESBL-producing *E. coli* strains

Plasmids found in association with E.coli CTX-M-15

Année	Plasmide	Ville	Résistances associées	Taille (kb)
1999	pC15-1av	Vancouver	Cip Gen Tob Sxt	92
2000	pC15-1a	Toronto	Cip Gen Tob Sxt	92
2000	pC15-2a	Vancouver	Cip Sxt	92
2000	pC15-2b	Toronto	Cip Gen Tob	92
2000	MDR id	Paris	Cip toujours	95
	à pC15-1a	Tunis	AG, Tet Sxt variable	à
2004		Bangui		130
2001		Paris	Cip AG, Tet variable	120
à				
2002				
Résistance quinolones non transférable				
¹ Boyd D.A et al AAC 2004;48:3758-64; ² Lavollay M et al AAC 2006;50:2433-8				
³ Leflon-Guibout V et al AAC 2004;48:3736-42				

CTX-M-15

Clonal expansion: Highly Virulent E.coli
O25:H4-ST131
Plasmid dissemination



Worldwide emergence of ESBL-producing *E. coli* community infections

- **Urinary tract infections (± bacteremia)**
 - in elderly subjects caused by CTX-M producing *E. coli*
 - 21% in Turkey; 5% in Spain
- Sporadic cases of infections in most countries
- Isolates most often resistant to ABs used for the treatment of UTI:
 - ciprofloxacin
 - trimethoprim/sulpha.
 - gentamicin
 - ceftriaxone

Faecal colonisation

Lebanon (2003): 16% patients
3% care workers
2% healthy subjects

Faecal colonisation increased:
Spain: 1% (1991) to 5%(2003) in outpatients
1% to 12% in hospitalised patients

70% of ESBLs EC in community UTI pts
17-27% household contacts



Risk factor for UTI by ESBL organism
Potential source of transmission among households

Rodriguez-Bano et al, J Clin Microbiol 2004;
Pitout et al, Clin Infect Dis 2004;
Munday et al, J Antimicrob Chemother 2004;
Woodford et al, J Antimicrob Chemother 2004
Moubareck et al JCM 2005
Coque eurosurveillance 2008

ESBL- E. coli in the community

- Significant increase in the number of CTX-M- E. coli including CTX-M-15 ST 131 in the community
- **Fecal carriage of ESBL-E. coli on healthy volunteers**
 - Valverde 2004 (n=108) Spain. **3,7%** (50% CTX-M)
 - Palechi 2005; Children in South America **1,7%** (predominance CTX-M-2 and CTX-M-15)
 - N-Chanoine 2008 (n=332); France **0,6%** (none CTX-M).
- **No data on the risk factors** of fecal carriage of CTX-M in healthy adults subjects
- **ST131** virulent clone, biofilms.
 - **Present in 7% of healthy subjects (no associated with CTX-M-15)**
(N Chanoine JCM 2008)

