Is AmpC-producing *Escherichia coli* an increasing problem?

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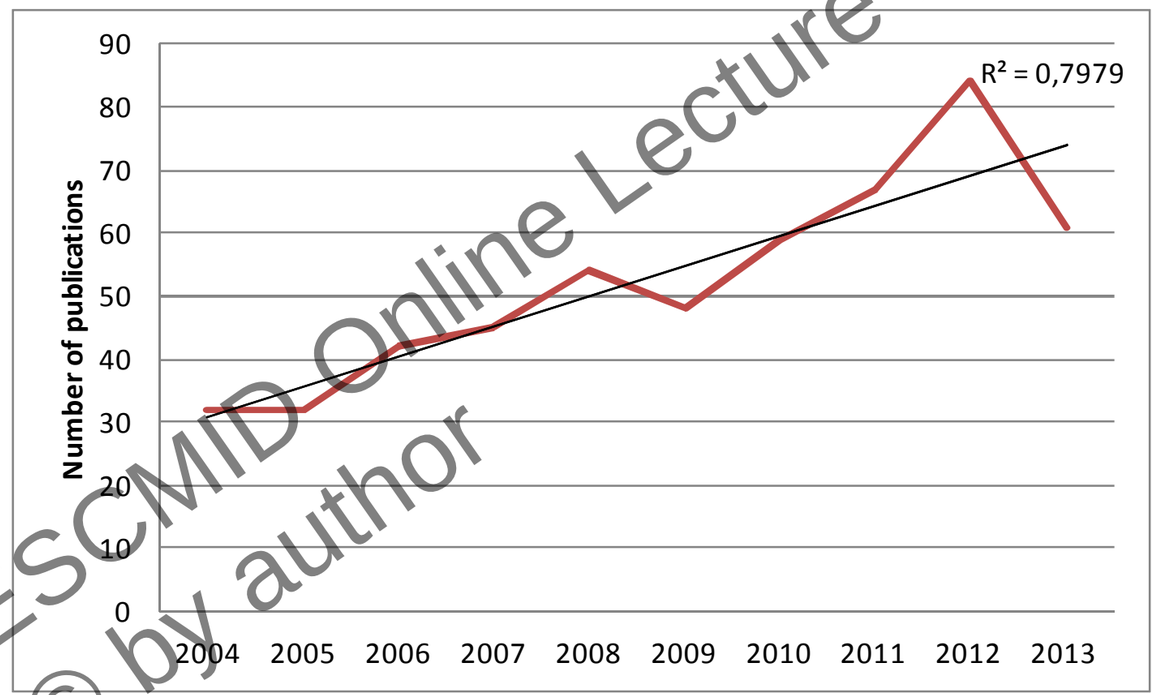
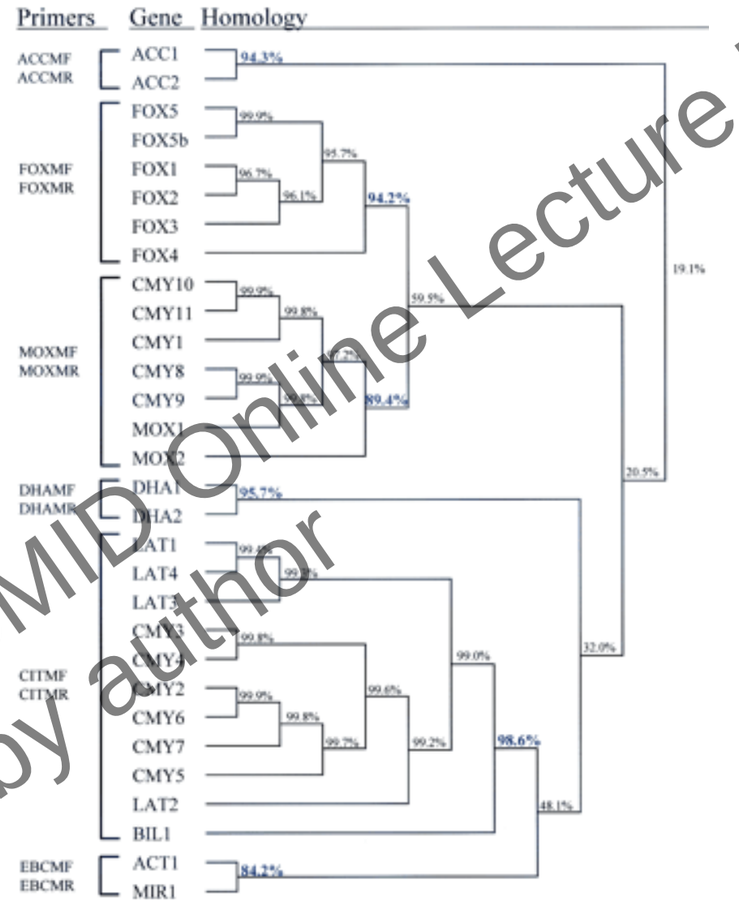
Barcelona, 21 November 2013

PubMed: AmpC AND plasmid



Introductory remarks on plasmid-mediated AmpC (pAmpC)

What are the genes to consider?



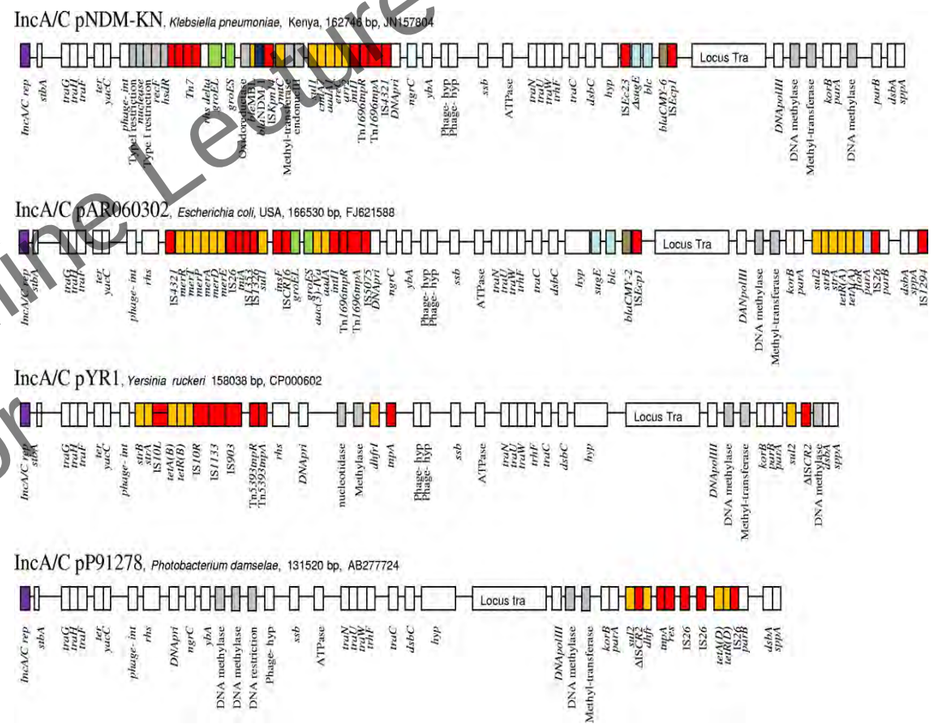
Perez-Perez and Hanson. JCM 2002

Recent reports on plasmid AmpC

Countries that have reported pAmpC in 2012 or 2013

Cephamycinases (CMY)

