

The Conjugative Ability of *Escherichia coli* bla_{CTX-M} Plasmids From Returning Travellers

Matthew J Powell, Edward R Bevan, Christopher M Thomas,
Laura JV Piddock & Peter M Hawkey



INSTITUTE OF
MICROBIOLOGY
AND INFECTION



@mattjpowell94 @BevanEdward



UNIVERSITY OF
BIRMINGHAM

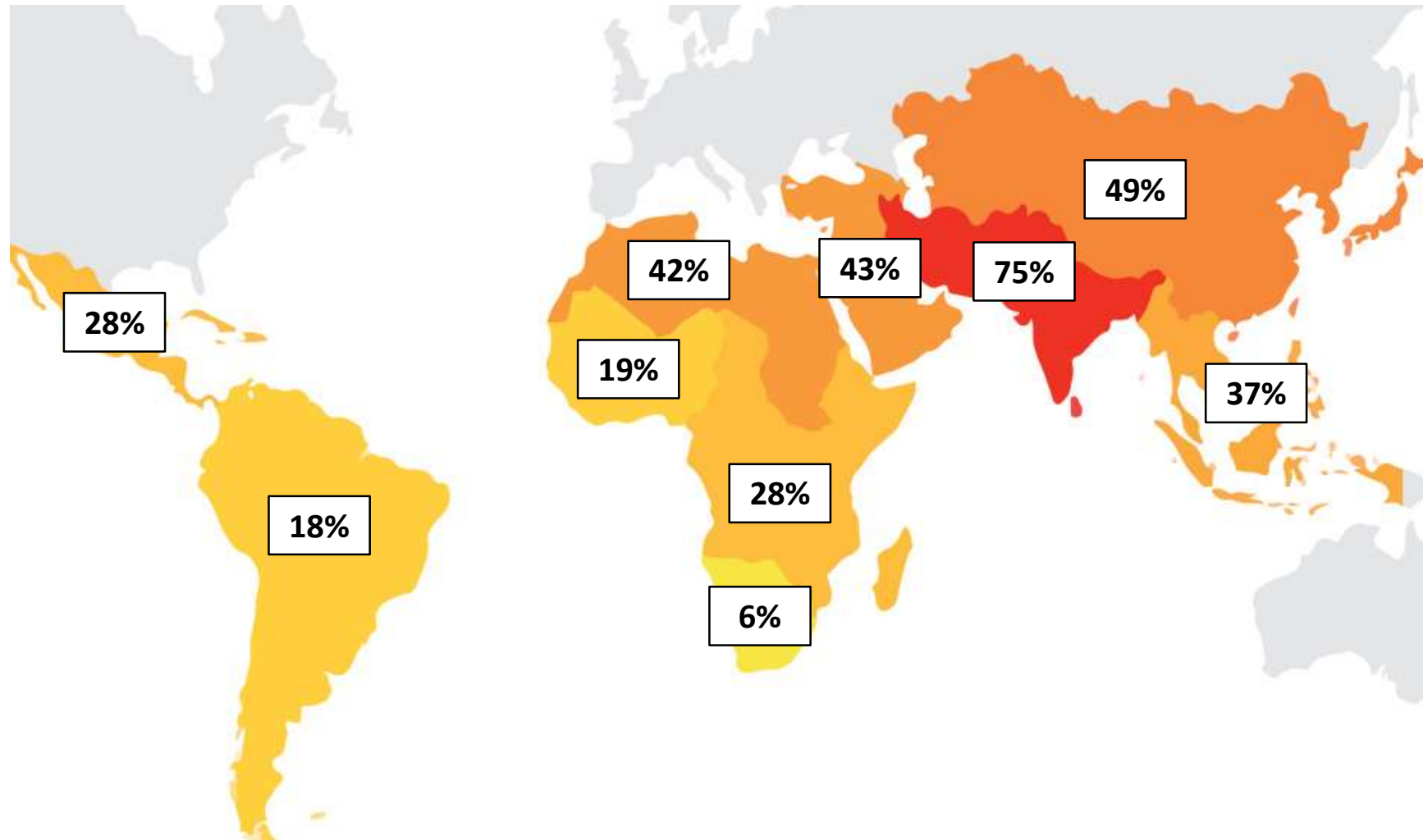
Background

- Antimicrobial resistance is a hugely significant and growing problem
- AMR is associated with excess mortality
- ESBL genes encode resistance to beta-lactam antibiotics - including 3rd generation cephalosporins
- *bla*_{CTX-M} is internationally disseminated in Enterobacteriaceae^{1,2}

