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Antibiotic resistance among clinical isolates of *Acinetobacter* in the UK, and *in vitro* evaluation of tigecycline (GAR-936)

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A survey was conducted of the antimicrobial susceptibilities of 595 *Acinetobacter* spp. isolated from routine clinical specimens in 54 sentinel laboratories throughout the UK during 2000. Isolates of the *Acinetobacter baumannii* complex (genomic groups 2, 3 and 13TU; *n* = 443) were distinguished from other genomic groups (*n* = 152) by PCR fingerprinting of tDNA spacer regions. MICs of amikacin, cefotaxime, ceftazidime, ciprofloxacin, colistin, gentamicin, imipenem, meropenem, minocycline, piperacillin, piperacillin/tazobactam, rifampicin, sulbactam and tetracycline were determined on IsoSensitest agar and interpreted, wherever possible, using BSAC breakpoints. Tigecycline (GAR-936), a new glycylcycline, was also tested. Resistance to cephalosporins, aminoglycosides and ciprofloxacin was widespread, but carbapenems, colistin, sulbactam, minocycline and tigecycline were each active against >80% of the isolates. Isolates of *A. baumannii* were more often resistant to cefotaxime, ceftazidime, piperacillin, piperacillin/tazobactam, ciprofloxacin, gentamicin and tetracyclines than those belonging to other genomic groups, but were less often resistant to colistin; no significant differences between genomic groups were noted in the susceptibilities to amikacin, carbapenems, rifampicin or sulbactam. The relative activities of the tetracyclines were minocycline > tigecycline > tetracycline. Thirteen carbapenem-resistant isolates (MICs ≥ 8 mg/L; 2.2%) were received from six centres; four centres sent single isolates; one sent three and one sent six. An allele of bla_{IMP} was detected in one of these isolates, but the other 12 isolates either had carbapenemase-independent resistance, or undetectable carbapenemase activity combined with other resistance mechanisms. In conclusion, carbapenems, colistin and minocycline retained greatest activity against the *Acinetobacter* isolates collected. Tigecycline was less active than minocycline, but both agents overcame most tetracycline resistance.

Introduction

Acinetobacter spp., particularly *A. baumannii*, are important nosocomial pathogens, especially in intensive care and burns units,[1] where they are frequent causes of ventilator-associated pneumonia and of bacteraemias.[2] They are often multiresistant to antibiotics, meaning that therapy and infection control are complicated. Carbapenems have retained anti-*Acinetobacter* activity better than most other antimicrobial classes, but carbapenemases belonging to β-lactamase classes B and D have begun to emerge in the genus, often in isolates already resistant to all other therapeutic antibiotics. Although carbapenemases are not always associated with high levels of phenotypic resistance in *Acinetobacter* spp.,[3] major outbreaks of resistant carbapenemase producers have occurred in a few centres worldwide,[4–6] and the International Network for the Study and Prevention of Emerging Antimicrobial Resistance (INSPEAR) has defined the emergence of carbapenem resistance in *Acinetobacter* as a 'global sentinel event'.[7]

To assess the prevalence of antibiotic resistance in the UK, we examined 595 *Acinetobacter* spp. isolated during 2000 from routine clinical specimens at 54 sentinel laboratories. Each isolate was tested with a panel of 14 estab-

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lished antimicrobial agents, including representatives of all the major antibiotic classes. There is a paucity of new drugs active against *Acinetobacter*—or any other Gram-negative pathogens—but tigecycline (glycylcycline GAR-936)[8] was included as a novel tetracycline known to evade both ribosomal- and efflux-mediated resistance to established analogues. Also included were the established carbapenems and colistin, which have been used against infections caused by multiresistant *Acinetobacter* strains.

Materials and methods

Study design

Fifty-four diagnostic laboratories with a good geographical spread across the UK (see Acknowledgements for a full listing) were each asked to collect up to 25 consecutive, non-replicate isolates of *Acinetobacter* from clinical specimens and to send these to the Antibiotic Resistance Monitoring & Reference Laboratory, CPHL, where susceptibility testing was performed. Species identification was confirmed centrally at Nottingham Public Health Laboratory. Data collected included the patient's age, sex, ward type, the site of isolation of organism and the susceptibility data generated by the source laboratory.

Identification

Genomic groups were assigned by amplification of the tDNA spacer regions,[9] followed by separation of products on 1.5% agarose gels.

