First report of *bla*$_{IMP-14}$ on a plasmid harboring multiple drug resistance genes in *Escherichia coli* ST131

Nicole Stoesser[a#], Anna E. Sheppard[a], Gisele Peirano[b,c], Robert Sebra[d], Tarah Lynch[c], Luke Anson[a], Andrew Kasarskis[d], Mary R. Motyl[e], Derrick W. Crook[a], Johann D. Pitout[b,c,f,g,h]

[a] Modernizing Medical Microbiology Consortium, Nuffield Department of Medicine, John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom

[b] Division of Microbiology, Calgary Laboratory Services, Calgary, Alberta, Canada

[c] Department of Pathology and Laboratory Medicine, University of Calgary, Alberta, Canada

[d] Icahn Institute and Department of Genetics and Genomic Sciences, Icahn School of Medicine, Mount Sinai, New York, USA

[e] Clinical Microbiology, Merck and Co Inc., Rahway, New Jersey, USA

[f] Department of Microbiology, Immunology, and Infectious diseases, University of Calgary, Alberta, Canada

[g] Snyder Institute for Chronic Diseases, University of Calgary, Alberta, Canada

[h] Department of Medical Microbiology, University of Pretoria, Pretoria, South Africa

#Address correspondence to Nicole Stoesser, nicole.stoesser@ndm.ox.ac.uk

Running title: *E. coli* ST131 and *bla*$_{IMP-14}$

Words: Abstract: 68/75, Main text: 944/1000

ABSTRACT

The *bla*$_{IMP-14}$ carbapenem resistance gene has largely previously been observed in *Pseudomonas aeruginosa* and *Acinetobacter* spp. As part of global surveillance and sequencing of carbapenem-resistant *E. coli*, we identified an ST131 strain harboring *bla*$_{IMP-14}$ within a class 1 integron, itself nested within a ~54kb multi-drug resistance region on an epidemic IncA/C_2 plasmid. The emergence of *bla*$_{IMP-14}$ in this context in the ST131 lineage is of potential clinical concern.

MAIN TEXT

The emergence of carbapenemases in clinically prevalent *Escherichia coli* lineages, such as sequence type (ST) 131, is a major problem for the management of patients infected by these strains(1, 2). Globally, five major transmissible carbapenemase enzymes predominate, represented by the KPC, OXA-48-like, NDM, VIM, and IMP families(2, 3).

An IMP metallo-beta-lactamase enzyme (IMP-1) was first detected in Japan in *Pseudomonas aeruginosa* in the late 1980s(4); since then, 52 genetically diverse bla_{IMP} gene variants 738-747bp in length have been identified(5). Most bla_{IMP} variants have been isolated from either *Pseudomonas* or *Acinetobacter* spp. and demonstrate a degree of geographic structuring(6); however, some, such as bla_{IMP-4} and bla_{IMP-8} have emerged successfully in Enterobacteriaceae and are distributed over wider geographic regions(6, 7). Associations of bla_{IMP} with *E. coli* ST131 have to date been restricted to bla_{IMP-4} and bla_{IMP-8} in Taiwan, China and Australia(8-11). As part of the Merck Study for Monitoring Antimicrobial Resistance Trends (SMART)(1), we identified an IMP-14-producing ST131 *E. coli* isolate, Ecol_732, from Bangkok, Thailand, isolated in 2012, which was sequenced in order to ascertain the genetic structures associated with this IMP variant in ST131.

Ecoli_732 was obtained from the urine of hospitalized elderly male with lower urinary tract infection. Minimum inhibitory concentrations (MICs) to ampicillin-sulbactam (SAM), piperacillin-tazobactam (TZP), cefoxitin (FOX), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), ertapenem (ERT), imipenem (IPM), amikacin (AMK) and ciprofloxacin (CIP) were determined using microdilution panels

prepared at International Health Management Associates (IHMA, Inc, Schaumburg, IL USA), following 2015 CLSI guidelines. It tested non-susceptible (i.e. either intermediate or resistant) to the above-mentioned agents. MICs to colistin and tigecycline (using E-tests) were 0.12 and 1 mg/L respectively.

