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A prospective surveillance study to determine the prevalence of 16S rRNA methyltransferase-producing Gram-negative bacteria in the UK

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Objectives: To determine the prevalence of 16S rRNA methyltransferase- (16S RMTase-) producing Gram-negative bacteria in patients in the UK and to identify potential risk factors for their acquisition.

Methods: A 6 month prospective surveillance study was conducted from 1 May to 31 October 2016, wherein 14 hospital laboratories submitted *Acinetobacter baumannii*, Enterobacterales and *Pseudomonas aeruginosa* isolates that displayed high-level amikacin resistance according to their testing methods, e.g. no zone of inhibition with amikacin discs. Isolates were linked to patient travel history, medical care abroad, and previous antibiotic exposure using a surveillance questionnaire. In the reference laboratory, isolates confirmed to grow on Mueller-Hinton agar supplemented with 256 mg/L amikacin were screened by PCR for 16S RMTase genes *armA*, *rmtA–rmtH* and *npmA*, and carbapenemase genes (bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$ and bla_{VIM}). STs and total antibiotic resistance gene complement were determined via WGS. Prevalence was determined using denominators for each bacterial species provided by participating hospital laboratories.

Results: Eighty-four isolates (44.7%), among 188 submitted isolates, exhibited high-level amikacin resistance (MIC >256 mg/L), and 79 (94.0%) of these harboured 16S RMTase genes. *armA* (54.4%, 43/79) was the most common, followed by *rmtB* (17.7%, 14/79), *rmtF* (13.9%, 11/79), *rmtC* (12.7%, 10/79) and *armA* + *rmtF* (1.3%, 1/79). The overall period prevalence of 16S RMTase-producing Gram-negative bacteria was 0.1% (79/71 063). Potential risk factors identified through multivariate statistical analysis included being male and polymyxin use.

Conclusions: The UK prevalence of 16S RMTase-producing Gram-negative bacteria is low, but continued surveillance is needed to monitor their spread and inform intervention strategies.

Introduction

16S rRNA methyltransferases (16S RMTases) are transferable resistance mechanisms that cause high-level resistance (MICs >256 mg/L) to all clinically relevant aminoglycosides via acquisition of a single gene. The 16S RMTase enzymes methylate 16S rRNA within the A-site of the ribosome at the ring nitrogen N-1 of residue A1408 or N-7 of residue G1405, preventing the binding of aminoglycosides to the A-site.[1] To date, 10 16S RMTase genes (*armA*, *rmtA-rmtH* and *npmA*) have been identified in Gram-negative bacteria such as *Acinetobacter baumannii*, Enterobacterales and *Pseudomonas aeruginosa*.[1]

There are several features of 16S RMTases that are of clinical concern. They are associated with mobile genetic elements, such as broad- and narrow-host range plasmids (e.g. IncL/M, IncA/C and IncF)[2] and transposons (e.g. Tn*1548* with *armA*),[3] which facilitate their horizontal spread between different strains and species of bacteria. Secondly, 16S RMTase genes are associated with other antibiotic resistance genes of public health importance, such as carbapenemases, ESBLs and fluoroquinolone resistance genes, which can seriously compromise the treatment options available against Gram-negative bacteria in both the clinical and veterinary settings.[2]

There has been no systematic surveillance for 16S RMTase-producing bacteria in the UK to date, but their prevalence appears to be quite low within Europe based on studies investigating non-duplicate Enterobacterales isolates, with rates of 0.12% (19/15 386) reported in Belgium[4] and 0.15% (6/4080) in Greece.[5] In comparison, the prevalence of 16S RMTase genes in non-duplicate Enterobacterales isolates appears to be much higher in the Far East with rates of 3.9% (38/985) reported in China.[6]

In this study, we aimed to determine the period prevalence rate of 16S RMTase genes in amikacin-resistant Gram-negative bacteria (*A. baumannii*, Enterobacterales and *P. aeruginosa*) isolated in the UK, and possible risk factors associated with acquisition of 16S RMTase-producing bacteria, by conducting a 6 month prospective surveillance survey involving NHS hospitals.

Materials and methods

Hospital laboratory recruitment

Thirty hospital laboratories were approached to participate in the study. These laboratories were chosen because, from January to October 2015, they had reported amikacin susceptibility data to PHE's Second Generation Surveillance System (SGSS)[7] for the highest numbers of *Escherichia coli* isolates and were a subset of 110 laboratories that routinely reported amikacin susceptibility. Fourteen of the 30 laboratories participated in the study.

