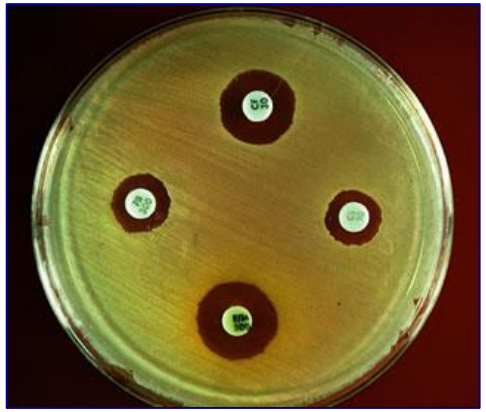

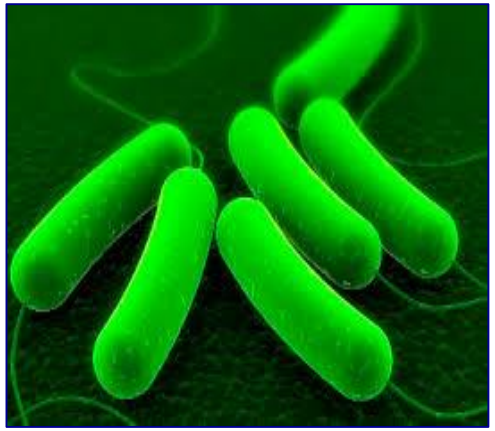

Genotypic and phenotypic characterisation of antimicrobial resistance encoding determinants of multidrug resistant *Escherichia coli*

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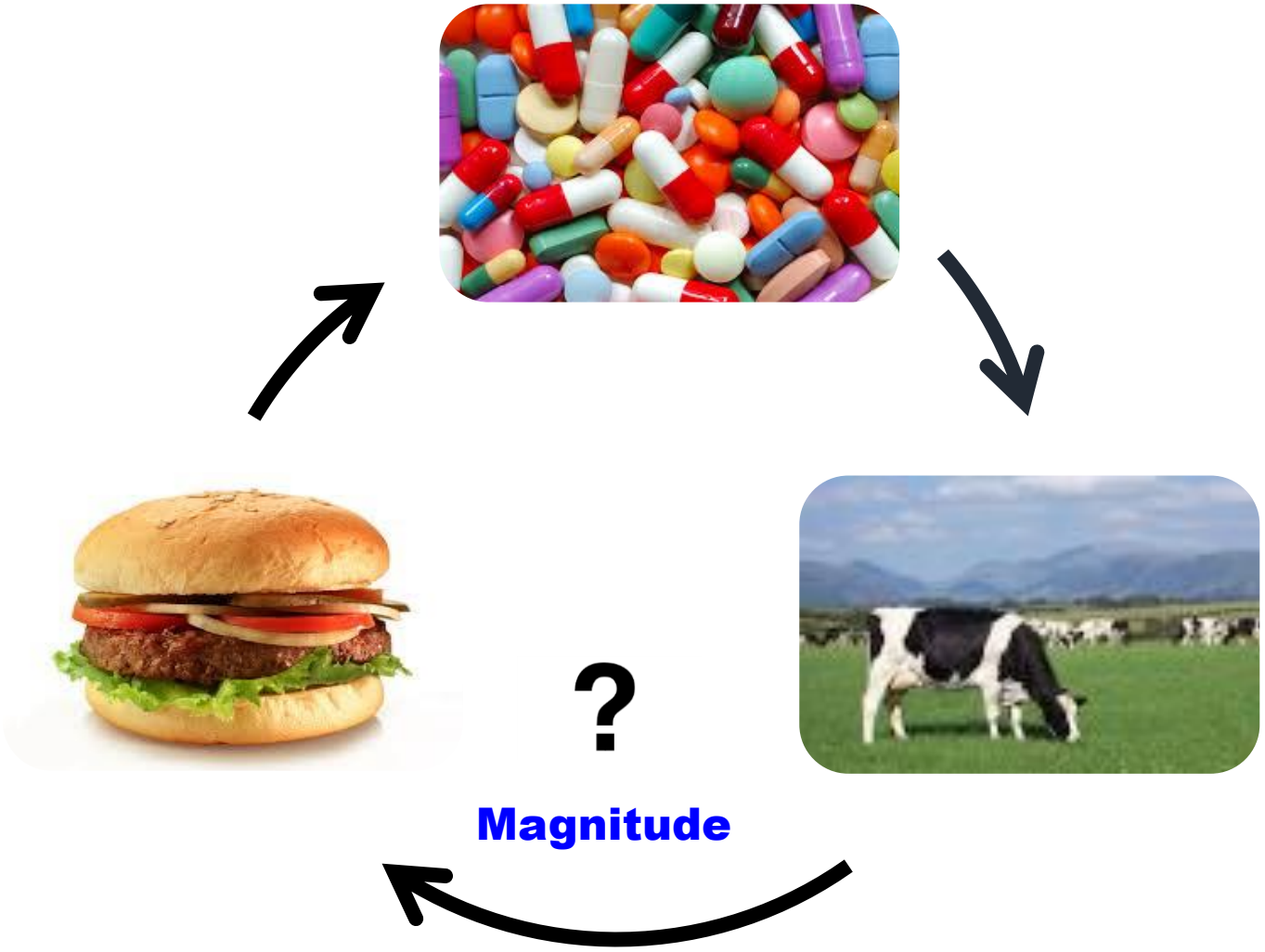
Bsc (Hons.) in Medical Science

