

Pan-aminoglycoside resistance: the emergence of 16S rRNA methyltransferases in the UK

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Conflict of interest: None



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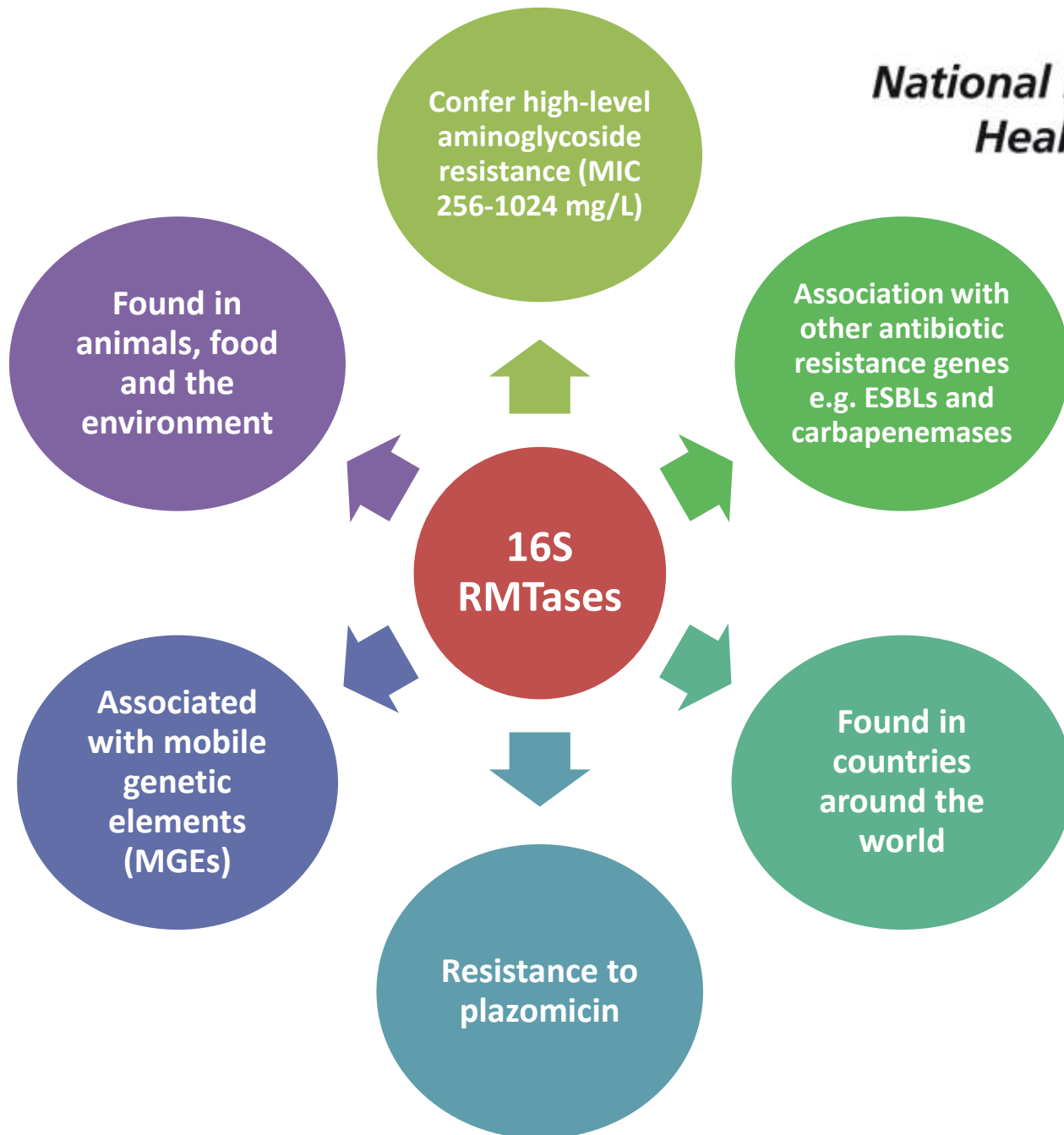
16S rRNA Methyltransferases (16S RMTases)