Nursing Homes as a Reservoir of FQ-R, ESBL- Producing *E. coli* in N. Ireland

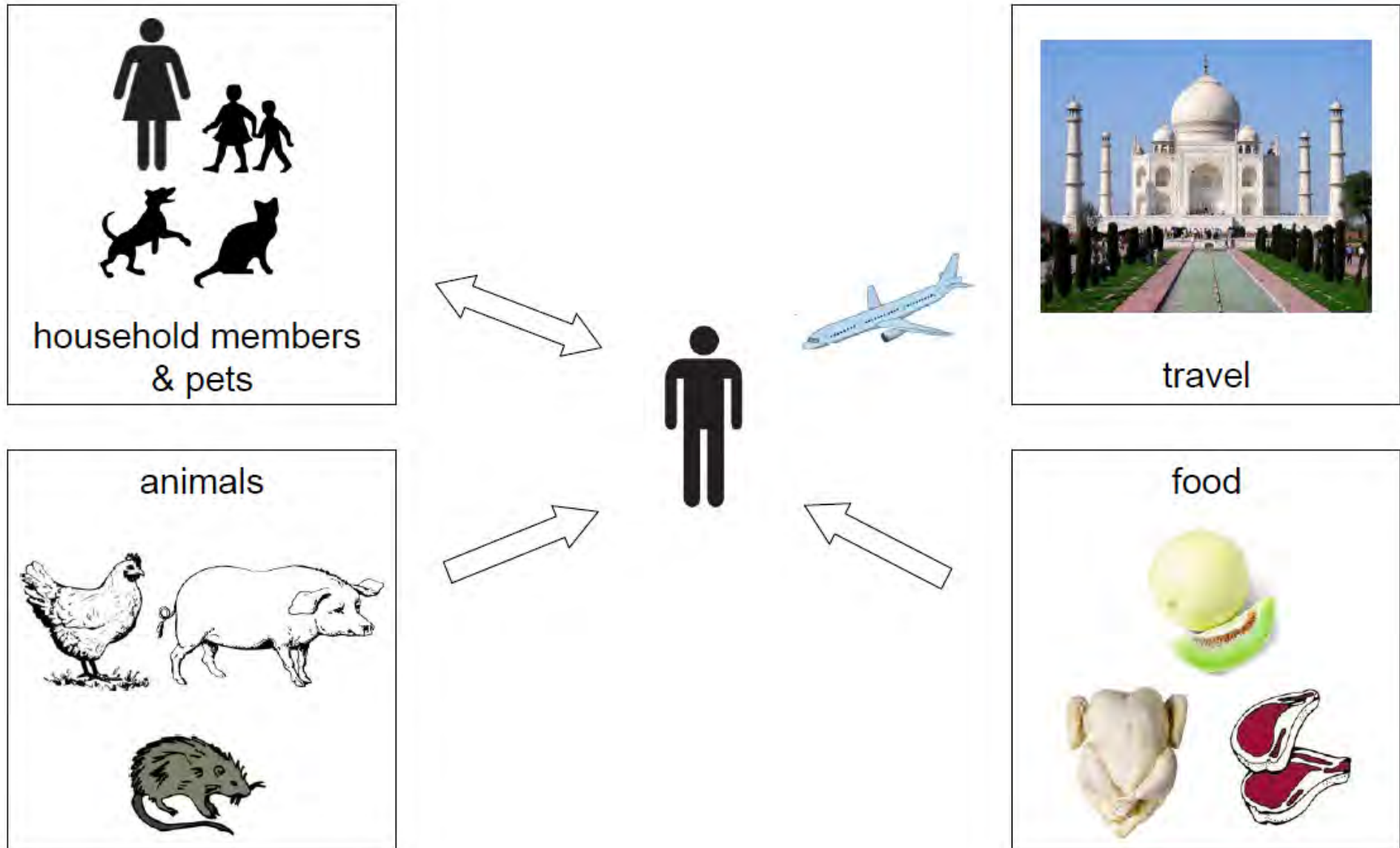
- **Prevalence:**

- 119/294 (40.5%) faecal samples grew FQ-R, ESBL-producing *E. coli*
- 58/119 (49%) belonged to Clone A (O25:H4-ST131-*bla*CTX-M-15)
- 51% of carriers had no history of recent hospital admission
- Only 13% of carriers had history of ESBL *E. coli* colonization / infection

