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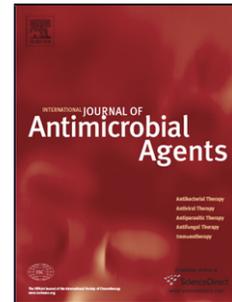
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**What remains against carbapenem-resistant Enterobacteriaceae?  
Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin,  
minocycline, nitrofurantoin, temocillin and tigecycline**

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## ABSTRACT

Carbapenem-resistant Enterobacteriaceae present an increasing and diverse problem, including strains of multiple species with metallo- $\beta$ -lactamases (IMP, NDM or VIM) and non-metallo KPC and OXA-48 enzymes as well as those combining an extended-spectrum  $\beta$ -lactamase (ESBL) or AmpC enzyme with porin loss. Most strains, except those with OXA-48 alone, are broadly resistant to  $\beta$ -lactams and have multiple aminoglycoside-modifying enzymes; those with NDM-1 carbapenemase typically also have 16S rRNA methylases, conferring complete aminoglycoside resistance. In this study, the activity of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline was evaluated against 81 carbapenem-resistant Enterobacteriaceae isolates from the UK. Testing was performed by the Clinical and Laboratory Standards Institute (CLSI) agar dilution method. Chloramphenicol, ciprofloxacin and nitrofurantoin inhibited <25% of the isolates at the breakpoint, whereas colistin was active against 75/81 isolates (92.6%), the exceptions being four *Klebsiella pneumoniae* and *Enterobacter cloacae* isolates along with members of inherently resistant genera. Fosfomycin was active against 49/81 isolates (60.5%), including 7/7 *Escherichia coli*, 16/20 *Enterobacter* and *Citrobacter* spp., but only 25/52 *Klebsiella* spp. Tigecycline was active against 38/81 isolates (46.9%) and was intermediate against another 27 (33.3%), with resistance scattered among *K. pneumoniae* and *Enterobacter* spp. The activity of colistin, fosfomycin and tigecycline was unrelated to the isolates' carbapenem resistance mechanisms. Temocillin was fully active [minimum inhibitory concentration (MIC)  $\leq$ 8 mg/L] against only 4/81 isolates (4.9%), but inhibited a further 22 isolates (27.2%) at the British Society for Antimicrobial Chemotherapy (BSAC) urinary breakpoint (32 mg/L), predominantly comprising those isolates with combinations of impermeability

and an ESBL or AmpC enzyme, along with 6/11 isolates producing KPC carbapenemases. Studies with transconjugants and transformants confirmed the small effect of KPC enzymes against temocillin, whereas OXA-48 and NDM-1 conferred clear resistance.

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## 1. Introduction

Carbapenem-resistant Enterobacteriaceae are increasingly prevalent in many parts of the world. They are diverse, variously producing metallo-carbapenemases (mostly NDM [1] or VIM [2] enzymes) or non-metallo-types (principally KPC [3] or OXA-48 [4]). Most carbapenemase-producers are almost completely resistant to  $\beta$ -lactam antibiotics except that: (i) those with OXA-48 alone remain susceptible to several oxyimino-cephalosporins [5]; and (ii) those with metallo-carbapenemases alone, lacking AmpC or extended-spectrum  $\beta$ -lactamases (ESBLs), remain susceptible to aztreonam [6]. Aminoglycoside resistance is extensive but more variable: strains with NDM-1 enzyme mostly co-produce an ArmA or RmtC 16S rRNA methylase, conferring broad resistance, whereas those with other carbapenemases have variable arrays of modifying enzymes, with isepamicin often spared and with gentamicin retaining activity against the internationally widespread KPC<sup>+</sup> sequence type (ST) 258 *Klebsiella pneumoniae* strain [7].

Here we present susceptibility data for chloramphenicol, ciprofloxacin, colistin, fosfomicin, nitrofurantoin, minocycline and tigecycline against carbapenemase-producers collected in the UK, selected as non- $\beta$ -lactam and non-aminoglycoside drugs. The  $\beta$ -lactam temocillin was also included as it has been suggested by others to have specific activity against strains with KPC carbapenemases [8].