- Aminoglycosides are broad-spectrum antibiotics that target 16S rRNA in bacterial ribosomes and disrupt protein synthesis.
- 16S RMTases were discovered in the aminoglycoside-producing Actinomycetes (e.g. *Streptomyces* spp.).
- 16S RMTases were first discovered in Gram-negative bacteria in late 1990s.
- They cause high level resistance to all clinically important aminoglycosides (MIC 256-1024 mg/L).
- There are 10 16S RMTase genes plus variants (*armA*, *rmtA-H* and *npmA*).



Streptomyces spp.

Year of Isolation	16S-RMTase gene	RMTase target	Bacterial Species	Country
1996	<i>armA</i>	G1405	<i>Citrobacter freundii</i>	Poland
1997	<i>rmtA</i>	G1405	<i>P. aeruginosa</i>	Japan
2002	<i>rmtB</i>	G1405	<i>Serratia marcescens</i>	Japan
2003	<i>rmtC</i>	G1405	<i>Proteus mirabilis</i>	Japan
2005	<i>rmtD</i>	G1405	<i>P. aeruginosa</i>	Brazil
2010	<i>rmtE</i>	G1405	<i>E. coli</i>	USA
2011	<i>rmtF</i>	G1405	<i>K. pneumoniae</i>	France
2011	<i>rmtG</i>	G1405	<i>K. pneumoniae</i>	Brazil
2009	<i>rmtH</i>	G1405	<i>K. pneumoniae</i>	Iraq
2003	<i>npmA</i>	A1408	<i>E. coli</i>	Japan



Retrospective analysis of PHE's AMRHAI isolate archive

- PHE's AMRHAI Reference Unit is the national reference laboratory for the investigation of antibiotic resistance in healthcare-associated bacterial pathogens
- **Retrospective study:** screened Enterobacteriaceae and *Acinetobacter baumannii* displaying pan-aminoglycoside resistance (amikacin, gentamicin and tobramycin with MICs of ≥ 64 , ≥ 32 and ≥ 32 mg/L, respectively).



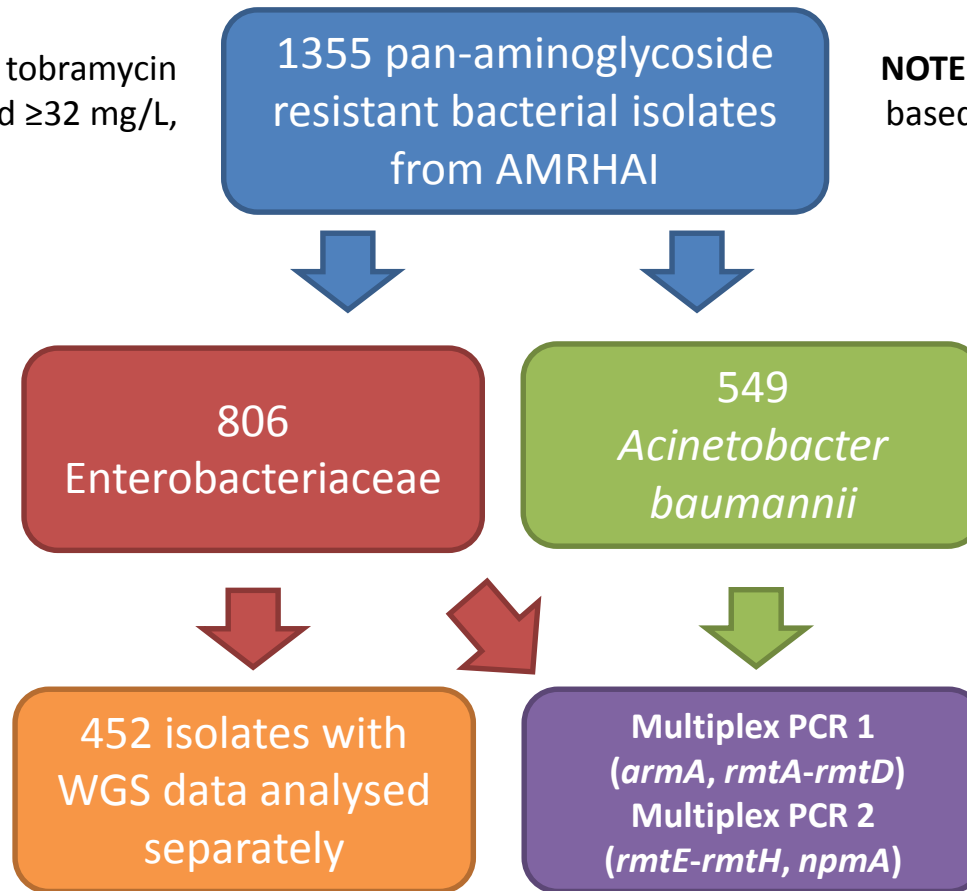
Pseudomonas aeruginosa data can be found on my e-poster 'First identification of 16S rRNA methyltransferases in *Pseudomonas aeruginosa* in the UK' (EV0474)