DNA (chromosomal plus plasmid) was extracted from pure, overnight sub-cultures of the isolate for both PacBio (long-read) sequencing and Illumina MiSeq (short-read) sequencing using the Qiagen Genomic tip 100/G kit and QIAamp DNA Mini kit (Qiagen, Valencia, CA; catalogue numbers 10243, 51304), respectively. Preliminary *de novo* assembly of PacBio reads using HGAP3 was performed; resulting contigs were annotated using PROKKA(12), and then trimmed on the basis of sequence/annotation overlaps in Geneious (Version 9.04)(13). 150 base, paired-end MiSeq reads for each of the isolates were trimmed using Trimmomatic (Version 0.35)(14), and then mapped to the corresponding PacBio assemblies using BWA mem (Version 0.7.9a-r786)(15). Read pileups were inspected to confirm the structural integrity of the contigs and correct any small errors in the assembled contigs. Unmapped MiSeq reads were assembled using A5MiSeq(16) in order to identify any small plasmids (<7kb) that may have been filtered out during the size selection process implemented as part of the PacBio library preparation. Additional annotation focused on resistance genes and insertion sequences was performed with reference to the ResFinder(17), PlasmidFinder(18) and ISFinder(19) databases.

The Ecol_732 genome consists of a 5,009,900 bp chromosome and six plasmids, five of which could be fully resolved. These included: pEC732-IMP14 (186,826 bp, IncA/C_2); pEC732_2 (129,154 bp, IncFII/FIA/FIB/col); pEC732_3 (82,588 bp,

IncB/O/K/Z); pEC732_4 (4,072 bp, untyped) and pEC732_5, (1,549 bp, untyped). A partial, sixth *mob* plasmid fragment was also present (4,204 bp, untyped). In pEC732-IMP14, bla_{IMP-14} differed from the reference sequence AY553332 by a single synonymous substitution (A249C), resulting in the same amino acid sequence. It was located within a 3,791bp class 1 integron, In687 (*intI1-*bla_{IMP-14}*-aac(6')-qacEdelta-sul1*). This integron is almost identical (single nucleotide difference, A925G) to that in *Achromobacter xylosoxidans* strain R4, cultured from a urine sample, also in Thailand (GenBank accession number: KJ406505).

The backbone of pEC732-IMP14 was highly similar to the prototype Type 1 IncA/C_2 plasmid, pRMH760 (RefSeq: NC_023898; from a *Klebsiella pneumoniae* strain) and other Type 1 IncA/C_2 plasmids, recently reviewed in(20), almost all of which also include a specific region, designated "ARI-A", which contains a variable array of resistance genes, 1,711bp upstream of *rhs*(20). Similarly, in pEC732-IMP14, the bla_{IMP-14}-harboring integron was part of a much larger, 54,454bp ARI-A-like region, containing antimicrobial, heavy metal and biocide resistance genes (Fig. 1), including those encoding resistance to beta-lactams (bla_{OXA-10}, bla_{IMP-14}), macrolides (drug efflux), rifampicin (*arr-2*), sulfonamides (*sul1*), aminoglycosides (*aadA1*, *aadB*, *aph(3')-IVa*, *aac(6')*) chloramphenicol (*cmlA7*), chromate (*srpC*), mercury (*mer* operon) and quaternary ammonium compounds (*qac*). Some of these were part of a second, novel integron, designated In1286 (*intI1-qacH-aadB-arr-2-cmlA7-*bla_{OXA-10}*-aadA1*).

