CMY-1/MOX-family AmpC β-lactamases MOX-1, MOX-2 and MOX-9 were mobilized independently from three Aeromonas species

J Antimicrob Chemother 2019; **74**: 1202–1206
doi:10.1093/jac/dkz025 Advance Access publication 7 February 2019

Journal of Antimicrobial Chemotherapy

CMY-1/MOX-family AmpC β-lactamases MOX-1, MOX-2 and MOX-9 were mobilized independently from three *Aeromonas* species

Stefan Ebmeyer[1,2], Erik Kristiansson[1,3] and D. G. Joakim Larsson[1,2]*

[1]*Center for Antibiotic Resistance Research, SE-40530 Göteborg, Sweden;* [2]*Department of Infectious Diseases, Institute of Biomedicine, University of Gothenburg, SE-41346 Göteborg, Sweden;* [3]*Mathematical Sciences, Chalmers University of Technology and the University of Gothenburg, SE-41296 Göteborg, Sweden*

*Corresponding author. Tel: +46 31 342 4625; E-mail: joakim.larsson@fysiologi.gu.se

Received 25 September 2018; returned 6 November 2018; revised 19 December 2018; accepted 8 January 2019

Objectives: To investigate the origin of CMY-1/MOX-family β-lactamases.

Methods: Publicly available genome assemblies were screened for CMY-1/MOX genes. The loci of CMY-1/MOX genes were compared with respect to synteny and nucleotide identity, and subjected to phylogenetic analysis.

Results: The chromosomal *ampC* genes of several *Aeromonas* species were highly similar to known mobile CMY-1/MOX variants. Annotation and sequence comparison revealed nucleotide identities >98% and conserved syntenies between MOX-1-, MOX-2- and MOX-9-associated mobile sequences and the chromosomal *Aeromonas sanarellii*, *Aeromonas caviae* and *Aeromonas media ampC* loci. Furthermore, the phylogenetic analysis showed that MOX-1, MOX-2 and MOX-9 formed three distinct monophyletic groups with the chromosomal *ampC* genes of *A. sanarellii*, *A. caviae* and *A. media*, respectively.

Conclusions: Our findings show that three CMY-1/MOX-family β-lactamases were mobilized independently from three *Aeromonas* species and hence shine new light on the evolution and emergence of mobile antibiotic resistance genes.

Introduction

Plasmid-encoded AmpC β-lactamases frequently cause β-lactam resistance in clinical strains. Their evolution has been thoroughly investigated and origins have been proposed for several known mobile AmpC enzymes, which occur in the chromosomes of species from several bacterial genera, such as *Enterobacter*, *Citrobacter*, *Morganella* and *Aeromonas*.[1–3] ISs are in several cases likely to be responsible for their mobilization through transposition of parts of the chromosomal *ampC* loci to mobile genetic elements (MGEs).[4]

The origins of some AmpC β-lactamases, including the CMY-1/MOX-family β-lactamases, are still unclear. CMY-1/MOX-family enzymes hydrolyse extended-spectrum cephalosporins and cephamycins, and, in contrast to other AmpC β-lactamases, several members of this family also provide increased resistance to moxalactam.[5,6] Although CMY-1/MOX enzymes are suspected to be derived from the chromosomal *ampC* gene of *Aeromonas* species,[6,7] critical evidence is lacking. Several variants of the CMY-1/MOX-family β-lactamases have been reported on plasmids in Gram-negative pathogens from Europe and East Asia (CMY-1/MOX-1/CMY-8/CMY-9/CMY-10/CMY-11/CMY-19, Japan/Korea/Taiwan, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marcescens* and *Klebsiella aerogenes*; MOX-2, Greece, *K. pneumoniae*; MOX-4, China, *Aeromonas caviae*; and MOX-9, Italy, *Citrobacter freundii*), whereas information on mobility is lacking for others (MOX-3/-5/-6/-7, Spain, *Aeromonas* spp. and *A. caviae*; and MOX-8, Thailand, *A. caviae*). Some CMY-1/MOX enzymes (CMY-1/MOX-1) are highly prevalent in East Asia and compromise the efficacy of β-lactam treatment,[8] whereas other variants (MOX-2 to MOX-9) are detected less frequently. Understanding from which species resistance genes were mobilized might help us to understand the events that led to their spread into the clinics. This may be valuable for future mitigation efforts, aiming to delay the arrival of novel antibiotic resistance genes at the clinics. The aim of this study was to identify the origin of the mobile CMY-1/MOX β-lactamases using a comparative genomics approach.