AMRHAI's isolate collection is **not** representative of the prevalence of 16S RMTases in the UK due to lacking denominator data.

Methods

Amikacin, gentamicin and tobramycin
MIC values of ≥ 64 , ≥ 32 and ≥ 32 mg/L,
respectively.

NOTE: Isolates were deduplicated
based on patient name, bacterial
species and MIC data.



Multiplex 1 = Arakawa *et al.* 2009

Multiplex 2 = This study

Acinetobacter baumannii

- 530/549 (96.5%) were positive for 16S RMTase genes:
 - 529 *armA* (96.4%)
 - 1 *rmtE* (0.2%)
- The most common carbapenemases were OXA-23 + OXA-51 (92.8%, 492/530 isolates)
- 477/530 (90.0%) isolates belonged to international clone II.

16S RMTase	Carbapenemase					
	Negative	OXA-23	OXA-23 + OXA-51	OXA-40 + OXA-51	NDM + OXA-23 + OXA-51	Other Carbapenemase
Negative	0	0	17	0	0	2
ArmA	23	5	492	3	3	3
RmtE	0	0	0	1	0	0
Total (%)	23 (4.2%)	5 (0.9%)	509 (92.7%)	4 (0.7%)	3 (0.5%)	5 (0.9%)

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Acinetobacter baumannii

- 67/530 (12.8%) patients had a travel history.
- 60/68 (88.2%) isolates belonged to international clone II.

Patient travel history	Number of isolates	16S RMTase gene	Carbapenemase (Number of isolates)	<i>A. baumannii</i> international clone II (Number of isolates)
China	3	<i>armA</i>	OXA-23 + OXA-51 (3)	Yes (3)
Egypt	3	<i>armA</i>	OXA-23 + OXA-51 (3)	Yes (2), No (1)
Greece	10	<i>armA</i>	OXA-23 + OXA-51 (10)	Yes (10)
India	6	<i>armA</i>	OXA-23 + OXA-51 (4) NDM + OXA-23 + OXA-51 (2)	Yes (5), No (1)
Italy	6	<i>armA</i>	OXA-23 + OXA-51 (5) OXA-40 + OXA-51 (1)	Yes (4), No (2)
Kuwait	5	<i>armA</i>	OXA-23 + OXA-51 (5)	Yes (4), Unknown (1)
Pakistan	4	<i>armA</i>	OXA-23 + OXA-51 (4)	Yes (3), No (1)
Thailand*	17	<i>armA</i>	OXA-23 + OXA-51 (16)	Yes (14), No (2), Unknown (1)
Turkey	3	<i>armA</i>	OXA-23 + OXA-51 (3)	Yes (3)
Other	16	<i>armA</i>	Negative (1), OXA-23 + OXA-51 (15)	Yes (13), No (2), Unknown (1)

