membrane impermeability, derive mainly from wild-type cephalosporinases by structural alterations in the R2 binding site that accommodates the R2 lateral side chain of β-lactams. Taken together, these findings suggest that acquired resistance to avibactam and extension of the hydrolysis spectrum of class C β-lactamases might result from the same structural changes. It prompted us to investigate the inhibitory activity of avibactam against extended-spectrum AmpC β-lactamases containing structural changes in the R2 binding site.

Six isogenic Escherichia coli recombinant clones—E. coli TOP10 pEC14, E. coli TOP10 pEC16, E. coli TOP10 pEC17, E. coli TOP10 pEC18, E. coli TOP10 pMEV and E. coli TOP10 pBER—that have been characterized in previous work—were tested in this study. They expressed extended-spectrum AmpC enzymes with representative structural alterations in the R2 binding site, such as the Val-298→Leu and His-296→Pro replacements, the Ser-287→Asn and Ser-287→Cys substitutions, the Ser-282 duplication and the tandem duplication of the alanine residues at positions 294 and 295. All these modifications occurred in the R2 loop or the H-9 and H-10 helices that are secondary structures surrounding the R2 binding site.

The MICs of ceftazidime, cefermpe and imipenem were determined using an agar dilution technique on Mueller–Hinton agar (Sanofi Diagnostics Pasteur, Paris, France) plates and Mueller–Hinton agar supplemented with 4 mg/L avibactam with an inoculum of 10⁶ cfu per spot, and were interpreted according to the guidelines of the CLSI. Results are shown in Table 1. Avibactam restored the susceptibility to ceftazidime and cefermpe of all recombinant clones, thus confirming its potency against these variant enzymes. These results are in agreement with the crystallographic study carried out by Lahiri et al., which showed the weak interaction between avibactam and the R2 binding site of the AmpC β-lactamase of Pseudomonas aeruginosa. According to that study, the carbonyl amide and sulphate groups of avibactam, and the carbonyl oxygen of the acylated compound, did not interact with the R2 binding site of the AmpC enzyme. By contrast, the hydrophobic side chain of the Ala-293 residue, which is located in the R2 loop, interacted with the piperidine ring of the inhibitor.

In conclusion, the present study demonstrates that avibactam, which is a novel covalent non-β-lactam β-lactamase inhibitor, remains active against extended-spectrum AmpC β-lactamases, suggesting that this molecule could be used in clinical practice with limited risk of selecting AmpC variants.

Acknowledgements
This study was presented in part at the Thirty-third Interdisciplinary Meeting of Anti-Infectious Chemotherapy, Paris, France, 2013 (Abstract 334).

We thank AstraZeneca Pharmaceuticals (Waltham, MA, USA) for providing avibactam. N. P.-O. has a predoctoral fellowship from Instituto de Salud Carlos III, Ministry of Economy and Competitivity of Spain.

Funding
This study was supported by internal funding.

Transparency declarations
None to declare.

References


In vitro activity of ceftaroline against Burkholderia pseudomallei

Samuel Maloney1,2, Cathy Engler2 and Robert Norton1,2*

1The Townsville Hospital, Townsville, Queensland 4814, Australia; 2Microbiology, Pathology Queensland, Townsville, Queensland 4814, Australia

*Corresponding author. Tel: +61-(07)-44331111; Fax: +61-(07)-44332415; E-mail: robert.norton@health.qld.gov.au

Keywords: MIC50s, MIC90s, epidemiological cut-off values

In conclusion, the present study demonstrates that avibactam, which is a novel covalent non-β-lactam β-lactamase inhibitor, remains active against extended-spectrum AmpC β-lactamases, suggesting that this molecule could be used in clinical practice with limited risk of selecting AmpC variants.
Sir,

We conducted a preliminary investigation into the in vitro susceptibility of *Burkholderia pseudomallei* to ceftaroline. In Northern Australia and similar regions, both melioidosis and methicillin-resistant *Staphylococcus aureus* (MRSA) are common causes of community-acquired pneumonia and sepsis. The clinical presentations of these infections are indistinguishable. This has led to first-line empirical treatment of life-threatening community-acquired infections in this region with a combination of ceftazidime/meropenem and vancomycin. Previous investigations have looked at the comparative activity of several agents to identify a potential alternative regimen.