¹Bevan ER, Jones AM, Hawkey PM, JAC, 2017 (in press)

²Hawkey PM, Jones AM, JAC, 2009

Rate of Acquisition of ESBL-Enterobacteriaceae by Dutch Travellers



Hypothesis

*bla*_{CTX-M} plasmids carried by *E.coli* acquired during travel are transferrable by conjugation in vitro

Aims of Study

1. Isolate ESBL *E. coli* from faeces before and after travel to Indian subcontinent
2. Investigate the conjugative ability of *E. coli bla*_{CTX-M} plasmids acquired during travel

Laboratory Methods

Post-travel CTX-M-producing *E. coli* cultured from stool samples

Isolates 'screened' for inclusion in conjugation experiment (n=109)

Overnight filter-mating with recipient J53 *E. coli* (n=64)

Sub-culture to rifampicin/cefotaxime MacConkey's agar & PCR check

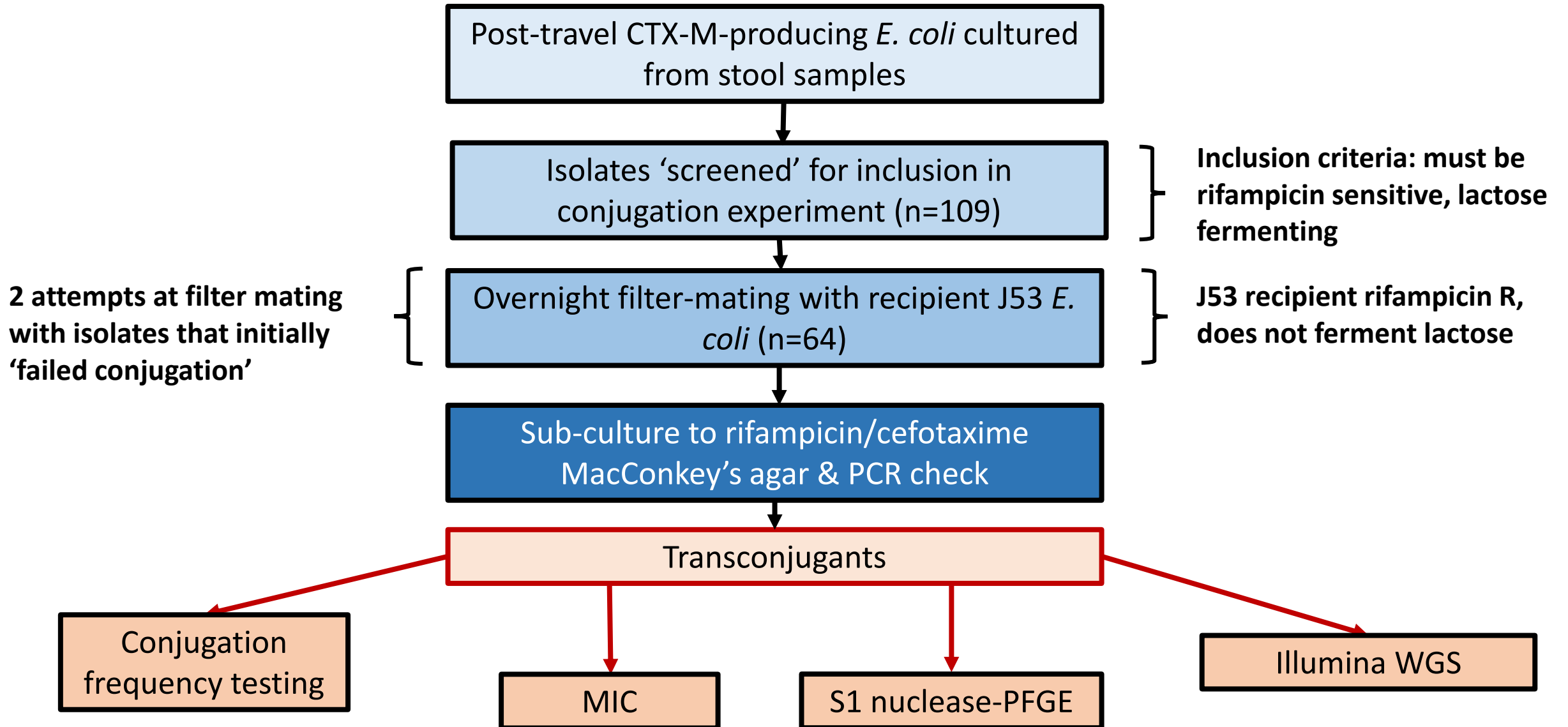
Inclusion criteria: must be rifampicin sensitive, lactose fermenting

