JOURNAL OF MEDICAL MICROBIOLOGY

SHORT COMMUNICATION

Taylor *et al.*, *Journal of Medical Microbiology* 2022;71:001531

DOI 10.1099/jmm.0.001531

MICROBIOLOGY SOCIETY

OPEN ACCESS

Novel 16S rRNA methyltransferase RmtE3 in *Acinetobacter baumannii* ST79

Emma Taylor[1,2]†, Elita Jauneikaite[1,3], Shiranee Sriskandan[1,4], Neil Woodford[1,2] and Katie L. Hopkins[1,2,5,*]

Abstract

Introduction. The 16S rRNA methyltransferase (16S RMTase) gene *armA* is the most common mechanism conferring high-level aminoglycoside resistance in *Acinetobacter baumannii*, although *rmtA*, *rmtB*, *rmtC*, *rmtD* and *rmtE* have also been reported.

Hypothesis/Gap statement. The occurrence of 16S RMTase genes in *A. baumannii* in the UK and Republic of Ireland is currently unknown.

Aim. To identify the occurrence of 16S RMTase genes in *A. baumannii* isolates from the UK and the Republic of Ireland between 2004 and 2015.

Methodology. Five hundred and fifty pan-aminoglycoside-resistant *A. baumannii* isolates isolated from the UK and the Republic of Ireland between 2004 and 2015 were screened by PCR to detect known 16S RMTase genes, and then whole-genome sequencing was conducted to screen for novel 16S RMTase genes.

Results. A total of 96.5% (531/550) of isolates were positive for 16S RMTase genes, with all but 1 harbouring *armA* (99.8%, 530/531). The remaining isolates harboured *rmtE3*, a new *rmtE* variant. Most (89.2%, 473/530) *armA*-positive isolates belonged to international clone II (ST2), and the *rmtE3*-positive isolate belonged to ST79. *rmtE3* shared a similar genetic environment to *rmtE2* but lacked an IS*CR20* element found upstream of *rmtE2*.

Conclusion. This is the first report of *rmtE* in *A. baumannii* in Europe; the potential for transmission of *rmtE3* to other bacterial species requires further research.

INTRODUCTION

Acinetobacter baumannii is a nosocomial pathogen known to cause serious infections such as bacteraemia, ventilator-associated pneumonia and urinary infections. *A. baumannii* infections can be difficult to treat due to the presence of genes encoding aminoglycoside-modifying enzymes, GES-, PER- and VEB-type extended-spectrum β-lactamases (ESBLs) and carbapenemases, as well as efflux pumps such as AdeABC, which confers resistance to aminoglycosides, chloramphenicol, fluoroquinolones, tetracyclines and trimethoprim [1].

16S rRNA methyltransferases (16S RMTases) are encoded by a single gene and confer high-level pan-aminoglycoside resistance (MICs >256 mg l^{-1}), with 11 16S RMTase genes (*armA*, *rmtA–rmtH, npmA* and *npmB*) identified to date [2, 3]. *armA* is the most commonly identified 16S RMTase gene reported in *A. baumannii* [2], although *A. baumannii* has been associated with *rmtA* and *rmtD* in India [4], *rmtB* in Vietnam [5] and *rmtC* in Uruguay [6].

Received 23 September 2021; Accepted 01 March 2022; Published 19 May 2022

Author affiliations: [1]National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Healthcare Associated Infections and Antimicrobial Resistance, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK; [2]Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit, Reference Services Division, UK Health Security Agency, London NW9 5EQ, UK; [3]School of Public Health, Imperial College London, London W2 1PG, UK; [4]MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London SW7 2DD, UK; [5]Healthcare Associated Infections, Fungal, Antimicrobial Resistance, Antimicrobial Usage and Sepsis Division, UK Health Security Agency, London NW9 5EQ, UK.

***Correspondence:** Katie L. Hopkins, katie.hopkins@phe.gov.uk

Keywords: 16S RMTase; aminoglycoside resistance; bla_{OXA-23}; genetic environment.

Abbreviations: AME, aminoglycoside-modifying enzyme; ESBL, extended-spectrum β-lactamase; LB, lysogeny broth; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; PHE, Public Health England; 16S RMTase, 16S rRNA methyltransferase; ST, sequence type; WGS, whole-genome sequencing.