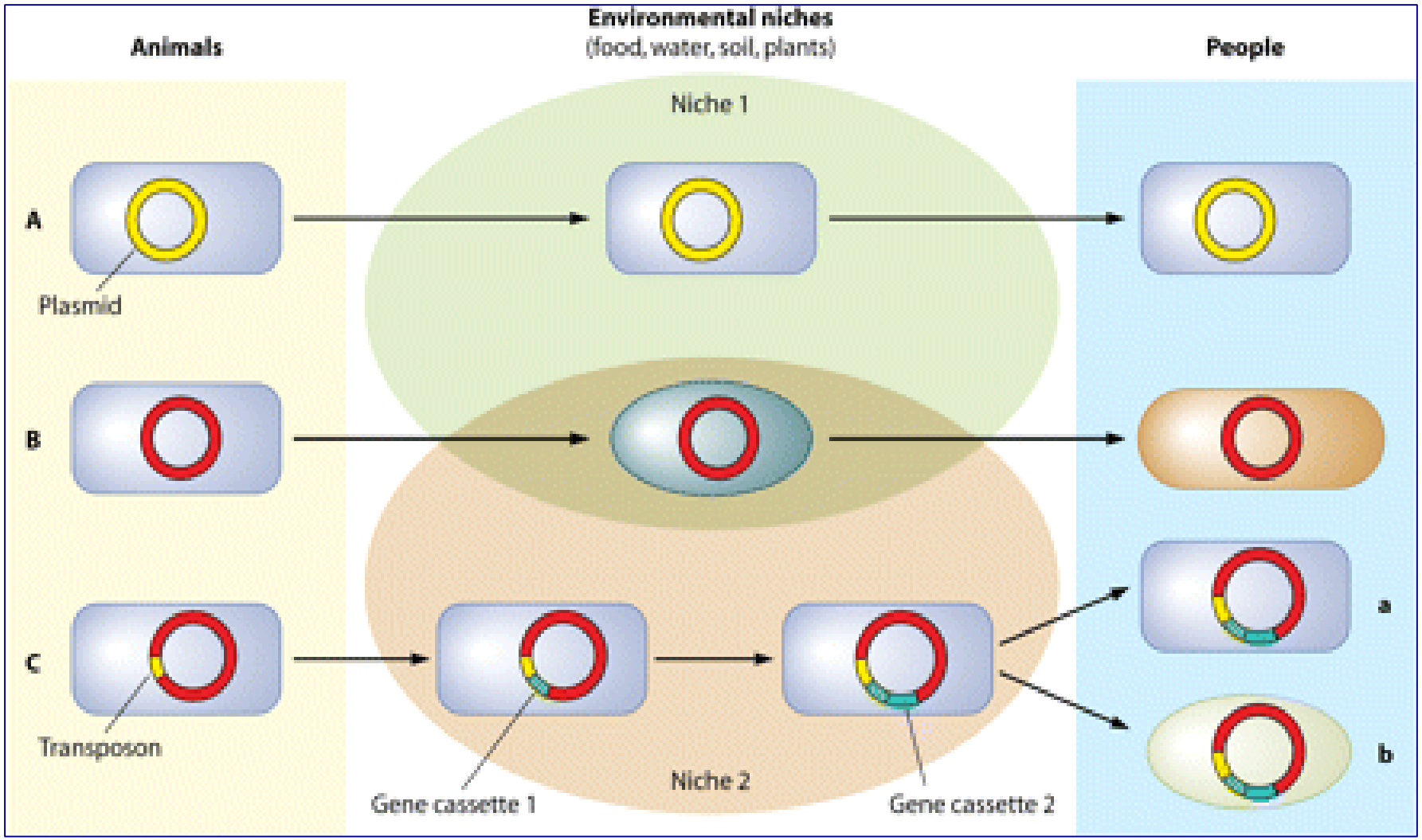
Galway-Mayo Institute of Technology

May, 2015

Antibacterial resistance- a serious problem for animal and human populations (WHO 2014) -

