IMP-13-producing Pseudomonas monteilii recovered in a hospital environment

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IMP-13 is a class B metallo-β-lactamase that was first described in a carbapenem-resistant clinical isolate of Pseudomonas aeruginosa from Italy.1 The IMP-13 gene was mostly described as part of the class 1 InPSG integron located on a Tn5051 transposon, and it was reported in intercontinentally disseminating P. aeruginosa of sequence type 621 causing outbreaks in Italy, Romania and Argentina.1–3 Multidrug-resistant P. aeruginosa are at the origin of outbreaks that are often difficult to control and to eradicate. The persistence of pools of transferable elements carrying antimicrobial resistance genes in hidden environmental reservoirs may further explain the resurgence of some epidemics, as well as their ability to quickly appear. The role of Pseudomonas putida as a metallo-β-lactamase reservoir was recently reinforced by showing its ability to transfer carbapenem resistance to P. aeruginosa.5 We briefly describe here the carriage of blaIMP-13 in Pseudomonas monteilii, a species closely related to P. putida, recovered from the clinical environment.

In the setting of an outbreak due to a GES-12-producing Acinetobacter baumannii occurring in 2009, the inanimate environment of an acute care burn unit of a Belgian hospital was regularly screened. In January 2011, the materials and surfaces in multiple hospital environments were sampled. In total, >70 different specimens were obtained from both dry inanimate surfaces (floor, bed frame, bed linen and computer pads) and wet surroundings (sinks, baths, toilets, toilet brushes and soap). This screening was carried out all over the burn unit as well as in the operating rooms, corridors and nurse restrooms.

Screening cultures were grown overnight at 35°C on a MacConkey plate onto which a 30 μg ceftazidime disc (Bio-Rad, Nazareth, Belgium) was placed. While no A. baumannii could be recovered from any of the specimens, culture of a toilet brush sample yielded the growth of a ceftazidime-resistant isolate that was presumptively identified as P. putida by Vitek® 2 GN-ID cards. However, this isolate was subsequently confirmed as P. monteilii both by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (Microflex LT, Bruker Daltonics, Leipzig, Germany) and by sequencing of the gene encoding 16S rRNA (100% identity with P. monteilii GAPP1, accession no. GU396289), emphasizing the difficulty of correctly distinguishing between P. putida and P. monteilii by conventional biochemical methods.9 Antimicrobial susceptibility testing by agar disc diffusion according to CLSI guidelines revealed a multidrug- and carbapenem-resistant isolate, which only remained susceptible to colistin and immediately susceptible to aztreonam. MICs, determined according to the CLSI microdilution reference method,9 confirmed the high-level resistance of this environmental isolate to extended-spectrum β-lactamases (ESBLs; MICs of ceftazidime and cefepime >128 mg/L, and MICs of imipenem and meropenem >32 mg/L). Double discs showed the presence of a synergy between imipenem (10 μg) and EDTA (465 μg; 10 μL of 0.125 M EDTA stock), which highly suggested the presence of a metallo-β-lactamase. PCR sequencing confirmed the presence of a blaIMP-13 gene located upstream of a mutated gene encoding an aminoglycoside-modifying enzyme, aacA4-2, embedded in a new class I integron, In449, which is slightly different (six mutations in 1889 nucleotides analysed; accession number JN091097) from the InPSG already described in P. aeruginosa.7 Moreover, PCR performed with primers specific to the transposase (tnpA) and the resolvase (tnpR) of Tn5051 were negative, indicating that this integron is located on a different and unknown genetic structure. Neither genes encoding ESBLs (blaSHV, BEL, VEB, PER and GES) nor oxacillinase of groups 1, 2 or 10 could be detected by PCR. Plasmid extractions performed using the Kieser method or with Qiagen midi preparation did not yield any plasmidic bands and several attempts to obtain IMP-13-expressing electroporants in PA01 wild-type P. aeruginosa failed, suggesting the possible location of blaIMP-13 on the bacterial chromosome. Subsequent to this screening, reinforced infection control measures were taken, including discarding of all toilet brushes, reviewing and extensive explanation to the housekeeping personnel of the standard operation cleaning/disinfection procedures, implementation of antibacterial filters on taps, reinforced practice of hand hygiene disinfection, daily cleaning of the entire unit, systematic environmental screening of infected/colonized patients’ rooms upon discharge, enhanced...
communication between the infection control staff and the medical and nursing teams, and organization of multiple educative workshop sessions. No IMP-13-producing isolates were detected before or after the detection of IMP-13-producing P. monteilii, either in the burn unit or in any other hospital ward. Up until now, P. monteilii infections had not occurred in this hospital and, in contrast to P. putida, are not yet reported in the literature.

The blaIMP-13 gene has been reported in association with multiple outbreaks of P. aeruginosa abroad, but no outbreak has occurred at this hospital. Instead, GES-12-expressing A. baumannii and ESBL-producing P. aeruginosa outbreaks (P. Bogaarts, personal data) were previously reported in this setting, while, ironically, such isolates could not be recovered from the hospital environment. To the best of our knowledge, only blaIMP-12 has been reported in a P. putida clinical isolate, whereas no outbreak due to a blaIMP-13-expressing P. aeruginosa has ever been reported. The fact that P. monteilii expressing IMP-13 was recovered in the environment, although unrelated to any IMP-13 outbreak, further underlines the potential role of several Pseudomonas spp. to act as silent environmental reservoirs of metallo-β-lactamase genes.

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Transparency declarations
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References


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High prevalence of multidrug-resistant tuberculosis in Zunyi, Guizhou Province of China

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Sir,

According to the WHO Report 2010, China had the most multidrug-resistant tuberculosis (MDR-TB) cases in 2008, followed by India and Russia, and was ranked 16th in terms of the estimated proportion of primary (5.7%) and acquired (previously treated) MDR-TB cases among the 27 MDR-TB high-burden countries.1 However, our recent drug-susceptibility surveillance results indicated that the prevalence of acquired MDR-TB cases in the Zunyi area, Guizhou province, China, was much higher (45.1%) than the average prevalence of acquired MDR-TB in China (25.6%), but lower than those in the top six MDR-TB high-burden countries (Tajikistan, Kazakhstan, Azerbaijan, Republic of Moldova, Uzbekistan and Lithuania).

To determine the prevalence and trends of drug-resistant TB in the Zunyi area, in order to improve the treatment outcomes of TB patients, 263 Mycobacterium tuberculosis clinical isolates were collected at the Affiliated Hospital of Zunyi Medical College from 2008 to 2010 and used in drug susceptibility testing against rifampicin, isoniazid, streptomycin and ethambutol. We used the proportion method on Löwenstein–Jensen media with different concentrations of drugs (rifampicin 40 mg/L, isoniazid 0.2 mg/L, streptomycin 4 mg/L and ethambutol 2 mg/L). The standard M. tuberculosis H37Rv strain was used as a control for all testing.

To compare our results with those from other provinces of China, we selected recent survey results from Shanghai2 and 4 out of the 10 provinces (covering 38% of the total Chinese population) with the highest rates of MDR among new TB cases (5.4%–10.4%),3 and combined them with ours in Table 1. Results indicated that the Zunyi area had the highest prevalence of MDR-TB among previously treated (45.1%) and the second highest among new (8.1%) TB cases in China. In addition, it was observed that the proportions of rifampicin and isoniazid resistance in previously treated (56.0% versus 59.3%) and new (12.2% versus 14.5%) TB cases from the Zunyi area were similar (Table 1), which indicated that isoniazid resistance might be related to rifampicin resistance and that screening for rifampicin resistance could be used as a marker for the detection