†Present address: Department of Bacteriology, Animal and Plant Health Agency, Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB, UK.

GenBank accession number MH572011.

In order to identify whether 16S RMTase genes, other than the most commonly identified *armA* gene, are circulating in *A. baumannii* in the UK and the Republic of Ireland, the presence of 16S RMTase genes was sought in a collection of *A. baumannii* isolates displaying high-level pan-aminoglycoside resistance that had been submitted to Public Health England's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit.

On 1 October 2021, the UK Health Security Agency (UKHSA) became fully operational, bringing together the health protection functions of Public Health England (PHE) and the National Health Service Test and Trace, including the Joint Biosecurity Centre. As this study was completed prior to this transition, we have retained the former Public Health England designations in the text.

METHODS

Bacterial isolates

A panel of 550 *A. baumannii* isolates exhibiting high-level pan-aminoglycoside resistance (amikacin, gentamicin and tobramycin MICs >64, >32 and >32 mg l^{-1}, respectively), as determined by agar dilution, was recovered from the AMRHAI Reference Unit's culture collection. Isolates had been submitted for investigation of unusual (primarily carbapenem) resistance between January 2004 and December 2015.

DNA extraction and detection of 16S RMTase genes

Crude DNA extracts were subjected to two multiplex PCRs to detect 16S RMTase genes *armA*, *rmtA–rmtH* and *npmA*, as previously described [7]. Following PCR, the DNA fragments were analysed via gel electrophoresis using 2% agarose gels.

Carbapenemase detection

The carbapenemase genes bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$ and bla_{VIM} were sought using in-house conventional [8–11] or real-time PCR [12]. The genes $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}40\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$ and $bla_{OXA\text{-}58\text{-}like}$, and $bla_{GES\text{-}5}$, bla_{IMI}, bla_{IMP}, bla_{GIM}, bla_{SIM}, bla_{SME}, and bla_{SPM} were identified as previously described [10, 13–16].

Bacterial typing

A. baumannii isolates were identified as belonging to international clone II using a multiplex PCR targeting the carbapenemase, porin and pilus assembly system genes $bla_{OXA\text{-}51\text{-}like}$, *ompA* and *csuE,* respectively, as previously described [17].

Plasmid extraction and electroporation

Plasmid extraction was conducted on the *rmtE3*-positive isolate using the PureYield Plasmid Miniprep System (Promega, Southampton, UK) according to the manufacturer's instructions and the electroporation of plasmid DNA into TOP10 *Escherichia coli* cells (Invitrogen) was attempted. Cell suspensions were incubated in 10 ml lysogeny broth(LB) with 50 mg l^{-1} amikacin on a shaker overnight at 37 °C before being plated on LB agar plates containing 50 mg l^{-1} amikacin to select for transformants.

Analysis of 16S RMTase gene PCR-negative isolates

Isolates that were PCR-negative for 16S RMTase genes were screened for high-level pan-aminoglycoside resistance by streaking the isolates onto Mueller–Hinton agar plates supplemented with 256 mg l^{-1} amikacin, gentamicin or tobramycin, followed by incubation overnight at 37 °C. Isolates that grew on all three plates were whole-genome sequenced and analysed as described below.

Whole-genome sequencing (WGS)

WGS was conducted on the *rmtE3*-positive isolate, and also on 16S RMTase gene PCR-negative isolates exhibiting high-level aminoglycoside resistance, using a HiSeq sequencing system (Illumina, San Diego, CA, USA) with 150 bp paired-end reads. Genomes were *de novo* assembled using Velvet with the pipeline and improvements found at https://github.com/sanger-pathogens/vr-codebase and https://github.com/sanger-pathogens/assembly_improvement [18]. Reads were uploaded to the European Nucleotide Archive (ENA) under the project number PRJEB23879 and accession number ERR3181642. The purity of the DNA sequences was confirmed using KmerFinder 3.0 (https://cge.cbs.dtu.dk/services/KmerFinder/) [19–21], with antibiotic gene content and sequence type determined using ResFinder 3.0 (https://cge.cbs.dtu.dk/services/ResFinder/) [22] and MLST 2.0 (https://cge.cbs.dtu.dk/services/MLST/) [23], respectively. Additionally, the variant *rmtE3* was identified by aligning its DNA sequence to *rmtE1* (accession number: GU201947) and *rmtE2* (accession number: KT428293) using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) [24]. The genetic environment of *rmtE3* was investigated using Artemis 16.0.0 (Wellcome Trust Sanger Institute, Cambridge, UK) and compared to that of *rmtE1* and *rmtE2* using Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi). Genetic environments were visualized using EasyFig version 2.2.2 (http://mjsull.github.io/Easyfig/) [25].