Materials and methods

All available CMY-1/MOX amino acid sequences were downloaded from the Comprehensive Antibiotic Resistance Gene Database (CARD).[9] CMY-1/MOX

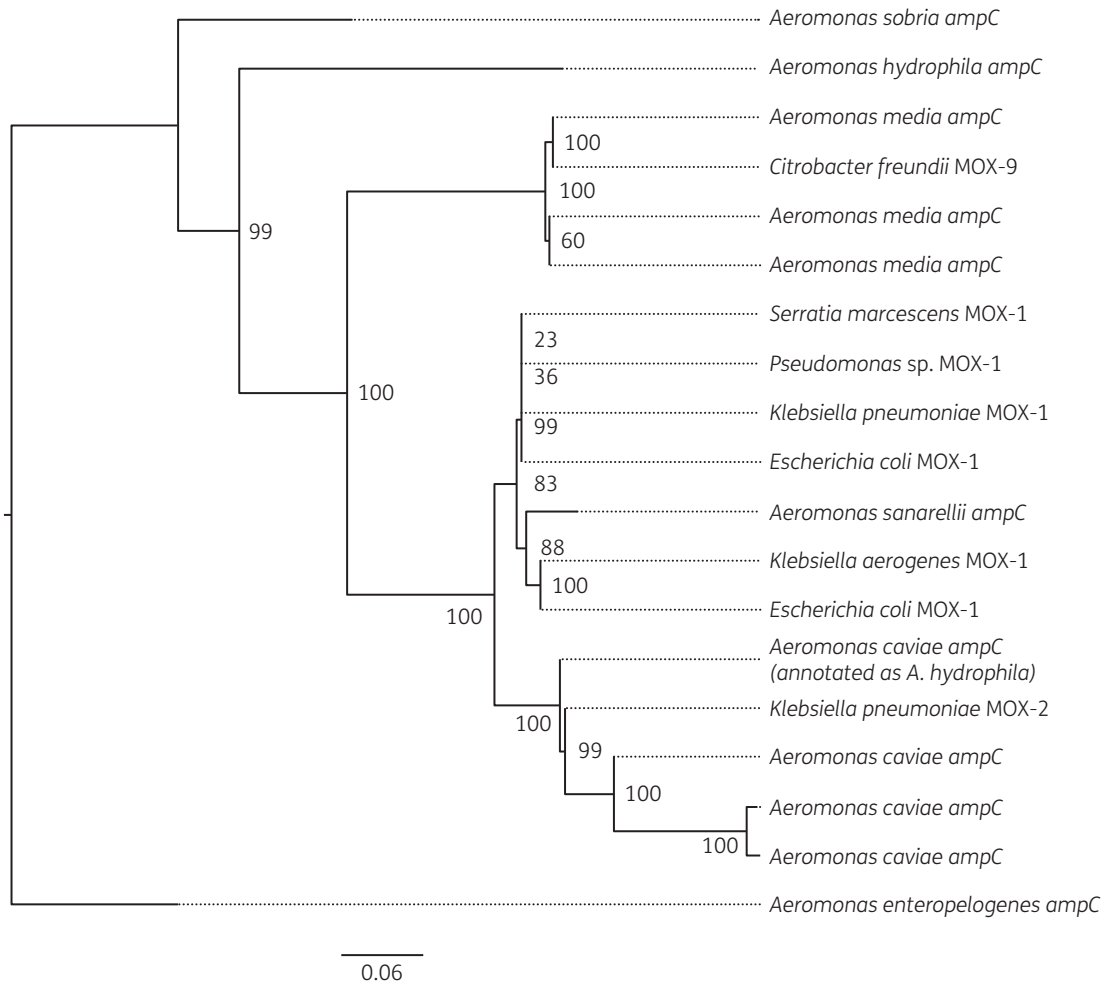

Figure 1. Phylogenetic analysis based on nucleotide sequences of mobile CMY-1/MOX sequences and CMY-1/MOX-like *Aeromonas ampC* genes, which were the only non-mobile genes with similarity of ≥85% to mobile CMY-1/MOX genes. The *ampC* genes of *A. sobria* and *A. enteropelogenes* were <90% identical to mobile CMY-1/MOX variants.