Susceptibility testing

MICs for each isolate were determined on IsoSensitest agar with an inoculum of 10^4 to 10^5 cfu. End-points were read after overnight incubation at 37°C. Antimicrobial agents and ranges tested were: amikacin (0.125–64 mg/L); cefotaxime (0.25–32 mg/L); ceftazidime (0.25–32 mg/L); ciprofloxacin (0.064–8 mg/L); colistin (0.064–32 mg/L); gentamicin (0.25–32 mg/L); imipenem (0.032–32 mg/L); meropenem (0.032–32 mg/L); minocycline (0.125–8 mg/L); piperacillin (2–64 mg/L); piperacillin/tazobactam (2–64 mg/L with tazobactam at a fixed concentration of 4 mg/L); rifampicin (2–64 mg/L); sulbactam (1–32 mg/L); tetracycline (0.5–32 mg/L); tigecycline (formerly GAR-936, 0.032–32 mg/L). All powders were obtained from the Sigma Chemical Company (Poole, Dorset, UK), except ceftazidime (GlaxoSmithKline, Uxbridge, UK), colistin (Alpharma, Copenhagen, Denmark), imipenem (Merck, Hoddesdon, UK), meropenem (AstraZeneca, Macclesfield, UK), sulbactam (Pfizer, Sandwich, UK), tazobactam (Wyeth, Taplow, UK) and tigecycline (Wyeth-Ayerst, St Davids, PA, USA). Confirmatory tests, where needed, were performed using Etest strips (Cambridge Diagnostics, Cambridge, UK).

Susceptibilities were interpreted, where possible, using the breakpoints recommended for *Acinetobacter* spp. by the BSAC Working Party.[10,11] Exceptions were sulbactam, with resistance defined as MIC $\geqslant$ 16 mg/L;[12] and tigecycline, with resistance provisionally defined by an MIC $\geqslant$ 8 mg/L and susceptibility by an MIC $\leqslant$ 2 mg/L (R. Testa, Wyeth-Ayerst, personal communication). No breakpoint is recommended for rifampicin versus *Acinetobacter* either by the BSAC[10,11] or by the NCCLS.[13]

Investigation for carbapenemases

Carbapenem-resistant isolates were screened by PCR for alleles of the gene families encoding known acquired carbapenemases. Published primers and amplification conditions were used for bla_{IMP},[14] bla_{VIM},[15] $bla_{\text{OXA-23}}$[5] and $bla_{\text{OXA-24}}$.[5] The isolates were examined for their ability to hydrolyse 0.1 mM imipenem by spectrophotometry at 297 nm as described previously,[16] except that the crude enzymes were released from the *Acinetobacter* cells by six alternate cycles of freezing and thawing.

Data handling and statistical analyses

All data were stored and analysed using Microsoft Access and Excel, WHONET 5.1 and the Statcalc component of EpiInfo 2000. The χ^2 test was used with Yates' correction; $P < 0.05$ was used to indicate significance.

Results

General

A total of 595 of 649 isolates referred by the 54 sentinel laboratories were confirmed as *Acinetobacter* spp.; 443 of these isolates belonged to genomic groups comprising the clinically important members of the *A. baumannii* complex (genomic groups 2 = *A. baumannii*, 3 and 13TU), and 152 isolates belonged to other genomic groups, including *Acinetobacter calcoaceticus* (genomic group 1). Nineteen (35%) of the 54 participating hospitals referred 444 (75%) isolates (range 15–29), comprising 343 (77%) isolates of the *A. baumannii* complex and 101 (66%) isolates of other genomic groups. These 'major referral sites' were used to assess the geographical distribution of resistance across the UK.

Isolates of the *A. baumannii* complex and non-*A. baumannii* isolates were similarly distributed with regard to patient age or sex (not shown). A greater proportion of *A. baumannii* complex isolates were from intensive care units (ICUs): 164 isolates (37%), compared with 15 non-*A. baumannii* isolates (10%) ($P < 0.0000001$). A greater proportion of *A. baumannii* complex isolates were from sputum: 85 isolates (19%), compared with nine non-*A. baumannii* isolates (6%) ($P = 0.00018$). Non-*A. baumannii* isolates were more likely to be from blood (82 isolates, 54%) than were isolates of the *A. baumannii* complex (109 isolates, 25%) ($P < 0.0000001$). It should be noted that

many UK laboratories only identify Gram-negative bacteria to species level if they have been isolated from blood, meaning that the collection as a whole probably over-represented bloodstream isolates.