An alignment of pEC732-IMP14, the prototype IncA/C_2 Type 1 plasmid, pRMH760, and the only publicly available Type 1 IncA/C_2 sequence from Thailand, pR148

(RefSeq: NC_019380, from *Aeromonas hydrophila*(21)), demonstrates the genetic similarity of these plasmids (Fig. 2). All three sequences were >99% similar in the 1-86,573 bp region, and in the ~27.5 kb region downstream of ARI-A (Fig. 2). Differences in pEC732-IMP14 include a region of clustered single nucleotide variants (SNVs) suggestive of a recombination event (region: 3,100-8,000kb), and the acquisition of two integrase subunits (regions: 86,573-89,203 and 90,138-200,167 bp; Fig. 2). Interestingly, the pR148-containing *A. hydrophila* strain was identified on a Thai tilapia fish farm that had successively used several antimicrobial classes(21).

To date, $bla_{\text{IMP-14}}$ has not been described in *E. coli* to our knowledge, and has largely previously been reported in *P. aeruginosa* and *Acinetobacter baumannii* strains from several hospital centers in Thailand, in some cases as part of clonal outbreaks(22-25). Although $bla_{\text{IMP-14}}$ is similarly associated with class 1 integrons in these cases, as in pEC732-IMP14, the wider plasmid contexts and sequence of these integrons in *P. aeruginosa* and *A. baumannii* strains have not been investigated. It is however conceivable that the $bla_{\text{IMP-14}}$-harboring integron observed in pEC732-IMP14 and the *A. xylosoxidans* strain R4 has been exchanged more widely with *Pseudomonas* and *Acinetobacter* spp. in Thailand. Class 1 integrons have been linked with the recent successful spread and expansion of another metallo-beta-lactamase, bla_{VIM}, in IncA/C_2 plasmids in Enterobacteriaceae in Greece(26), and bla_{VIM} and bla_{IMP} in Spain(27).

The presence of the extensively drug-resistant region observed here on an epidemic IncA/C_2 plasmid in an *E. coli* ST131 strain from Thailand is therefore of concern, and may represent wider, regional, horizontal dissemination of $bla_{\text{IMP-14}}$ mediated by

mobile genetic elements across bacterial families. The homology of pEC732-IMP14 with an *A. hydrophila* plasmid found on a fish farm, and the presence of bla_{IMP}-harboring plasmids in *E. coli* in other environmental(28) and animal sampling frames(29), suggests that the transmission network for IMP-positive *E. coli* may extend beyond the healthcare setting. Broad surveillance and control measures which are targeted at both community and healthcare contexts may be required to monitor and limit bla_{IMP} dissemination.

Nucleotide sequence accession numbers

Complete sequence data for Ecol_732 have been deposited in GenBank under the BioProject number PRJNA316786. Accession numbers for the sequences are as follows: CP015138 (chromosome), CP015139 (pEC732-IMP14), CP015140 (pEC732_2), CP015141 (pEC732_3), CP015142 (pEC732_4), CP015143 (pEC732_5), and CP015144 (pEC732_6 [partial sequence only]).

Acknowledgements

The authors are grateful for the support of the Modernizing Medical Microbiology Informatics Group (MMMIG). We also wish to thank Dr. Daryl Hoban and the Merck SMART surveillance team.

Funding information

NS is currently funded through a Public Health England/University of Oxford Clinical Lectureship; the sequencing work was also partly funded through a previous Wellcome Trust Doctoral Research Fellowship (#099423/Z/12/Z). Additional funding support was provided by a research grant from Calgary Laboratory Services

(#10006465), and by the Health Innovation Challenge Fund (a parallel funding partnership between the Wellcome Trust [WT098615/Z/12/Z] and the Department of Health [grant HICF-T5-358]). The research was supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre Programme.