J53 recipient rifampicin R, does not ferment lactose

2 attempts at filter mating with isolates that initially 'failed conjugation'



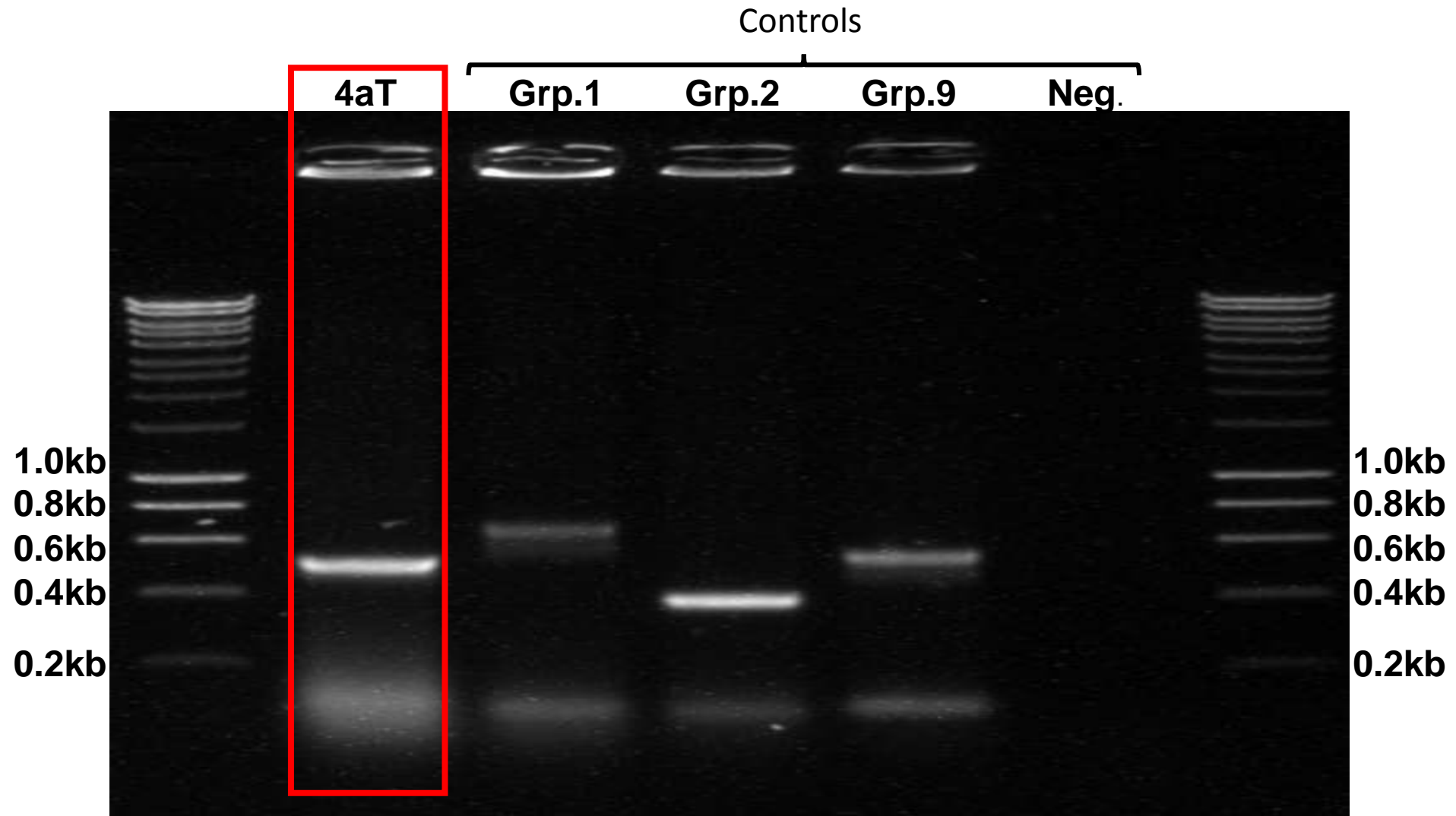
Laboratory Methods