## 2. Materials and methods

### 2.1. Bacteria

The strain set has been described previously [6]. It represents a diversity of carbapenem resistance types and host species, including organisms with carbapenemases and with combinations of ESBL or AmpC and impermeability, but does not comprise consecutive producer isolates, nor was it matched to the (ever-changing) relative prevalence of different resistance types. Transformants and transconjugants of *Escherichia coli* DH5 $\alpha$  and J62-1 with carbapenemase-encoding plasmids were prepared as described previously [7].

### 2.2. Antibiotics

Chloramphenicol, ciprofloxacin, colistin, disodium fosfomicin, minocycline and nitrofurantoin were all from Sigma (Poole, UK), temocillin was from Eumedica (Brussels, Belgium) and tigecycline was from Pfizer (Sandwich, UK).

### 2.3. Susceptibility testing

Antibiotics were tested by the Clinical and Laboratory Standards (CLSI) agar dilution method [9] using Mueller–Hinton agar from Oxoid/Thermo Fisher (Basingstoke, UK); glucose-6-phosphate (25 mg/L) was added for tests with fosfomicin.

## 3. Results and discussion

Minimum inhibitory concentration (MIC) distributions are shown in relation to carbapenem resistance mechanism in Table 1 and to host species in Table 2. Table

2 omits temocillin since, uniquely among the compounds tested, it is a  $\beta$ -lactam directly affected by some of the carbapenemases; it is therefore implausible that species would be the major determinant of its activity. Table 3 shows the MICs of temocillin for *E. coli* DH5 $\alpha$  transformants and J62-1 transconjugants with acquired carbapenemase genes.

Colistin had the most consistent activity among the compounds tested, with MICs generally  $\leq 1$  mg/L, although resistance was seen in 3/52 *Klebsiella* spp., comprising single isolates with KPC or OXA-48 enzymes and one with a combination of porin loss and an ESBL, as well as in a single *Enterobacter cloacae* with an NDM carbapenemase. Inherent colistin resistance was also seen in the single *Morganella morganii* (with NDM-1 enzyme) and *Serratia marcescens* (with SME-1 enzyme) isolates included.

The other two compounds with frequent activity were tigecycline and fosfomycin. Both had consistent activity at European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints against all seven carbapenem-resistant *E. coli* (6 of which had NDM-1 enzyme) but were more variable against the larger numbers of carbapenem-resistant *Enterobacter* and, particularly, *Klebsiella* isolates, with MICs scattered either side of the breakpoint. Among 20 isolates of *Enterobacter* spp. and *Citrobacter freundii*, 10 were susceptible to tigecycline (MIC  $\leq 1$  mg/L), five were intermediate (MIC = 2 mg/L) and five were resistant (MIC > 2 mg/L); corresponding numbers among 52 *Klebsiella* spp. were 21 susceptible, 22 intermediate and 9 resistant. In the case of fosfomycin, 16/20 carbapenem-resistant *Enterobacter* spp. and *C. freundii* were susceptible at the EUCAST/British Society for Antimicrobial

Chemotherapy (BSAC) systemic and urinary breakpoint of 32 mg/L, whereas four were resistant; corresponding proportions for *Klebsiella* spp. were 25/52 susceptible and 27/52 resistant. The ten *K. pneumoniae* with the highest level of fosfomicin resistance (MIC  $\geq$  256 mg/L) were a mixed group; five had combinations of an ESBL and impermeability, three had IMP enzymes, and single isolates had KPC and VIM carbapenemases.