- CMY-2 by far the most common pAmpC
- Frequently associated with IncA/C broad range plasmids
- Such plasmids isolated both from humans and animals – both in *E. coli* and *Salmonella* spp.
- Recently shown that NDM-1 may have been acquired by such plasmids as backbones are highly similar



Carattoli AAC 2012

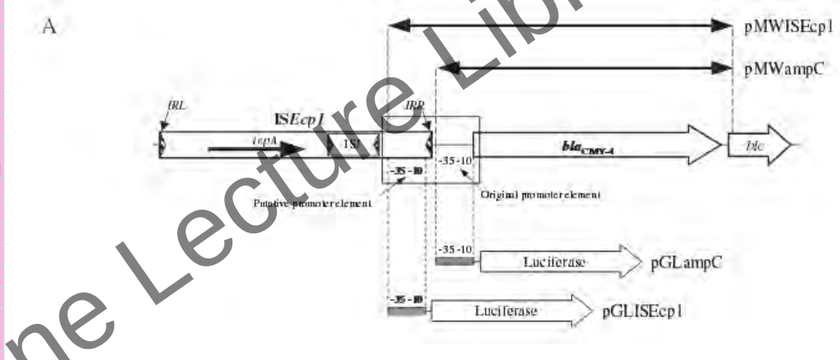
CMY and linkage to plasmids

Enzyme	Replicon(s)	Species	No. of plasmids	Country(ies)	Source(s)
CMY-2	A/C	Escherichia coli, S. enterica	155	Canada, France, Honduras, Iraq, Ireland, UK, USA	Humans, cattle, pigs, poultry
CMY-2	I1	E. coli, S. enterica	30	Canada, France, Gambia, Italy, UK, USA	Humans, cattle, horses, dogs, pigs, poultry
CMY-2	FIA-FIB	E. coli	1	UK	Humans
CMY-2	NT	E. coli, S. enterica	6	Canada, UK	Cattle, poultry, pigs
CMY-4	A/C	S. enterica	1	UK	Humans
CMY-7	I1	E. coli	11	Pakistan, UK	Humans
CMY-8 (CTX-M-3)	HI2	Klebsiella pneumoniae	1	Taiwan	Humans
CMY-21	I1	E. coli	1	UK	Humans
CMY-31	ColE	S. enterica	1	USA	Humans
CMY-36	ColE	K. pneumoniae	1	Greece	Humans

Carattoli. AAC 2009

Link of CMY-2 to IS*Ecp*1

bla AmpC	ST	PG	Genetic support	Plasmid number	Size AmpC plasmid	Replicon typing
CMY-2	117	D	IS*Ecp1*	2	80 kb	A/C
CMY-2	127	B2	IS*Ecp1*	2	80 kb	I1
CMY-2	131	B2	IS*Ecp1*	3	80 kb	–
CMY-2	131	B2	IS*Ecp1*	3	80 kb	I1
CMY-2	131	B2	IS*Ecp1*	4	140 kb	I1
CMY-2	131	B2	IS*Ecp1*	2	80 kb	I1
CMY-2	359	A	IS*Ecp1*	2	80 kb	A/C-FII
CMY-2	636	B2	IS*Ecp1*	2	80 kb	I1
CMY-2	976	A	IS*Ecp1*	1	80 kb	I1-A/C-FII
CMY-2	10	A	IS*Ecp1*	2	80 kb	I1-FII
CMY-2	10	A	IS*Ecp1*	2	80 kb	I1-FII
CMY-7	167	A	IS*Ecp1*	2	80 kb	I1
CMY-2	155	A	IS*Ecp1*	4	130 kb	–
CMY-2	155	B1	–	1	80 kb	I1-FII
CMY-2	93	A	IS*Ecp1*	3	80 kb	–
DHA-1	23	A	*qacEΔ1 sul1*	3	NT	–
CMY-2	88	A	IS*Ecp1*	1	130 kb	A/C-FII
CMY-2	88	B1	IS*Ecp1*	3	80 kb	–
CMY-2	393	D	IS*Ecp1*	NT	NT	–
CMY-2	38	D	IS*Ecp1*	1	NT	C[g]
CMY-2	778	D	IS*Ecp1*	1	NT	C[g]
CMY-2	778	D	IS*Ecp1*	1	NT	C[g]
CMY-2	778	D	IS*Ecp1*	2	NT	C[g]
CMY-2	778	D	IS*Ecp1*	3	NT	C[g]
CMY-2	963	D	–	1	80 kb	I1
CMY-2	981	D	–	1	80 kb	I1
CMY-2	405	D	IS*Ecp1*	1	NT	C[g]
CMY-2	448	B1	IS*Ecp1*	2	NT	C[g]
CMY-2	448	A	IS*Ecp1*	1	NT	C[g]
CMY-2	448	A	IS*Ecp1*	3	NT	C[g]
CMY-2	448	B1	IS*Ecp1*	3	NT	C[g]
CMY-2	448	B1	IS*Ecp1*	2	140 kb	FII
DHA-1	977	B1	–	–	NT	–
CMY-2	420	D	IS*Ecp1*	3	80 kb	–
CMY-2	648	D	IS*Ecp1*	3	80 kb	FII
CMY-2	69	D	IS*Ecp1*	3	130 kb	A/C-FII
CMY-2	453	A	IS*Ecp1*	2	140 kb	A/C-FII
CMY-2	979	B2	IS*Ecp1*	3	250 kb	A/C