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

REFERENCES

1. **Peirano G, Bradford PA, Kazmierczak KM, Badal RE, Hackel M, Hoban DJ, Pitout JD.** 2014. Global incidence of carbapenemase-producing *Escherichia coli* ST131. Emerg Infect Dis **20:**1928-1931.
2. **Mathers AJ, Peirano G, Pitout JD.** 2015. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clin Microbiol Rev **28:**565-591.
3. **Nordmann P, Naas T, Poirel L.** 2011. Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis **17:**1791-1798.
4. **Watanabe M, Iyobe S, Inoue M, Mitsuhashi S.** 1991. Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother **35:**147-151.
5. **Bush KP, T.; Jacoby, G.** 2015. ß-Lactamase Classification and Amino Acid Sequences for TEM, SHV and OXA Extended-Spectrum and Inhibitor Resistant Enzymes. http://www.lahey.org/studies/. Accessed
6. **Cornaglia G, Giamarellou H, Rossolini GM.** 2011. Metallo-beta-lactamases: a last frontier for beta-lactams? Lancet Infect Dis **11:**381-393.
7. **Sidjabat HE, Townell N, Nimmo GR, George NM, Robson J, Vohra R, Davis L, Heney C, Paterson DL.** 2015. Dominance of IMP-4-producing *Enterobacter cloacae* among carbapenemase-producing Enterobacteriaceae in Australia. Antimicrob Agents Chemother **59:**4059-4066.
8. **Ho PL, Cheung YY, Wang Y, Lo WU, Lai EL, Chow KH, Cheng VC.** 2016. Characterization of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* from a healthcare region in Hong Kong. Eur J Clin Microbiol Infect Dis **35:**379-385.

9. **Yan JJ, Tsai LH, Wu JJ.** 2012. Emergence of the IMP-8 metallo-beta-lactamase in the *Escherichia coli* ST131 clone in Taiwan. Int J Antimicrob Agents **40:**281-282.

10. **Zhang F, Zhu D, Xie L, Guo X, Ni Y, Sun J.** 2015. Molecular epidemiology of carbapenemase-producing *Escherichia coli* and the prevalence of ST131 subclone H30 in Shanghai, China. Eur J Clin Microbiol Infect Dis **34:**1263-1269.

11. **Sidjabat HE, Robson J, Paterson DL.** 2015. Draft Genome Sequences of Two IMP-4-Producing *Escherichia coli* Sequence Type 131 Isolates in Australia. Genome Announc **3**.

12. **Seemann T.** 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics **30:**2068-2069.

13. **Kearse MM, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, A.; Markowitz, S.; Duran, C.; Thierer, T.; Ashton, B.; Metjies, P.; Drummond A.** 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28:**1647-1649.

14. **Bolger AM, Lohse M, Usadel B.** 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30:**2114-2120.

15. **Li H, Durbin R.** 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics **25:**1754-1760.

16. **Coil D, Jospin G, Darling AE.** 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics **31:**587-589.

17. **Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV.** 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother **67:**2640-2644.

18. **Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H.** 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother **58:**3895-3903.

19. **Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M.** 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res **34:**D32-36.

20. **Harmer CJ, Hall RM.** 2014. pRMH760, a precursor of A/C(2) plasmids carrying blaCMY and blaNDM genes. Microb Drug Resist **20:**416-423.

21. **Del Castillo CS, Hikima J, Jang HB, Nho SW, Jung TS, Wongtavatchai J, Kondo H, Hirono I, Takeyama H, Aoki T.** 2013. Comparative sequence analysis of a multidrug-resistant plasmid from *Aeromonas hydrophila*. Antimicrob Agents Chemother **57:**120-129.

22. **Khuntayaporn P, Montakantikul P, Santanirand P, Kiratisin P, Chomnawang MT.** 2013. Molecular investigation of carbapenem resistance among multidrug-resistant *Pseudomonas aeruginosa* isolated clinically in Thailand. Microbiol Immunol **57:**170-178.

23. **Piyakul C, Tiyawisutsri R, Boonbumrung K.** 2012. Emergence of metallo-beta-lactamase IMP-14 and VIM-2 in *Pseudomonas aeruginosa* clinical isolates from a tertiary-level hospital in Thailand. Epidemiol Infect **140:**539-541.