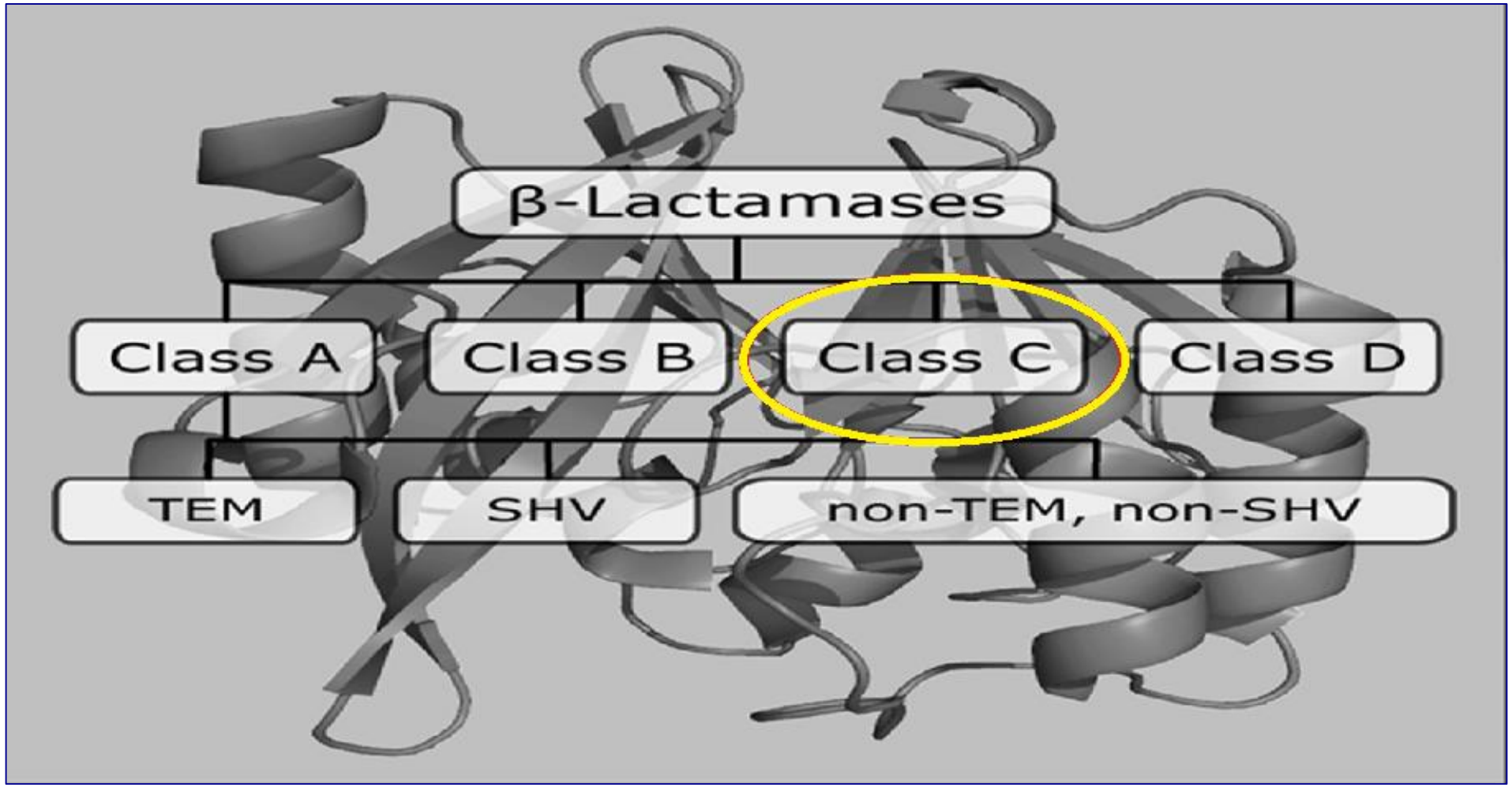


Plasmids- have a major role to play in transmission of resistance



(Levy and Marshall 2011)