Antimicrobial susceptibilities

Susceptibility distributions are shown in Tables 1 and 2. Resistance to ciprofloxacin, gentamicin, rifampicin, sulbactam, tetracycline and, to a lesser extent, amikacin was unequivocal, with bimodal MIC distributions divided by the BSAC breakpoints, whereas resistance to other drugs was defined by the BSAC breakpoint dividing a 'tail' of resistant or susceptible organisms from a majority population with the converse phenotype.

Over 75% of the isolates were resistant to cefotaxime and ceftazidime, and over 30% to ciprofloxacin, gentamicin, piperacillin and piperacillin/tazobactam. The only established drugs active against over 90% of the isolates at the BSAC breakpoints were the two carbapenems, colistin and sulbactam. Minocycline should perhaps be added to this list: although only 82% of isolates were susceptible at the BSAC breakpoint of 0.5 mg/L, 97% were susceptible at the NCCLS breakpoint of 4 mg/L (see below).

Statistically, isolates of the *A. baumannii* complex were more often resistant than non-*A. baumannii* isolates to cephalosporins, ciprofloxacin, gentamicin, piperacillin and piperacillin/tazobactam. However, *A. baumannii* complex isolates were less often resistant to colistin. Isolates of the *A. baumannii* complex were neither more nor less often resistant than non-*A. baumannii* isolates to amikacin, carbapenems, rifampicin or sulbactam.

Antimicrobial resistance was geographically scattered across the UK; 18 of the 19 'major referral sites' submitted gentamicin-resistant isolates; 17 submitted isolates resistant to amikacin; 17 submitted isolates resistant to ciprofloxacin; four submitted isolates resistant to imipenem; and two submitted isolates resistant to meropenem. The extent of multiresistance among *Acinetobacter* spp. was investigated by analysing resistance to eight antimicrobial agents: amikacin, ciprofloxacin, colistin, gentamicin, imipenem, meropenem, minocycline and sulbactam (Table 3). Multiresistance was more frequently associated with isolates of the *A. baumannii* complex than with other genomic groups; nevertheless, 206 (47%) *A. baumannii* complex isolates and 85 (56%) other isolates were susceptible to all of the eight selected agents. Isolates with each of the most frequently observed multiresistance patterns were referred from multiple sentinel laboratories (Table 3).

Relative activity of tigecycline and established tetracycline analogues

On a weight-for-weight basis, the relative activities of the tetracyclines were minocycline > tigecycline > tetracycline. For the entire collection, the MIC_{50} and MIC_{90} of tigecycline were 0.5 and 2 mg/L, compared with 0.125 and 1 mg/L of minocycline, and 4 and >32 mg/L of tetracycline. Calculation of resistance prevalence to tetracyclines, however, presents some difficulty. The BSAC adopts a single breakpoint of 1 mg/L for most established analogues except minocycline, to which it attributes a breakpoint of 0.5 mg/L.[10] The NCCLS recommends identical criteria for all licensed tetracycline analogues, with susceptible and resistant categories defined by MIC $\leqslant$ 4 and $\geqslant$16 mg/L, respectively.[13] These differences, together with the manufacturer's provisional breakpoints of $\leqslant$2 and $\geqslant$8 mg/L of tigecycline, distort attempts to compare susceptibilities, so uninterpreted distributions are shown in Tables 1 and 2. Using BSAC criteria, 84% of isolates were resistant to tetracycline, although this fell to 30% resistant and 10% intermediate if the NCCLS breakpoints were used. It should be noted, however, that tests reported here were not performed in accordance with NCCLS recommended methodology. Similarly, 18% of total isolates were resistant to minocycline according to BSAC criteria, but none was considered resistant and just 3% were intermediate using the NCCLS breakpoints. Sixteen isolates (2.7%) from 10 centres were resistant to tigecycline (at the provisional breakpoint of MIC $\geqslant$ 8 mg/L); these all belonged to the *A. baumannii* complex. Twenty-six isolates (4.4%) showed intermediate susceptibility to tigecycline (MIC 4 mg/L); 22 belonged to the *A. baumannii* complex and four to other genomic groups. Irrespective of breakpoints, *A. baumannii* complex isolates were more often resistant to tetracyclines than non-*A. baumannii* isolates.