Isolate collection

Participating laboratories were asked to submit any *A. baumannii*, Enterobacterales and *P. aeruginosa* isolated between 1 May to 31 October 2016 that displayed high-level resistance to amikacin, i.e. no zone of inhibition around 30 μg amikacin discs, gradient strip MIC >256 mg/L or those at the top of the tested MIC range when using automated susceptibility testing methods.

Bacterial identification and strain typing

Bacterial identification was confirmed by MALDI-ToF (Bruker, Bremen, Germany). *Klebsiella pneumoniae* and *P. aeruginosa* isolates were typed using variable-number tandem-repeat (VNTR) analysis.[8,9] *A. baumannii* and other Enterobacterales were typed by PFGE using the restriction enzymes ApaI and XbaI,[10] respectively. STs were derived from WGS data of 16S RMTase-producing isolates using MLST 2.0.[11]

Antibiotic susceptibility testing and 16S RMTase gene detection

Antibiotic susceptibility testing was performed by agar dilution[12] against PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) standard Gram-negative antibiotic panel and the data were interpreted using EUCAST clinical breakpoints (version 8.1).[13] Only isolates that were amikacin-resistant based on the clinical breakpoint for resistance (MIC >16 mg/L) were included in the study. Isolates that grew on Mueller-Hinton (MH) agar plates containing 256 mg/L amikacin, indicating likely 16S RMTase production, were screened for 16S RMTase genes using two multiplex PCRs to detect *armA*, *rmtA-rmtH* and *npmA*.[14]

Detection of carbapenemase and other antibiotic resistance genes

All isolates were screened for carbapenemase genes bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$ and bla_{VIM} using a real-time PCR as previously described.[15] Other antibiotic resistance genes were detected in 16S RMTase-producing isolates via ResFinder v3.0[16] following WGS using the Illumina HiSeq 2500 (Illumina, USA) with 150 bp paired-end reads.

Analysis of 16S RMTase gene-negative isolates

Bacterial isolates exhibiting high-level amikacin resistance (MIC >256 mg/L) but that were PCR-negative for 16S RMTase genes were screened with ResFinder,[16] following WGS, to confirm negativity. Pfam 31.0[17] was used to screen for motifs that could be associated with novel 16S RMTase genes. Following a six-frame translation of contig DNA, two searches were performed for the presence of motifs associated with the two protein families that 16S RMTases belong to [FmrO (Pfam ID: PF07091) for ArmA and RmtA-RmtH and the Methyltransf_4 family (Pfam ID: PF02390) for NpmA]. The E-value cut-offs used were the default E-value of 1.0 and 1×10^{-5} (for increased stringency).

Determination of the period prevalence rate of 16S RMTase bacteria

The period prevalence of 16S RMTase-producing isolates was calculated using the total number of *A. baumannii*, Enterobacterales and *P. aeruginosa* isolates screened with amikacin over the study period, regardless of the susceptibility testing result, in each individual laboratory as the denominator. Prevalence rates were calculated per laboratory and for all participating laboratories collectively.

Identification of possible risk factors for acquisition of 16S RMTase-producing bacteria

Laboratories were asked to complete an anonymized questionnaire (Figure S1, available as Supplementary data at *JAC* Online) for each isolate referred, to provide information regarding the patient and isolate, such as bacterial identification, patient age, sex, ward location, country of residence, previous antibiotic use, and history of travel and/or medical care abroad.

Statistical analysis

Carbapenemase production was compared between 16S RMTase-producing and non-producing isolates using the Chi-squared test (Excel, 2016).

Additionally, the likelihood-ratio test was performed comparing patient questionnaire data from patients positive for 16S RMTase-producing bacteria to patients with amikacin-resistant, 16S RMTase-negative bacteria, via Stata MP version 15 (StataCorp LP), to identify risk factors associated with the acquisition of 16S RMTase-producing bacteria.

Variables with $P \leq 0.2$ in the single-variable analysis were selected for multivariable logistic regression analysis, which was performed to control for confounding and identify variables that may act as risk factors for the acquisition of 16S RMTase-producing bacteria. The final multivariable logistic regression analysis model only contained those variables that were statistically significant or substantial confounders. ORs, 95% CIs and *P* values were calculated for univariate logistic regression and multivariable logistic regression, where $P \leq 0.05$ was statistically significant.

Ethics approval

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

Results

Hospital laboratory recruitment

Fourteen laboratories participated in the study. The geographical distribution of the 14 laboratories was as follows: Scotland (28.6%, 4/14), London (21.4%, 3/14), North West (21.4%, 3/14) and one laboratory (7.1%) each in the East of England, East Midlands, Wales and West Midlands.