*Two isolates are from the same patient. Other = Albania (n=1), Bangladesh (n=1), Croatia (n=1), Cyprus (n=1), Dubai (n=1), Fiji (n=1), Hong Kong (n=1), Indonesia (n=1), Mauritius (n=1), Montenegro (n=1), Myanmar (n=1), Romania (n=1), South Korea (n=1), Sri Lanka (n=1), Tunisia (n=1) and Vietnam (n=1).

Enterobacteriaceae

- 762/806 (94.5%) Enterobacteriaceae were positive for 16S RMTase genes:
 - 340 *armA* (44.6%)
 - 87 *rmtB* (11.4%)
 - 146 *rmtC* (19.1%)
 - 137 *rmtF* (18.0%)
 - 2 *armA* + *rmtB* (0.3%)
 - 23 *armA* + *rmtC* (3.0%)
 - 17 *armA* + *rmtF* (2.2%)
 - 2 *rmtB* + *rmtC* (0.3%)
 - 5 *rmtB* + *rmtF* (0.7%)
 - 3 *rmtC* + *rmtF* (0.4%)
- 729/762 (95.7%) isolates were positive for carbapenemase genes.
- NDM was the most common carbapenemase (81.3%, 593/729 isolates).

16S RMTase	Carbapenemase					
	Negative	NDM	OXA-48-like	NDM + OXA-48-like	KPC	Other carbapenemase
Negative	26	2	6	0	8	2
ArmA	29	248	31	26	4	2
RmtB	5	64	3	12	1	2
RmtC	3	129	1	12	0	0
RmtF	12	54	65	6	0	0
Two 16S RMTases	2	35	12	3	0	0
Total (%)	77 (9.6%)	533 (66.1%)	118 (14.6%)	59 (7.3%)	13 (1.6%)	6 (0.7%)

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- 25 *E. coli* STs and 30 *K. pneumoniae* STs were identified from the Enterobacteriaceae WGS data.
- The most common STs were:
 - *E. coli* ST405 (19/87; 14 hospitals)
 - *K. pneumoniae* ST14 (111/314; 25 hospitals)
- 16.3% (124/762) patients had a travel history.

Country	Number of isolates	16S RMTase genes (Number of isolates)	Carbapenemases
Bangladesh	4	<i>armA</i> (2), <i>rmtC</i> (2)	NDM
D.R of Congo	2	<i>armA</i>	Negative (1), NDM (1)
Egypt	6	<i>rmtB</i> (1), <i>rmtC</i> (1), <i>rmtF</i> (4)	NDM (3), OXA-48-like (1), NDM + OXA-48-like (2)
Greece	2	<i>rmtB</i>	KPC (1), VIM (1)
India	72	<i>armA</i> (13), <i>rmtB</i> (15), <i>rmtC</i> (29), <i>rmtF</i> (11), <i>armA</i> + <i>rmtF</i> (2), <i>rmtB</i> + <i>rmtF</i> (1), <i>rmtC</i> + <i>rmtF</i> (1)	Negative (2), NDM (60), OXA-48-like (6), NDM + OXA-48 (4)
Middle East	2	<i>armA</i>	NDM (1), OXA-48-like (1)
Pakistan	23	<i>armA</i> (7), <i>rmtB</i> (1), <i>rmtC</i> (13), <i>armA</i> + <i>rmtC</i> (1), <i>armA</i> + <i>rmtF</i> (1)	NDM (21), OXA-48-like (1), NDM + OXA-48-like (1)
Singapore	2	<i>rmtF</i> (1), <i>armA</i> + <i>rmtF</i> (1)	Negative (1), OXA-48-like (1)
Other	11	<i>armA</i> (7), <i>rmtC</i> (2), <i>rmtF</i> (1), <i>rmtB</i> + <i>rmtF</i> (1)	Negative (1), NDM (5), OXA-48-like (4), NDM + OXA-48-like (1)

Other: Asian continent (n=1), Equatorial Guinea (n=1), Kenya (n=1), Kuwait (n=1), Nigeria (n=1), Oman (n=1), Saudi Arabia (n=1), Sri Lanka (n=1), Syrian Arab republic (n=1), Thailand (n=1), Vietnam (n=1).