To try to identify the origin of *rmtE3*, 24 high-quality genomes of ST79 *A. baumannii* submitted to pubMLST (https://pubmlst.org/organisms/acinetobacter-baumannii) were included in a phylogenetic analysis, as well as the *rmtE3*-positive ST2 *A. baumannii*

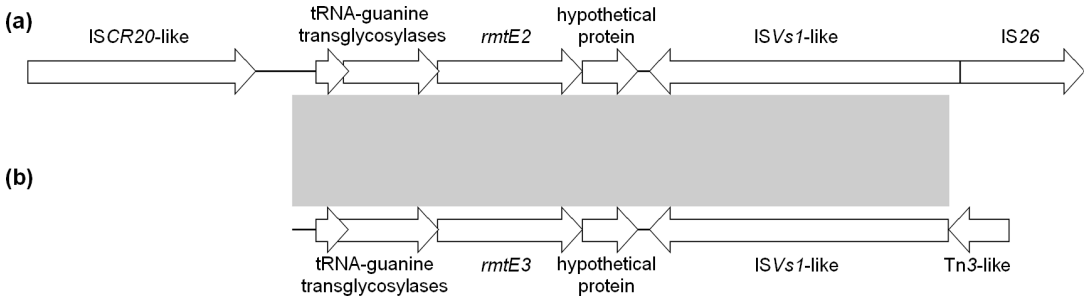

Fig. 1. Alignment of the genetic environments of *rmtE2* and *rmtE3*. (a) *rmtE2*-positive *E. coli* strain S68 plasmid pS68 (accession number: KU130396.1). (b) *rmtE3*-positive *A. baumannii* ST79. The grey shading indicates 99.0% sequence homology.

isolate from Venezuela (accession number: NZ_ULHD01000074.1). Core single-nucleotide polymorphisms (SNPs) for 26 *A. baumannii* genomes [24 ST79 genomes identified from pubMLST, including strain AbH12o-A2 (accession number: CP009534) used as reference, the *rmtE3*-positive isolate from this study and the *rmtE3*-positive isolate from Venezuela] were called using Snippy v4.6.0 (https://github.com/tseemann/snippy) against strain AbH12o-A2. The whole-genome alignment was cleaned using the snippy-clean_full_aln function available as part of Snippy. Gubbins v2.4 [26] was used to identify recombination before processing alignment with SNP sites [27] to obtain recombination-free core SNPs file. Then, a maximum-likelihood phylogenetic tree was constructed using IQ-TREE v.2.0.3 [28] with the 'GTR+G+ASC' model and 1000 bootstrap replicates. The phylogenetic tree was visualized using iTOL v.5 [29]. SNP pairwise distances were calculated using MEGA X v.10.2.4 [30].

To rule out the presence of novel 16S RMTase genes, DNA sequences of the contigs were analysed with Pfam 31.0 (https://pfam.xfam.org/) [31] to screen for protein motifs that could be associated with novel 16S RMTase genes. Following a six-frame translation of the DNA, two searches were carried out to determine the presence of motifs associated with the two protein families 16S RMTases belonging to [FmrO (Pfam ID: PF07091) for ArmA and RmtA–RmtH and Methyltransf_4 family (Pfam ID: PF02390) for NpmA]. The E-value cut-offs used were the default E value of 1.0 and one with an E value of 1×10^{-5} (to increase stringency).

RESULTS AND DISCUSSION

Five hundred and thirty-one (96.5%) of 550 *A. baumannii* isolates displaying high-level pan-aminoglycoside resistance were positive for 16S RMTase genes, where 99.8% (530/531) were positive for *armA* and 1 isolate was positive for *rmtE* (0.2%). No *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtF*, *rmtG*, *rmtH* or *npmA* genes were detected in this study.