24. **Kansakar P, Dorji D, Chongtrakool P, Mingmongkolchai S, Mokmake B, Dubbs P.** 2011. Local dissemination of multidrug-resistant *Acinetobacter baumannii* clones in a Thai hospital. Microb Drug Resist **17:**109-119.

25. **Samuelsen O, Toleman MA, Sundsfjord A, Rydberg J, Leegaard TM, Walder M, Lia A, Ranheim TE, Rajendra Y, Hermansen NO, Walsh TR, Giske CG.** 2010. Molecular epidemiology of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* isolates from Norway and Sweden shows import of international clones and local clonal expansion. Antimicrob Agents Chemother **54:**346-352.

26. **Papagiannitsis CC, Dolejska M, Izdebski R, Giakkoupi P, Skalova A, Chudejova K, Dobiasova H, Vatopoulos AC, Derde LP, Bonten MJ, Gniadkowski M, Hrabak J.** 2016. Characterisation of IncA/C2 plasmids carrying an In416-like integron with the blaVIM-19 gene from *Klebsiella pneumoniae* ST383 of Greek origin. Int J Antimicrob Agents **47:**158-162.

27. **Zamorano L, Miro E, Juan C, Gomez L, Bou G, Gonzalez-Lopez JJ, Martinez-Martinez L, Aracil B, Conejo MC, Oliver A, Navarro F.** 2015. Mobile genetic elements related to the diffusion of plasmid-mediated AmpC beta-lactamases or carbapenemases from Enterobacteriaceae: findings from a multicenter study in Spain. Antimicrob Agents Chemother **59:**5260-5266.

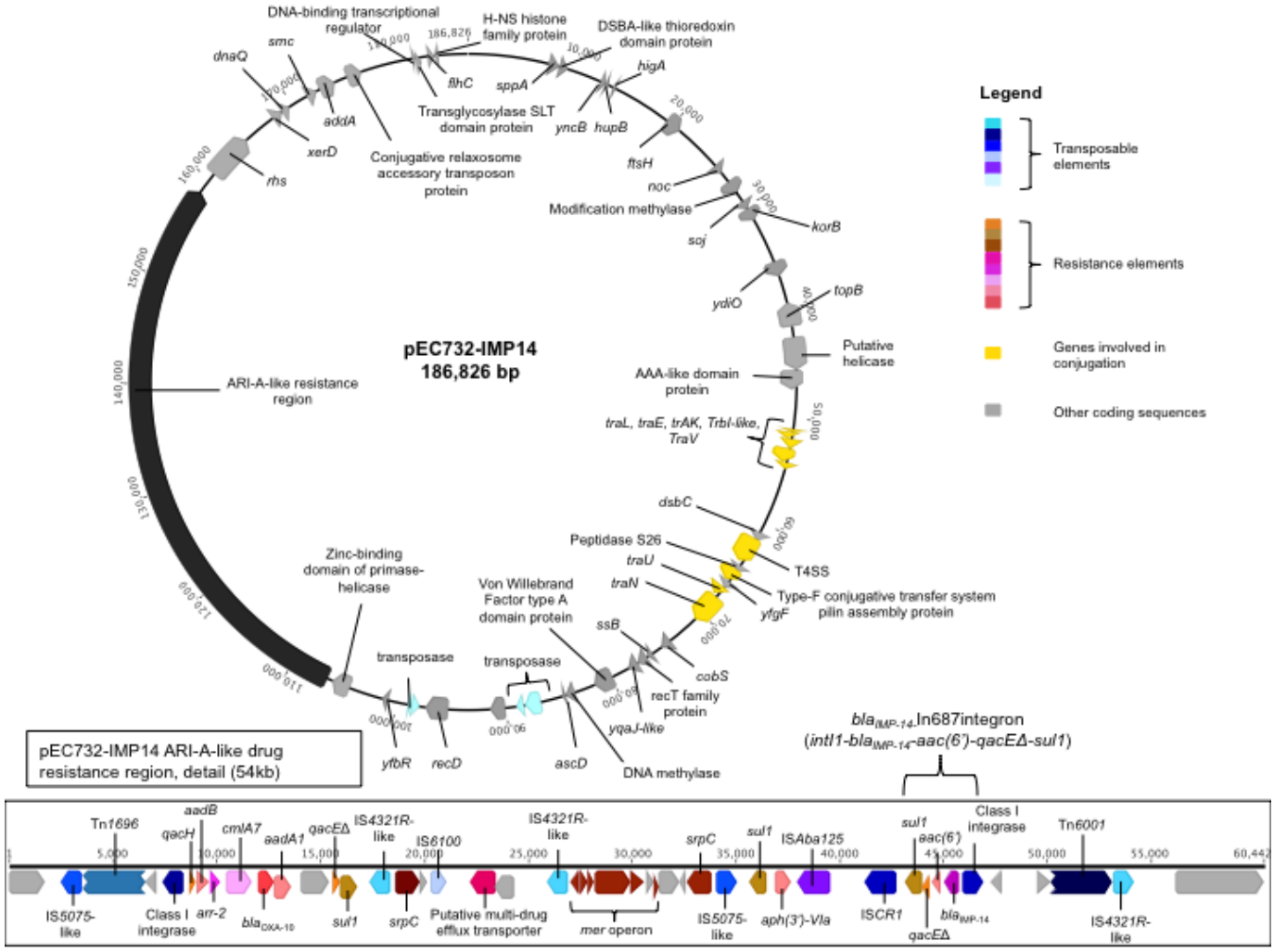
28. **Kieffer N, Poirel L, Bessa LJ, Barbosa-Vasconcelos A, da Costa PM, Nordmann P.** 2016. VIM-1, VIM-34, and IMP-8 Carbapenemase-Producing *Escherichia coli* Strains Recovered from a Portuguese River. Antimicrob Agents Chemother **60:**2585-2586.