Resistance to carbapenems

Thirteen carbapenem-resistant isolates (MICs $\geqslant$ 8 mg/L; 2.2%) were received from six centres (Table 4); eight belonged to the *A. baumannii* complex and five to other genomic groups. Single resistant isolates were referred from four centres; one centre sent three isolates (all non-*A. baumannii*); and one sent six (five *A. baumannii* and one of another genomic group). Based on BSAC breakpoints, nine isolates were resistant to imipenem only, two to meropenem only and two to both carbapenems. The imipenem-resistant, meropenem-susceptible isolates all required raised meropenem MICs of 1 to 4 mg/L, indicating reduced susceptibility; the two meropenem-resistant, imipenem-susceptible isolates required elevated imipenem MICs (2 to 4 mg/L) as compared with typical isolates (Table 4). The carbapenem-resistant isolates were all multiresistant, but mostly remained susceptible to colistin at $\leqslant$4 mg/L (11/13 isolates), tigecycline at $\leqslant$2 mg/L (11/13), minocycline at $\leqslant$4 mg/L (13/13) and sulbactam at $\leqslant$8 mg/L (9/13). A bla_{IMP} PCR product was obtained from one meropenem-resistant, imipenem-susceptible isolate (Table 4). None of the other 12 isolates yielded products with primers for genes known to encode carbapenemases, or detectably hydrolysed imipenem in spectrophotometric assays.

Table 1. Distributions of antibiotic susceptibilities for isolates of the *A. baumannii* complex (n = 443)

	MIC (mg/L)[a]													
Antibiotic	<=0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	No. (%) resistant
Amikacin			3	12	80	104	109	40	**16**	**18**	**20**	**28**	**13**	95 (21)
Gentamicin				130	76	48	**21**	**18**	**13**	**12**	**63**	**62**		189 (43)
Cefotaxime				5	6	6	**18**	**13**	**56**	**101**	**64**	**174**		426 (96)
Ceftazidime				4	4	12	30	**61**	**128**	**74**	**50**	**80**		393 (89)
Ciprofloxacin		27	25	61	105	22	**6**	**15**	**20**	**162**				203 (46)
Colistin			7	147	222	52	3	2	**2**	**2**	**3**	**3**		10 (2)
Imipenem	31	51	125	145	52	11	9	12	**5**	**1**		**1**		7 (2)
Meropenem	20	11	27	160	131	53	21	18	**2**					2 (0.5)
Piperacillin							11	12	31	72	**86**	**65**	**166**	317 (72)
Piperacillin/ tazobactam							142	56	30	44	**46**	**54**	**71**	171 (39)
Rifampin							108	200	114	1	3	8	9	–
Sulbactam						192	143	50	6	**37**	**13**	**2**		52 (12)
Tigecycline	3	3	20	120	95	61	103	22	11	5				–
Minocycline			225	55	62	48	24	10	18					–
Tetracycline					9	48	97	76	44	29	24	116		–

[a]Bold type indicates isolates resistant to individual antibiotics according to BSAC criteria (see text). No breakpoint is available for rifampicin against *Acinetobacter* spp. No interpretation is made for tetracycline analogues owing to the incompatibility of the provisional value for tigecycline with BSAC values for minocycline and tetracycline.

Table 2. Distributions of antibiotic susceptibilities for isolates of *Acinetobacter* spp. not belonging to the *A. baumannii* complex (n = 152)

	MIC (mg/L)[a]													
Antibiotic	<=0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	No. (%) resistant
Amikacin			4	15	42	22	24	17	**10**	**10**	**7**	**1**		28 (18)
Gentamicin				84	12	24	**24**	**1**		**1**	**2**	**3**		31 (20)
Cefotaxime				4	2	15	**11**	**34**	**45**	**24**	**11**	**6**		131 (86)
Ceftazidime				5	3	10	23	**40**	**42**	**18**	**7**	**4**		111 (73)
Ciprofloxacin		28	36	35	37	2	**1**	**4**	**4**	**5**				14 (9)
Colistin		1	4	46	58	12	3	7	**3**	**4**	**6**	**8**		21 (14)
Imipenem	33	21	39	43	10	1	1		**1**	**1**		**2**		4 (3)
Meropenem	20	15	13	53	34	10	3	2	**2**					2 (1)
Piperacillin							3	10	39	38	**31**	**18**	**13**	62 (41)
Piperacillin/ tazobactam							113	10	10	11	**4**	**3**	**1**	8 (5)
Rifampin							73	57	20		1		1	–
Sulbactam						119	17	5	1	**1**	**2**	**7**		10 (7)
Tigecycline		2	17	58	44	18	9	4						–
Minocycline			93	43	7	2	4	2	1					–
Tetracycline					6	32	43	47	14	4	2	4		–

[a]Bold type indicates isolates resistant to individual antibiotics according to BSAC criteria (see text). No breakpoint is available for rifampicin against *Acinetobacter* spp. No interpretation is made for tetracycline analogues owing to the incompatibility of the provisional value for tigecycline with BSAC values for minocycline and tetracycline.