sequences were clustered at 95% identity threshold using USEARCH v8.0.1445.[10] All plasmid sequences and genome assemblies were downloaded from the NCBI nucleotide and assembly databases and searched for CMY-1/MOX sequences using DIAMOND[11] blastx with an 85% identity cut-off. The *ampC* nucleotide sequences of *Aeromonas enteropelogenes*, *Aeromonas hydrophila* and *Aeromonas sobria* were also downloaded from the nucleotide database (accession no.s EU046614.1, NC_008570.1 and NG_047381.1). From each contig containing a CMY-1/MOX gene, 10 kbp upstream and downstream of the gene were extracted and annotated using PROKKA.[12] Proteins denoted as 'hypothetical' by PROKKA were searched against the UniProtKB protein sequence database using DIAMOND (50% id). To identify ISs or transposase-like genes at the extracted CMY-1/MOX loci, the sequences were searched against the ISFinder database.[13] A multiple sequence alignment of the identified CMY-1/MOX genes and *Aeromonas ampC* genes was computed using MAFFT[14] (–adjustdirectionaccurately –maxiterate 1000 –globalpair). Phylogenetic analysis was conducted using RAxML[15] (raxmlHPC, 1000 times rapid bootstrapping) with the GTRCAT model. Unique mobile MOX-1, MOX-2 and MOX-9 sequences were included, as well as the CMY-1/MOX *Aeromonas ampC* genes that reflected the within-species diversity of the *ampC* locus (meaning that if, for example, several *A. caviae ampC* genes had different nucleotide similarities to the respective mobile CMY-1/MOX locus, a sequence representing each specific similarity towards the most similar mobile CMY-1/MOX locus was included). The extracted loci were compared at the nucleotide level using the NCBI online blastn[16] suite. Global average nucleotide identity (gANI) and alignment fraction (AF; fraction of orthologous genes between two genomes) as measures of genetic relatedness were calculated using ANIcalculator_v1.[17]

Results

Cluster analysis revealed six distinct clusters: MOX-1, CMY-1, -8, -9, -10, -11, -19; MOX-2, -4, -8; MOX-6, -7; MOX-3; MOX-5; and MOX-9. For simplicity, members of the first cluster will be referred to as MOX-1 hereafter.

The blastx search identified 35 CMY-1/MOX-encoding assembled (contigs or scaffolds) and plasmid sequences: *A. caviae* (15) (MOX-7/8: ASM78377v1, ASM170247v1, PRJEB7046, ASM72185v1, ASM173020v1, ASM127076v1, ASM81347v1, ASM173021v1, ASM20882v1, ASM118359v1, ASM78369v1, ASM78371v1, ASM95970v2, PRJEB7024 and ASM95970v1), *Aeromonas media* (4) (MOX-9: ASM75491v1, ASM28721v3, PRJEB7032 and ASM75492v1), *A. hydrophila* (2) (MOX-2: Aero_hydr_BWH65_V1 and Aero_hydr_SSU_V1), *Aeromonas*