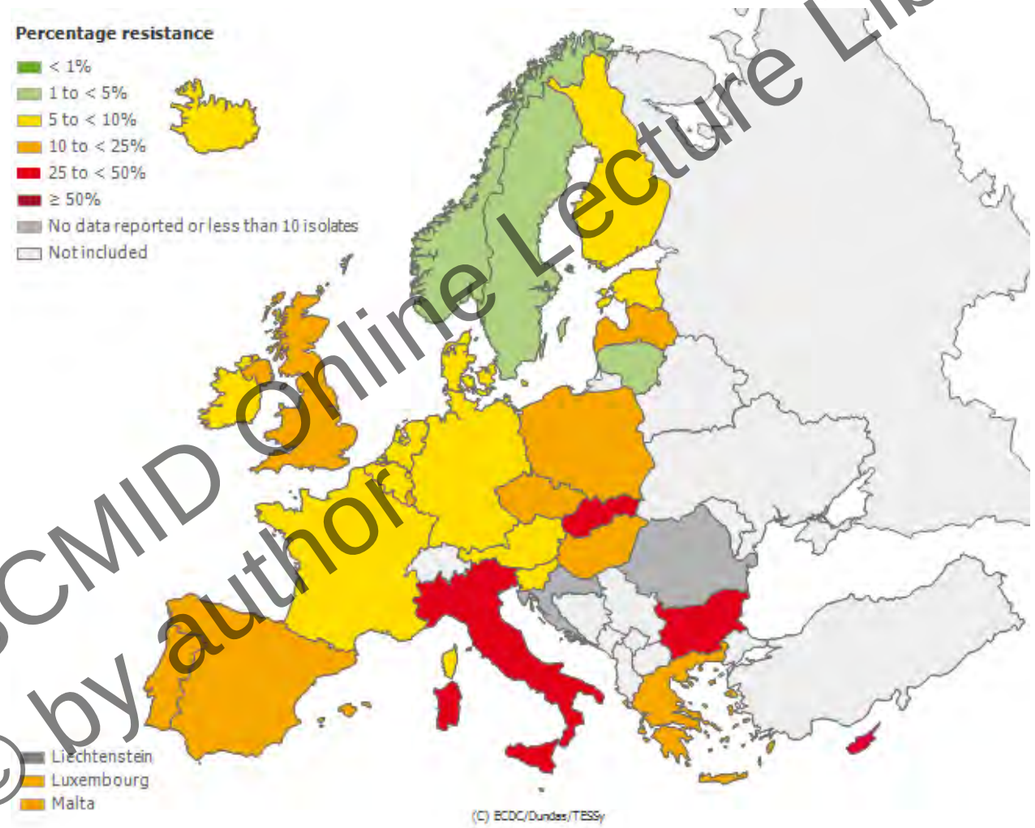


- IS*Ecp*1 provides the promotor regions that drive high-level expression of blaCMY-4
- Nakano et al. J Infect Chemother 2007

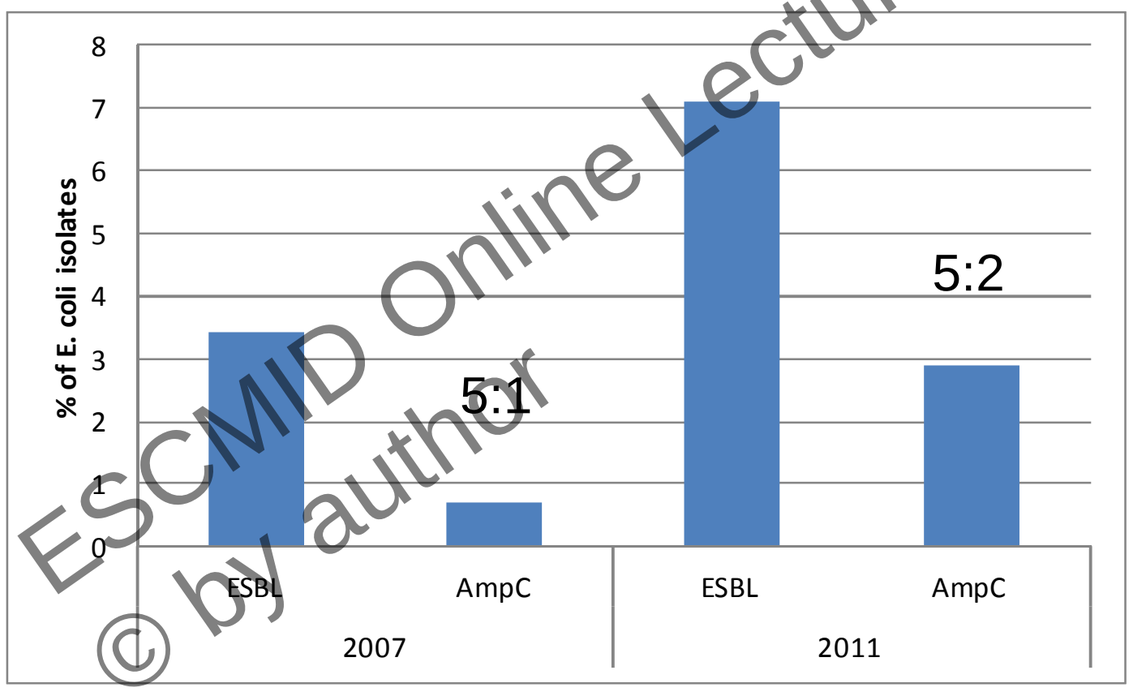
Naseer. CMI 2010

Some recent data on international prevalence

EARS-Net 2012: *E. coli* resistant to 3rd gen cephs



CANWARD national surveillance study 2007-2011 (5,451 *E. coli*)

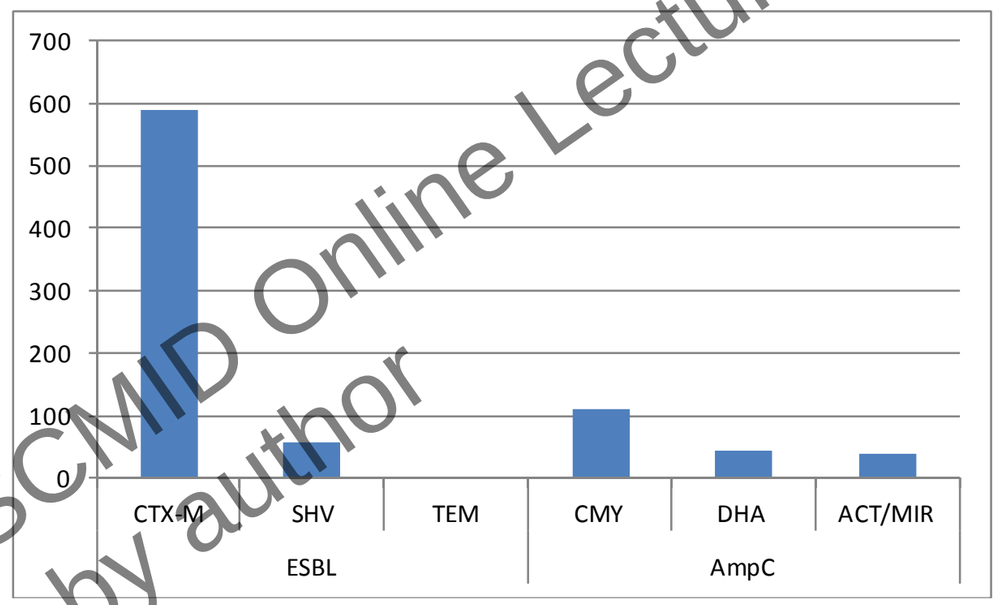


ST131: 55.8% of ESBL vs 28.7% of AmpC (p=0.001)