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Greece	2	<i>rmtB</i>	KPC (1), VIM (1)
India	72	<i>armA</i> (13), <i>rmtB</i> (15), <i>rmtC</i> (29), <i>rmtF</i> (11), <i>armA</i> + <i>rmtF</i> (2), <i>rmtB</i> + <i>rmtF</i> (1), <i>rmtC</i> + <i>rmtF</i> (1)	Negative (2), NDM (60), OXA-48-like (6), NDM + OXA-48 (4)
Middle East	2	<i>armA</i>	NDM (1), OXA-48-like (1)
Pakistan	23	<i>armA</i> (7), <i>rmtB</i> (1), <i>rmtC</i> (13), <i>armA</i> + <i>rmtC</i> (1), <i>armA</i> + <i>rmtF</i> (1)	NDM (21), OXA-48-like (1), NDM + OXA-48-like (1)
Singapore	2	<i>rmtF</i> (1), <i>armA</i> + <i>rmtF</i> (1)	Negative (1), OXA-48-like (1)
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Total results

National Institute for Health Research

95.3% (1292/1355) of the pan-aminoglycoside resistant bacteria had 16S RMTases.

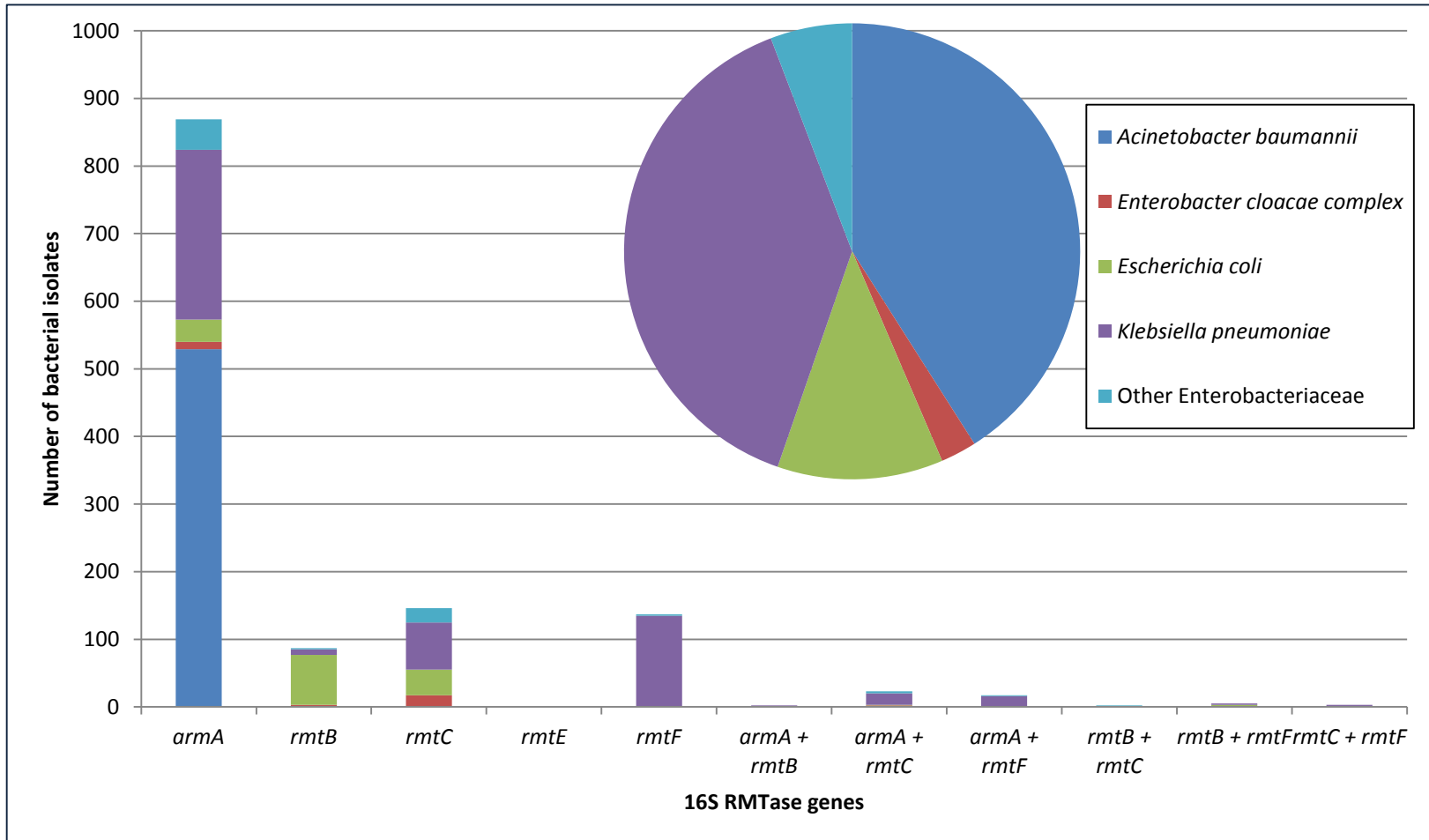


Figure 1: Total number of Gram-negative bacterial isolates with 16S RMTases *armA* and *rmtB-rmtF* from the AMRHAI WGS database and isolate archives between 2004-2015.

■ Total isolates ■ Referring hospitals ■ *armA* ■ *rmtB* ■ *rmtC* ■ *rmtE* ■ *rmtF* ■ Two 16S RMTases