Strain	Location	Year	Isolation site	Accession number
Ab421_GEIH-2010	Spain	2010	Human source	CP014266
AbH12o-A2 (reference)	Spain	2014	NA	CP009534
This study	UK	2014	Rectal swab	MH572011
UH12208	USA	2008	Trachea	AYGK00000000
AB030	Canada	2010	Blood	CP009257
CR-S16	Croatia	2016	Environment	NA
837_16	Brazil	2016	Blood	NZ_JABLWF000000000
461_16	Brazil	2016	Urine	NZ_JABLWR000000000
213_16	Brazil	2016	Trachea	NZ_JABLWL000000000
168_16	Brazil	2016	Trachea	NZ_JABLVV000000000
572_16	Brazil	2016	Trachea	NZ_JABLVX000000000
180_17	Brazil	2017	Urine	NZ_JABLWU000000000
397_16	Brazil	2016	Blood	NZ_JABLWE000000000
403_16	Brazil	2016	Urine	NZ_JACDYB000000000
917_16	Brazil	2016	Abdominal fluid	NZ_JACDYX000000000
915_16	Brazil	2016	Abdominal fluid	NZ_JABLVE000000000
830_16	Brazil	2016	Trachea	NZ_JABLWD000000000
93_16	Brazil	2016	Rectal swab	NZ_JABLVJ000000000
478_16	Brazil	2016	Trachea	NZ_JABLVH000000000
398_16	Brazil	2016	Blood	NZ_JABLWJ000000000
961_16	Brazil	2016	Blood	NZ_JABLVZ000000000
541_17	Brazil	2017	Blood	NZ_JACDYG000000000
808_16	Brazil	2016	Blood	NZ_JABLVY000000000
512_16	Brazil	2016	Eye	NZ_JABLWM000000000
464_16	Brazil	2016	Cerebrospinal fluid	NZ_JABLVB000000000

Fig. 2. Maximum-likelihood phylogenetic tree of the *rmtE3*-positive isolate from this study and 24 ST79 *A. baumannii* isolates. *A. baumannii* strain AbH12o-A2 (accession number: CP009534) was used as a reference strain. NA, not available.

Table 1. Genes encoding aminoglycoside modifying enzyme (AME) genes identified in 16S RMTase-negative *A. baumannii* isolates (*n*=15) that demonstrate high-level resistance to amikacin, gentamicin and tobramycin from the AMRHAI's strain collection, 2003–2015

No. of isolates	ST	AME genes					
		aph(3')-VIa*	***ant(2')-Ia*†‡**	***aac(3)-IIa*†‡**	***aac(6')-Ib-cr**‡**	***aac(3)-IId*†‡**	***aac(6')-Ib**‡**
2	1	2	1	–	–	–	–
1	1	1	1	–	1	–	1
1	2	1	–	1	–	–	–
1	16	1	1	–	–	1	–
1	23	1	1	–	–	–	–
2	94	2	1	–	–	–	–
1	113	1	1	–	–	–	–
5	624	1	1	–	–	–	–
1	718	1	1	–	–	–	–
Total		19	14	1	1	1	1

Enzymes confer resistance to *, amikacin; †, gentamicin and ‡, tobramycin.

The majority of *armA*-positive isolates harboured acquired carbapenemase genes (95.5% (506/530), where $bla_{\text{OXA-23}}$ (99.0%, 501/506) was the most common, and 89.2% (473/530) isolates belonged to international clone II, which is a worldwide 'high-risk' bacterial clone known to be associated with both *armA* and $bla_{\text{OXA-23}}$ [32]. These isolates were not characterized further.

The *rmtE*-positive isolate was isolated in 2014 from a patient in the East Midlands with no declared history of travel outside of the UK. To our knowledge, this is the first report of *rmtE* in *A. baumannii* in Europe and represents the earliest known *rmtE*-positive isolate of *A. baumannii*, as *rmtE* was reported in *A. baumannii* in Venezuela (accession no.: NZ_ULHD01000074.1) subsequent to this study. *rmtE* has also been reported in *E. coli* [33] and S*almonella enterica* subsp. *enterica* serovar Braenderup (accession no: NZ_QDSH01000020.1) in the USA as well as *Pseudomonas aeruginosa* in Myanmar [34] and Thailand (accession no: NSPO01000054.1). The variant *rmtE2* has been identified in *E. coli* in PR China [35] and *Enterobacter hormaechei* in Myanmar (accession no: LC511997.1) and uncharacterized variants of *rmtE* have been identified in *E. hormaechei* in Thailand (accession no: NZ_NPZP01000120.1) and *Klebsiella pneumoniae* in Colombia (accession no: NZ_NCOO01000022.1).