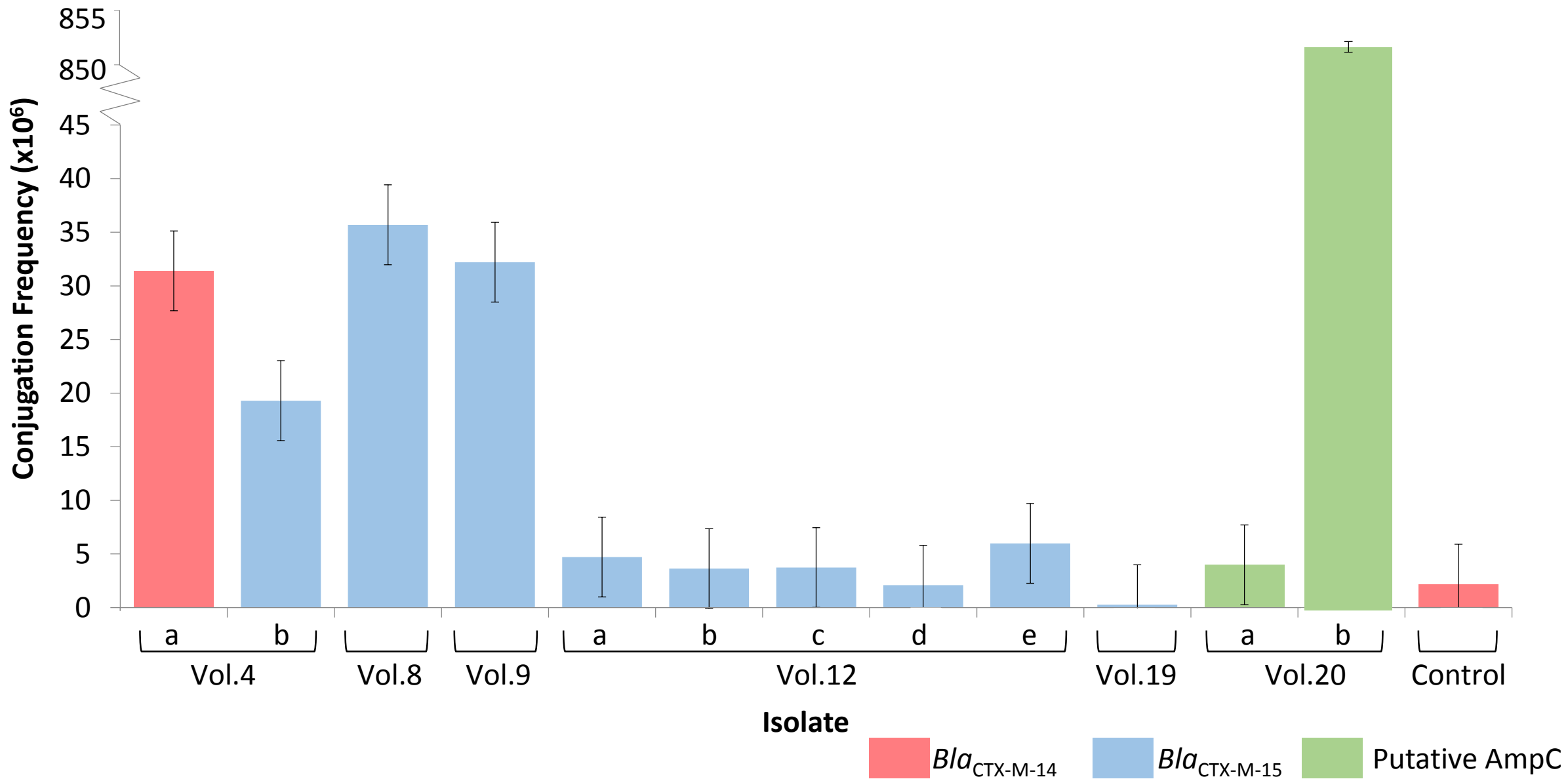
Results

- ESBL *E. coli* from 67% (8/12) of travellers transferred $bla_{\text{CTX-M}}$ to a plasmid-free recipient
- Incidental finding: Isolate screening, 50% travellers (6/12) carried non-lactose fermenting *E. coli* (33/109 isolates screened)

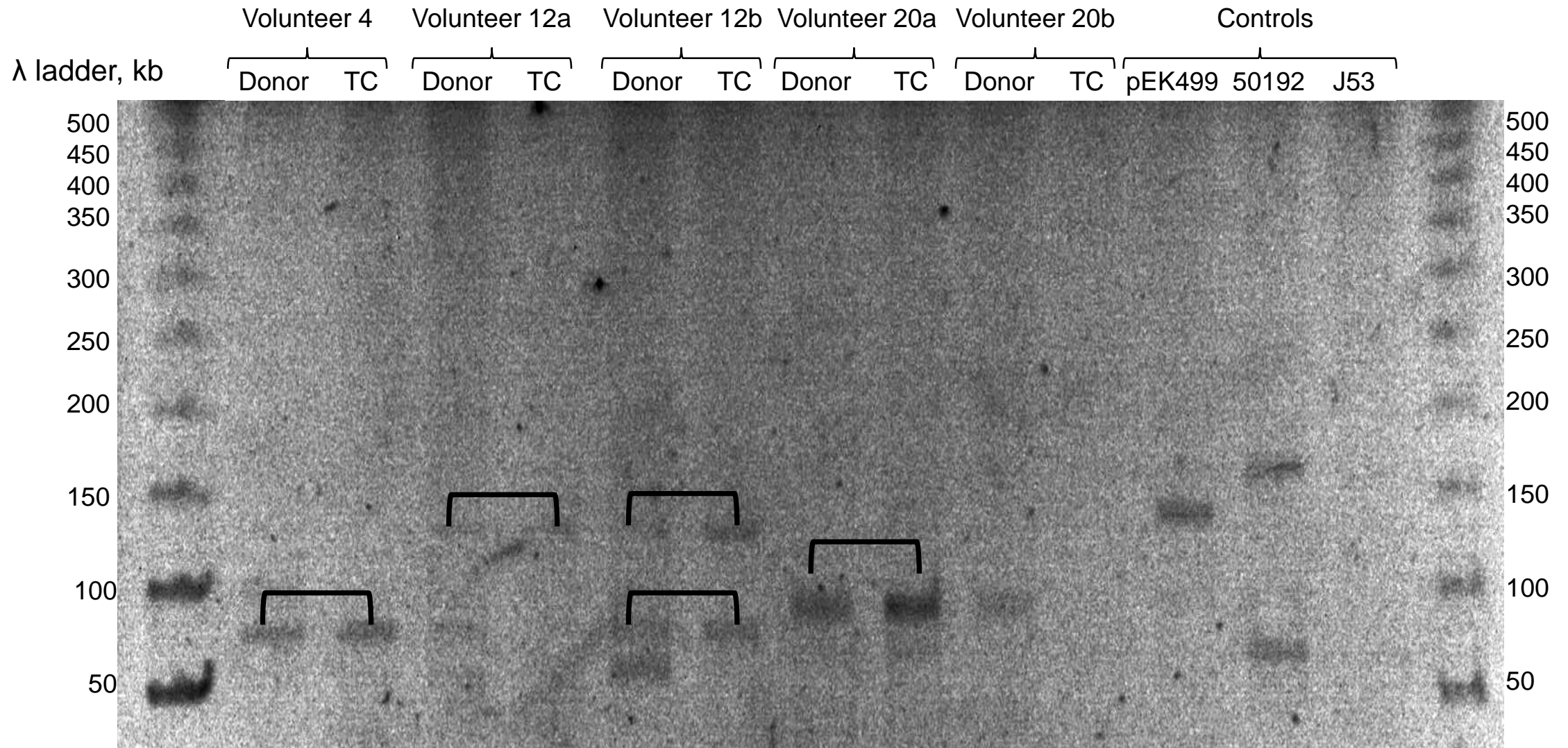
PCR of transconjugant 4aT showed that this strain carried
a group 9 *bla*_{CTX-M} gene