Classification of beta-lactamase enzymes -



(Pleiss 2015)

Objectives of this study -

- Investigate for the presence of *ampC*-encoding genotypes in 3 cefoxitin resistant isolates
- Establish the plasmid incompatibility (Inc) types and profiles of 47 multi-drug resistant *Escherichia coli*
- Determine the minimum inhibitory concentration (MIC) of colistin in a sub-set of positive isolates
- Establish the gentic basis for resistance to colistin
- Discuss the significance of all these findings

Methods used in this project -

Plasmid isolation and purification

Agarose Gel Electrophoresis

Pulsed-Field Gel Electrophoresis (PFGE)

Polymerase Chain Reaction (PCR)

Broth Microdilution

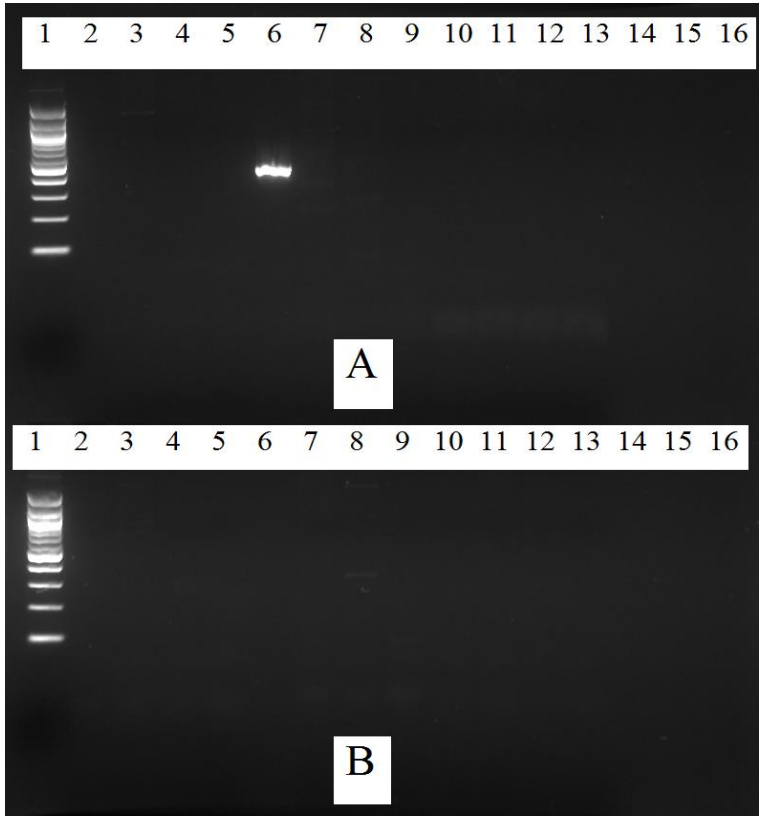
Isolate No.	Isolate ID	Approx Small Plasmid size (kb)	PFGE results (147kb / 63kb)	Replicon Types													Colistin Susceptibility Testing			
				A/C	B/O	FIA	FIB	FIC	HI1	P	I1	K/B	FIIS	Y	HI2	W	X	MIC (A) (mg/L)	MIC (B) (mg/L)	MIC © (mg/L)	Sensitive/ Resistant
M1	XX-2219	>36																0.125	0.125	0.125	Sensitive
M2	-22130	>36, >36					■											<0.0625	<0.0625	0.125	Sensitive
M4	-22132	>36			■	■				■								0.125	0.125	0.125	Sensitive
M5	-22133	>36, >36, <2.0, <2.0														■		<0.0625	<0.0625	<0.0625	Sensitive
M6	-22134	>36, >36																8	8	8	Resistant
M9	-22509	25, 10			■		■			■								0.125	0.125	0.0625	Sensitive
M10	-22510	>36, >36, 20			■		■			■								0.0625	0.0625	0.0313	Sensitive
M11	-22511	>36, >36, >36					■			■								0.0313	0.0313	0.0156	Sensitive
M12	-22512	>36																0.125	0.125	0.125	Sensitive
M13	-22513	>36, <2			■					■								<0.0625	<0.0625	0.125	Sensitive
M14	-22514	>36, 25				■	■											<0.0625	<0.0625	<0.0625	Sensitive
M15	-22515	None				■	■											0.0625	0.0625	0.0625	Sensitive
M17	-22517	>36, 25, 10				■	■											0.0156	0.0313	0.0625	Sensitive
M18	-22518	None				■	■											<0.0625	<0.0625	<0.0625	Sensitive
M19	-22733	>36			■													0.0313	0.0625	0.75	Sensitive
M20	-22734	>36																0.0313	0.0625	0.125	Sensitive
M22	-22736	>36			■													0.125	0.25	0.25	Sensitive
M23	-22737	>36, >36, 25			■							■						0.0625	0.0313	0.0313	Sensitive
M26	-29712	>36, >36, 36			■	■	■			■								0.5	0.125	0.0313	Sensitive
M28	-29714	>36			■													<0.0625	<0.0625	0.125	Sensitive