- **Factors associated with risk of carrying FQ-R, ESBL-producing *E. coli*:**

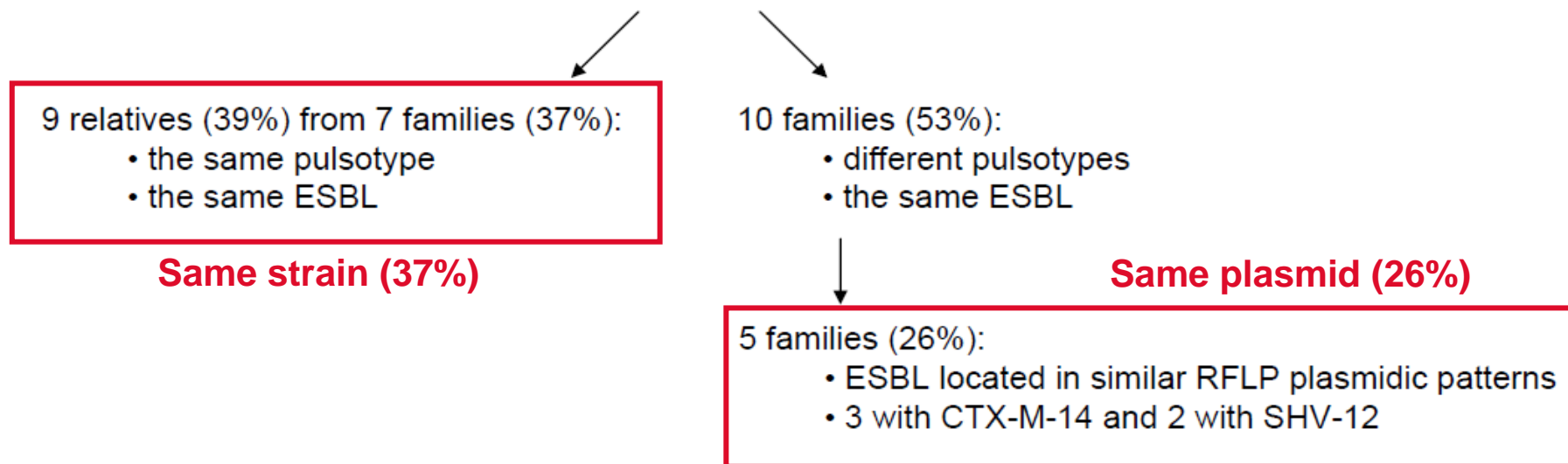
Characteristic	Multivariate analysis:	
	OR (95% CI)	P value
Days of fluoroquinolone use	1.33 (1.04-1.69)	0.02
History of UTI	2.56 (1.37-4.78)	0.003

ESBL transmission



ESBL transmission among families

- Molecular analysis of ESBL dissemination in *E. coli* within families:
 - 19 families of patients with UTI, caused by ESBL-producing *E. coli*, where at least one other faecal carrier within each family was identified
 - 19 patients with UTI + 23 relatives



→ Common source or horizontal transmission of the same ESBL-producing strain or plasmid was found in 63% of the families with ESBL-producers carriers

Intrafamilial transmission of ESBL-producing *E. coli* and *S. enterica* among families of adopted children

- High prevalence of multidrug-resistant (MDR) bacteria in developing countries → High risk for carriage and infection
- French study: 24/25 adoptees (Mali) positive for ESBL-producing *Enterobacteriaceae* (E-ESBL)
- Transmission of E-ESBL demonstrated for 5/22 (22%) families in which at least one family member other than the adoptee was found positive for E-ESBL

β-Lactam resistance genes	ESBL-producing <i>Enterobacteriaceae</i> :	
	<i>E. coli</i> , n (%)	<i>Salmonella spp.</i> , n (%)
CTX-M-15	6 (12.2)	-
CTX-M-15/TEM-1	36 (73.5)	-
SHV-12/TEM-1	4 (8.2)	4 (100.0)
SHV-2/TEM1	1 (2.0)	-
Unknown*	2 (4.1)	-
TOTAL	49 (100.0)	4 (100.0)

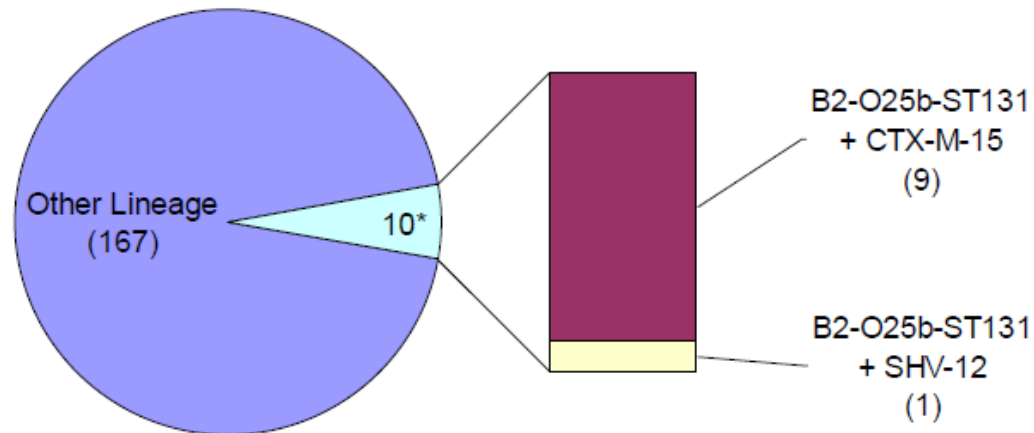
*: Isolates could not be recultured

Household Members & Pets

- Extensive sharing between household members
- Companion animals:
 - Cats & dogs (ST131; CTX-M-15)
 - Probably transmitted from owners