Conjugation Frequencies



S1 nuclease PFGE of donor and transconjugants (TC)



*bla*_{CTX-M} Epidemiology in South Asia

- *bla*_{CTX-M-15} (group 1) predominates in South Asia⁴
- 13% of ESBLs in urinary isolates in Sri Lanka are group 9 *bla*_{CTX-M}⁵
- One traveller to Sri Lanka in this study acquired *bla*_{CTX-M-14} (group 9)

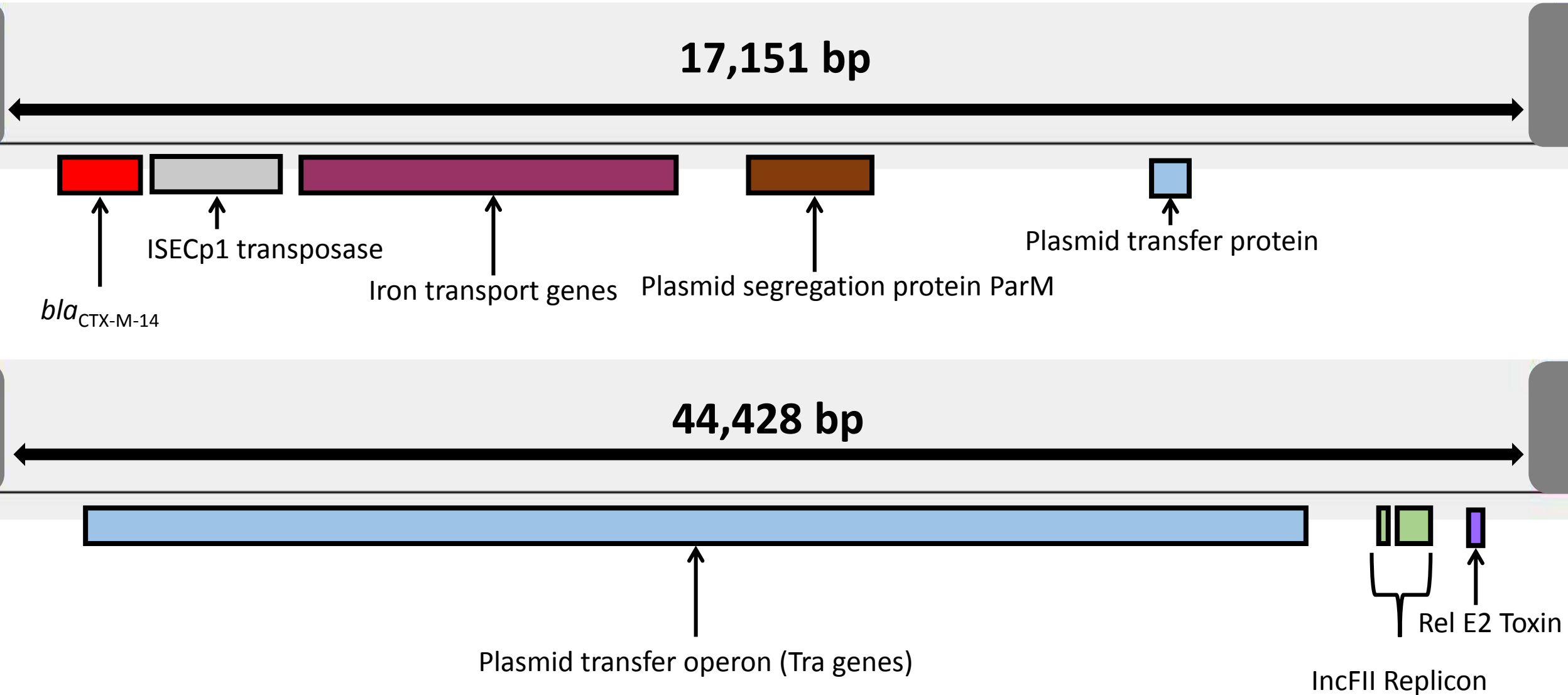
⁴Sheng WH, Antimicrob Agents Chemother, 2013