M32	-29956	>36								■								0.25	0.25	0.0313	Sensitive
M33	-29957	>36, >36								■					■			4	4	4	Resistant
M34	-29958	>36, >36				■	■			■								<0.0625	<0.0625	0.125	Sensitive
M35	-30391	>36, >36									■							<0.0625	0.25	0.25	Sensitive
M39	-30545	>36, >36, <2.0			■					■								0.0625	0.125	0.25	Sensitive
M44	-30550	None				■	■									■		0.125	0.125	0.125	Sensitive
M45	-30551	>36, >36, 36					■			■	■							0.0313	0.0625	0.125	Sensitive
M48	-30554	None																<0.0625	<0.0625	<0.0625	Sensitive
M49	-30555	None				■	■									■		0.5	0.5	0.25	Sensitive
M50	-30556	>36, >36			■		■				■							<0.0156	0.0625	0.25	Sensitive
M52	-747	<2.0													■			2	2	4	Intermediate
M53	-748	>36, 36, 22, <2.0					■											0.0313	0.0313	0.0625	Sensitive
M54	-749	None			■					■								0.0313	0.0313	0.125	Sensitive
M61	-1725	>36, 28, 20, <2.0				■												0.125	0.125	0.25	Sensitive
M69	-1733	>36, 25, 15, <2.0				■	■											0.0313	0.0625	0.125	Sensitive
M73	-1896	>36, 28, 22, 15, <2.0					■				■							8	8	8	Resistant
M74	-1897	>36, >36			■	■	■											0.0625	0.0625	0.125	Sensitive
M75	-1898	>36, >36, 25																<0.0625	<0.0625	<0.0625	Sensitive
M85	-22423	>36, 20																0.0313	0.0625	0.125	Sensitive
M88	-22426	None								■								0.0313	0.0313	0.0625	Sensitive
M92	-22430	>36, 28				■	■											0.0313	0.0313	0.125	Sensitive
M95	-23002	>36, >36, 30, 22					■											<0.0156	0.0313	0.0625	Sensitive
M99	-23013	28														■		0.125	0.25	0.5	Sensitive
M117	-30447	>36, >36, >36, 25, <2			■		■			■						■		0.0625	0.125	0.25	Sensitive
M121	-30451	None			■													<0.0625	<0.0625	0.125	Sensitive
M139	11-01562	>36, 30					■											<0.0625	<0.0625	0.125	Sensitive
M147	11-01917	<2.0					■				■							0.125	0.125	0.0313	Sensitive
ATCC 25922 E.coli	N/A	N/A		■	■	■	■	■	■	■	■	■	■	■	■	■	■	<0.0625	<0.0625	<0.0625	Target Range (0.25-2)

Comparison of plasmid Inc types in two studies -

Incompatibility type	Current Project (% positive *E. coli* isolated from bovine samples)	Wang *et al* (2013) (% positive *E. coli* isolated from human, lamb and chicken samples)
IncFIA	28%	22%
IncFIB	49%	62.5%
IncI1	11%	67%
IncB/O	32%	9%

ampC genotypes identified by PCR -

PCR results for samples M14, M23 and M69 for *ampC* genes MOX, CIT, DHA, ACC, EBC and FOX