Cefaroline is a relatively new fourth-generation cephalosporin with activity against MRSA that retains a broad Gram-negative spectrum. However, its activity against *B. pseudomallei*, the causative agent of melioidosis, is unknown. Cefaroline represents a promising potential alternative to the two-agent empirical regimen currently employed, and we undertook a preliminary investigation into its potential usefulness in this area.

Specialized M.I.C.Evaluator (M.I.C.E.) strips, with doubling MIC graduations between 0.002 and 32 mg/L, were obtained from Thermo Fisher (Thermo Fisher Scientific Australia Pty Ltd, Thebarton, South Australia, Australia). Thirty-nine randomly selected clinical strains from a 17 year local collection of sporadic cases of melioidosis (previously serologically and biochemically characterized and stored on latex beads at −70°C) were thawed and inoculated onto horse blood agar (HBA) plates (bioMérieux, Baulkham Hills, NSW, Australia). These plates were incubated aerobically at 36°C for 18 h before single colonies of the isolates were subcultured onto another HBA plate and incubated for a further 24 h. These plates were inspected visually for purity and morphology, an oxidase reaction was performed and ID was checked using a Remel RapID kit (Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK).

M.I.C.E. strips were set up in accordance with the manufacturer’s instructions. Briefly, normal saline was inoculated with individual colonies to give a turbidity equivalent to that of a 0.5 McFarland standard. Mueller–Hinton (MH) agar plates (bioMérieux) were inoculated using sterile cotton swabs immersed in the inoculated saline using a three-directional streaking pattern. The M.I.C.E. strips were placed on the MH plates using forceps and the plates were then incubated for 16–18 h at 36°C in an aerobic atmosphere.

All isolates revived from storage were pure, had a typical morphology, were oxidase positive and were identified as *B. pseudomallei* using Remel RapID NF. Laboratory Gram-negative quality-control organisms had MICs within the reference range for the M.I.C.E. batch. Thirty-nine isolates had their ceftaroline MIC determined (see Figure 1).

An MIC<sub>50</sub> of 8 mg/L and an MIC<sub>90</sub> of 32 mg/L were determined. A histogram was produced; however, no epidemiological cut-off value could be suggested as four of the isolates had an MIC above the maximum level tested. For the same reason, a meaningful IQR could not be calculated.

Our preliminary investigation suggests that ceftaroline would not be an appropriate single-agent replacement for empirical vancomycin plus ceftazidime/meropenem for serious infections during the wet season in Northern Australia and similar regions. The MIC<sub>50</sub>, MIC<sub>90</sub> and minimum MIC are all higher than the EUCAST pharmacokinetic/pharmacodynamic breakpoint of 0.5 mg/L for ceftaroline. With normal dosing, the peak concentration (C<sub>peak</sub>) of ceftaroline is 18.96–21.02 mg/L. Using our susceptibility figures this would give a C<sub>peak</sub>/MIC<sub>90</sub> of 0.5925 and make very unlikely the clinical achievement of 100% T > 4–5 × MIC.

**Funding**

This study was carried out as part of our routine work. AstraZeneca provided the funding for the strips that were obtained from Thermo Fisher. All other laboratory disposables and equipment were provided by Pathology Queensland.

**Transparency declarations**

None to declare.

**References**


Synergistic activity and effectiveness of a double-carbapenem regimen in pandrug-resistant Klebsiella pneumoniae bloodstream infections

Alessandra Oliva†, Alessandra D’Abramo†, Claudia D’Agostino†, Marco Iannetta†, Maria T. Mascellino†, Carmela Gallinelli†, Claudio M. Mastroianni†,2* and Vincenzo Vullo†

1Dipartimento di Sanità Pubblica e Malattie Infettive, Sapienza Università, Piazzale Aldo Moro, 00185 Rome, Italy; 2UOC Malattie Infettive, Fondazione Eleonora Lorillard Spencer Cenci, Sapienza Università, Corsa della Repubblica, 04100 Latina, Italy

†Corresponding author. Tel: +39-0649970313-0773653745; Fax: +39-0649972625; E-mail: claudio.mastroianni@uniroma1.it
†Alessandra Oliva and Alessandra D’Abramo equally contributed to the manuscript.