Chloramphenicol, ciprofloxacin (the quinolone with greatest inherent activity against Enterobacteriaceae) and nitrofurantoin had activity against only 15–25% of the isolates, again with little relationship to species or carbapenem resistance mechanism; minocycline was much less active than tigecycline, with 60/81 isolate MICs  $\geq$  8 mg/L. Temocillin also had limited activity, with only 4/81 isolates susceptible at the BSAC systemic breakpoint of susceptible  $\leq$ 8 mg/L and resistant  $>$ 8 mg/L; however, a total of 26/81 were susceptible at the BSAC urinary breakpoint of  $\leq$ 32/ $>$ 32 mg/L. These included all of the organisms with carbapenem resistance contingent on combinations of porin loss with an AmpC or ESBL enzyme and 6/11 with KPC carbapenemases. By contrast, 18/19 isolates with OXA-48 enzyme and 32/35 with a metallo-carbapenemases (i.e. IMP, NDM and VIM types) were resistant to temocillin even at the BSAC urinary breakpoint. MIC determinations with *E. coli* constructs confirmed that acquisition of a KPC carbapenemase caused only a one dilution rise in the temocillin MIC, whereas NDM (metallo-) and OXA-48 enzymes had a greater effect (Table 3).

The present results, along with previously published data for aminoglycosides [7], underscore the considerable resistance of many carbapenemase-producers.

Although colistin, fosfomycin and tigecycline were active or intermediately active against more than one-half of the study population, all have significant limitations. Colistin is increasingly used but has nephrotoxicity and uncertain efficacy in pulmonary infections, although it appears more consistently effective in other infection types [10,11]. Moreover, in some cases, colistin resistance is selected during therapy, and this may apply for the resistant *E. cloacae* with NDM-1 enzyme in the present study, which was from a child who had already received colistin for *Acinetobacter* and *Pseudomonas* infections [12]. Furthermore, colistin-resistant variants of the international KPC<sup>+</sup> ST258 *K. pneumoniae* strain are circulating in Greece and Hungary [13,14], with a few cases also seen in the UK. In the case of tigecycline, there are positive case reports of its use in infections due to *K. pneumoniae* with VIM and KPC carbapenemases in Greece [15], but also of resistance emerging during therapy, albeit in an off-label setting (nosocomial pneumonia) [16]. Moreover, it is licensed for use only in intra-abdominal and complicated skin and skin-structure infections, and proved inferior to imipenem/cilastatin in ventilator-associated pneumonia, providing a caution against off-label use in this infection [17]. Tigecycline is also probably not appropriate in urinary infections owing to largely biliary excretion and low urinary levels. Fosfomycin, by contrast, may be highly appropriate for urinary infections, where it has a good reputation for efficacy [18]. On the other hand: (i) fosfomycin is not readily available in many countries, or is available only as an oral formulation for uncomplicated urinary infections; and (ii) a question mark hangs over its use as monotherapy for severe infections at other body sites in view of the borderline susceptibility of many *Klebsiella* spp. and the potential for resistance to emerge by mutation, although this may be less frequent in vivo than in vitro [18].

Temocillin only showed potential when reviewed against urinary breakpoints and, on these criteria, had activity against all of the isolates with carbapenem resistance contingent on combinations of ESBL or AmpC and impermeability, as well as one-half those with KPC enzymes. The former activity is surprising since temocillin carries a double negative charge, which would be expected to impede uptake [19]; nevertheless, the behaviour was consistent for all porin-deficient strains. The limited effect of KPC enzymes, as confirmed with transconjugant studies (Table 3), is in keeping with the findings of Adams-Haduch et al. [8]. Although the maximum licensed dosage of 2 g intravenous twice daily can only justify a systemic breakpoint of 8 mg/L, a 2 g thrice daily regimen is under evaluation and may justify a higher breakpoint, as may 6 g daily by continuous infusion [20]. Moreover, temocillin is a close analogue of ticarcillin, which is used at dosages of up to 18 g/day, and we would urge that safety evaluations of temocillin at such dosages as a preliminary to clinical evaluation against systemic infections due to bacteria with KPC carbapenemases.

