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First report of OXA-23 carbapenemase in clinical isolates of *Acinetobacter* species in the Irish Republic

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Sir,

Meropenem and imipenem are carbapenems that remain active against organisms carrying most Ambler classes A and C β -lactamases which include many Gram-negative bacilli, including *Acinetobacter* spp. However, carbapenem resistance is increasingly encountered in *Acinetobacter* isolates worldwide.¹ Mechanisms of carbapenem resistance include the loss of porins, increase in efflux activity and the production of Ambler class B metallo- β -lactamases such as VIM and IMP enzymes. Another mechanism is the production of Ambler class D oxacillinas (OXA enzymes) with carbapenem-hydrolysing activity, such as OXA-23 and OXA-51 carbapenemases. A multidrug-resistant OXA-23 carbapenemase-producing *Acinetobacter baumannii* clone has spread rapidly among UK hospitals in recent years.² We report two *Acinetobacter* isolates producing OXA-23 enzyme in the Irish Republic.

Two meropenem-resistant *Acinetobacter* isolates were encountered in our hospital in 2005. The first isolate (05/29540) was isolated from a biliary drain fluid specimen of a 62-year-old patient who underwent a laparotomy for gall bladder perforation and associated intra-abdominal sepsis. She had underlying pancreatic carcinoma and diabetes mellitus. Recent antimicrobial therapy included piperacillin/tazobactam, ciprofloxacin and vancomycin. The patient did not receive a carbapenem prior to the isolation of the organism. The second isolate (05/12659) was cultured from a sputum specimen of a 49-year-old patient with a history of complicated inflammatory bowel disease associated with recent intensive care (ICU) admission for intra-abdominal sepsis and methicillin-resistant *Staphylococcus aureus* vascular catheter-related bloodstream infection. Recent antimicrobial treatment included meropenem, ciprofloxacin, vancomycin and caspofungin. He was also on immunosuppressants, methotrexate and infliximab, and was neutropenic (neutrophil count of $<0.1 \times 10^9/L$) at the time of the organism's isolation. The two cases were epidemiologically unrelated in time or space.

Both isolates were presumptively identified as *A. baumannii* using the Vitek-2 automated identification and susceptibility test system (bioMerieux, Basingstoke, UK), but amplified ribosomal DNA restriction analysis (ARDRA) subsequently confirmed both

isolates to be *Acinetobacter* genomic species 3. Both isolates demonstrated resistance to β -lactams including meropenem (MIC ≥ 16 mg/L) and to quinolones. 05/29540 was also resistant to gentamicin and co-trimoxazole but susceptible to tobramycin and amikacin. 05/12659 was susceptible to gentamicin, tobramycin, amikacin and co-trimoxazole. Antimicrobial susceptibility results with agar dilution and Etest (AB Biodisk, Solna, Sweden) methods using CLSI guidelines are shown in Table 1.

Using Etest metallo- β -lactamase (MBL) strips (imipenem MIC: imipenem + EDTA MIC) (AB Biodisk), ratios of ≥ 24 and ≥ 12 were obtained for 05/29540 and 05/12659, respectively, suggesting the presence of MBL activity. However, PCR using VIM and IMP primers did not reveal the presence of relevant amplicons on agarose gel electrophoresis. Isoelectric focusing revealed a β -lactamase with a pI value of 6.7 for both isolates as well as the OXA-23 positive control, suggesting the presence of OXA-23 carbapenemase activity. PCR using OXA-23-like primers revealed a single amplicon in the region between 800 and 900 bp in size for both isolates and the OXA-23 positive control.³ Nucleotide sequencing of the amplicons demonstrated $>99.5\%$ and 100% homology with the *bla*_{OXA-23} carbapenemase gene (GenBank database accession number AJ132105), from bp 24 to bp 822 for 05/29540 and from bp 31 to bp 822 for 05/12659.³ Nucleotide sequences in the regions of variation between *bla*_{OXA-23}, *bla*_{OXA-27} and *bla*_{OXA-49} are all consistent with *bla*_{OXA-23} for both isolates. PCR with OXA-51-like primers did not reveal the presence of a *bla*_{OXA-51}-like carbapenemase gene in either isolate,⁴ while amplicons of the expected size were obtained with the positive control as well as the *A. baumannii* ATCC 19606 strain. PCR also did not detect the presence of class 1 integrons in both isolates.² DNA macro-restriction followed by PFGE revealed the two isolates' profiles to be distinct from one another, suggesting that they are not clonally related.

