Emergence of VIM-1-carbapenemase-producing Enterobacter cloacae in Tyrol, Austria

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The rapid emergence and dissemination of carbapenemase-producing Enterobacter species and other members of the Enterobacteriaceae poses a considerable threat to the care of hospitalized patients and to public health. In this study, Enterobacter isolates demonstrating decreased susceptibility to carbapenems detected at the Division of Hygiene and Medical Microbiology, Innsbruck Medical University, between January 2006 and December 2010 were tested for blaVIM-1, blaNDM-1, blaIMP, blaKPC and blaOXA-48 using a multiplex PCR with published primers. PFGE was performed to determine the genetic relatedness. In total, 33 isolates (28 Enterobacter cloacae and 5 Enterobacter aerogenes) were collected during the study period. From 2006 to 2009, between two and seven isolates were found per year. In 2010, a significant increase of carbapenem-resistant strains was observed (n=12). The blaVIM-1 gene was detected in all 28 isolates of E. cloacae. Typing of E. cloacae by PFGE revealed three distinct clusters, the biggest of which contained 18 isolates. These findings demonstrate the emergence of VIM-1-producing Enterobacter in Tyrol, western Austria. The clonal relationship confirms the risk of spread of these organisms and their possible persistence over time.

INTRODUCTION

Carbapenems play an important role in the treatment of nosocomial infections caused by multidrug-resistant Gram-negative bacteria. They are stable to hydrolysis by most β-lactamases, including extended-spectrum β-lactamases (ESBLs) (Paterson & Bonomo, 2005). However, the emergence of carbapenemases and other resistance mechanisms affecting carbapenems, such as high expression of AmpC or CTX-M ESBLs in combination with porin alteration, is becoming a therapeutic challenge (Cornaglia et al., 2007).

The carbapenemases fall into three classes according to their amino acid sequence: Ambler class A (serine carbapenemases); Ambler class B (metallo-β-lactamases; MBLs) and Ambler class D (OXA carbapenemases). These classes are divided further into subgroups and new variants are frequently encountered (Queenan & Bush, 2007).

Enterobacter species are significant causes of nosocomial infections and are intrinsically resistant to aminopenicillins, cefazolin and cefoxitin due to the production of constitutive chromosomal AmpC β-lactamases. Moreover, ESBL-producing Enterobacter species, particularly Enterobacter cloacae, have been identified in the United States (Levison et al., 2002) and Europe (Tzelepi et al., 2000), and carbapenems are considered the drug of choice in these cases. Carbapenem resistance in clinical isolates of E. cloacae is unusual but has been described in strains with porin alterations combined with the hyperproduction of chromosomal cephalosporinase (Lee et al., 1991), and in strains producing class A carbapenem-hydrolysing non-MBLs (Rasmussen et al., 1996). MBLs have been reported in clinical isolates of E. cloacae from various geographical areas (Aschbacher et al., 2011; Falcone et al., 2009; Queenan & Bush, 2007; Tato et al., 2007; Treviño et al., 2009).

In this study, Enterobacter isolates collected at the Division of Hygiene and Medical Microbiology, Innsbruck Medical University, demonstrating decreased susceptibility to imipenem, meropenem, ertapenem or doripenem were evaluated for the production of MBLs (VIM, NDM-1 and IMP), serine β-lactamases (KPC) and OXA carbapenemases (OXA-48) using PCR tests.

Additionally, the isolates were epidemiologically typed by PFGE to determine the genetic relatedness.

METHODS

Bacterial isolates. From January 2006 to December 2010, strains of Enterobacter species with reduced susceptibility to at least one of the carbapenems from various clinical samples were collected from the University Hospital of Innsbruck (UH), district hospitals and general
practitioners in Tyrol (one isolate per species and patient). Meropenem and ertapenem were used as indicator carbapenems; the screening breakpoint was set at 0.5 mg l\(^{-1}\) as recommended by Cohen Stuart & Leverstein-Van Hall (2010). Isolates of \textit{E. cloacae} ATCC 23355 and six carbapenem-susceptible clinical isolates were used as reference strains for typing.

Species identification and antimicrobial susceptibility testing were performed using the Vitek 2 system (bioMérieux) and the Etest (AB Biodisk), respectively. The results were interpreted according to EUCAST guidelines (EUCAST, 2011).

**DNA extraction.** \textit{Enterobacter} isolates were subcultured on MacConkey agar (Oxoid) for 24 h at 37 °C and DNA was extracted using the UltraClean Microbial DNA Isolation kit (MO BIO Laboratories) following the manufacturer’s instructions. The DNA concentration was quantified by spectrophotometer analysis (Gene Quant II; Pharmacia) and adjusted to approximately 25 ng µl\(^{-1}\).

**Characterization of carbapenemases.** The presence of five relevant carbapenem-resistance genes was investigated by PCR as described by Gröbner et al. (2009) (\textit{blaVIM}, \textit{blaOXA-16}, and \textit{blaOXA-23}) and Poirel et al. (2011) (\textit{blaNDM-1}). Sequencing of all amplicons was carried out by using a 3500 Genetic Analyzer (Applied Biosystems).

**PFGE.** Typing of clinical isolates with the extraction of genomic DNA and digestion with \textit{XbaI} (Promega) was carried out according to the standardized PulseNet protocol for \textit{Escherichia coli} O157 of the Centre for Disease Control and Prevention, Atlanta, USA (http://www.cdc.gov/pulentnet/protocols.htm), as described by Ribot et al. (2006). The subsequent PFGE procedure was performed on a CHEF Drive II system (Bio-Rad). Banding patterns were analysed using BioNumerics software (Applied Maths). The relatedness of isolates was calculated on the basis of the Dice coefficient and analysed according to the criteria of Tenover et al. (1995).

**RESULTS**

**Characterization of the strains**

During the period January 2006–December 2010, 33 patients were found to be colonized or infected with \textit{E. cloacae} (\(n=28\)) or \textit{Enterobacter aerogenes} (\(n=5\)) with reduced susceptibility to carbapenems. From 2006 to 2009, between two and seven isolates per year were collected. In 2010, a significant increase was observed (\(n=12\)). The isolates were collected mostly from the UH (\(n=28\)), four isolates originated from district hospitals and only one originated from a general practitioner.

The majority of isolates (\(n=14\); 44.1%) were collected from patients on intensive care units (ICUs), eight were collected from patients on surgical wards (23.6%) and seven were from medical wards (20.6%). The remaining four isolates were obtained from outpatients (11.7%). Three of these patients had been hospitalized earlier.

The isolates were mainly recovered from urine (\(n=10\)), throat or nose swabs (\(n=5\)) and respiratory secretions (\(n=5\)); they were less frequently found in blood cultures (\(n=3\)), wound or vaginal swabs (\(n=3\)), tissue (\(n=3\)), catheters (\(n=2\)), pus (\(n=1\)) and bile (\(n=1\)).

**Antimicrobial susceptibility testing and detection of carbapenemases**

Forty-seven per cent of isolates were resistant to imipenem, 88% to ertapenem, 20% to meropenem and 50% to doripenem. Twenty-one per cent of the isolates were found to have intermediate resistance to imipenem, 12% to ertapenem, 24% to meropenem and 26% to doripenem. The MIC ranges of imipenem (0.75–32 µg ml\(^{-1}\)), ertapenem (1–32 µg ml\(^{-1}\)), meropenem (0.5–32 µg ml\(^{-1}\)) and doripenem (0.5–32 µg ml\(^{-1}\)) were very wide for \textit{E. cloacae} isolates, with several values below the EUCAST susceptibility breakpoint or in the intermediate range. In contrast, all \textit{E. aerogenes} isolates displayed high MICs irrespective of VIM production. Most of the \textit{E. cloacae} and \textit{E. aerogenes} isolates were resistant to aztreonam, ciprofloxacin, co-trimoxazole and gentamicin (94%, 94%, 79% and 88%, respectively). Colistin remained active for all isolates, whereas 23% were resistant and 24% intermediately resistant to tigecycline.

Specific PCR for \textit{blaVIM} yielded 29 positive isolates (all 28 \textit{E. cloacae}, one \textit{E. aerogenes}). The remaining four isolates (\textit{E. aerogenes}) had negative results for molecular detection of the carbapenemases tested (VIM, NDM-1, IMP, KPC and OXA).

**PFGE**

Among the 28 \textit{E. cloacae} isolates, PFGE identified three clusters (1, 2 and 3) with 18, five and two isolates, respectively (Fig. 1). Cluster 1 mostly contained isolates collected from one particular ICU at the UH before. All five isolates grouped in cluster 2 were collected from one particular ICU at the UH within a 2 year period (April 2006–October 2008). Two further isolates showed identical banding patterns and formed a small cluster (cluster 3). Three \textit{E. cloacae} isolates each showed distinct PFGE patterns. The reference strain ATCC 23355 and the five \textit{E. aerogenes} isolates had PFGE patterns that were clearly different from those of \textit{E. cloacae}. All six carbapenem-susceptible clinical isolates showed individual PFGE patterns and were grouped outside of the clusters (data not shown).