The *rmtE*-positive isolate belonged to ST79 and harboured $bla_{\text{OXA-65}}$ + $bla_{\text{OXA-72}}$, which encode OXA-51-like and OXA-24-like carbapenemases, respectively. *A. baumannii* ST79 harbouring $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-72}}$ carbapenemase genes has been reported in South America [36] and has previously been identified harbouring *rmtC* in Uruguay [6].

Analysis of WGS data from the *rmtE*-positive isolate identified that this isolate also harboured *sul2* (conferring sulphonamide resistance) as well as *strA* and *strB* (conferring streptomycin resistance). Comparison with the sequences of *rmtE1* (accession number: GU201947) and *rmtE2* (accession number: KT428293) indicated that the identified *rmtE* gene had two SNPs: one at nucleotide 20 (T→C, V7A) and another at nucleotide 141 (T→A, N47K). As the latter mutation was not found in either *rmtE1* or *rmtE2*, the new allele was designated *rmtE3* (accession no: MH572011), based on the nomenclature proposed by Doi *et al.* [37].

Analysis of the genetic environment of *rmtE3* identified the gene on a 4290 bp contig, which consisted of two tRNA-guanine transglycosylases, *rmtE3*, a hypothetical protein, an IS*Vs1*-like transposase and a Tn*3*-like transposase. BLAST analysis found that *rmtE3* shared a similar genetic environment with the *rmtE2* gene reported on plasmid pS68 in *E. coli* strain S68 (accession number: KU130396.1). Unlike *rmtE2*, the IS*CR20*-transposase gene found upstream of the two tRNA-guanine transglycosylases was missing for *rmtE3* and a Tn*3*-like transposase was found downstream of *rmtE3* instead of an IS*26* transposase gene (Fig. 1). IS*CR20* was not found elsewhere in the genome, indicating that another mobile genetic element may be involved in the transposition of *rmtE3*. Transfer of *rmtE3* to TOP10 *E. coli* by electroporation of plasmid DNA failed despite repeated attempts, hence the genetic location of *rmtE3* could not be identified.

In addition to our study, *rmtE3* was identified in *A. baumannii* strain 12918, which belonged to ST2 and was isolated from Venezuela in 2016 (accession number: NZ_ULHD01000074.1), on a similarly sized contig (4035 bp) with an identical genetic environment also lacking IS*CR20* or any other mobile genetic element upstream of *rmtE3*; it is unknown if *rmtE3* was chromosomally or plasmid encoded. A maximum-likelihood phylogenetic tree generated to compare the 2 *rmtE3*-positive *A. baumannii* isolates with published genomes of 24 *A. baumannii* ST79 isolates confirmed that there was no close relationship between the 2 *rmtE3*-positive isolates (27047 SNP difference; due to this distance the *rmtE3*-positive isolate from Venezuela was not included in the maximum-likelihood phylogenetic tree). Furthermore,

the *rmtE3*-positive isolate from our study was not closely related to the other *A. baumannii* ST79 isolates (85–179 SNP difference; Fig. 2). The origin of *rmtE3* is therefore currently unknown.

Out of the 19 16S RMTase PCR-negative *A. baumannii* isolates, only 15 (78,9%) exhibited high-level amikacin, gentamicin and tobramycin resistance (MICs >256 mg l^{-1}) on repeated screening. Following WGS, no novel 16S RMTase genes were identified but multiple genes encoding aminoglycoside-modifying enzymes (AMEs) were found, which would confer resistance to all three aminoglycosides when their phenotypes were combined (Table 1). High-level aminoglycoside resistance (MICs >128 mg l^{-1}) due to the presence of multiple AME genes has also been reported in two *A. baumannii* isolates in Brazil [38].

In conclusion, whilst the majority of pan-aminoglycoside *A. baumannii* isolates in our collection belonged to international clone II and consequently harboured *armA*, we detected a novel RMTase gene, *rmtE3*, in an isolate belonging to ST79. As *rmtE3* has only been reported in *A. baumannii* to date, additional research is required to identify whether *rmtE3* is plasmidic or chromosomal in order to assess its potential for spread to other bacterial genera.