OXA carbapenemases are increasingly encountered worldwide, especially in the nosocomial setting. To our knowledge, these are the first reported isolates of OXA-23 carbapenemases in *Acinetobacter* spp. in the Irish Republic. They were associated with risk factors for antimicrobial resistance such as prior antibiotic treatment, ICU admission, immunosuppression and severe underlying diseases. *Acinetobacter* genomic species 3, like *A. baumannii*, has been associated with nosocomial cross-infection and is the predominant *Acinetobacter* sp. in some institutions.⁵ In view of their roles in nosocomial outbreaks, accurate speciation of *Acinetobacter* spp. is essential. Thus we would like to highlight the problem of misidentification of *Acinetobacter* spp. by commercial systems utilizing phenotypic tests (e.g. Vitek-2) and the need for further molecular typing for accurate speciation.

Interestingly, both isolates met the screening criterion (imipenem to imipenem/EDTA ratio of ≥ 8) for MBL activity using the Etest MBL strips, even though subsequent PCR assays did not indicate the presence of *bla*_{VIM} or *bla*_{IMP} genes. Such a phenomenon has also been observed by Segal *et al.*⁶ However, the imipenem to imipenem/EDTA ratios of 05/29540 and 05/12659 were lower (8 and 4, respectively) with the agar dilution method, thus suggesting that the latter method may be more specific for the detection of MBL activity. Therefore, Etest for the detection of MBL in *Acinetobacter* spp. must be used with caution and requires further validation before a positive result is conclusive.

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Table 1. Antimicrobial susceptibility patterns of the *Acinetobacter* isolates

Antibiotic	05/29540	05/12659
MICs (mg/L) using the agar dilution method		
Ampicillin	>128	>128
Piperacillin	>128	>128
Amoxicillin/clavulanate	>128	>128
Piperacillin/tazobactam	128	128
Cefoxitin	128	>128
Cefotaxime	64	32
Ceftazidime	32	16
Meropenem	64	128
Meropenem + EDTA (320 mg/L)	8	32
Imipenem	128	128
Imipenem + EDTA (320 mg/L)	16	32
Nalidixic acid	>128	>128
Ciprofloxacin	8	4
Gentamicin	32	1
Amikacin	0.5	2
MICs (mg/L) using the Etest method		
Meropenem	≥32	≥32
Imipenem (MBL test)	24	12
Imipenem + EDTA (MBL test)	≤1	≤1
Tigecycline	0.25	0.25
Colistin	0.25	0.5

The presence of OXA-23 carbapenemase-producing *Acinetobacter* spp. in the Irish Republic has not yet been associated with outbreak problems as seen in the UK. Nevertheless, the emergence of such a resistance mechanism in *Acinetobacter* isolates represents a worrying trend, although it is probably unsurprising given the recent trends in carbapenem resistance elsewhere in the world. We would like to reiterate the importance of prudence in antimicrobial prescription and adherence to infection control measures in the efforts to control the rise of such resistance mechanisms, as well as the urgent need for new antimicrobial agents in the face of pan-β-lactam resistance.

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Transparency declarations

We have no affiliations with the pharmaceutical industry or any related commercial concerns.

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- Antibiotic susceptibility of 50 clinical isolates of *Burkholderia pseudomallei* from Singapore**
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- Sir,
- Burkholderia pseudomallei* is the aetiological agent of melioidosis, a potentially fatal disease in humans and animals. In Singapore, a high incidence of melioidosis cases was observed in early 2004 with a high mortality rate (40%).¹ Prevention of exposure is difficult as this organism is common in the soil of many parts of south-east Asia. In the absence of a vaccine, antibiotic prophylaxis for those predisposed to melioidosis could be explored. Others have shown that oral doxycycline/ciprofloxacin could prevent melioidosis in experimentally infected mice.² We thus examined the *in vitro* susceptibility of 50 clinical isolates obtained from five local hospitals in Singapore, between the years 1996 and 2004, of which 31 were from the outbreak in 2004,¹ to four oral antibiotics, namely amoxicillin/clavulanic acid, doxycycline, ciprofloxacin and co-trimoxazole.
- MICs were determined by the Etest (AB Biodisk, Solna, Sweden) method using Mueller-Hinton (MHII) agar (Oxoid, Basingstoke, UK) and the plates were read after incubation at 37°C for 24 h. The MIC of each antibiotic (in mg/L) for each *B. pseudomallei* isolate was reported as susceptible, intermediate or resistant as per CLSI³ guidelines: ≤8/4, 16/8 and ≥32/16 for amoxicillin/clavulanic acid, ≤4, 8 and ≥16 for doxycycline, ≤2/38, – and ≥4/76 for co-trimoxazole and ≤1, 2 and ≥4 for ciprofloxacin. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa*