carbapenem-resistant K. pneumoniae. Here, during the hospital stay, Patient A received successively piperacillin/tazobactam with vancomycin, ceftazidime with ciprofloxacin, and imipenem with ciprofloxacin, but Patient B only received amoxicillin/clavulanate (Figure 1). It is well established that K. pneumoniae isolates are an important reservoir of β-lactamases. Thus, the dissemination of the KPC carbapenemase has been linked to the dispersion of a clonal ST258-type K. pneumoniae strain. Nevertheless, it appears that the rapid emergence of blaOXA-48 in K. pneumoniae would be explained by the horizontal transmission of an OXA-48-encoding plasmid within strains belonging to different STs.

Our experience raises concern about a possible rapid rise in carbapenem resistance in enteric bacteria through the spread of blaOXA-48-positive plasmids and/or strains. Outbreaks involving different OXA-48-producing species have already been described. Early detection by sensitive screening methods is needed, with targeted surveillance and consideration given to infection control measures, to prevent this spread.

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Transparency declarations
None to declare.

References

Emergence of metallo-β-lactamases GIM-1 and VIM in multidrug-resistant Pseudomonas aeruginosa in North Rhine–Westphalia, Germany

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Sir,
Little is known about the emergence and dissemination of metallo-β-lactamase (MBL)-producing Pseudomonas aeruginosa in Germany. In the last decade only a few sporadic cases have been reported. In 2004 the detection of the unique MBL-type GIM-1 in five identical P. aeruginosa isolates from a German hospital was described.1 Several reports on P. aeruginosa possessing the MBLs VIM-1, VIM-2 and VIM-16 were published in 2005 and 2008.2,3 Here we report on the molecular characterization of GIM-1 and VIM MBLs in clinical isolates of P. aeruginosa.

Between March 2009 and August 2010 we isolated 3000 clinical strains of P. aeruginosa from 37 hospitals in the federal state of North Rhine–Westphalia, Germany. Included in the present study were all multidrug-resistant P. aeruginosa isolates possessing resistance to β-lactams (piperacillin/tazobactam), cephalosporins (ceftazidime) and fluoroquinolones (ciprofloxacin) and being additionally resistant to carbapenems (imipenem and meropenem). Among the 3000 isolates, 18 (0.6%) consecutive non-duplicate strains were found fulfilling the criteria. Furthermore, these isolates were resistant to gentamicin but remained susceptible to polymyxin B. MBL production was confirmed for 8 of the 18 P. aeruginosa isolates by Etest MBL (bioMérieux, Nürtingen, Germany).

Molecular screening was performed for the common MBL genes blaGIM, blaVIM, and blaNDM-1 and for the locally occurring genes blaGIM and blaGPM and blaSJM. Since most MBL genes are integrated at specific sites in class 1 integrons (intI1), the gene sequence of the variable region was determined by PCR mapping. PCR and sequencing of relevant MBL genes revealed the presence of blaGIM in five isolates, blaGPM in two isolates and
bla<sub>BLAM</sub>-16 in one isolate. PCR mapping analyses showed that all MBL genes were located within different gene cassettes embedded in class 1 integrons (Figure 1). The nucleotide sequences of the bla<sub>GIM</sub>-1-containing integron structures have been registered in the GenBank database under accession numbers GU390399, GU390401, GU390404, GU390403 and JF414726.

Transfer of β-lactam resistance was performed by broth mating assays using the GIM-1-producing <i>P. aeruginosa</i> isolates and a sodium azide-resistant <i>Escherichia coli</i> J53 recipient. However, we did not obtain any transconjugants. Furthermore, transformation of plasmids isolated from all GIM-1-producing <i>P. aeruginosa</i> isolates using the Qiagen Plasmid Mini Kit (Qiagen, Hilden, Germany) into the electrocompetent recipients <i>P. aeruginosa</i> ATCC 27853 and <i>P. aeruginosa</i> NCTC 10662 was unsuccessful. These observations correspond to the report of the first <i>P. aeruginosa</i> isolate from 2002 describing the detection of bla<sub>GIM</sub>-1 on a non-transferable plasmid.1

The two <i>P. aeruginosa</i> isolates in the present study harbouring bla<sub>BLAM</sub>-16 showed integron structures identical to those that were recently described in outbreak strains from Greece, designated as In59.2.6 Both strains were recovered from two hospitals within the city of Cologne. However, both strains showed identical macrorestriction patterns in PFGE analysis (data not shown). In another <i>P. aeruginosa</i> (MG1222) we identified the MBL gene bla<sub>VIM</sub>-15. The int1 structure corresponded with a partially sequenced integron from a <i>P. aeruginosa</i> strain isolated in Munich, Germany, in 2005.3 Additional strain typing by PFGE revealed that <i>P. aeruginosa</i> MG1222 and the strain from Munich are very closely related.