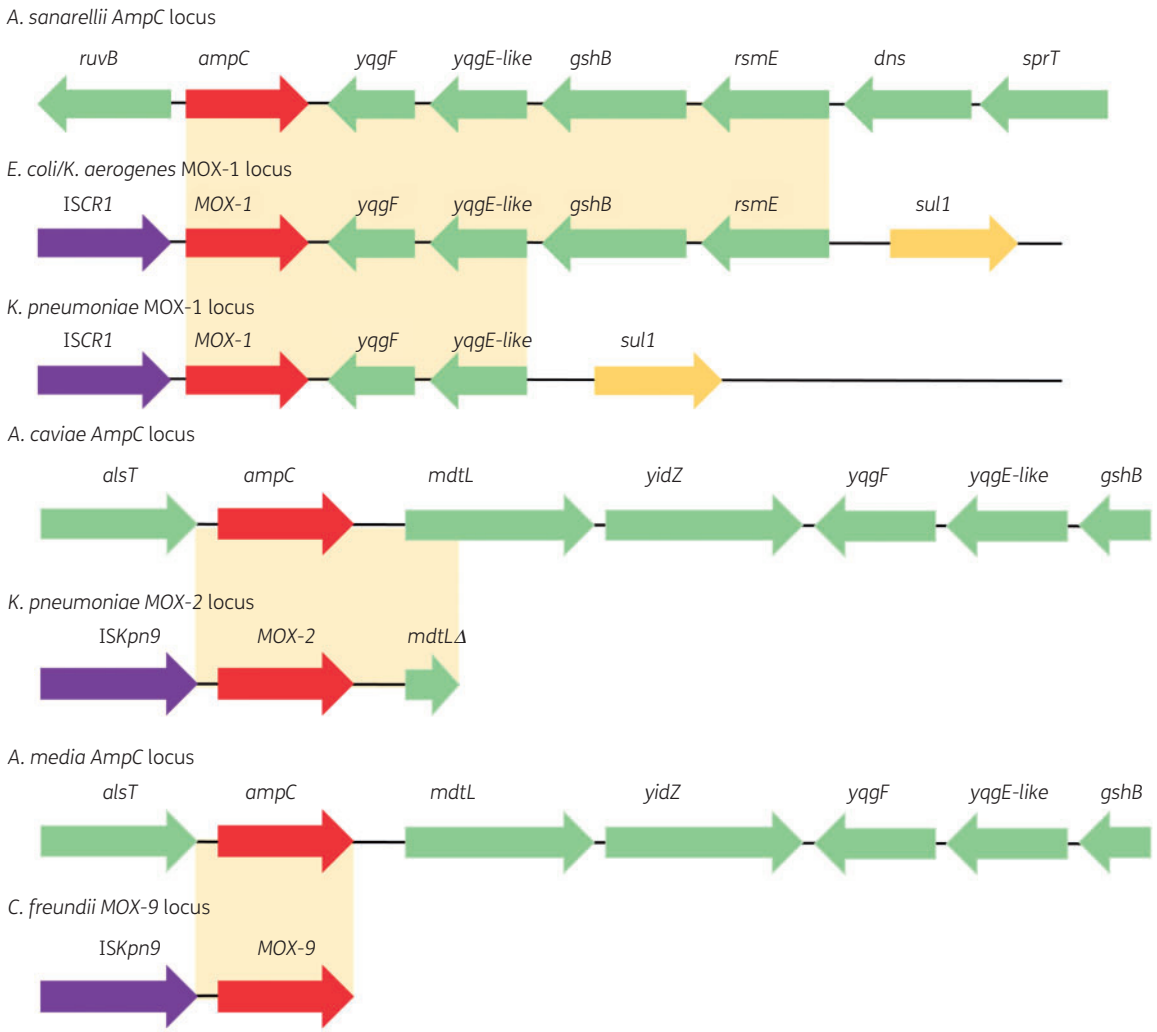

Figure 2. Sequence comparison between chromosomal *Aeromonas ampC* loci and mobile CMY-1/MOX loci. Aligning regions are highlighted. Nucleotide identities for whole aligning sequences: *A. sanarellii–K. aerogenes/E. coli*, 98%; *A. sanarellii–K. pneumoniae* MOX-1 locus, 97%; *A. caviae–K. pneumoniae* MOX-2 locus, 99%; and *A. media–C. freundii*, 98%–99%. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

sanarellii (1) (MOX-1: PRJEB7037), *Aeromonas dhakensis* (1) (MOX-1: A_dhakensis_SSU), unclassified *Aeromonas* species (3) (MOX-7: AeroHZM_1.0, ASM189858v1 and ZOR0002.1), unclassified *Pseudomonas* species (2) (MOX-1: ASM175389v1 and ASM175393v1), *Klebsiella pseudomonas/aerogenes* (3) (MOX-1/2: EF382672, FJ004895 and AJ276453), *E. coli* (2) (MOX-1: FJ763641 and AB061794), *S. marcescens* (2) (MOX-1: AP013064 and ASM82877v1) and *C. freundii* (1) (MOX-9: KJ746495.1).

In the phylogenetic analysis, MOX-1, MOX-2 and MOX-9 each formed a monophyletic group, supported by bootstrap values >99, with the *ampC* gene of only one *Aeromonas* species (Figure 1). Previously suggested CMY-1/MOX-origins, the *ampC* genes from *A. hydrophila* and *A. sobria*, formed single branches. Annotation of the 21 kbp MOX loci showed that in all species except for aeromonads, CMY-1/MOX genes were encoded on MGEs and associated with IS*CR1* or IS*Kpn9*, as previously reported.[6,18] The CMY-1/MOX loci consisted of similar genes in all aeromonads (as shown in Figure 2).