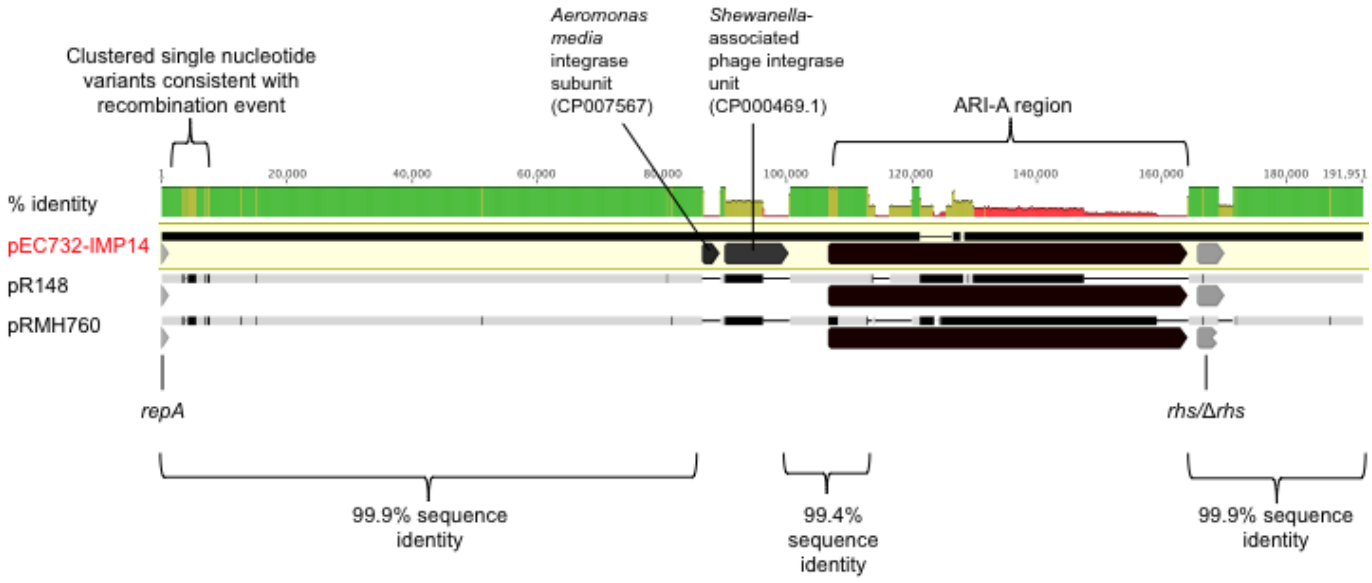
29. **Dolejska M, Masarikova M, Dobiasova H, Jamborova I, Karpiskova R, Havlicek M, Carlile N, Priddel D, Cizek A, Literak I.** 2016. High