Emergence of human pandemic O25:H4-ST131 CTX-M-15
ESBL-producing *E. coli* among companion animals



*: 9 strains from dogs, 1 strain from a horse

Sharing of *Escherichia coli* Sequence Type ST131 and Other Multidrug-Resistant and Urovirulent *E. coli* Strains among Dogs and Cats within a Household^{∇†}

James R. Johnson,^{1*} Sybille Miller,^{2‡} Brian Johnston,¹ Connie Clabots,¹ and Chitrita DebRoy³

VA Medical Center and University of Minnesota, Minneapolis, Minnesota¹; Veterinary Internal Medicine Practice of Northern Virginia, Manassas, Virginia²; and Escherichia coli Reference Center, Pennsylvania State University, University Park, Pennsylvania³



- Human-associated variant of *E. coli* ST131 (O25:H4) colonized two cats and dog
- Urovirulent
- Cephalosporin-susceptible, FQ-R or CTX-M-15 FQ-R
- ST131 in companion animals and suggest host-to-host transmission of ST131 among household pets
- Possible human-to-animal or animal-to-human transmission

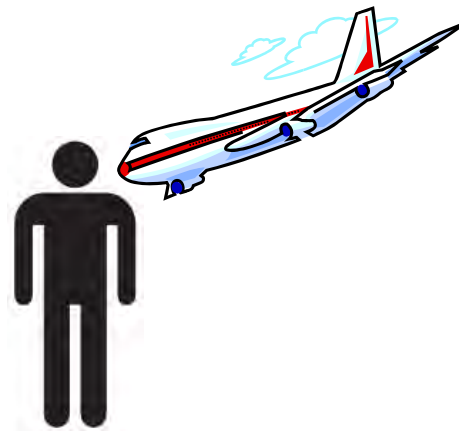
Letters to the Editor

Detection of the Pandemic O25-ST131 Human Virulent *Escherichia coli* CTX-M-15-Producing Clone Harboring the *qnrB2* and *aac(6′)-Ib-cr* Genes in a Dog[∇]

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Santander, Spain

ESBL transmission



Travel

Prevalence of CTX-M β -Lactamase producing Enterobacteriaceae from healthy subjects in Thailand

- Stool samples from healthy individuals in rural area of Thailand
 - Plating of stool samples on McConkey agar with 2 mg/L cefotaxime
 - Detection of ESBL phenotype by double-disc synergy test
 - Genotyping of CTX-M-type ESBL by PCR
- 82/141 (58%) of the specimens with CTX- M β -lactamase producing Enterobacteriaceae
- Majority of CTX-M-producing bacteria were *E. coli* (85%)

Conclusion: High prevalence of CTX-M-producing Enterobacteriaceae in the healthy population

Colonization in Travellers

- High frequency of faecal colonization with ESBL-producing *Enterobacteriaceae* (ESBL-E) among Swedish persons after travelling outside the Scandinavian countries:

	After travel:		↔	Before travel:	
	Carrier	Non-carrier		Carrier	Non-carrier
N° of persons	73/231 (32%)	158/231 (68%)		9/231 (4%)	222/231 (96%)
Symptoms during travel:					
Diahorrea	54%	38%			
Other abdominal symptoms	25%	13%			
Reason for travelling:					
Business	12%	6%			
Backpacking	16%	8%			
Travel destinations:					
India	82%	18%			
Egypt	57%	43%			
Thailand	37%	63%			
Peru	36%	64%			
South Africa	27%	73%			
Tanzania	24%	76%			

Distribution of carriers vs. non-carriers of ESBL-PE before and after travel, and associated symptoms and factors