Table 3. Prevalence and distribution of common resistance and multiresistance patterns among *Acinetobacter* spp. in the UK

Resistance pattern[a]						*A. baumannii* complex (n = 443)		Other genomic groups (n = 152)	
						number of isolates	number of referring laboratories	number of isolates	number of referring laboratories
Susceptible to all eight selected agents						206	44	85	31
–	GEN	CIP	MIN	–	–	43	12	2	2
AMK	GEN	CIP	–	–	–	42	11	1	1
–	GEN	CIP	–	–	–	25	12	-	-
–	–	CIP	–	–	–	18	14	3	3
AMK	GEN	CIP	MIN	SUL	–	14	6	–	–
–	GEN	CIP	MIN	SUL	–	14	4	–	–
–	–	CIP	MIN	–	–	11	4	–	–
AMK	GEN	CIP	–	SUL	–	10	3	–	–
AMK	GEN	CIP	MIN	–	–	10	6	–	–
AMK	GEN	–	–	–	–	5	5	11	8
AMK	–	–	–	–	–	3	3	11	10
–	–	–	–	–	COL	8	5	9	7
–	GEN	–	–	–	COL	–	–	7	7
–	–	–	MIN	SUL	–	1	1	6	6
–	GEN	–	–	–	–	9	8	5	4

[a]Patterns shown were represented by ⩾10 isolates of the *A. baumannii* complex or ⩾5 isolates of other genomic groups. Drugs selected for this analysis were amikacin (AMK), gentamicin (GEN), ciprofloxacin (CIP), minocycline (MIN), sulbactam (SUL), colistin (COL), imipenem and meropenem.

Table 4. Isolation of carbapenem-resistant *Acinetobacter* spp.

Hospital	Genomic group	Imipenem MIC (mg/L)	Meropenem MIC (mg/L)	Ward type	Carbapenemase gene(s) detected[a]
A	*A. baumannii* complex	16	8	burns unit	–
A	*A. baumannii* complex	8	4	ICU	–
A	*A. baumannii* complex	8	4	burns unit	–
A	*A. baumannii* complex	8	4	burns unit	–
A	*A. baumannii* complex	8	4	burns unit	–
A	other	8	8	burns unit	–
B	other	>32	4	ICU	–
B	other	>32	1	general medical	–
B	other	16	2	general medical	–
C	*A. baumannii* complex	>32	4	ICU	–
D	*A. baumannii* complex	8	4	burns unit	–
E	*A. baumannii* complex	4	8	general medical	–
F	other	2	8	nutrition	bla_{IMP}

[a]Genes sought were: bla_{IMP}, bla_{VIM}, bla_{OXA-23} and bla_{OXA-24}.

Discussion

All of the *Acinetobacter* included in this survey were isolated from clinical specimens and were considered to be significant by the referring laboratory. The *A. baumannii* complex accounted for 75% (443/595) of those collected and we confirmed the association between isolates belonging to this complex and ICUs. Furthermore, the high isolation rate of *A. baumannii* complex isolates from sputa is consistent with their association with lower respiratory tract infections.[1,2] A small majority of the isolates belonging to other genomic groups were recovered from blood, indicating that they also may cause serious infections, albeit less frequently than isolates of the *A. baumannii* complex; however, this association with blood could also reflect the fact that non-*A. baumannii* isolates are ubiquitous members of the normal human skin flora and are therefore prone to cause contamination of blood cultures. The isolates represented a wide distribution across the UK, but 75% were referred from only 35% of the sentinel laboratories. This bias may reflect the incidence of clonal outbreaks. Molecular epidemiological typing of the isolates is ongoing to investigate this possibility, to determine the extent of any intra- and inter-hospital spread of strains, and to compare resistant and susceptible isolates from individual sentinel laboratories. These studies will be reported separately.