⁵Tillekeratne LG, Infect Chemother, 2016

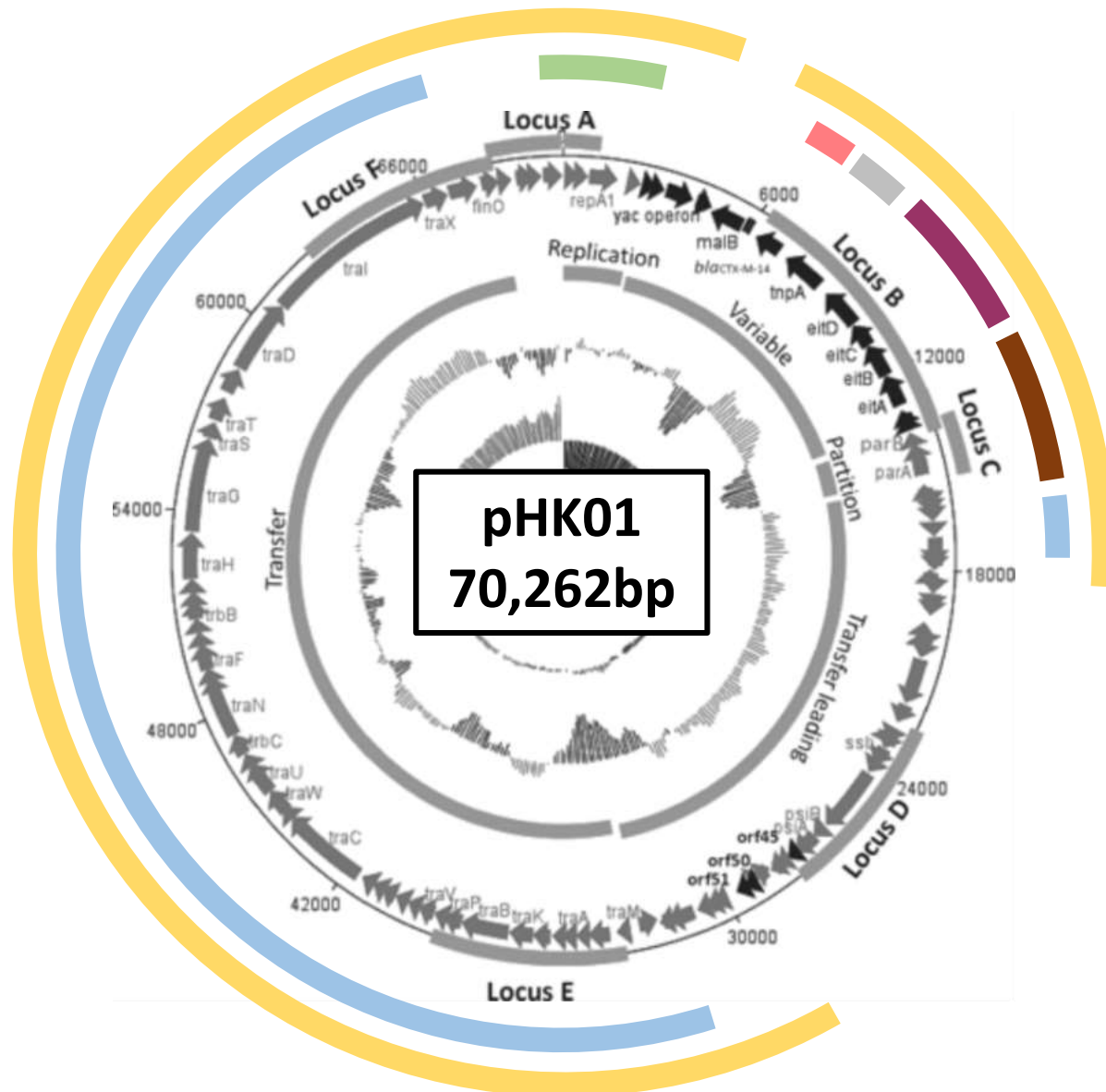
Transconjugant (TC) WGS Outputs

ISOLATE	MLST	RESISTANCE GENE FINDER	PLASMID FINDER
J53 recipient	ST10	None	None
4a donor	ST2732	<i>bla</i> _{CTX-M-14}	IncFII (p4aT)
4a TC	ST10	<i>bla</i> _{CTX-M-14}	IncFII (p4aT)
20a donor	ST6438	<i>bla</i> _{DHA-1} , QnrB4, mphA, sul1, dfrA17	IncFII, IncFIC, IncFIB, IncB/O/K/Z
20a TC	ST10	<i>bla</i> _{DHA-1} , QnrB4, mphA, sul1, dfrA17	IncFII, IncB/O/K/Z

Partial assembly of p4aT (approx. 68kb)



Plasmid fragments show homology to pHK01



- Homologous sequences
- Plasmid transfer proteins (tra genes)
- IncFII replicon
- bla*_{CTX-M-14}
- ISECp1 transposase