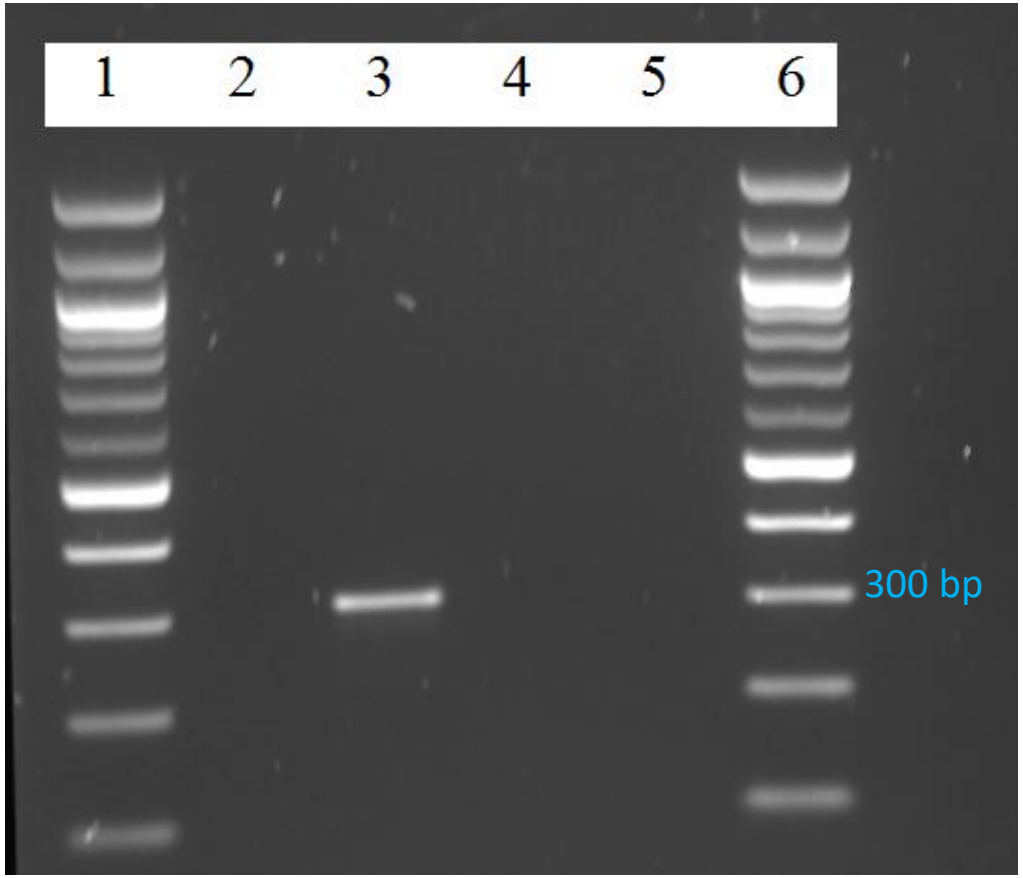
Low molecular weight ladder -	M14	M23	M69	Negative control -	AmpC gene family-	*ampC* gene(s) detected-
1 A	2 A	3 A	4 A	5 A	MOX	MOX-1, MOX-2,CMY-1, CMY-8 to CMY-11
N/A	6 A	7 A	8 A	9 A	CIT	LAT-1 to LAT-4, CMY-2 to CMY-7, BIL-1
N/A	10 A	11 A	12 A	13 A	DHA	DHA-1, DHA-2
1 B	2 B	3 B	4 B	5 B	ACC	ACC
N/A	6 B	7 B	8 B	9 B	EBC	MIR-1T, ACT-1
N/A	10 B	11 B	12 B	13B	FOX	FOX-1 to FOX-5b

Sequencing and BLAST of this positive PCR product matched it as a CMY-2 b-lactamase

Colistin susceptibility results -

- In November, 2015 a paper published details describing a gene known as *mcr-1*
- First known transmissible plasmid-mediated colistin resistance gene
- Three putative colistin isolates were detected among 47 MDR *E. coli* isolates with MIC values ranging from 4-8 mg/L
- The genotypes of these isolates were studied

PCR of 3 colistin resistant isolates revealed the presence of *mcr-1* -



Lane number	Sample
1 and 6	100 bp ladder
2	-22134
3	-29957
4	-1896
5	Negative control

Other similar studies have shown how broad spectrum cephalosporins may have the potential to co-select for colistin resistance and *vice-versa* (Haenni *et al* 2016)

Particular association with IncHI2 plasmids

Conclusions -

- the relationship between plasmid genotypes and resistant phenotypes
- the need for standardisation at a global level to track resistance at the food-production level
- food chain risk for resistance gene spread
- the risk of uncontrolled antibiotic usage in the development of multidrug resistance spread

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