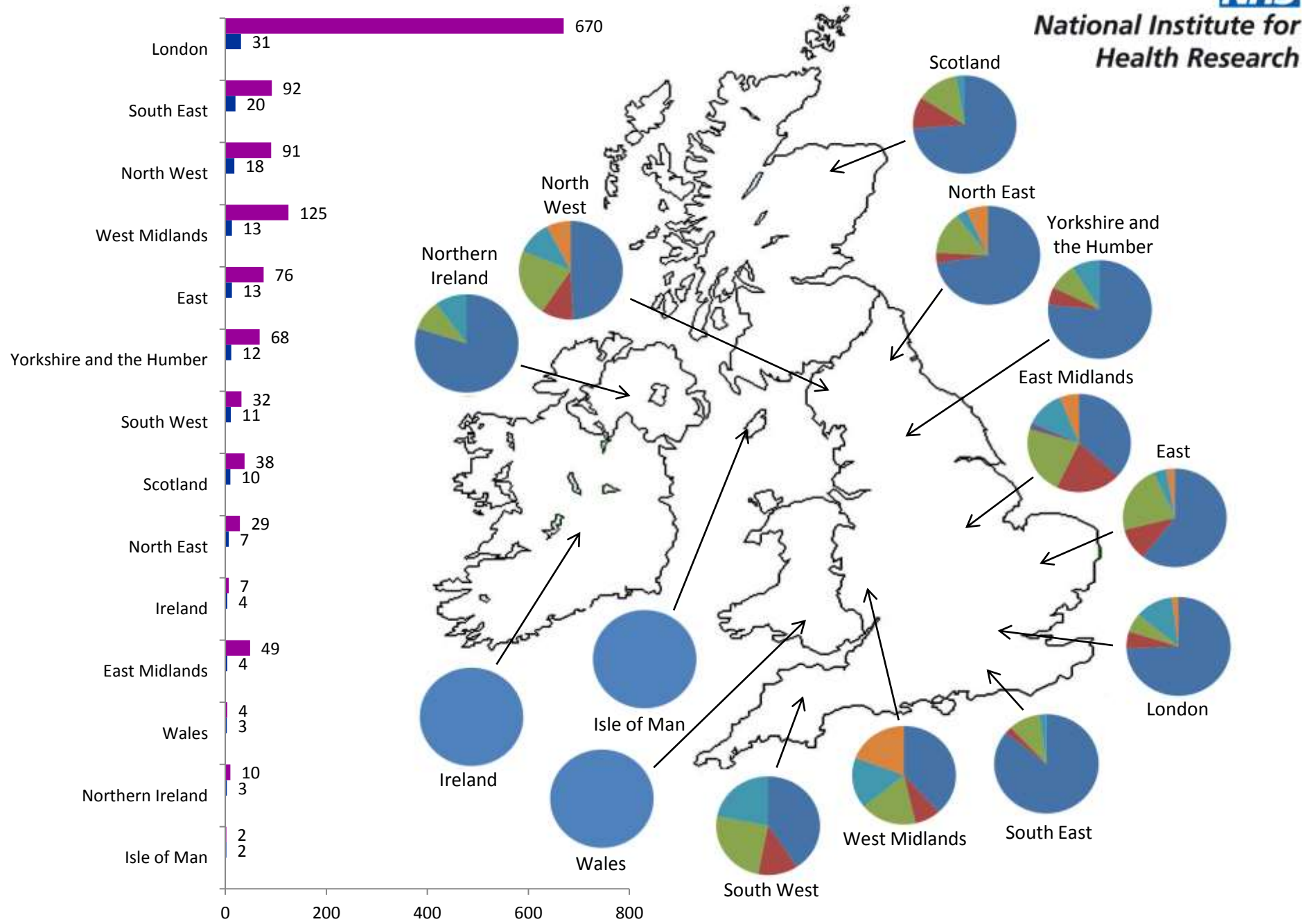


Figure 2: Regional distribution of 16S RMTase-positive bacterial isolates in the UK