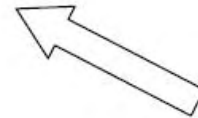
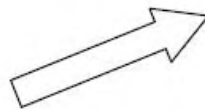
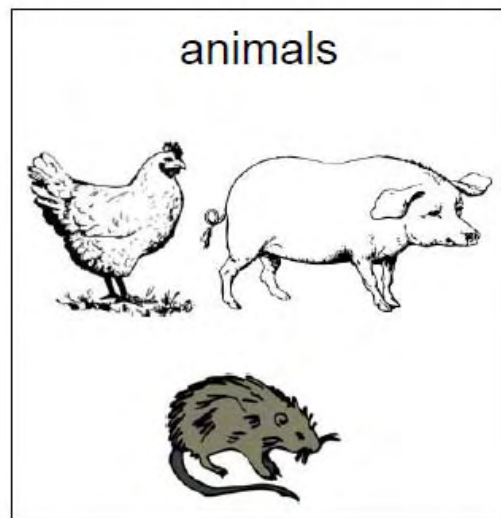
- ➡ Travel increases the risk of colonization by ESBL-E
- ➡ Acquisition of ESBL-E during travel is more often associated with abdominal symptoms (ie: diarrhea)

Travel as a risk factor for development of community-onset ESBL-producing *E. coli* Infections

- Associated with travelers' diarrhea
- Destinations (CTX-M-15 sources):
 - India
 - Middle East
 - Africa

Travel Destination	N° of cases/ Total (%)	Relative Risk (95% Confidence Interval)	p-value
Overseas travel	71/163 (44)	5.7 (4.1-7.8)	<0.001
India	14/163 (9)	145.6 (77.7-252.1)	<0.001
Middle-East	9/163 (6)	18.1 (8.1-35.2)	<0.001
Africa	5/163 (3)	7.7 (2.8-17.2)	0.002
South America	4/163 (2)	3.5 (1.0-9.2)	0.035
Asia	22/163 (13)	3.4 (2.1-5.4)	<0.001
Mexico	13/163 (8)	2.1 (1.1-3.7)	0.020

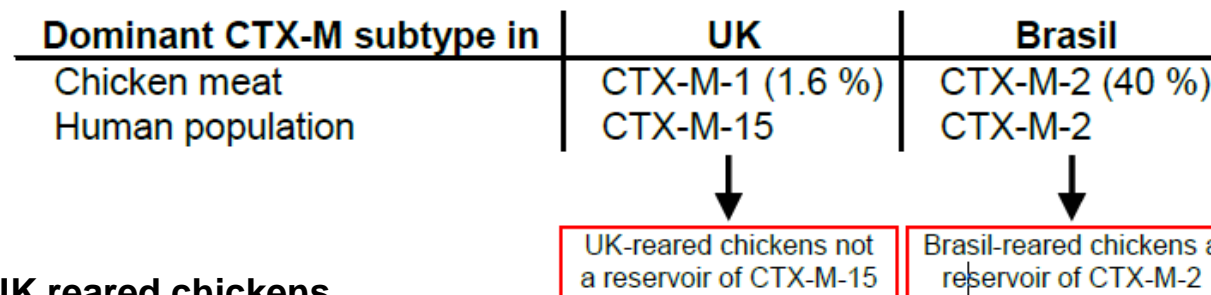
ESBL transmission



Domestic / Imported chicken meat in the UK as a potential source of ESBL-producing *E. coli*

E. coli isolates with CTX-M ESBLs from chicken breast, by country of origin

Origin	Total positive/ total tested	CTX-M gene present:			
		CTX-M-1	CTX-M-2	CTX-M-8	CTX-M-14
UK	1/62	1	0	0	0
Ireland	0/3	0	0	0	0
Brazil	4/10	0	4	0	0
Brazil/Poland/France	3/4	0	3	0	0
Poland	0/4	0	0	0	0
The Netherlands	2/2	0	2	0	0
Spain, France, Denmark and Germany	0/4	0	0	0	0
Unknown	7/40	0	1	1	5
Total	17/129	1	10	1	5



No CTX-M-15 in UK reared chickens

CTX-M-2 ofte found in chicken reared in South America

ECCMID 2010: High carriage rate in meat



Extend spectrum beta-lactamase producing Enterobacteriaceae (ESBL) in retail meat.