Clinical isolates

Two hundred and seventy-one isolates were submitted from 12 laboratories; the two remaining laboratories did not identify any amikacin-resistant isolates during the study period. Eighty-three (30.6%) isolates were excluded from the study: 55 (66.3%) were susceptible to amikacin (MICs ≤16 mg/L), 14 (16.9%) were non-viable, 13 (15.7%) were an incorrect species [*Achromobacter* spp. ($n=5$), *Enterococcus* spp. ($n=4$), *Chryseobacterium* sp. ($n=1$), *Delftia* sp. ($n=1$), *Staphylococcus* sp. ($n=1$) and *Stenotrophomonas* sp. ($n=1$)], and one (1.2%) was isolated outside the timeframe of the study.

The remaining 188 isolates included *K. pneumoniae* (34.6%, 65/188), *E. coli* (21.8%, 41/188), *P. aeruginosa* (19.7%, 37/188), *A. baumannii* (8.5%, 16/188), *Enterobacter cloacae* complex (5.3%, 10/188), *Citrobacter freundii* (4.8%, 9/188), *Klebsiella oxytoca* (2.1%, 4/188), *Serratia marcescens* (1.6%, 3/188) and single isolates of *Citrobacter amalonaticus, Morganella morganii* and *Providencia stuartii*.

Identification of 16S RMTase-producing bacteria

Among the 188 remaining isolates, which had confirmed amikacin resistance (MIC >16 mg/L), 84 (44.7%) exhibited high-level amikacin resistance (MIC >256 mg/L) of which 79 (94.0%) were positive for 16S RMTase genes (Table 1). This included *armA* (54.4%, 43/79), *rmtB* (17.7%, 14/79), *rmtF* (13.9%, 11/79), *rmtC* (12.7%, 10/79) and *armA* + *rmtF* (1.3%, 1/79). The genes *rmtD, rmtE, rmtG, rmtH* and *npmA* were not detected. No 16S RMTase genes were identified in the remaining 104 isolates that were amikacin-resistant (MIC >16 mg/L) but not high-level amikacin-resistant (MIC 256 mg/L). Analysis of the five PCR-negative isolates exhibiting high-level amikacin resistance (MIC 256 mg/L) did not identify protein motifs associated with a possible novel 16S RMTase, but high-level amikacin resistance could be attributed to combinations of aminoglycoside-modifying enzymes (Table 2).

The 16S RMTase-producing isolates (Table 1) included *K. pneumoniae* (51.9%, 41/79), *E. coli* (16.5%, 13/79), *A. baumannii* (13.9%, 11/79), *C. freundii* (7.6%, 6/79), *E. cloacae* complex (3.8%, 3/79), *K. oxytoca* (2.5%, 2/79), *P. aeruginosa* (2.5%, 2/79) and *P. stuartii* (1.3%, 1/79).

The most common isolation site was 'screening swab' (58.2%, 46/79), where 41 (89.1%) were rectal swabs, one (2.2%) was a throat swab, one (2.2%) was a wound swab and three (6.5%) were 'screening swabs' of unknown origin. Other isolation sites included tissue or fluid (20.3%, 15/79), urine (13.9%, 11/79), respiratory (3.8%, 3/79), blood or line tip (3.8%, 3/79) and faeces (1.3%, 1/79). Further information on isolation site for each bacterial species can be found in Table S1.

16S RMTase-producing isolates were submitted from eight laboratories located in five UK regions, including three laboratories from London (68.4%, 54/79), two from Scotland (8.9%, 7/79) and one each from the East of England (10.1%, 8/79), East Midlands (1.3%, 1/79) and the North West (11.4%, 9/79).

Antibiotic susceptibility of 16S RMTase producers

In addition to the expected high-level resistance to amikacin, gentamicin and tobramycin (MICs >64 mg/L, >32 mg/L and >32 mg/L, respectively), all 16S RMTase producers were resistant to multiple antibiotic classes. All 11 *A. baumannii* isolates were resistant to imipenem, meropenem, ceftazidime and ciprofloxacin, but all were susceptible to colistin. Enterobacterales isolates were resistant to cefotaxime (98.5%, 65/66), ceftazidime (98.5%, 65/66), ertapenem (92.4%, 61/66), ciprofloxacin (92.4%, 61/66), meropenem (62.1%, 41/66), imipenem (54.5%, 36/66), colistin (18.2%, 12/66) and tigecycline (16.7%, 11/66). The two *P. aeruginosa* isolates were resistant to imipenem, meropenem, ceftazidime and ciprofloxacin, but were susceptible to colistin.