Keywords: meropenem, ertapenem, KPC, bacteraemia, nosocomial infections

Sir,

Infections due to carbapenemase-producing Klebsiella pneumoniae (CP-Kp) are associated with a high mortality rate.1,2 Therapeutic options are limited, especially when associated with colistin resistance.3 In this setting, a double-carbapenem regimen has been shown to be effective and safe.3,5

Herein, we evaluated through antibiotic kill studies the in vitro synergistic activity of meropenem plus ertapenem against pandrug-resistant CP-Kp isolated from three patients with bacteraemia who were successfully treated with double-carbapenem therapy.

For each patient, informed consent to participate in the study was obtained.

Case 1

An adult patient underwent aortic endoprosthesis placement. One year later, he developed periprosthetic infection that was treated with antimicrobial therapy and prosthesis replacement.

During hospitalization, the patient had fever (39°C) and three blood cultures were positive for CP-Kp resistant to colistin (ertapenem, meropenem and colistin MICs of 128, 256 and ≥16 mg/L, respectively). Therapy with 3 g of fosfomycin every 6 h and 50 mg of tigecycline every 12 h was started without response. Therefore, 2 g of meropenem every 8 h plus 1 g of ertapenem every 24 h was given for 21 days with complete recovery.

Case 2

An adult patient underwent aorto-bisiliac graft placement. Four years later, the patient was hospitalized for revascularization of the left lower limb and after 2 days he developed fever (38.5°C). Three blood cultures were positive for CP-Kp resistant to colistin (ertapenem, meropenem and colistin MICs of 256, 256 and ≥16 mg/L, respectively). Meropenem (1 g) every 12 h (adjusted for creatinine clearance) plus 6 mg/kg daptomycin every 24 h was given without any clinical response. Subsequently, therapy was changed to 500 mg of ertapenem every 24 h and 1 g of meropenem every 12 h according to creatinine clearance. The patient became afebrile after 48 h of treatment and blood cultures were sterile. However, he died 2 days later due to acute heart failure.

Case 3

An adult patient was hospitalized because of arterial embolization due to renal haematoma. One day later, the patient had fever (39°C) and 4.5 g of piperacillin/tazobactam every 8 h was started with no clinical response. Blood cultures were positive for CP-Kp resistant to colistin (ertapenem, meropenem and colistin MICs of 256, 128 and ≥16 mg/L, respectively). Meropenem (1 g) every 8 h plus 50 mg of tigecycline every 12 h was given with partial response. Therapy was changed to 2 g of meropenem every 8 h plus 1 g of ertapenem every 24 h and administered for 24 days with complete recovery.

Phenotypic analyses showed that the three isolates were all KPC producers, in agreement with a previous report indicating that the circulating strain in our hospital is KPC-3.6,7 The activity of meropenem, alone and plus ertapenem, was investigated by time–kill studies using an initial inoculum of ~5 × 10⁸ cfu/mL for all isolates. At 2, 4, 6, 8 and 24 h timepoints, the number of cfu was determined. Bactericidal activity was defined as ≥99.9% reduction of the initial bacterial count at each timepoint. Synergy was defined as a >100-fold decrease in cfu/mL between the combination and its most active constituent after 24 h.

The time–kill analysis showed that ertapenem or meropenem alone exhibited an initial reduction in log cfu/mL followed by a significant regrowth at 24 h in all the patients (Figure 1). When the double-carbapenem combination was assessed, a bactericidal and synergistic activity was achieved at 4, 6 and 8 h and maintained at 24 h at concentrations of meropenem 0.5× MIC plus ertapenem 1× MIC, meropenem 1× MIC plus ertapenem 1× MIC and meropenem 2× MIC plus ertapenem 1× MIC in all the patients (Figure 1).

In the setting of CP-Kp infections, the role of double-carbapenem regimens containing ertapenem has been recently reviewed.6,9 In fact, ertapenem, whose activity is greatly affected...