- Iron transport proteins
- Plasmid segregation protein ParM

Conclusions

- Horizontal gene transfer of *bla*_{CTX-M} in vitro is important as probably occurs in vivo as well
- Plasmid transfer supports worldwide dissemination of *bla*_{CTX-M}
- This work is a model for the study of other resistance genes, e.g. *bla*_{KPC}, *bla*_{NDM-1} and *mcr-1*
- Such a model would help inform public health policy and perhaps slow the wider dissemination of these resistance genes

Acknowledgements

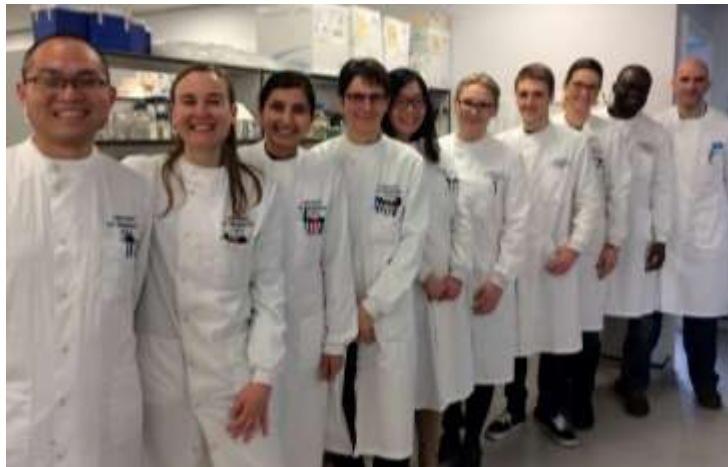
Dr Edward R. Bevan

Prof. Peter M. Hawkey

Prof. Laura J.V. Piddock

Prof. Christopher M. Thomas

Antimicrobials Research Group



Public Health
England