Funding information
This research was funded by the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Healthcare Associated Infections and Antimicrobial Resistance at Imperial College London in partnership with Public Health England (HPRU-2012-10047).

Acknowledgements
We would like to thank Professor Bruno González-Zorn, Dr Laurent Poirel and Dr Yohei Doi for providing bacterial positive controls for *rmtA* +*rmtE*+ rmtF, *rmtG* and *rmtH*, respectively. We would like to thank staff from the AMRHAI Reference Unit at PHE for their assistance with strain characterization. Finally, we would like to thank the Wellcome Trust Sanger Institute for carrying out Illumina sequencing on the bacterial isolates. The research was funded by the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Healthcare Associated Infections and Antimicrobial Resistance at Imperial College London in partnership with Public Health England. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, the Department of Health or Public Health England. Part of this work was previously presented at the 27th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) in Vienna, Austria (session number OS0298).

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Following an internal review at PHE, this study was classified as surveillance and as no ethical issues were identified there was no requirement for a full review by an ethics committee.

References

1. Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: Mechanisms and epidemiology. *Int J Antimicrob Agents* 2015;45:568–585.
2. Doi Y, Wachino JI, Arakawa Y. Aminoglycoside resistance: the emergence of acquired 16S ribosomal RNA methyltransferases. *Infect Dis Clin North Am* 2016;30:523–537.
3. Kawai A, Suzuki M, Tsukamoto K, Minato Y, Doi Y. Functional and structural characterization of acquired 16S rRNA methyltransferase NpmB1 conferring pan-aminoglycoside resistance. *Antimicrob Agents Chemother* 2021;65:e0100921.
4. Upadhyay S, Khyriem AB, Bhattacharya P, Bhattacharjee A, Joshi SR. High-level aminoglycoside resistance in *Acinetobacter baumannii* recovered from intensive care unit patients in Northeastern India. *Indian J Med Microbiol* 2018;36:43–48.
5. Tada T, Miyoshi-Akiyama T, Kato Y, Ohmagari N, Takeshita N, *et al*. Emergence of 16S rRNA methylase-producing *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates in hospitals in Vietnam. *BMC Infect Dis* 2013;13:251.
6. Bado I, Papa-Ezdra R, Delgado-Blas JF, Gaudio M, Gutiérrez C, *et al*. Molecular characterization of carbapenem-resistant *Acinetobacter baumannii* in the intensive care unit of Uruguay's university hospital identifies the first *rmtC* gene in the species. *Microb Drug Resist* 2018;24:1012–1019.
7. Taylor E, Sriskandan S, Woodford N, Hopkins KL. High prevalence of 16S rRNA methyltransferases among carbapenemase-producing Enterobacteriaceae in the UK and Ireland. *Int J Antimicrob Agents* 2018;52:278–282.
8. Hidalgo L, Hopkins KL, Wareham DW, Gutierrez B, González-Zorn B. Association of extended-spectrum β-lactamase VEB-5 and 16S rRNA methyltransferase armA in *Salmonella enterica* from the United Kingdom. *Antimicrob Agents Chemother* 2012;56:4985–4987.
9. Mushtaq S, Irfan S, Sarma JB, Doumith M, Pike R, *et al*. Phylogenetic diversity of *Escherichia coli* strains producing NDM-type carbapenemases. *J Antimicrob Chemother* 2011;66:2002–2005.
10. Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum (beta)-lactamases. *J Antimicrob Chemother* 2006;57:154–155.
11. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, *et al*. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2001;45:1151–1161.
12. Ellington MJ, Findlay J, Hopkins KL, Meunier D, Alvarez-Buylla A, *et al*. Multicentre evaluation of a real-time PCR assay to detect genes encoding clinically relevant carbapenemases in cultured bacteria. *Int J Antimicrob Agents* 2016;47:151–154.
13. Turton JF, Hyde R, Martin K, Shah J. Genes encoding OXA-134-like enzymes are found in *Acinetobacter lwoffii* and *A. schindleri* and can be used for identification. *J Clin Microbiol* 2012;50:1019–1022.
14. Poirel L, Le Thomas I, Naas T, Karim A, Nordmann P. Biochemical sequence analyses of GES-1, A novel class A extended-spectrum beta-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2000;44:622–632.
15. Rasmussen BA, Bush K, Keeney D, Yang Y, Hare R, *et al*. Characterization of IMI-1 beta-lactamase, A class A carbapenem-hydrolyzing enzyme from *Enterobacter cloacae*. *Antimicrob Agents Chemother* 1996;40:2080–2086.
16. Queenan AM, Torres-Viera C, Gold HS, Carmeli Y, Eliopoulos GM, *et al*. SME-type carbapenem-hydrolyzing class A beta-lactamases from geographically diverse *Serratia marcescens* strains. *Antimicrob Agents Chemother* 2000;44:3035–3039.
17. Turton JF, Gabriel SN, Valderrey C, Kaufmann ME, Pitt TL. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. *Clin Microbiol Infect* 2007;13:807–815.