Identification of carbapenemase and other antibiotic resistance genes

Carbapenemase genes were identified in more 16S RMTase-producing isolates (87.3%, 69/79) compared with 36.7% (40/109) 16S RMTase-negative isolates ($P<0.05$). WGS analysis of the 16S RMTase-producing isolates identified carbapenemase gene variants $bla_{\text{NDM-1}}$ (42%, 29/69), $bla_{\text{OXA-23}}$ (14.5%, 10/69), $bla_{\text{NDM-5}}$ (10.1%, 7/69), $bla_{\text{OXA-48}}$ (7.2%, 5/69), $bla_{\text{OXA-181}}$ (4.3%, 3/69), $bla_{\text{OXA-232}}$ (4.3%, 3/69), $bla_{\text{NDM-5}}$ + $bla_{\text{OXA-181}}$ (4.3%, 3/69), $bla_{\text{NDM-1}}$

Table 1. Sequence types (STs) associated with 16S RMTase and carbapenemase genes

Species	16S RMTase gene(s)	Carbapenemase gene(s)	STs
A. baumannii	*armA* (*n* = 11)	bla_{OXA-23} (*n* = 10)	2 (*n* = 9) and 113 (*n* = 1)
		bla_{NDM-1} + bla_{OXA-23} (*n* = 1)	622 (*n* = 1)
C. freundii	*armA* (*n* = 2)	bla_{OXA-48} (*n* = 2)	11 (*n* = 1) and 111 (*n* = 1)
	rmtC (*n* = 4)	bla_{NDM-1} (*n* = 4)	18 (*n* = 2), 22 (*n* = 1) and 170 (*n* = 1)
E. cloacae complex	*armA* (*n* = 2)	Negative (*n* = 2)	265 (*n* = 2)
	rmtC (*n* = 1)	bla_{NDM-1} (*n* = 1)	275 (*n* = 1)
E. coli	*armA* (*n* = 4)	bla_{NDM-1} (*n* = 3)	405 (*n* = 3)
		Negative (*n* = 1)	636 (*n* = 1)
	rmtB (*n* = 7)	bla_{NDM-5} (*n* = 4)	405 (*n* = 2), 1585 (*n* = 1) and 2450 (*n* = 1)
		bla_{NDM-1} (*n* = 1)	90 (*n* = 1)
		bla_{NDM-7} (*n* = 1)	448 (*n* = 1)
		Negative (*n* = 1)	167 (*n* = 1)
	rmtC (*n* = 2)	bla_{NDM-1} (*n* = 1)	6870 (*n* = 1)
		bla_{NDM-1} + $bla_{OXA-181}$ (*n* = 1)	167 (*n* = 1)
K. oxytoca	*armA* (*n* = 2)	bla_{NDM-1} (*n* = 1)	84 (*n* = 1)
		bla_{OXA-48} (*n* = 1)	354 (*n* = 1)
K. pneumoniae	*armA* (*n* = 22)	bla_{NDM-1} (*n* = 13)	78 (*n* = 5),14 (*n* = 3), 15 (*n* = 2), 147 (*n* = 2) and 29 (*n* = 1)
		bla_{KPC-2} (*n* = 1)	15 (*n* = 1)
		bla_{NDM-11} (*n* = 1)	16 (*n* = 1)
		bla_{OXA-48} (*n* = 1)	15 (*n* = 1)
		bla_{NDM-1} + bla_{OXA-48} (*n* = 1)	147 (*n* = 1)
		bla_{NDM-1} + $bla_{OXA-232}$ (*n* = 2)	14 (*n* = 2)
		Negative (*n* = 3)	14 (*n* = 1), 29 (*n* = 1) and 307 (*n* = 1).
	rmtB (*n* = 4)	bla_{NDM-5} (*n* = 3)	138 (*n* = 2) and 1248 (*n* = 1)
		Negative (*n* = 1)	231 (*n* = 1)
	rmtC (*n* = 3)	bla_{NDM-1} (*n* = 3)	29 (*n* = 1), 89 (*n* = 1) and 307 (*n* = 1)
	rmtF (*n* = 11)	bla_{NDM-5} + $bla_{OXA-181}$ (*n* = 3)	147 (*n* = 3)
		$bla_{OXA-232}$ (*n* = 3)	231 (*n* = 3)
		$bla_{OXA-181}$ (*n* = 2)	147 (*n* = 2)
		bla_{OXA-48} (*n* = 1)	383 (*n* = 1)
		Negative (*n* = 2)	336 (*n* = 1) and 231 (*n* = 1)
	armA + *rmtF* (*n* = 1)	$bla_{OXA-181}$ (*n* = 1)	231 (*n* = 1)
P. stuartii	*rmtB* (*n* = 1)	bla_{VIM-1} (*n* = 1)	NA
P. aeruginosa	*rmtB* (*n* = 2)	bla_{NDM-1} (*n* = 2)	773 (*n* = 2)

NA, not available.