55.8% CMY-2

Denisuik JAC 2013

SMART study – intraabdominal infections Asia-Pacific 2008-2009



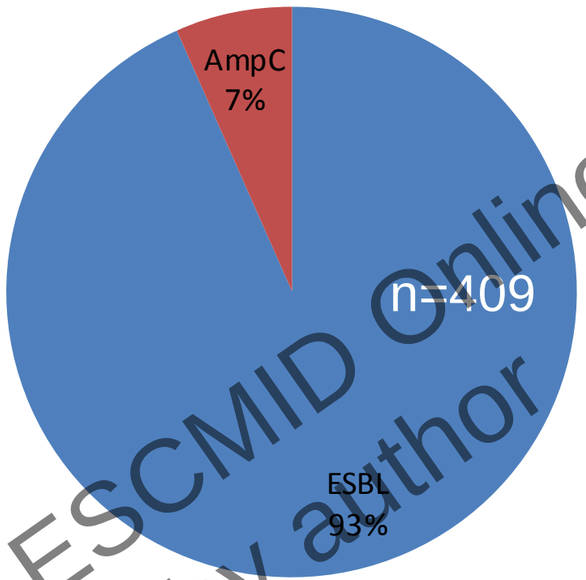
N=699 isolates with extended-spectrum cephalosporin resistance
Sheng et al. AAC 2013

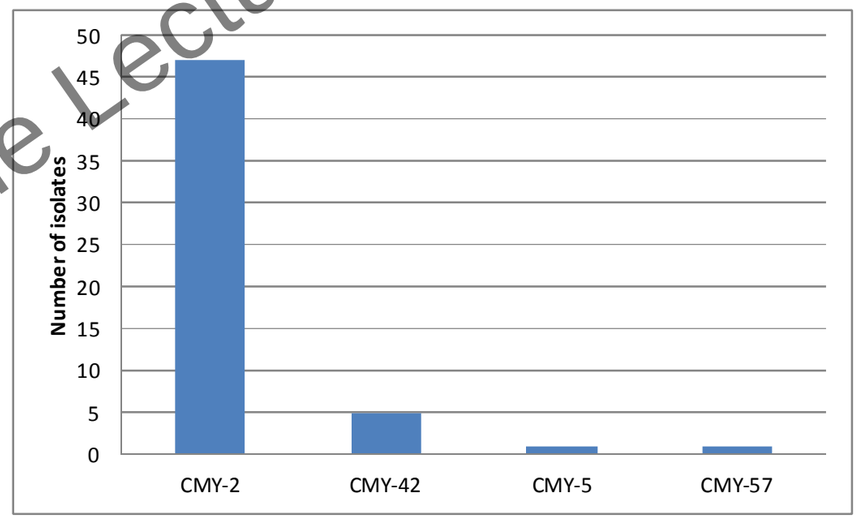
SENTRY U.S. 2010

- 3,061 Enterobacteriaceae bloodstream isolates were consecutively collected from 26 hospitals located in 20 U.S. states during 2010 as part of the SENTRY Program
- 0.5% of the isolates had plasmid-mediated AmpC (Castanheira AAC 2013)

CMY-2, CTX-M-14	1	New York (1)	E. coli (1)
CMY-2, SHV-33	1	Vermont (1)	K. pneumoniae (1)
CMY-2, TEM-1	5	Colorado (1), Kentucky (1), Michigan (1), New Jersey (1), Texas (1)	E. coli (5)
CMY-like,[b] TEM-1	1	Iowa (1)	E. coli (1)
DHA-1, TEM-1	1	Hawaii (1)	P. mirabilis (1)
FOX-5, PSE-like[a]	3	Florida (1), Massachusetts (1), Virginia (1)	E. cloacae (1), K. pneumoniae (1), P. mirabilis (1)
FOX-5, SHV-11	1	Wisconsin (1)	K. pneumoniae (1)
FOX-5, PSE-like,[a] SHV-11	1	Kentucky (1)	K. pneumoniae (1)