18. Page AJ, De Silva N, Hunt M, Quail MA, Parkhill J, *et al*. Robust high-throughput prokaryote *de novo* assembly and improvement pipeline for Illumina data. *Microb Genom* 2016;2:e000083.
19. Hasman H, Saputra D, Sicheritz-Ponten T, Lund O, Svendsen CA, *et al*. Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. *J Clin Microbiol* 2014;52:139–146.
20. Larsen MV, Cosentino S, Lukjancenko O, Saputra D, Rasmussen S, *et al*. Benchmarking of methods for genomic taxonomy. *J Clin Microbiol* 2014;52:1529–1539.
21. Clausen P, Aarestrup FM, Lund O. Rapid and precise alignment of raw reads against redundant databases with KMA. *BMC Bioinformatics* 2018;19:307.
22. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, *et al*. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012;67:2640–2644.
23. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, *et al*. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol* 2012;50:1355–1361.
24. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, *et al*. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 2011;7:539.
25. Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. *Bioinformatics* 2011;27:1009–1010.
26. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, *et al*. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res* 2015;43:e15.
27. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, *et al*. *SNP-sites*: rapid efficient extraction of SNPs from multi-FASTA alignments. *Microb Genom* 2016;2:e000056.
28. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, *et al*. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol* 2020;37:1530–1534.
29. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 2019;47:W256–W259.
30. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.
31. Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, *et al*. The Pfam protein families database: towards a more sustainable future. *Nucleic Acids Res* 2016;44:D279-85.
32. Tada T, Miyoshi-Akiyama T, Shimada K, Nga TTT, Thu LTA, *et al*. Dissemination of clonal complex 2 *Acinetobacter baumannii* strains co-producing carbapenemases and 16S rRNA methylase ArmA in Vietnam. *BMC Infect Dis* 2015;15:433.
33. Davis MA, Baker KNK, Orfe LH, Shah DH, Besser TE, *et al*. Discovery of a gene conferring multiple-aminoglycoside resistance in *Escherichia coli*. *Antimicrob Agents Chemother* 2010;54:2666–2669.
34. Tada T, Hishinuma T, Watanabe S, Uchida H, Tohya M, *et al*. Molecular characterization of multidrug-resistant *Pseudomonas aeruginosa* isolates in hospitals in Myanmar. *Antimicrob Agents Chemother* 2019;63:e02397-18.
35. Xia J, Sun J, Li L, Fang L-X, Deng H, *et al*. First report of the IncI1/ST898 conjugative plasmid carrying *rmtE2* 16S rRNA methyltransferase gene in *Escherichia coli*. *Antimicrob Agents Chemother* 2015;59:7921–7922.
36. Camargo CH, Tiba MR, Saes MR, Vasconcellos FM de, Santos LFD, *et al*. Population structure analysis of carbapenem-resistant *Acinetobacter baumannii* clinical isolates from Brazil reveals predominance of clonal complexes 1, 15, and 79. *Antimicrob Agents Chemother* 2016;60:2545–2547.
37. Doi Y, Wachino J, Arakawa Y. Nomenclature of plasmid-mediated 16S rRNA methylases responsible for panaminoglycoside resistance. *Antimicrob Agents Chemother* 2008;52:2287–2288.
38. Ballaben AS, Andrade LN, Galetti R, Ferreira JC, McElheny CL, *et al*. Diversity of high-level aminoglycoside resistance mechanisms among gram-negative nosocomial pathogens in Brazil. *Antimicrob Agents Chemother* 2018;62:e01550-18.