Three final points should be made. First, these results provide only a wide-angle snapshot. Carbapenem-resistant isolates from individual cases and outbreaks may be different from those examined here. For example, most ST258 *K. pneumoniae* with KPC carbapenemases are susceptible to gentamicin, tigecycline and colistin but, as already noted, some variants are resistant even to colistin [13,14], whereas non-ST258 *K. pneumoniae* isolates presently circulating in north-west England, and not included in the present study, often are susceptible to multiple aminoglycosides and fluoroquinolones (Health Protection Agency, data on file). Second, the present results

suggest formulary-based interventions, e.g. preferential use of tigecycline in mild-to-moderate intra-abdominal infections and of fosfomicin (or temocillin if KPC enzymes are the concern) for urinary infections, by which a hospital might mitigate selection pressure for carbapenemase-producers. Such strategies deserve evaluation, both for their efficacy and ecological consequences. Last, for severe infections it may be appropriate to combine the few agents that remain active to maximise the chance of efficacy and to minimise selection of further resistance.

### **Funding**

The Health Protection Agency (HPA) received funding from Eumedica to evaluate temocillin against carbapenemase-producers and, in another context, has a research contract with Meiji, who market fosfomicin in Japan.

**Competing interests:** The HPA received funding from Eumedica to evaluate temocillin against carbapenemase-producers and, in another context, has a research contract with Meiji, who market fosfomicin in Japan. DML has accepted sponsorship to attend conferences and to speak for Pfizer and Eumedica; and holds shares in AstraZeneca, Merck, Pfizer and Dechra, amounting to <10% of all portfolio holdings.

### **Ethical approval**

Not required.

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**Table 1**

Minimum inhibitory concentrations (MICs) of antibiotics in relation to carbapenemase type <sup>a</sup>

Antibiotic/carbapenemase	No. isolates with indicated MIC (mg/L):												
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
<b>Chloramphenicol</b>													
IMP								3	2		4		4
NDM							2	1	3	1	1	1	8
VIM							1			1		3	
KPC								1	1	2	4		3
SME-1											1		
OXA-48							5	3				4	7
Impermeability + ESBL							2	1		2	1		3
Impermeability + AmpC								2	3	1			
<b>Ciprofloxacin</b>													
IMP	2		1	1	1	1	1	3			3	1	
NDM	1 <sup>b</sup>								2	1	1	4	4
VIM					2					1	1		1
KPC										2	3	5	1
SME-1	1 <sup>b</sup>												
OXA-48	2 <sup>b</sup>	1		1			7	1		1	1	2	3
Impermeability + ESBL							1	1	1	3	3		



NDM			3	3	4	7
VIM				1	1	3
KPC						11
SME-1						1
OXA-48					3	16
Impermeability + ESBL						9
Impermeability + AmpC				1	2	3
Temocillin						
IMP				1	6	5
NDM	1	1			4	1
VIM						1
KPC			2	4	4	1
SME-1			1			
OXA-48			1			18
Impermeability + ESBL			1	1	7	
Impermeability + AmpC			5	1		
Minocycline						
IMP			2	6	3	1
NDM	1	3	1	5	3	2
VIM			1	3	1	
KPC				6	1	1
						3 <sup>c</sup>

SME-1						1	
OXA-48		2	3	8	2	3	1 <sup>c</sup>
Impermeability + ESBL			4	2	2	1	
Impermeability + AmpC			4		1		1 <sup>c</sup>
Tigecycline							
IMP		1	4	4	4		
NDM	1 <sup>b</sup>	5	4	3	3	1	
VIM			3	1	1		
KPC			2	6	2	1	
SME-1					1		
OXA-48		3	6	9	1		
Impermeability + ESBL		1	3	3	2		
Impermeability + AmpC			5	1			

ESBL, extended-spectrum  $\beta$ -lactamase.

<sup>a</sup> Unshaded indicates susceptible, dark shading indicates resistant and light shading indicates intermediate (tigecycline) or susceptible at British Society for Antimicrobial Chemotherapy (BSAC) urinary breakpoint (temocillin).

<sup>b</sup> MIC  $\leq$  indicated value.

<sup>c</sup> MIC  $\geq$  indicated value.