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Abstract

Objectives

Worldwide there is a rapid increase of resistance in Enterobacteriaceae causing infections in humans, especially of the Extended spectrum Beta-lactamase (ESBL) resistance pattern. Potential sources for infections caused by ESBL producing micro-organisms in humans are food-products like meat. The aim of this study is to determine the prevalence of ESBL-producing Enterobacteriaceae in meat used for consumption.

Methods

A prospective observational study was performed between 17th august and 30th October, 2009. In this period parts of raw, non-spiced meat which were bought for consumption, were collected. The meat was inoculated into a TSB broth which was incubated at 37 ° C for at least 16 hours. Subsequently part of the initial broth was transferred to another TSB broth which contained 8 mg/L Vancomycin and 0.25 mg/L Cefotaxime. After at least 16 hours of incubation 10 ml of the broth was inoculated on a selective chromogenic agar (Biomerieux, Marcy l'Etoile, France) and incubated again for at least 16 hours. Determination was done by Vitek2; and confirmation of the presence of ESBL was done by e-test.

Results

A total of 262 meat samples were included in this study (mean weight: 11,9g). From 42.7% of the samples ESBL producing micro-organisms were recovered. The animal sources of the meat were 89 (34.0%) chicken; 85 (32.4%) beef; 57 (21.8%) pork and 31 (11.8%) were from other or mixed source (Table 1). ESBL producing Enterobacteriaceae were most frequently recovered from chicken (86.5%, $p < 0.001$). Beef and pork had statistically significant lower recovery rates, 17.6% and 17.5% respectively. 95.0% of the meat included in the survey was not produced biologically. Non-biological produced meat had a higher rate of ESBL-carriage than biological produces meat (45.2% vs. 30.8%), but this was not statistical significant.

Conclusion

Almost half of the meat in retail stores contained ESBL producing bacteria. However, chicken contained significantly more often ESBL than pork or beef. This is a potential source of the current pandemic of ESBL and further investigations are warranted to elucidate the role of antimicrobial use in food production animals.

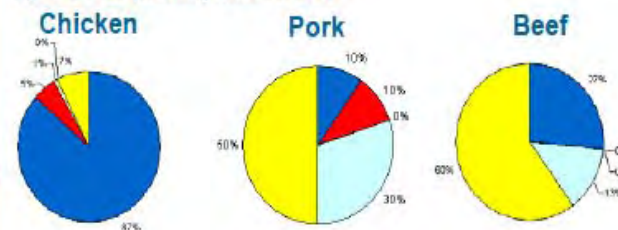
Background

Although the Extended spectrum Beta-lactamase (ESBL) resistance pattern was initially considered as a problem related to hospitals, recent studies show that patients frequently carry ESBL producing micro-organisms on admission to the hospitals¹. This suggests a reservoir of ESBL in the community. Food production animals have been identified as a potential source². This may cause contamination of the meat and thus introduce ESBL producing strains in the food chain.

- 1) Friedman R, Ravelli D, Zarbet E, Rudenski B, Beside E, Atlas D, Yinon AM. Prospective Evaluation of Colonization with Extended-spectrum β -lactamase (ESBL)-Producing Enterobacteriaceae Among Patients at Hospital Admission and of Subsequent Colonization with ESBL-producing Enterobacteriaceae Among Patients During Hospitalization. *Infect Control Hosp Epidemiol*. 2009;30:534-42
- 2) Smet A, Merel A, Pearson D, Dewulf J, Heyndrickx M, Coby B, Hermier L, Haesebrouck F, Bouaya P. Diversity of Extended-Spectrum β -lactamases among Cloacal *Escherichia coli* Isolates on Belgian Broiler Farms. *Antimicrob Agents Chemother*. 2008;52:1238-43

Results

Figure 1: ESBL producing species in meat



ESBL producing strains in Humans:

According to Friedman et al¹, in human the main ESBL producing species are *Escherichia coli* (42.4%) and *Klebsiella* species (33.9%).

