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 b-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-b-lactamases such as VIM and IMP enzymes. Another mechanism is the production of Ambler class D oxacillinases (OXAs) 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 with underlying pancreatic carcinoma and diabetes mellitus. Recent antimicrobial treatment included meropenem, ciprofloxacin, 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-b-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 b-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 blaOXA-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 blaOXA-23, blaOXA-27 and blaOXA-40 are all consistent with blaOXA-23 for both isolates. PCR with OXA-51-like primers did not reveal the presence of a blaOXA-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 macrorestriction 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. Their widespread distribution results in 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.3 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 blaVIM or blaIMP 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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References


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%).

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.

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, 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 (MMH) 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 CLSI 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 were used as quality controls.

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.