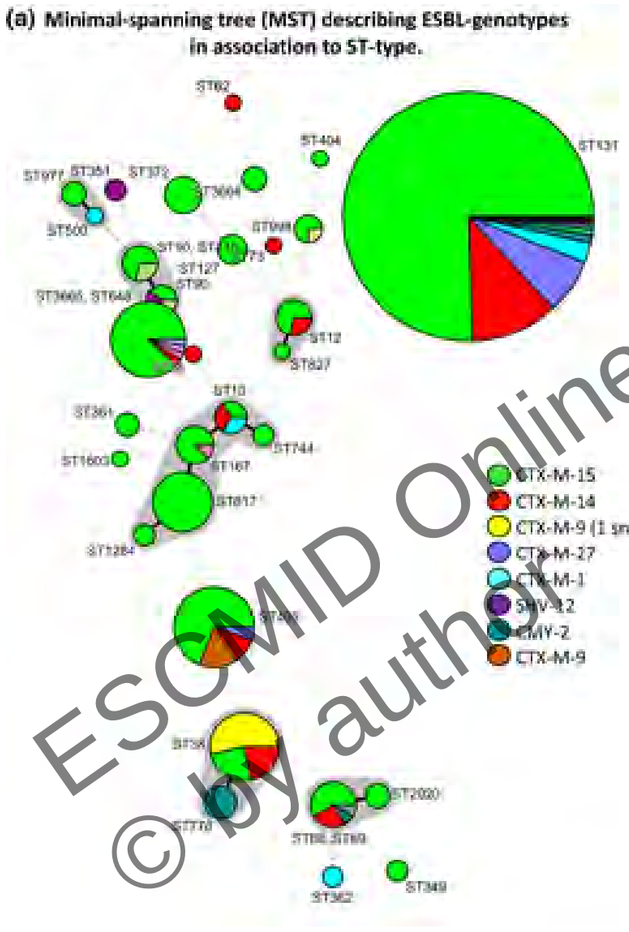
National Swedish point prevalence study

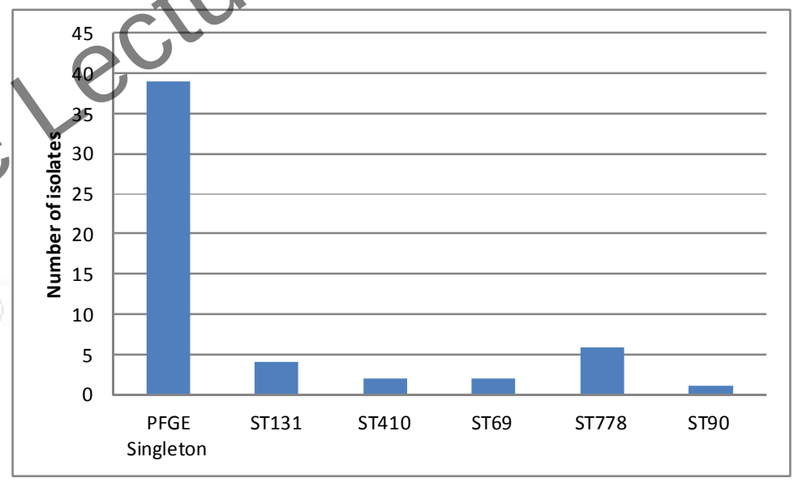




Brolund A et al. CMI 2013 Epub

Sequence types Sweden

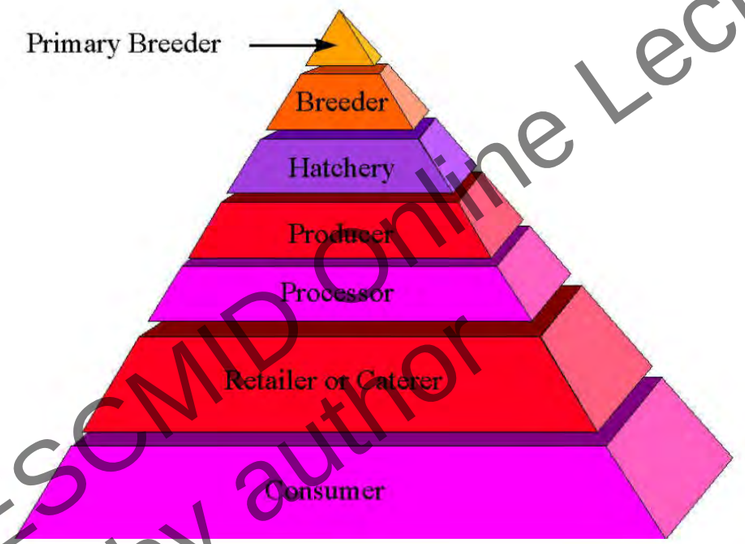




Brolund A et al. CMI 2013 Epub

Plasmid-mediated AmpC among animals

Poultry breeding pyramid



British Veterinary Poultry Association

- Apex: very small population of elite breeding birds
- 1 male selected by the primary breeder could give rise to 20 million offspring
- Purpose: rapid dissemination of genetic progress

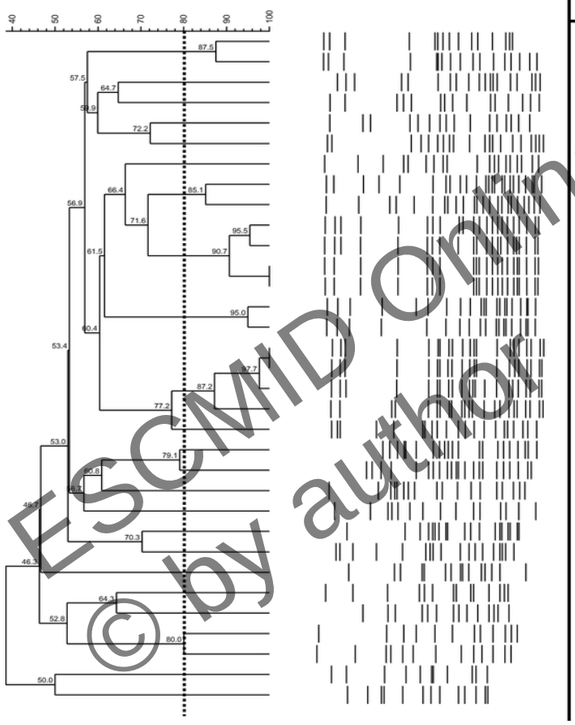
AmpC and ESBLs in the broiler production, The Netherlands

1 Upon arrival in NL 2 days				2 Rearing farm 18 wks†				3 Production farm 31 wks			
Code		Caeca (n)	Prevalence ESBL/AmpC, % (95%CI)*	Poultry house	GPS chickens (n)	Cloaca swabs (n)	Prevalence ESBL/AmpC, % (95%CI) (phenotypes)	Poultry house	GPS chickens (n)	Cloaca swabs (n)	Prevalence ESBL/AmpC, % (95%CI) (phenotypes)
11-1	♂	10	20 (3-56)	1	2100 ♂	41	0 (0-9)	2	400 ♂ (PH 1) & 4000 ♀ (PH 4)	41	2 (0-13) (1 ESBL)
11-2	♂	10	0 (0-31)								
22-1	♀	10	10 (0-45)	4	4200 ♀	41	0 (0-9)				
22-2	♀	10	70 (35-93)								
44-1	♀	10	60 (26-88)	3'	8250 ♀	25	100 (86-100) (24 AmpC, 1 ESBL)	4	535 ♂ (PH 2b) & 5050 ♀ (PH 3)	41	24 (12-40) (10 AmpC)
33-1	♂	10	0 (0-31)	2b	3800 ♀	41	44 (28-60) (18 ESBL)	3	380 ♂ (PH 2b) & 500 ♀ (PH 2a) & 3100 ♀ (PH 3)	41	27 (14-43) (8 AmpC, 3 ESBL)
33-2	♂	10	10 (0-45)								
44-2	♀	10	10 (0-45)	2a	2730 ♀	41	2 (0-13) (1 ESBL)	1	230 ♂ (PH 2b)& 2200 ♀ (PH 2a)	41	7 (2-20) (3 ESBL)

Arrows show how broilers were mixed between poultry houses with time
Only a few primary breeding companies at the top of the production pyramid, and pAmpC is present already at this level (Dierikx et al. PloSOne 2013)

AmpC among Swedish broilers

- 2010 Sweden: AmpC (CMY-2) and ESBL (CTX-M-1) among 34% of Swedish broilers, most likely related to imported breeding stocks
- No evidence of clonal spread between animals and humans



Isolate ID	MLST	Plasmid replicon types	Non-beta-lactam Resistance pattern
H70	N.D.	K, FIB, FII	CiTc
H30	N.D.	K, FIB, FII	KmTc
H27	N.D.	K, FIB, FII, N	
H44	N.D.	K, FIB, FII, I1	CiNaSuTm
H97	N.D.	K, FIB, FII	CiNa
C20	ST69	K, FIB, FII	KmSmSuTc
H20	N.D.	K, FIB, FII	CiNal
C55	ST115	K, FIB, F	
C23	ST10	K, FIB	
C68	ST68	K, FIB, F	CiNa
C05	ST68	K, FIB, F	CiNa
C78	ST68	K, FIB, F	CiNa
C97	ST68	K, FIB, F	CiNa
C82	ST770	K, FIB, F	
C27	ST770	K, FIB, F	
C38	ST2167	K, FIB, P	Su
C79	ST2167	K, FIB, P	Su
C84	ST2167	K, FIB, P	Su
C19	ST2167	K, FIB, P	Su
C98	ST57	K, FIB, F	Su
C75	ST10	K, FIB	
C63	ST10	K, FIB	
H52	N.D.	K, I1	
H07	N.D.	K, FIB, FII, N	CiNa
H77	N.D.	K, FIB, FII	
H66	N.D.	K, FIA, FIB, FII, Y	CiNaGmSmSu
H33	N.D.	K, FIB, FII, X	CCiNaFf
H08	N.D.	K, FIB, X	
H14	N.D.	K, FIB, FII, N, X	CiTc
H80	N.D.	K, FIA, FIB, FII, I, Y	CCiNaGmKmSmSuTmTc
H17	N.D.	K, FIB, FII, Y, N	CiNaSmSuTmTc
H22	N.D.	K, FIB, FII, HI2	CCiNaFf
H32	N.D.	K, FIB, FII	SmSuTm

Prevalence of AmpC and ESBL in healthy and diarrheic cats and dogs

		Dog		Cat	
	ESBL/AmpC coding gene	**Healthy**	**Diarrheic**	**Healthy**	**Diarrheic**
No. of animals carrying	$bla_{CTX-M-1}$	4	2	–	–
	$bla_{CTX-M-15}$	–	–	–	1
	$bla_{CTX-M-14}$	–	1	–	–
	$bla_{CTX-M-1}$ + $bla_{CTX-M-14}$	–	1	–	–
	$bla_{CTX-M-1}$ + $bla_{TEM-52-StPaul}$	–	–	–	1
	$bla_{CTX-M-1}$ + bla_{CMY-2}	–	–	–	1
	$bla_{CTX-M-1}$ + bla_{CMY-2} + *ampC* type-3	–	–	–	1[f]
	$bla_{CTX-M-1}$ + bla_{CMY-2} + bla_{TEM-35}	–	1[a]	–	–
	$bla_{CTX-M-1}$ + bla_{CMY-2} + unknown[b]	1	–	–	–
	$bla_{CTX-M-1}$ + *ampC* type-3	–	1	–	–
	bla_{SHV-12} + bla_{CMY-2}	–	–	–	1
	bla_{CMY-2}	2	2	–	–
	ampC type-3[c]	1	1	–	–
	ampC type-new[d]	0	1	–	–
	Unknown[b]	1	1	–	–
	>1 R gene[e]	1	3	0	4
	E. coli	9	11	0	4
	E. coli + *P. mirabilis*	0	0	0	1[f]

[a] *This animal carried one E. coli harboring both a bla_{TEM-35} gene and a bla_{CMY-2} gene. The $bla_{CTX-M-1}$ gene and another bla_{CMY-2} gene were found in separate E. coli.*
[b] *Isolates negative on Check-MDR CT-103 array, and no ampC type-3.*
[c] *Classification as previously described (Mulvey et al., 2005).*
[d] *New ampC variant compared to previously described variants (Mulvey et al., 2005).*
[e] *Number of animals with more than one type of ESBL/pAmpC gene.*
[f] *This animal carried a P. mirabilis harboring a bla_{CMY-2} gene. All other genes reported in this table were found in E. coli.*

Hordijk. Front Microbiol 2013

How do you detect plasmid-mediated AmpC?

EUCAST guidelines

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

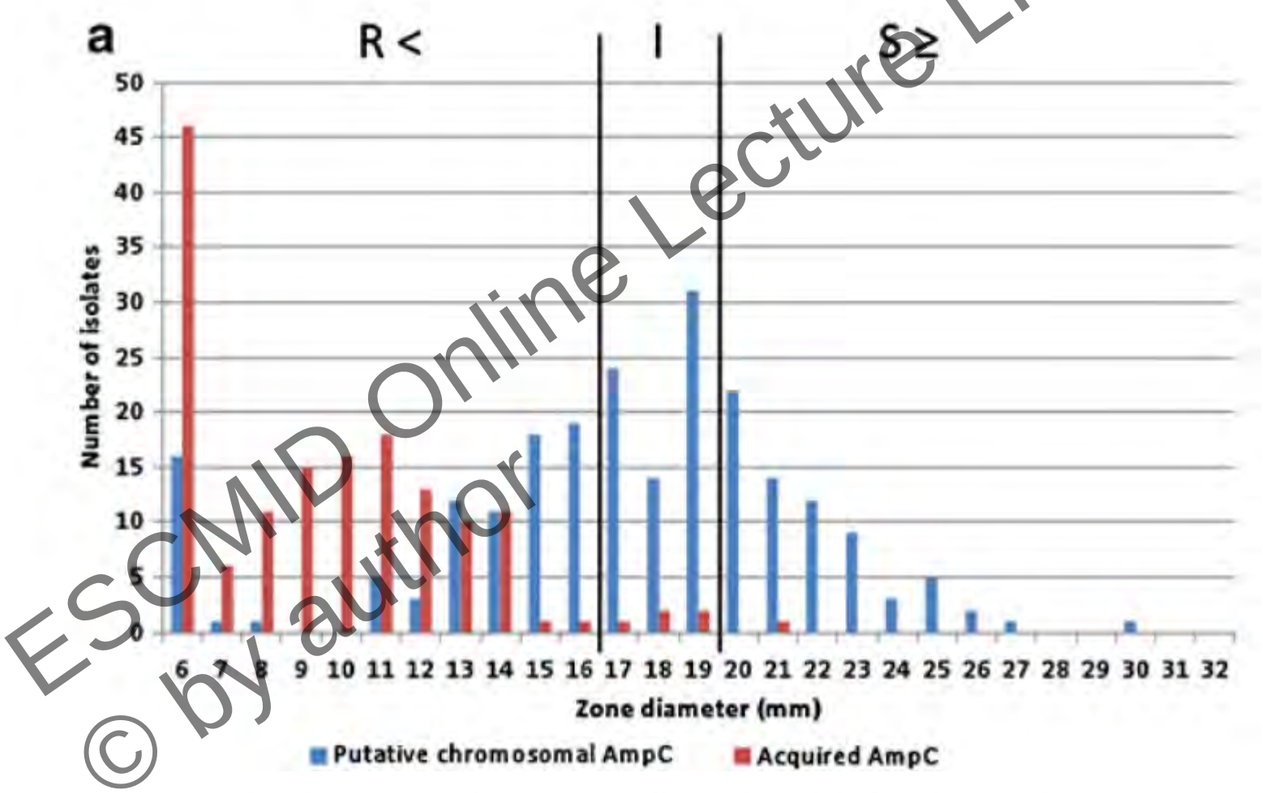
European Society of Clinical Microbiology and Infectious Diseases