**Table 2**

Minimum inhibitory concentrations (MICs) of antibiotics in relation to bacterial species <sup>a,b</sup>

Antibiotic/species	No. isolates with indicated MIC (mg/L):												
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
<b>Chloramphenicol</b>													
<i>Klebsiella</i> spp. <sup>c</sup>							9	7	1	4	8	7	16
<i>Enterobacter</i> spp./ <i>Citrobacter freundii</i> <sup>d</sup>							1	3	6	3	2	5 <sup>g</sup>	
<i>Escherichia coli</i> <sup>e</sup>								1	1			1	4
<b>Ciprofloxacin</b>													
<i>Klebsiella</i> spp.	5 <sup>f</sup>	1		2	2		8	5	1	1	6	7	7
<i>Enterobacter</i> spp./ <i>C. freundii</i>	4 <sup>f</sup>		1		1		1	1	1	2	5	1	2
<i>E. coli</i>								1			4	1	1
<b>Colistin</b>													
<i>Klebsiella</i> spp.				36 <sup>f</sup>	13				1	2			
<i>Enterobacter</i> spp./ <i>C. freundii</i>				16 <sup>f</sup>	3			1					
<i>E. coli</i>				6 <sup>f</sup>	1								
<b>Fosfomicin</b>													
<i>Klebsiella</i> spp.							2 <sup>f</sup>	2	7	5	9	10	7
<i>Enterobacter</i> spp./ <i>C. freundii</i>							2 <sup>f</sup>	3	3	4	4	3	1
<i>E. coli</i>							5 <sup>f</sup>	1	1				
<b>Nitrofurantoin</b>													

<i>Klebsiella</i> spp.			1 <sup>f</sup>	2	1		9	39 <sup>g</sup>
<i>Enterobacter</i> spp./ <i>C. freundii</i>				1		4	4	11 <sup>g</sup>
<i>E. coli</i>				2		1	2	2 <sup>g</sup>
Minocycline								
<i>Klebsiella</i> spp.			3	10	22	10	6	1 <sup>g</sup>
<i>Enterobacter</i> spp./ <i>C. freundii</i>			1	5	5	3	1	5 <sup>g</sup>
<i>E. coli</i>			1	1		3		1 <sup>g</sup>
Tigecycline								
<i>Klebsiella</i> spp.		6	15	22	9			
<i>Enterobacter</i> spp./ <i>C. freundii</i>		1	9	5	3	2		
<i>E. coli</i>	1	3	3					

<sup>a</sup> Excludes single isolates of (i) *Morganella morganii* with NDM-1 enzyme: MICs, chloramphenicol 16 mg/L, ciprofloxacin 128 mg/L, colistin >32 mg/L, fosfomycin 512 mg/L, nitrofurantoin 64 mg/L, temocillin 8 mg/L, minocycline >32 mg/L and tigecycline 4 mg/L; and (ii) *Serratia marcescens* with SME-1 enzyme: MICs, chloramphenicol 64 mg/L, ciprofloxacin <0.03 mg/L, colistin >32 mg/L, fosfomycin 16 mg/L, nitrofurantoin 256 mg/L, temocillin 8 mg/L, minocycline 8 mg/L and tigecycline 4 mg/L.

<sup>b</sup> Unshaded indicates susceptible, dark shading indicates resistant and light shading indicates intermediate.

<sup>c</sup> Comprising 7 with IMP, 7 with KPC, 6 with NDM-1, 19 with OXA-48 and 4 with VIM carbapenemases plus 9 with combinations of an ESBL and impermeability.

<sup>d</sup> Comprising five with IMP, four with KPC, four with NDM-1 and one with a VIM carbapenemase plus six with combinations of AmpC and impermeability.

<sup>e</sup> Comprising one with an IMP carbapenemase and six with NDM-1.

<sup>f</sup> MIC  $\leq$  indicated value.

<sup>g</sup> MIC  $\geq$  indicated value.

**Table 3**

Minimum inhibitory concentrations (MICs) of temocillin for *Escherichia coli* transconjugants and transformants with acquired carbapenemases

Host and enzyme	MIC (mg/L)
DH5 $\alpha$	8
DH5 $\alpha$ NDM	64
DH5 $\alpha$ OXA-48	>128
J62-1	8
J62-1 KPC-3	16

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