+ $bla_{OXA-232}$ (2.9%, 2/69), and one isolate (1.4%) each with bla_{KPC-2}, bla_{NDM-1} + bla_{OXA-23}, bla_{NDM-1} + bla_{OXA-48}, bla_{NDM-1} + $bla_{OXA-181}$, bla_{NDM-11}, bla_{NDM-7} or bla_{VIM-1} (Table 1).

ESBL genes were identified in 79.7% (63/79) of 16S RMTase-producing isolates, with $bla_{CTX-M-15}$ (82.5%, 52/63) being the most common, followed by bla_{SHV-39} (15.9%, 10/63), $bla_{SHV-100}$ (14.3%, 9/63), bla_{SHV-12} (9.5%, 6/63), $bla_{CTX-M-14b}$ (3.2%, 2/63), bla_{PER-7} (3.2%, 2/63), bla_{VEB-1} (3.2%, 2/63), $bla_{CTX-M-33}$ (1.6%, 1/63), $bla_{CTX-M-64}$ (1.6%, 1/63), bla_{SHV-5} (1.6%, 1/63) and $bla_{TEM-168}$ (1.6%, 1/63). Plasmid-mediated fluoroquinolone resistance genes were identified in 79.7% (63/79) 16S RMTase-producing isolates including *aac(6′)-Ib-cr* (88.7%, 55/62), *qnrB1* (40.3%, 25/62), *qnrS1* (16.1%, 10/62), *qnrB38* (3.2%, 2/62), *qnrVC1* (3.2%, 2/62), *qepA1* (1.6%, 1/62), *qnrB9* (1.6%, 1/62) and *qnrB35* (1.6%, 1/62). Colistin resistance genes were identified in 5.1% (4/79) 16S RMTase-positive isolates including *mcr-1* (50.0%, 2/4) and *mcr-9* (50.0%, 2/4). The antibiotic resistance genes in each 16S RMTase-positive isolate can be found in Table S1.

Bacterial typing using MLST, PFGE and VNTR

Analysis of the MLST data identified that 16S RMTase producers belonged to diverse STs (Table 1). The most common were *A. baumannii* ST2 (81.8%, 9/11), *C. freundii* ST18 (33.3%, 2/6), *E. cloacae* ST265 (66.7%, 2/3), *E. coli* ST405 (38.5%, 5/13), *K. pneumoniae* ST147 (19.5%, 8/41) and *P. aeruginosa* ST773 (100%, 2/2). PFGE analysis identified variation in all the most common *A. baumannii* and Enterobacterales STs except for two pairs of

Table 2. Genes encoding aminoglycoside-modifying enzymes (AMEs) identified in 16S RMTase-negative isolates ($n = 5$) that demonstrate high-level resistance to amikacin

Species (individual isolates)	ST	Aminoglycoside modifying enzyme genes					
		aac(6')-Ib-cr[a, c]	*aac(3)-IIa*[b, c]	*aph(3')-VIb*[a]	*aac(3)-Id*[b]	*aac(6')-Ib*[a, c]	*aph(3')-VIa*[a]
C. freundii	113	1	1	1	–	–	–
E. coli	405	1	1	–	–	–	–
K. pneumoniae	11	1	1	1	–	1	–
P. aeruginosa	233	–	–	1	1	–	–
P. aeruginosa	357	1	–	–	–	–	1
Total		4	3	3	1	1	1

[a]Confers amikacin resistance.
[b]Confers gentamicin resistance.
[c]Confers tobramycin resistance.

Table 3. Period prevalence rates of 16S RMTases between 1 May to 31 October 2016

Hospital	No. of isolates screened with amikacin during study period	No. of 16S RMTase positive isolates	Prevalence rate
East_1	16526	8	0.1%
East_Midlands_1	3120	1	0.1%
London_1	10235	17	0.2%
London_2	6959	26	0.4%
London_3	4862	11	0.2%
North West_1	3696	9	0.2%
North West_2	2297	0	0.0%
North West_3	2148	0	0.0%
West Midlands_1	7155	0	0.0%
Scotland_1	1657	4	0.2%
Scotland_2	3354	0	0.0%
Scotland_3	3462	3	0.1%
Scotland_4	2532	0	0.0%
Wales_1	3060	0	0.0%
Total	71063	79	0.1%