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance

Version 1.0
November 2013

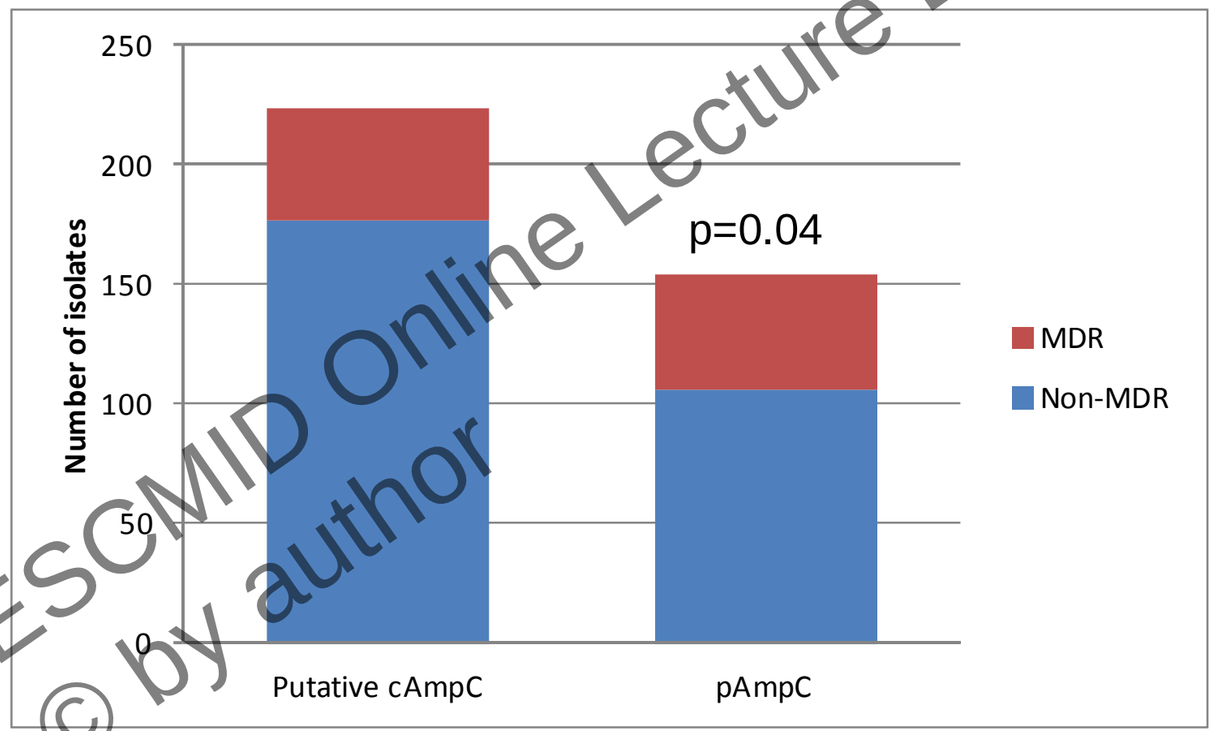
Contents

EUCAST disk diffusion – CTX 5 µg



Edquist P et al. Eur J Clin Microbiol Infect Dis 2013

Prevalence of MDR (2/3 of GEN, CIP, TSU)



Edquist P et al. Eur J Clin Microbiol Infect Dis 2013

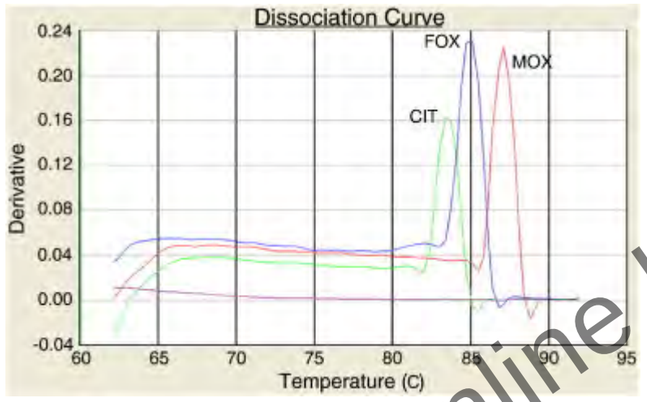
Comparison of methods

Method	Sensitivity	Specificity*
In-house FOX+cloxacillin	98%	52%
Rosco CTX and CAZ+cloxacillin	97%	54%
Etest cefotetan + cloxacillin	93%	69%

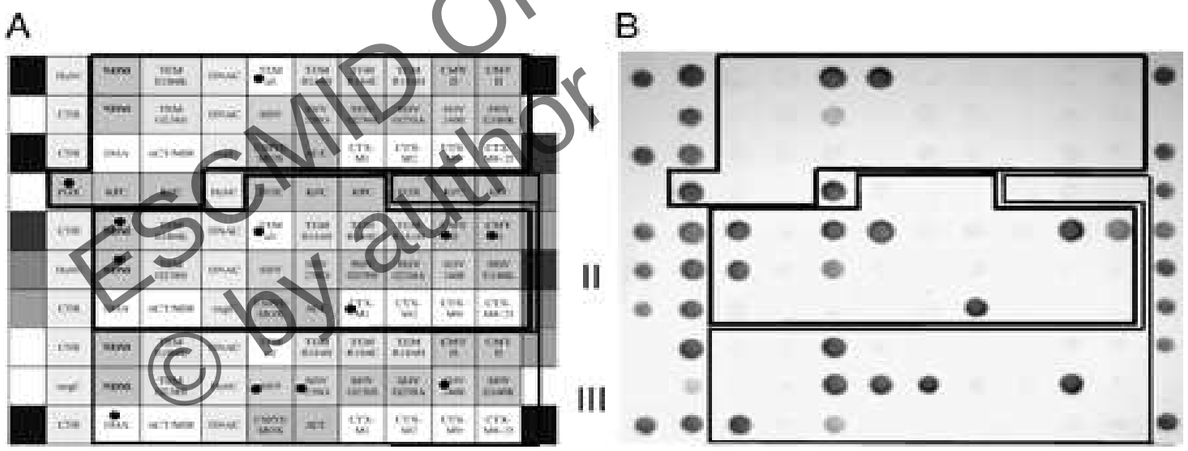
* Specificity calculated as specificity for pAmpC-detection