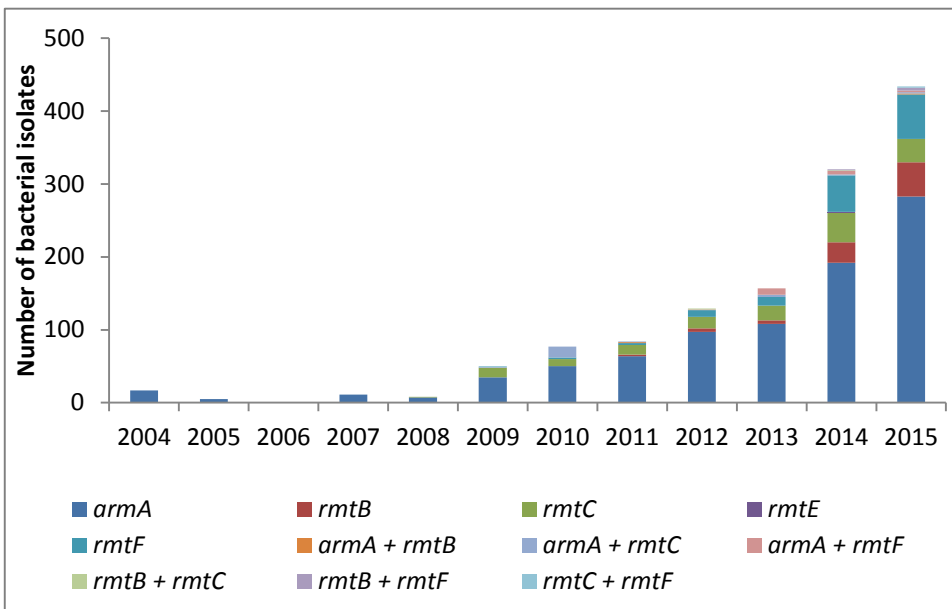


Figure 3: Number of 16S RMTase-positive bacterial isolates from the isolate collection between 2004-2015 at AMRHAI, PHE.