Comparison of gene synteny showed that the mobile MOX-1 loci from *Serratia*, *Pseudomonas*, *Klebsiella* and *Escherichia* spp. matched the gene arrangement of the chromosomal *A. sanarellii ampC* locus, which differed from other *Aeromonas ampC* loci. The *A. sanarellii* synteny did not match with the gene arrangements at the mobile MOX-2 locus (Figure 2), which instead matched the *A. hydrophila*, *A. caviae* and *A. media* synteny.

Nucleotide sequence alignment revealed that the mobile MOX-1 loci from *K. aerogenes* and *E. coli* were 98% identical to the *A. sanarellii ampC* locus, over a length of ∼3980 bp. This region included the *ampC*/MOX-1 gene and the genes *yqgF*, *yqgE*-like, *gshB* and *rsmE*Δ. The alignment included all intergenic regions, with the exception of 89 bp between *gshB* and *rsmE*Δ. The IS*CR1* upstream of MOX-1 in *K. aerogenes* was absent in *A. sanarellii*.

The other MOX-1-containing mobile sequences from *Klebsiella* spp., *Pseudomonas* spp. and *S. marcescens* were 97% similar over ~2649 bp, but lacked the *gshB* and *rsmE* genes. The mobile MOX-2 locus from the *K. pneumoniae* plasmid was only 90% similar to the *A. sanarellii ampC* locus, and the alignment over 1160 bp included only the *ampC* gene. The mobile *C. freundii* sequence containing MOX-9 was only 80% similar to the *A. sanarellii ampC* locus over 1123 bp, including only the *ampC* gene.

Alignment of the *Aeromonas ampC* loci against the mobile MOX-2 locus showed identities from 91% to 97% over 1629 bp for all *A. caviae* isolates and 99% over 1629 bp for one *A. hydrophila* (Aero_hydr_BWH65_V1) isolate. The alignment included the *ampC* gene, 109 bp of the *mdtL* efflux pump encoded downstream of the *A. hydrophila ampC* gene and the intergenic region of 293 bp. IS*Kpn9*, the IS associated with mobile MOX-2, was absent at the *A. hydrophila* (Aero_hydr_BWH65_V1) and *A. caviae ampC* loci. The gANI between Aero_hydr_BWH65_V1 and the *A. caviae* isolates ASM95970v1, ASM81347v1 and ASM173021 was >98% and AF was 0.89–0.93, whereas gANI between Aero_hydr_BWH65_V1 and *A. hydrophila* ASM1480v1 was only 88% and AF was 0.83. Hence, Aero_hydr_BWH65_V1 originates from a misclassified *A. caviae* isolate.

The Aero_hydr_BWH65_V1 sequence was only 82% identical over 1100 bp to the mobile MOX-9 locus, with the alignment only including the *ampC* gene. Aligning the *Aeromonas ampC* loci against the mobile *C. freundii* MOX-9 sequence revealed 98%–99% nucleotide identity over ~1285 bp to all four *A. media ampC* loci. The alignment included the *A. media ampC* gene and the 138 bp located between MOX-9 and IS*Knp9*, the IS associated with MOX-9 in the *C. freundii* mobile context. The IS*Kpn9* on the MOX-9-encoding *C. freundii* and the MOX-2-encoding *K. pneumoniae* MGEs were 99% similar, whereas MOX-9 and MOX-2 were only 82% similar. No similarity was found in the regions between IS*Kpn9* and the MOX genes on these mobile elements.

Discussion

We have identified *A. sanarellii*, *A. caviae* and *A. media* as the origins of MOX-1, MOX-2 and MOX-9, respectively, based on phylogenetic analysis of the nucleotide sequences of both mobile CMY-1/MOX and *Aeromonas ampC* genes, conserved synteny and nucleotide identities ≥98% between chromosomal and mobile CMY-1/MOX loci. The IS/IS*CR* sequences associated with mobile CMY-1/MOX variants were absent from the *Aeromonas ampC* loci.