Edquist P et al. Eur J Clin Microbiol Infect Dis 2013

Genotypic detection



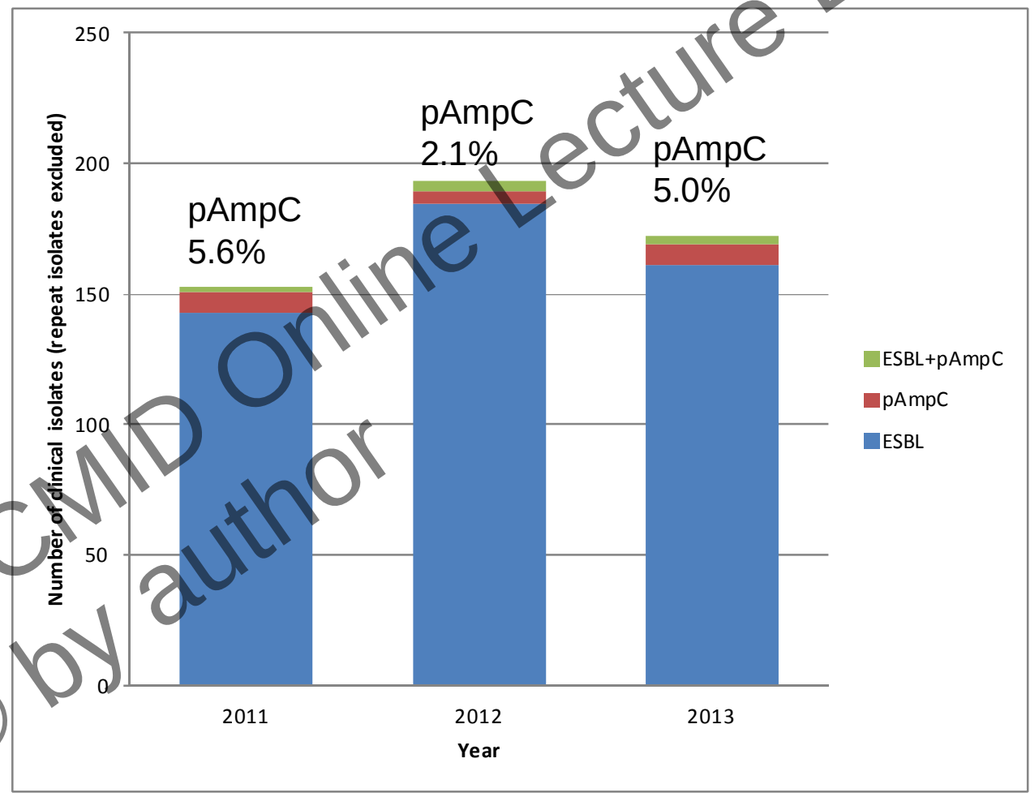
Brolund A.
J Microbiol
Methods 2012



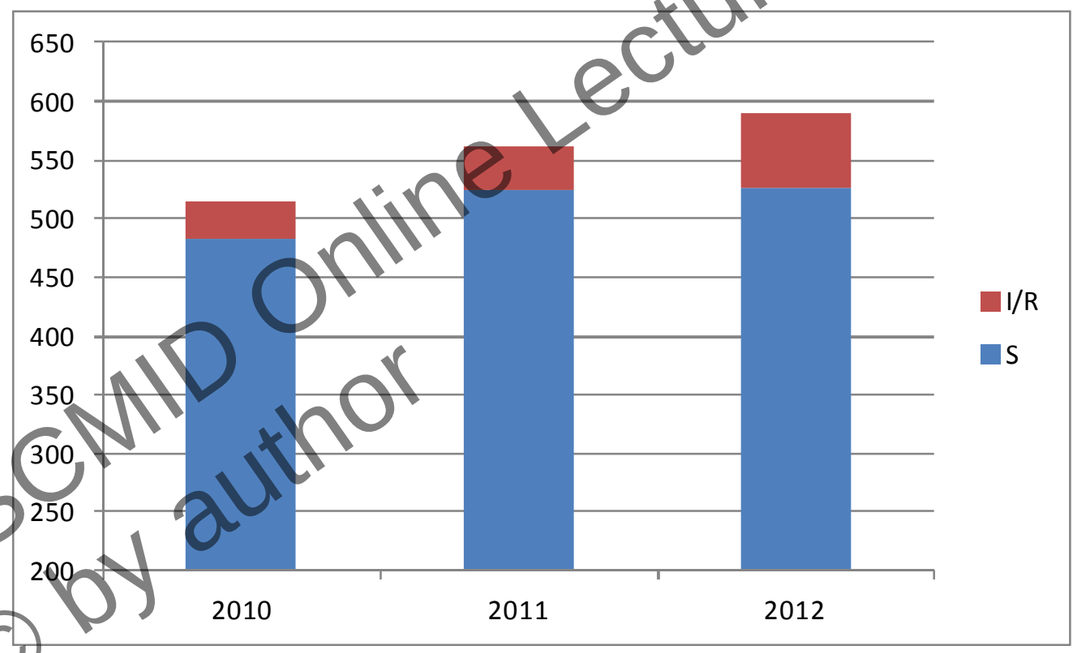
Bogaerts P.
AAC 2011

What do you find when you search beyond point prevalence studies?

Transferable cephalosporin resistance in *E. coli* at Karolinska 2011-2013



3rd gen ceph R *E. coli* are increasing the burden of disease; blood cultures at Karolinska



Conclusions

- No evidence supporting a rapid increase of pAmpC in *E. coli*
- Slow increase observed in most areas where these enzymes are actively searched for
- In many areas pAmpC are far more common than the minor ESBLs (SHV- and TEM-ESBLs)
- CMY-2 common among food-producing and companion animals
- CMY-2 has all the hallmarks of a successful enzyme, including the link to IS*Ecp1*, broad-range plasmids, and to ST131
- pAmpC-testing in blood culture isolates of *E. coli* with extended-spectrum cephalosporin resistance/negative ESBL-test may be warranted

Thank you for your attention!