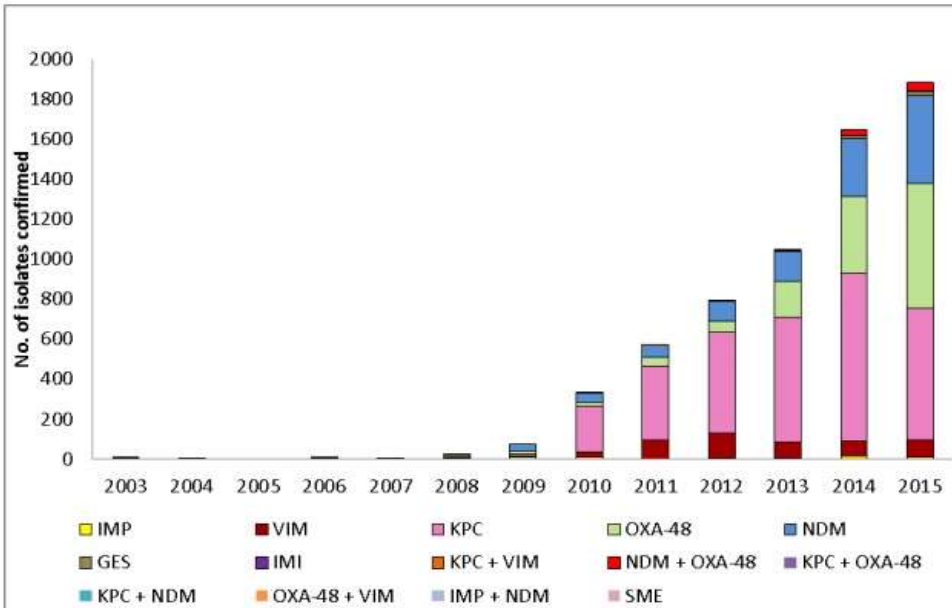


Figure 4: Number of isolates referred from UK hospital microbiology laboratories confirmed as carbapenemase-producing Enterobacteriaceae by AMRHAI, 2003-2015.

- There has been a year-on-year rise in the number of 16S RMTase-positive isolates.
- This mirrors the increase in carbapenemases identified by AMRHAI, PHE.
- This year-on-year increase is likely due to their association with carbapenemases.

Future Work

- Analyse the genetic environment of the positive isolates to identify if they are emerging due to:
 - Association with carbapenemases (or other antibiotic resistance genes)
 - Mobile genetic elements (e.g. plasmids or transposons)
 - Their occurrence in successful bacteria clones e.g. *E. coli* ST405
- Complete a prospective study involving UK hospitals to find true prevalence of 16S RMTase genes in the UK:
 - High-level amikacin-resistant isolates from hospitals were screened for 16S RMTase genes over a six month period (May 2016-October 2016)



Conclusion

- 16S RMTases were present in 95.3% pan-aminoglycoside resistant isolates:
 - 530/549 (96.5%) *A. baumannii*
 - 762/806 (94.5%) Enterobacteriaceae
- 1194/1292 (92.4%) were carbapenemase producers.
- 16S RMTases appear to be emerging in the UK due to their association with carbapenemases as well as through high-risk bacterial clones known to carry carbapenemases.



Acknowledgements

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- **Funding:** NIHR HPRU in Healthcare Associated Infection and Antimicrobial Resistance at Imperial College London.
- Dr Yohei Doi for supplying an *rmtH*-positive bacterial isolate to use as a positive control in this study.

Thank
you 