- *Escherichia coli*
- *Klebsiella* species
- *Proteus* species
- *Rahnella aquatilis*
- *Serratia* species

Table 1: ESBL in meat

Meat type	Number of samples	ESBL positive
	N (%)	N (%)
Chicken	89 (34.0)	77 (86.5)
Beef	85 (32.4)	15 (17.6)
Pork	57 (21.8)	10 (17.5)
Mixed meat, part Beef, part Pork	22 (8.4)	9 (40.9)
Other	9 (3.4)	1 (11.1)
Total	262 (100)	112 (42.7)

Table 2: risk factors ESBL carriage in meat samples

Potential risk factor	N	Number (%) positive	Relative risk	95% ci		P-value
				Lower	Upper	
Meat source						
Chicken	89	77 (86.5)	4.3	3.3	5.2	<0.001
All other	173	35 (20.2)				
Store						
Grocery	210	100 (47.6)	2.3	1.3	4.5	0.002
Butchery	39	8 (20.5)				
Production						
Non-biological	217	98 (45.2)	1.9	0.8	3.6	0.39
Biological	13	4 (30.8)				

After logistic regression analysis, including type of meat, type of store and biological production, the only statistical significant determinant was chicken ($p < 0.001$).

Discussion

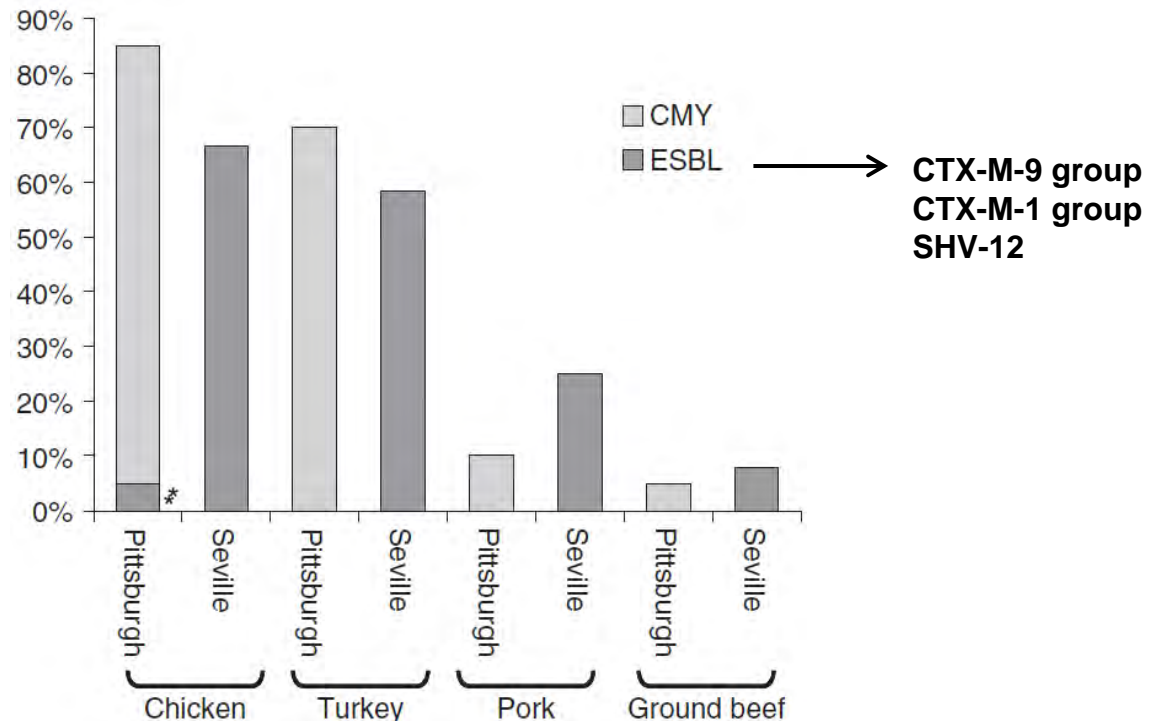
- 86.5% of Chicken meat contains ESBL producing bacteria
- Chicken was the only independent determinant of ESBL
- Large variation in kind of species between Chicken and Pork or Beef.
- Meat can be a potential source of the current ESBL pandemic

86% of chicken meat source contains ESBL-E in retail meat in The Netherlands

ESBL- and CMY-type-producing *E. coli* in clinical samples and retail meat from USA & EU

- 67% (8/12) and 85% (17/20) of retail chickens positive in Sevilla and Pittsburg, respectively. Similar ESBL and CMY genes in clinical and food isolates.