Nucleotide identities of >98% between mobile and non-mobile genes and their genetic environments conform to the hypothesis that the mobilizations and fixations of these genes are evolutionarily recent events that happened during the antibiotic era. *Aeromonas* species are primarily associated with aquatic environments, but are known to cause gastroenteritis or wound infections in humans after ingestion/contact with contaminated water, possibly triggering antibiotic use. As IS/IS*CR1* can both mobilize[19] and increase expression of adjacent genes,[20] insertion next to the *Aeromonas ampC* may increase its responsiveness to antibiotic selection pressure. Our findings are hence consistent with the hypothesis that MOX-1, MOX-2 and MOX-9 were mobilized, selected for and transferred to species of the human microbiome during *Aeromonas* infections treated with β-lactams. Having said that, we cannot exclude that the critical horizontal transfer events took place in, for example, aquaculture settings or other aquatic environments where aeromonads thrive.

Funding

This work was supported by the Swedish research council for the Environment, Agricultural Sciences and Spatial Planning (grant number 942-2015-750) and the Swedish research council Vetenskapsrådet (grant numbers 2015-02492 and 521-2013-8633).

Transparency declarations

None to declare.

References

1 Reisbig MD, Hanson ND. The ACT-1 plasmid-encoded AmpC β-lactamase is inducible: detection in a complex β-lactamase background. *J Antimicrob Chemother* 2002; **49**: 557–60.

2 Wu SW, Dornbusch K, Kronvall G. Characterization and nucleotide sequence of a *Klebsiella oxytoca* cryptic plasmid encoding a CMY-type β-lactamase: confirmation that the plasmid-mediated cephamycinase originated from the *Citrobacter freundii* AmpC β-lactamase. *Antimicrob Agents Chemother* 1999; **43**: 1350–7.

3 Barlow M, Hall BG. Origin and evolution of the AmpC β-lactamases of *Citrobacter freundii*. *Antimicrob Agents Chemother* 2002; **46**: 1190–8.

4 Nadjar D, Rouveau M, Verdet C *et al*. Outbreak of *Klebsiella pneumoniae* producing transferable AmpC-type β-lactamase (ACC-1) originating from *Hafnia alvei*. *FEMS Microbiol Lett* 2000; **187**: 35–40.

5 Oguri T, Furuyama T, Okuno T *et al*. Crystal structure of Mox-1, a unique plasmid-mediated class C β-lactamase with hydrolytic activity towards moxalactam. *Antimicrob Agents Chemother* 2014; **58**: 3914–20.

6 Raskine L, Borrel I, Barnaud G *et al*. Novel plasmid-encoded class C β-lactamase (MOX-2) in *Klebsiella pneumoniae* from Greece. *Antimicrob Agents Chemother* 2002; **46**: 2262–5.

7 Jacoby GA. AmpC β-lactamases. *Clin Microbiol Rev* 2009; **22**: 161–82.

8 Lee SH, Jeong SH, Park YM. Characterization of bla_{CMY-10} a novel, plasmid-encoded AmpC-type β-lactamase gene in a clinical isolate of *Enterobacter aerogenes*. *J Appl Microbiol* 2003; **95**: 744–52.

9 Jia B, Raphenya AR, Alcock B *et al*. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res* 2017; **45**: D566–73.

10 Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010; **26**: 2460–1.

11 Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. *Nat Methods* 2014; **12**: 59–60.

12 Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014; **30**: 2068–9.

13 Siguier P, Perochon J, Lestrade L *et al*. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 2006; **34**: D32–6.

14 Katoh K, Misawa K, Kuma K *et al*. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 2002; **30**: 3059–66.

15 Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014; **30**: 1312–3.

16 Altschul SF, Gish W, Miller W *et al*. Basic local alignment search tool. *J Mol Biol* 1990; **215**: 403–10.

17 Varghese NJ, Mukherjee S, Ivanova N *et al*. Microbial species delineation using whole genome sequences. *Nucleic Acids Res* 2015; **43**: 6761–71.

18 Horii T, Arakawa Y, Ohta M *et al*. Plasmid-mediated AmpC-type β-lactamase isolated from *Klebsiella pneumoniae* confers resistance to broad-spectrum β-lactams, including moxalactam. *Antimicrob Agents Chemother* 1993; **37**: 984–90.

19 Toleman MA, Bennett PM, Walsh TR. IS*CR* elements: novel gene-capturing systems of the 21st century? *Microbiol Mol Biol Rev* 2006; **70**: 296–316.

20 Lallement C, Pasternak C, Ploy MC *et al*. The role of IS*CR1*-borne P_{OUT} promoters in the expression of antibiotic resistance genes. *Front Microbiol* 2018; **9**: 2579.