Concerning antimicrobial susceptibility, the isolates within a cluster were very diverse with a wide spectrum of MICs for carbapenems. For example, the largest PFGE cluster showed MIC ranges for imipenem from 0.75 to >32 µg ml\(^{-1}\), for ertapenem from 1 to >32 µg ml\(^{-1}\), for meropenem from 0.38 to >32 µg ml\(^{-1}\) and for doripenem from 0.5 to >32 µg ml\(^{-1}\).

**DISCUSSION**

The rapid emergence and spread of carbapenemase-producing members of the \textit{Enterobacteriaceae} is mainly...
Caused by epidemics of bacteria bearing plasmid-mediated KPC (class A) and OXA-48 (class D) enzymes and MBLs (class B) (Deshpande et al., 2006). MBLs have been categorized in two major groups: IMP- and VIM-type enzymes. However, other groups, such as SPM, GIM and SIM, have also been reported. A novel carbapenemase, New Delhi MBL 1 (NDM-1), has recently been described on the Indian subcontinent and is spreading across Europe, where it is frequently linked to a history of health-care abroad, but also to emerging nosocomial transmission (Struelens et al., 2010).

IMP and VIM derivatives have been described worldwide, IMP-type MBLs are predominant in South-East Asia, whereas VIM-type MBLs are more common in Europe. The most affected bacterial species are usually *Klebsiella* species and *E. cloacae* (Walsh et al., 2005).

Although the prevalence of *Enterobacter* species resistant to carbapenems in Tyrol is low (between 0.16% and 0.98% from 2006 to 2010, unpublished data), the present study shows an emergence of these isolates at the University Hospital of Innsbruck and in district hospitals in Tyrol in the last 5 years. In our study, VIM-1 was the most important carbapenem-resistance mechanism in *E. cloacae*, whereas no carbapenemases were detected in most *E. aerogenes* isolates, thus suggesting the presence of other resistance mechanisms.
Data from other countries show the risk of spread of VIM-1-producing organisms. Very high rates of VIM-1-positive *Klebsiella pneumoniae* can be observed in Greece. One study showed an increase of *K. pneumoniae* resistant to imipenem from less than 1% in 2001 to 20% in isolates from hospital wards and to 50% in isolates from ICUs in 2006 (Vatopoulos, 2008). Another study from Greece reported that 37.6% of 178 *K. pneumoniae* blood isolates were positive for *bla*<sub>VIM-1</sub>; 77.8% of these were from ICUs (Psichogiou et al., 2008).

MBL-producing members of the *Enterobacteriaceae* often exhibit low MICs of carbapenems. In our study, the MICs of doripenem, imipenem and meropenem were below the susceptible breakpoints for 24%, 32% and 56% of isolates, respectively. Ertapenem showed the highest MICs; there were no susceptible isolates. The in vivo efficacy of carbapenems for carbapenemase-producing isolates with breakpoints below the MIC was not investigated in this study. The published data on this issue are controversial (Cohen Stuart & Leverstein-Van Hall, 2010; Daikos et al., 2007, 2009; Lomaestro et al., 2006; Mathers et al., 2009). As MBL production is typically associated with resistance to non-β-lactam antibiotics as a result of the presence of other resistance genes in the same integron, there are usually only a few antibiotic alternatives such as tigecycline or colistin, alone or in combination (Cobo et al., 2008). Our data showed that only 53% of the isolates were susceptible to tigecycline whereas colistin remained active in 100%. Therefore, in our region, colistin seems to be the only reliable antibiotic alternative for severe infections with VIM-producing members of the *Enterobacteriaceae*, but its use is limited because of adverse toxicity effects. In addition, there are several reports of resistance to colistin from other countries (Livermore et al., 2011; Meletis et al., 2011).

PFGE revealed that the spread of VIM-1-producing *E. cloacae* in Tyrol is mainly due to dissemination of one genotype. These clonally related isolates (*n* = 18) were obtained from different hospital locations and over a period of 5 years, suggesting an endemic situation rather than patient-to-patient transmission or a common source. Another cluster of clonally related VIM-1-producing *E. cloacae* isolates contained five isolates from the same ICU collected between April 2006 and October 2008. This finding demonstrates that nosocomial transmission may occur and that clones may persist over time.

Thus to prevent dissemination of carbapenemase-producing isolates and to restrict a tendency toward endemicity, the implementation of epidemiological control measures such as surveillance cultures, strict contact isolation of patients harbouring carbapenem-resistant members of the *Enterobacteriaceae* and recommendation of reduced carbapenem use is necessary.

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**REFERENCES**