The BSAC Working Party on Susceptibility Testing has advocated identical interpretative breakpoints for *Acinetobacter* spp. and the Enterobacteriaceae.[11] We used these values wherever possible, although the criteria for sulbactam and tigecycline were taken from other sources (see Materials and methods). Resistance and multiresistance to many agents, including aminoglycosides, cephalosporins and ciprofloxacin, were frequent and geographically scattered, especially among *A. baumannii* isolates. Among the established agents tested, the carbapenems, colistin, minocycline and sulbactam retained greatest activity. Carbapenems are increasingly the drugs of choice against infections caused by the genus and colistin has been perceived as a drug of last resort, generally active *in vitro*, though with variable efficacy and significant toxicity *in vivo*. Uniquely, colistin retained better activity versus *A. baumannii* isolates than those of other genomic groups. The potential role of rifampicin as a synergist in the treatment of *Acinetobacter* infections is interesting.[17–19] Hogg *et al.*[17] reported synergy between colistin and rifampicin against 11/13 multiresistant *A. baumannii* isolates, even though nine isolates were categorized as resistant to rifampicin (MIC $\geqslant$ 4 mg/L). The BSAC Working Party does not recommend interpretative criteria for rifampicin versus Gram-negative bacteria other than *Neisseria* spp., for which resistance is defined as MIC $\geqslant$ 2 mg/L.[11] Similarly, *N. meningitidis* and *Haemophilus* spp. are the only Gram-negative bacteria for which the NCCLS gives interpretative criteria for rifampicin; these being MIC $\leqslant$ 1 mg/L (susceptible) and $\geqslant$4 mg/L (resistant).[13] How such values relate to the potential for rifampicin to act as an adjunct in anti-*Acinetobacter* treatment is unknown. Tentatively, 76% of isolates belonging to the *A. baumannii* complex and 52% of isolates belonging to other genomic groups were considered resistant (MICs $\geqslant$ 4 mg/L) to rifampicin.

The MIC distribution for tetracycline was clearly bimodal, whereas those for minocycline and tigecycline were unimodal with tails of organisms with resistance or reduced susceptibility. Although we did not investigate the molecular basis of resistance, these distributions are consistent with the wide distribution of the Tet(A) and Tet(B) efflux proteins in clinical isolates of *A. baumannii* reported previously.[20] Tet(A) confers resistance to tetracycline, but

not to minocycline or glycylcyclines, while Tet(B) confers resistance to tetracycline and minocycline, but not to glycylcyclines.[21] Comparison of resistance rates among the tetracyclines is, however, difficult. The BSAC breakpoints for established tetracyclines[10] are four- to eight-fold lower than those recommended by the NCCLS,[13] and only a provisional breakpoint value is available for tigecycline. If, however, the BSAC breakpoints were applied, 66% of isolates (390/595) collected in this survey were resistant to tetracycline, and a further 18% (110 isolates) were resistant to both tetracycline and minocycline. We found tigecycline (GAR-936) to be less active than minocycline, but both agents overcame most tetracycline resistance. The MIC_{50} and MIC_{90} of tigecycline determined in this study were 0.5 mg/L and 2 mg/L, agreeing with values for *Acinetobacter* spp. from a previous study.[22] Using the provisional tigecycline breakpoint of 2 mg/L for susceptibility, we identified 42 insusceptible isolates (16 with full resistance, and 26 with intermediate susceptibility). The genetic basis of this resistance will be examined further; mutations in either Tet(A) or Tet(B) potentially may lead to glycylcycline resistance.[21,23]

Although increasing concern has been expressed about the emergence of class B and D carbapenemases in *Acinetobacter* spp.,[3,7] carbapenem resistance evidently remains very rare among *Acinetobacter* in the UK, seen in only 13 (2.2%) of 595 isolates. A bla_{IMP} allele was detected in one isolate with low-level meropenem resistance (MIC 8 mg/L), but susceptibility to imipenem (MIC 2 mg/L). This represents the first confirmed isolation in the UK of a bacterium with an IMP β-lactamase. Detailed characterization of the particular allele is being presented separately.[24] Genes encoding enzymes belonging to known carbapenemase families were not detected in any of the other 12 carbapenem-resistant isolates, and extracts prepared from them did not hydrolyse imipenem. Thus, they may have carbapenemase-independent resistance, or very weak carbapenemases linked with other resistance factors such as impermeability or efflux. The isolate with the IMP enzyme was barely resistant to meropenem (MIC 8 mg/L) and apparently susceptible to imipenem (MIC 2 mg/L) confirming international results that production of even a potent metallo-carbapenemase does not necessarily confer high levels of phenotypic resistance to carbapenems in *Acinetobacter* spp. or other Gram-negative bacteria.[5]

In conclusion, carbapenems, colistin and minocycline retained greatest activity against the *Acinetobacter* isolates collected. Tigecycline was less active than minocycline, but both agents overcame most tetracycline resistance.

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