AmpC (CMY-2) as frequent as ESBL in retail meat



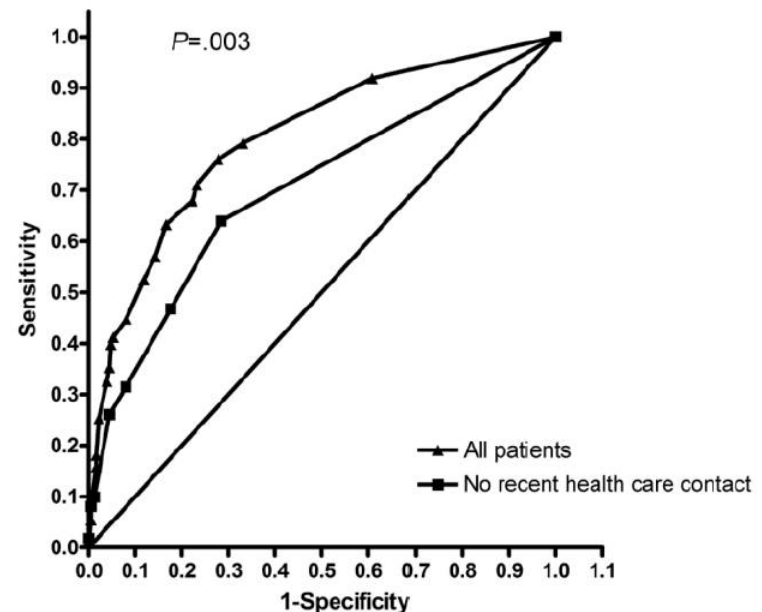
Distribution of ESBL-producing or CMY-producing *E. coli* cases according to the place of acquisition

Food Reservoir for *E. coli* Causing UTI

- Comparison of *E. coli* isolates in Montréal (2005-2007):
 - Women with UTI (n = 353)
 - Retail meat (n = 417)
 - Restaurant/ready-to-eat foods (n = 74)
- *E. coli* isolates, closely related to those causing UTI, present in:
 - retail chicken (O25:H4-ST131 and O114:H4-ST117)
 - honeydew melon (O2:H7-ST95)
- Conclusion: strong support for the role of food reservoirs in the dissemination of *E. coli* causing community-acquired UTIs

Risk factors for infection with ESBL-producing Enterobacteriaceae in non-hospitalised patients

- Significant risk factors (multivariate analysis):
 - Recent antibiotic use
 - Residence in a long-term care facility
 - Recent hospitalization
 - Age ≥ 65 years
 - Male sex



- But 65% of 339 patients had no recent health care contact
 - Area under the ROC curve: 0.70

Conclusions (1)

- **Rapid dissemination of CTX-M-15 *E.coli* (phylogroup B2) belonging to international ST131 clone**
 - Striking increase between 2006 and 2008
 - Needs to be monitored closely in the clinic (community/hospital)
- **Major potential threat for public health**
 - Frequent among commensal flora
 - Most common pathogen of urinary tract infection in community (UTI)
 - Multi-resistant character (quinolones, co-trimoxazole) may lead to therapeutic dead end
- **In Belgium like in most European Countries, dissemination of CTX-M-15 *E. coli* due:**
 - Clonal diffusion of pandemic ST131, O25:H4, subtype B2
 - Horizontal transfer through mobilisation by several genetic platforms :
 - IS26 in plasmid pEK499
 - ISEcp1 (heterogen pool of plasmids related to PEK516 or pC15)

Conclusions (2)

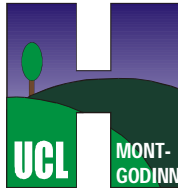
- ESBL-producing *Enterobacteriaceae* increasing worldwide
 - Associated with multidrug resistance
 - Increased virulence
 - Positive selection
 - Adoptive strategies
 - Vertical and horizontal transmission,
- Difficult to control !
- Next to ESBL, emergence of even more resistant organisms

Need for multidisciplinary approach (Task Force)



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- Olivier Denis

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- Patrice Nordmann

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- Bea Jans
- Boudewijn Catry



Belgian Infection control Society (BICS)

