Rapid communications

FIRST IDENTIFICATION OF METALLO-BETA-LACTAMASE-PRODUCING *PSEUDOMONAS AERUGINOSA* IN THE CZECH REPUBLIC

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Since 2005, invasive isolates of *Pseudomonas aeruginosa* have been collected in the Czech Republic as part of the European Antibiotic Resistance Surveillance System (EARSS). Forty-eight microbiology laboratories throughout the country including approximately 81% of the population provide consecutive isolates from blood and cerebrospinal fluid. Surprisingly, no metallo-beta-lactamase (MBL) was found in 1,259 invasive isolates tested over the past three years until the detection of two MBL-producing strains in mid-2008. Both strains were isolated from patients hospitalised in one regional hospital. The MBL was identified as IMP-7, which had been seen previously in Canada, Japan, Malaysia and Slovakia.

Metallo-beta-lactamases (MBL) belong to the group of clinically Important carbapenemases produced by Gram-negative bacteria. They have been found mostly in *Pseudomonas* spp., *Acinetobacter* spp. and *Enterobacteriaceae* [1]. Due to increasing diversity, the rapid spread of these enzymes, and the fact that they are often encoded on mobile genetic elements (integrations, transposons, plasmids) together with other resistance genes, MBL-producers belong to the group of clinically relevant multidrug-resistant bacteria [1]. Treating infections caused by such strains is a therapeutic challenge because of the limitations posed by high-level resistance to various groups of antibiotics [1].

Since 2005, invasive isolates of *Pseudomonas aeruginosa* have been collected in the Czech Republic by the National Reference Laboratory (NRL) for Antibiotics of the National Institute of Health in Prague (NIPH) within the European Antibiotic Resistance Surveillance System (EARSS). Forty-eight microbiology laboratories provide consecutive isolates from blood and cerebrospinal fluid (CSF). These laboratories are geographically spread throughout the country. As of 2007, the catchment population of the cooperating laboratories totalled approximately 81% of the Czech population [2].

The minimum inhibitory concentrations (MIC) of 12 antibiotics are determined by broth microdilution method, in accordance with recommendations by the Clinical and Laboratory Standards Institute (CLSI) [3]. MBL production in strains showing resistance to meropenem is verified by the standard synergy test with ethylenediaminetetraacetic acid (EDTA) and meropenem, imipenem and ceftazidime [1,4].

Case description

In 2008, the NRL for antibiotics identified for the first time two MBL-producing strains of *P. aeruginosa* in isolates from blood. Both isolates had been collected from patients hospitalised in the same hospital unit in Hospital A in the North Bohemian region.

The first strain was isolated in July 2008 from a patient with obstructive hydrocephalus complicated with shunt meningitis. The patient, a 25 year-old man whose first cerebral shunt had been implanted in 1994 had been admitted to the department of neurosurgery three times since April 2008 to have his shunt repositioned. During his third hospitalisation in July, the patient developed meningitis complicated with sepsis caused by MBL-producing *P. aeruginosa*. After recovery, the patient was transferred to another hospital in August 2008.

The second strain was identified in November 2008 in a 71 year-old woman with a diagnosis of neurological ependymoma. In July 2008, the patient had been admitted to the department of neurosurgery in Hospital A during the same time period as the patient described above. After surgery, the patient had developed obstructive hydrocephalus treated by use of a shunt. She was sent first to a different hospital and subsequently to a nursing home. In November, she was again transferred to the department of infectious diseases of Hospital A for treatment of a brain abscess. During this hospitalisation, she developed sepsis and an MBL-producing strain of *P. aeruginosa* was isolated from her blood. The patient ultimately died as a result of organ failure.

Because the patient was hospitalised in many different health-care settings, it is unfortunately not possible to describe complete data about antibiotic therapy.

Laboratory analysis

MBL production in the isolates from both cases was verified by the spectrophotometric method described by Lauretti *et al.* [5]. Preliminary identification of MBL was based on multiplex PCR according to Ellington *et al.* [6]. Subsequently, the entire coding regions of the class 1 integron were amplified (as proposed by Fiett *et al.* [7]) and sequenced. Sequencing revealed the MBL enzyme to be IMP-7, encoded on the class 1 integron in both isolates. The *blaIMP-7* gene cassette was found together with other resistance

genes (*aac(6)-Ib*). As only a partial sequence of the integron is available, it is not known at this stage whether the two strains encode further resistance genes.

These are the first two documented appearances of IMP-7 in the Czech Republic.

Pulsed-field gel electrophoresis (PFGE) was used to type the IMP-7 producers, as described by Struelens *et al.* [8], using the restriction enzyme *Xba*I; banding patterns were interpreted according to Tenover *et al.* [9]. The PFGE patterns in both isolates were indistinguishable.

Conclusions

The only hospital unit in which both patients had been simultaneously hospitalised was the department of neurosurgery of hospital A. However, as no epidemiological data or isolates are available from that unit, it was not possible to identify a reservoir or source of the MBL producers. The second patient may have become infected when returning to Hospital A in November 2008, but may also have been colonised already since her stay in that hospital in July 2008. We can therefore not be certain whether the *Pseudomonas* strain was present in Hospital A between July and November or only in July. Control epidemiological screening was proposed in the department of neurosurgery and the department of infectious diseases in Hospital A. At the time of publication of this report, we have no data from this screening.

Because both patients had been hospitalised in several health-care institutions in the North-Bohemian region, the potential risks of spreading MBL-producing bacteria in this geographical area are high.

MBL-producing strains have been described in all countries bordering the Czech Republic. Surprisingly, no MBL had been responsible for resistance to carbapenems in the 1,259 invasive isolates referred to the National Reference Laboratory for Antibiotics in the past three years.

IMP-7 MBL was first described in Canada in strains of *P. aeruginosa* that had caused nosocomial outbreaks in two rehabilitation wards in 1995 and 1996 [10]. Later, this enzyme was found in Malaysia [11], and most recently in Japan [12] and Slovakia [13]. Integron typing has been presented only in the first publication by Gibb *et al.* [10]. It is not clear if this enzyme is alone responsible for the resistance to carbapenems, because when cloned into an *E. coli* laboratory strain, it provided only slight protection against meropenem and no protection against imipenem [10].

With the exception of Italy, IMP-type-producing bacteria seem to be less common in Europe than VIM-type producers [14]. Where and how the IMP-7 MBL entered the Czech Republic therefore remains unclear.

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