A. baumannii ST2 isolates from one London laboratory and two *E. cloacae* ST265 isolates from one laboratory in Scotland (acquired from a single patient), which shared an identical PFGE profile. VNTR analysis identified that the two 16S RMTase-producing *P. aeruginosa* ST773 isolates, sent by two laboratories in London, shared an identical profile (12,4,6,5,3,1,10,9,12). However, VNTR typing found *K. pneumoniae* ST147 isolates belonged to two VNTR profiles: 5,3,5,14,14,2,4,2,5,3,6 ($n = 5$) and 5,3,5,14,14,2,4,2,1,–,– ($n = 3$). Isolates with the former VNTR profile were referred by two laboratories each in London and the North West, and one in the East Midlands. The isolates with VNTR profile 5,3,5,14,14,2,4,2,1,–,– were submitted from the two laboratories in London and one laboratory from the North West.

Calculation of period prevalence of 16S RMTases in the UK

The laboratory with the highest prevalence of 16S RMTase-producing isolates was London_2 (0.4%, 26/6959) (Table 3). Overall, during the study period, the total prevalence of 16S RMTase producers in the participating laboratories was 0.1% (79/71 063).

The prevalence of 16S RMTase genes was highest in *A. baumannii* (3.3%, 11/338), followed by the Enterobacterales (0.1%, 66/56 172) and *P. aeruginosa* (0.01%, 2/14 553).

Patient demographics

The 188 amikacin-resistant isolates were obtained from 166 patients, and 16S RMTase-producing isolates originated from 65 (39.2%) patients. Out of the latter, eight (12.3%) patients had multiple isolates, where five (62.5%) had bacterial isolates belonging to different species, five (62.5%) had bacterial isolates belonging to same species but had different MICs and one (12.5%) patient had bacterial isolates from different isolation sites. The average age of patients with 16S RMTase-producing bacteria was 67 years (ranging from 23 to 89 years), and 46 (70.8%) patients were male. Fifty-eight (89.2%) patients were hospital inpatients, three (4.6%) were hospital outpatients, two (3.1%) were community patients and patient type was unknown for two patients. Fifty-eight (89.2%) patients lived in the UK with the remainder living in Kuwait (28.6%, 2/7), Pakistan (28.6%, 2/7), Egypt (14.3%, 1/7), Greece (14.3%, 1/7) and India (14.3%, 1/7). Two patients had an unknown country of residence.

Forty-four (67.7%) patients with 16S RMTase-producing bacteria had received antibiotics in the two years prior to this study (but the timing of antibiotic usage was not known). The most commonly prescribed antibiotics were piperacillin/tazobactam (45.5%, 20/44), meropenem (31.8%, 14/44) and vancomycin (27.3%, 12/44). Aminoglycosides were administered to 10 patients (22.7%). Seven patients (10.8%) had received no antibiotics and antibiotic usage history was unknown for 14 (21.5%) patients.

Travel history was only known for 41 (63.1%) patients with 16S RMTase-producing bacteria, where 22 (53.7%) had travelled within 2 years prior to inclusion in this study and 19 (46.3%) had not travelled during this period. Travel history was not known for 24 (36.9%) patients. The countries that were most frequently visited were Kuwait (18.2%, 4/22) and India (18.2%, 4/22), followed by Pakistan (13.6%, 3/22), Egypt (9.1%, 2/22), Russia (9.1%, 2/22) and one patient each visited Crete, France, Greece, Nigeria, Philippines and Thailand. One patient had travelled abroad but the location was unknown. Eighteen of these patients (81.8%) had received medical treatment while abroad with surgical treatment in intensive care (38.9%, 7/18) being the most common.

Identification of possible risk factors for acquisition of 16S RMTase-producing bacteria

Univariate logistic regression analysis identified the following variables as potential risk factors ($P < 0.05$): age, gender, patient type, UK residency, the use of medical treatment abroad as well as aminoglycoside use. Analysis of the ORs indicated that patients had higher odds of acquiring 16S RMTase-producing bacteria if they were male, over 65 years of age, had travelled abroad, particularly to Asia or Africa, received medical treatment abroad or had been prescribed polymyxins (Table 4). In contrast, patients had lower odds of acquiring 16S RMTase-producing bacteria if they were hospital outpatients or community patients rather than hospital inpatients, UK residents or if they had been prescribed aminoglycosides (Table 4).