prevalence of Salmonella and IMP-4-producing Enterobacteriaceae in the silver gull on Five Islands, Australia. J Antimicrob Chemother **71:**63-70.

Figure 1. Plasmid pEC732-IMP14, with a detailed view of the ARI-A-like 54kb resistance region (lower panel).

Figure 2. Alignment of pEC732-IMP14, pR148 and pRMH760, demonstrating mean % pairwise identity over alignment columns (green = 100%, olive = 30-70%, red <30%, no colour = 0%). Vertical black lines in the sequence bars represent nucleotide differences between the sequences with respect to the pEC732-IMP14 reference; thin horizontal black lines deleted regions. The *repA* gene, variable ARI-A resistance region, and *rhs* gene are indicated, as are the region of clustered single nucleotide variants downstream of the *repA* region and two additional mobile genetic elements present in pEC732-IMP14 and absent in the other two sequences.


pEC732-IMP14
186,826 bp
ARI-A-like resistance region
DNA-binding transcriptional regulator
H-NS histone family protein
DSBA-like thioredoxin domain protein
dnaQ
smc
addA
xerD
rhs
fliC
sppA
higA
yncB
hupB
Transglycosylase SLT domain protein
Conjugative relaxosome accessory transposon protein
ftsH
noc
Modification methylase
soj
korB
ydiO
topB
Putative helicase
AAA-like domain protein
traL, traE, trAK, TrbI-like, TraV
dsbC
Peptidase S26
traU
traN
T4SS
yfgF
Type-F conjugative transfer system pilin assembly protein
ssB
cobS
recT family protein
yqaJ-like
DNA methylase
ascD
Von Willebrand Factor type A domain protein
transposase
transposase
Zinc-binding domain of primase-helicase
yfbR
recD
Legend
Transposable elements
Resistance elements
Genes involved in conjugation
Other coding sequences
pEC732-IMP14 ARI-A-like drug resistance region, detail (54kb)
blaIMP-14 In687integron
(intI1-blaIMP-14-aac(6')-qacEΔ-sul1)
Tn1696
aadB
qacH
cmlA7
aadA1
qacEΔ
IS4321R-like
IS6100
IS4321R-like
srpC
sul1
ISAba125
sul1
aac(6')
Class I integrase
Tn6001
IS5075-like
Class I integrase
arr-2
blaOXA-10
sul1
srpC
Putative multi-drug efflux transporter
mer operon
IS5075-like
aph(3')-VIa
ISCR1
qacEΔ
blaIMP-14
IS4321R-like


Clustered single nucleotide variants consistent with recombination event
Aeromonas media integrase subunit (CP007567)
Shewanella-associated phage integrase unit (CP000469.1)
ARI-A region
1
20,000
40,000
60,000
80,000
100,000
120,000
140,000
160,000
180,000
191,951
% identity
pEC732-IMP14
pR148
pRMH760
repA
rhs/Δrhs
99.9% sequence identity
99.4% sequence identity
99.9% sequence identity