The <i>P. aeruginosa</i> isolates MG1370 and MG3918 showed different macrorestriction patterns but identical gene cassettes, with bla<sub>GIM</sub>-1 inserted at the first position. Downstream, three aminoglycoside resistance genes were identified: the adenyltransferase gene adaB and the two acetyltransferase genes aacC and aacA7 (Figure 1a). The int1 structures of two further GIM-1-producing <i>P. aeruginosa</i> strains (MG3404 and MG2736) were related to each other and both lacked the genes aacC and aacA7 (Figure 1b). The isolates had identical PFGE patterns but showed no genetic relationships to the other GIM-1-producing strains. Both strains were isolated in two different hospitals in Mönchengladbach, Germany.

Sequence analysis of the fifth isolate (MG4737) harbouring bla<sub>GIM</sub>-1 revealed a cassette array identical to that found in the first isolated GIM-1-producing <i>P. aeruginosa</i> strain in 2002 in Düsseldorf, Germany (Figure 1c).1 The PFGE pattern of isolate MG4737 was unique compared with the other four isolates. Unfortunately, there was no opportunity to investigate the clonal relatedness with the strain from the outbreak in 2002.

In the present study we found MBL in 8 of 18 carbapenem-resistant <i>P. aeruginosa</i> isolates, indicating that other resistance mechanisms, such as up-regulated efflux, are more prevalent than MBL production in this species. The five bla<sub>GIM</sub>-1-containing <i>P. aeruginosa</i> strains were found in hospitals in four different cities within a radius of 40 km around Düsseldorf, the place where the first GIM-1-producing isolate was detected 9 years ago. The clonal diversity of strains with an identical integron structure indicates the capability to spread. Although the detection rate is likely underestimated, the occurrence of GIM-1 seems to be localized, in contrast to VIM-type MBL, which also occur in other parts of Germany. Thus, further molecular epidemiological investigations are needed to increase knowledge about the frequency, geographical distribution and origin of MBL-producing strains in Germany.

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References


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Investigation of Enterobacteriaceae isolates found to have a raised meropenem MIC by Vitek 2

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Sir,

In the UK prior to 2008, resistance to carbapenems was generally confined to non-fermenters.1 In recent years carbapenemases have been increasingly seen in Enterobacteriaceae, particularly Escherichia coli and Klebsiella pneumoniae. Following an alert from the Antibiotic Resistance & Monitoring Reference Laboratory (ARMRL) in January 2009 regarding the increase in carbapenemase-producing Enterobacteriaceae in the UK,1 draft guidance issued from Health Protection Scotland recommended the investigation of Enterobacteriaceae isolates with an MIC of >0.5 mg/L by the modified Hodge test and the use of combination discs. Similar recommendations are made in other European guidelines.2

We use Vitek 2 (bioMérieux, Marcy l’Etoile, France) for susceptibility testing, and, for Enterobacteriaceae with a meropenem MIC >0.5 mg/L, we then perform a meropenem Etest (bioMérieux), and in some cases a modified Hodge test. In order to determine the optimal procedure for investigating such isolates in our laboratory we analysed data from a retrospective search of the laboratory database from 1 September 2009 to 31 August 2010. A total of 48 isolates were found to have had a meropenem MIC >0.5 mg/L by Vitek 2 and also had an Etest. Results were interpreted according to CLSI methodology.3

The organisms identified were Escherichia coli (n=23), Enterobacter spp. (n=12), Klebsiella spp. (n=6), Proteus spp. (n=2), Serratia spp. (n=2), Citrobacter spp. (n=1), Providencia stuartii (n=1) and Hafnia alvei (n=1). The specimen types these organisms were isolated from were urine (n=39), general swabs and fluids (n=4), respiratory samples (n=4) and CSF (n=1).

Vitek 2 meropenem MICs ranged from 1 to ≥16 mg/L, including 23 isolates (48%) with MICs in the susceptible range (≤2 mg/L). Forty-one isolates were confirmed as meropenem susceptible (≤2 mg/L) by Etest and seven isolates had meropenem MICs >2 mg/L by Etest.

In 44 cases the Etest MIC was lower than the Vitek 2 MIC; of these, 21 isolates were susceptible by both methods. 18 were resistant when measured by Vitek 2 and susceptible by Etest. Five isolates were resistant by both methods and one isolate had the same MIC by both Vitek 2 and Etest. In these cases the Etest MIC was greater than the Vitek 2 MIC; of these, one isolate was susceptible by both methods and two isolates were resistant by both methods.

Modified Hodge tests were performed on 12 isolates; 11 were negative and 1 was positive. The positive isolate was an Enterobacter cloacae subsp. cloacae strain (n=8) with a meropenem MIC of 8 mg/L and an Etest MIC of 32 mg/L. The meropenem Etest MIC range for isolates that had a

Table 1. Isolates referred to ARMRL

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Vitek 2 MIC</th>
<th>Etest MIC</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>≥16</td>
<td>0.023</td>
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<tr>
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<tr>
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<td>≥16</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>32</td>
</tr>
</tbody>
</table>

ARMRL report
confirmed E. coli; meropenem MIC <0.06 mg/L (susceptible); resistance to carbapenems not confirmed
confirmed E. cloaceae; meropenem MIC 8 mg/L (resistant); VIM gene detected
confirmed E. cloaceae; meropenem MIC 4 mg/L (intermediate); VIM gene detected
confirmed H. alvei; KPC and OXA-48 genes not detected