The final multivariable logistic regression analysis model included the following variables: age (continuous variable), gender, patient type, UK residency, aminoglycoside use and polymyxin use. The model was only based on data from 65.1% (108/166) patients as those with data missing for any of the model variables were excluded. The only variables associated with reduced risk were previous aminoglycoside use and UK residency ($P < 0.05$). Odds of acquiring 16S RMTase-producing bacteria were increased in patients who were male or who had been prescribed polymyxins

Table 4. ORs, 95% CIs and *P* values obtained from single and multivariable analyses of patient questionnaire data using the likelihood ratio test

		Single-variable analysis				Multivariable analysis			
		RMTase				RMTase			
Variable		Positive ($n = 65$)	Negative ($n = 101$)	OR (95% CI)	*P* value	Positive ($n = 42$)	Negative ($n = 66$)	OR (95% CI)	*P* value
Age, years (min, median, max)		23, 67, 89	0.9, 60, 93	1.0 (1.0–1.0)	0.008	23, 64, 89	0.9, 63, 92	1.0 (1.0–1.0)	0.3
Age, years	≤1–17	0	4	NE	0.09			ND	
	18–45	7	20	Baseline 1				ND	
	46–65	24	37	1.7 (0.6–5.0)				ND	
	66–85	30	36	2.5 (0.9–7.0)				ND	
	86+	4	4	2.1 (0.4–11.6)				ND	
Gender	Male	46	55	2.1 (1.1–4.2)	0.03	29	37	2.0 (0.8–5.1)	0.1
	Female	19	46			13	29		
Patient type	Inpatient	58	78	Baseline 1	0.03	40	57	Baseline 1	0.6
	Outpatient	3	17	0.2 (0.05–0.8)		2	8	0.8 (0.1–4.6)	
	Community	2	6	0.6 (0.9–3.9)		0	1	NE	
UK Residency	Yes	58	98	0.09 (0.01–0.8)	0.008	37	66	NE	0.003
	No	7	1			5	0		
Previous travel history	Yes	22	10	2.3 (0.9–6.2)	0.09			ND	
	No	19	20					ND	
Travel to Asia	Yes	13	6	2.3 (0.7–7.0)	0.2			ND	
	No	29	30					ND	
Travel to Europe	Yes	5	4	0.9 (0.2–4.1)	0.9			ND	
	No	37	31					ND	
Travel to Africa	Yes	3	1	2.7 (0.3–28.6)	0.4			ND	
	No	39	34					ND	
Medical treatment abroad	Yes	18	7	3.0 (1.1–8.5)	0.03			ND	
	No	23	27					ND	
Aminoglycoside use	Yes	10	35	0.3 (0.1–0.6)	0.001	10	32	0.3 (0.1–0.7)	0.006
	No	34	31			32	31		
Polymyxin use	Yes	8	6	2.2 (0.7–6.9)	0.2	8	6	2.8 (0.7–10.5)	0.1
	No	36	60			34	60		

NE, not estimated; ND, not done.
Baseline 1 is the reference category, which was used for comparison with the other categories.

and were reduced if prescribed aminoglycosides or if patients were hospital outpatients compared with hospital inpatients (Table 4). ORs and 95% CIs could not be calculated for community patients or UK residency due to the reduced sample size.

Discussion

This is the first systematic evaluation of 16S RMTase-producing Gram-negative bacteria in hospital laboratories in the UK; the period prevalence was 0.1% (79/71 063). This is lower than in China[18] and Bulgaria,[19] where rates of 26.0% (193/741) and 1.2% (20/1649) were reported in non-duplicate clinical Gram-negative bacterial isolates. The UK has an apparently higher prevalence rate than Japan,[20] where 16S RMTase genes were identified in 0.03% (26/87 626) non-duplicate clinical Gram-negative bacterial isolates. However, the Japanese study only screened isolates that grew on LB agar supplemented with 500 mg/L arbekacin, so may have under-estimated prevalence if 16S RMTase-positive isolates with lower MICs were excluded. Additionally, the rate of 16S RMTase genes may be higher in the UK than Japan due to the high level of association between 16S RMTase genes and carbapenemase genes. Japan appears to have low levels of carbapenemases,[21,22] with bla_{IMP} genes the most commonly identified compared with other metallo-β-lactamases, which are more commonly associated with 16S RMTase genes.[1]

In the current study, the most common 16S RMTase genes identified were *armA* (54.4%, 43/79) and *rmtB* (17.7%, 14/79), which are also the two most frequently reported 16S RMTase genes worldwide.[1] *armA* was widely distributed among bacterial species, particularly *K. pneumoniae* (52.3%, 23/44), but other 16S RMTase genes were more specific to certain bacterial species such as *rmtF*, which was only found in *K. pneumoniae*, although it has been reported in other species.[23–25] *rmtB* was mainly found in *E. coli* and *rmtC* was mostly found in *C. freundii*.

A high percentage of 16S RMTase-producing bacteria harboured carbapenemase genes (87.3%, 69/79), with the bla_{NDM} family being the most common (66.7%, 46/69). This result supports findings from our previous study[14] where 93.4% (712/762) 16S RMTase-producing isolates harboured carbapenemase genes [with bla_{NDM} being the most common (83.1%, 592/712)], demonstrating that 16S RMTase genes are frequently associated with carbapenemase genes in the UK. Other antibiotic resistance genes that were frequently associated with 16S RMTase genes in this study were *aac(6′)-Ib-cr* and $bla_{CTX\text{-}M\text{-}15}$, which have previously been reported as associated with *armA* and *rmtB*.[26–28] The close association between carbapenemase, ESBL and fluoroquinolone resistance genes and 16S RMTase genes suggests these genes may be co-located on plasmids as previously described in the literature.[28–31] This association limits available treatment options for Gram-negative infections, leaving only last-line antibiotics, such as colistin or tigecycline, or newly developed agents, such as cefiderocol.[32,33]

A number of 'high-risk' clones were associated with 16S RMTase genes, including *A. baumannii* ST2, *K. pneumoniae* ST147, *E. coli* ST405 and *P. aeruginosa* ST773, which have all been reported to carry carbapenemase genes, such as $bla_{OXA\text{-}23\text{-}like}$ (*A. baumannii* ST2),[34–36] bla_{NDM} (*K. pneumoniae* ST147 and *E. coli* ST405),[37–40] $bla_{OXA\text{-}48\text{-}like}$ (*K. pneumoniae* ST147 and *E. coli* ST405)[37,41] and bla_{VIM} (*P. aeruginosa* ST773),[42,43] although in this study *P. aeruginosa* ST773 carried $bla_{NDM\text{-}1}$. However, based on the strain diversity observed via MLST, VNTR and PFGE it can be assumed that clonal expansion is not the only contributor to the emergence of 16S RMTase genes within the UK and other factors such as plasmid spread are involved.

Age, gender, patient type, UK residency, medical treatment abroad and aminoglycoside use were all found to be statistically significant in the single-variable analysis indicating they may act as factors that impact the risk of acquiring 16S RMTase-producing bacteria. Importantly the risk factors identified may serve as surrogate markers of at-risk populations. Polymyxins are last-line antibiotics, shown in this study to be active against 16S RMTase-producing bacteria, that are used to treat multidrug-resistant bacteria including carbapenemase producers. This is likely to explain the weak association between polymyxin exposure and increased odds of acquiring 16S RMTase-producing bacteria, as multiple studies[14,44,45] have shown a high level of association between 16S RMTase and carbapenemase genes.

Multivariate analysis revealed that patients who were UK residents had 10-fold lower risk of acquiring 16S RMTase-producing bacteria, while those previously prescribed aminoglycosides had 3-fold lower risk, refuting a major role for aminoglycoside use in selection for 16S RMTase-producing bacteria. It is possible that aminoglycoside use is a surrogate marker of UK residency or good antimicrobial stewardship; it may also be that those patients already known to carry aminoglycoside-resistant bacteria are not prescribed aminoglycosides.

To our knowledge, this is the first study to identify potential risk factors for the acquisition of 16S RMTase-producing bacteria; such knowledge could support antimicrobial selection. A limitation of this study was the inability to recruit hospital laboratories in all regions of the UK including Northern Ireland and the South West of England, thus the UK prevalence rate reported here is not definitive and must be considered an indication. Additionally, the prevalence rate may be an overestimation of clinically relevant prevalence as 58.2% samples were from screening swabs. Furthermore, missing data from a number of patients meant it was not possible to adequately evaluate the role of antibiotic use, overseas travel and treatment abroad. Patients who have received medical treatment abroad are at risk of carrying carbapenemase-producing bacteria;[46,47] due to the observed high level of association it seems likely such patients may be at increased risk of 16S RMTase-producing bacterial carriage also.

In conclusion, the prevalence of 16S RMTase producers between 1 May to 31 October 2016 in the 14 participating hospital laboratories was 0.1%, indicating that 16S RMTases do not currently pose a significant threat to aminoglycoside use in the UK. Although requiring further research, potential risk factors were identified and indicate that clinicians should consider the possibility of carriage of 16S RMTase producers in patients who live outside of the UK, have recently travelled abroad (especially to Asia or Africa), or have received medical treatment abroad. As such isolates are also likely to harbour carbapenemases, which may be aiding their spread within the UK, 16S RMTases pose a future threat

to the treatment of Gram-negative bacterial infections by limiting available treatment options.

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Supplementary data

Figure S1 and Table S1 are available as Supplementary data at *JAC* Online.

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