ORIGINAL ARTICLE

An Outbreak of Infection Due to Metallo-β-Lactamase-Producing *Proteus mirabilis* in the Surgical Intensive Care Unit

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ABSTRACT

Background. We described an emerging outbreak of infection caused by metallo-β-lactamase (MBL)-producing *Proteus mirabilis* that occurred in the surgical ICU of a Serbian university hospital, and assessed this outbreak in a retrospective observational study.

Methods. Records from patients in this ICU who had MBL-producing *P. mirabilis* isolates were reviewed retrospectively. All enterobacterial isolates from clinical specimens (one per patient) were tested for MBL production. We used a multiplex PCR assay to detect and differentiate each of the MBL gene families: IPM, VIM, SPM, GIM and SIM. In July and in November 2008, we conducted a point prevalence survey of rectal colonization with MBL-producing *P. mirabilis*.

Results. From June through November, 2008, nine patients in the surgical ICU were infected by MBL-producing *P. mirabilis*. These isolates exhibited multi-drug resistance. The outbreak was discovered in June and expanded rapidly; ten of twelve (83%) patients in ICU were colonized with outbreak strains in July. Seven cases of bacteremia, including 3 intravascular catheter related, one surgical site infection, and one urinary tract infection were identified. Six of nine MBL-positive *P. mirabilis* strains belonged to the IPM family, and three others belonged to the VIM family. Actual mortality was 56%, but we could not determine mortality indirectly attributable to the infection.

Conclusion. The rapid emergence of MBL-producing *P. mirabilis* within a Serbian hospital created a challenge for clinicians, microbiologists, and epidemiologists. This resistant infection added further to the established cases of antimicrobial resistance within the hospital.

KEY WORDS

Proteus mirabilis, outbreak of infection, surgical ICU, antimicrobial resistance, mortality

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Because of their rapid spread, increasing diversity, and broad hydrolytic spectrum, the acquired metallo-β-lactamases (MBLs) are an emerging threat (1). Carbapenems are used to treat severe hospital infections caused by multi-drug resistant organisms, but carbapenem-resistant bacteria present an increasing therapeutic challenge (1, 2). Five types of acquired MBLs have been identified: VIM, IPM, SPM, and SIM-type enzymes (1,2,3). The recently described New Delhi metallo-beta-lactamase 1 (NDM-1) is a growing problem worldwide (4). Two dominant groups of acquired MBLs are recognized: the IPM and VIM types (5). Moreover, since the MBL genes are linked to other resistance determinants, MBL-producing organisms are commonly multidrug resistant. MBLs have been spread throughout the world, with an overall trend moving from *Pseudomonas aeruginosa* into *Enterobacteriaceae* (5). Several recent reports described the emergence of VIM-producing *Klebsiella pneumoniae*, mainly in Southern Europe (5). MBL-positive isolates among other members of the family *Enterobacteriaceae* such as *P. mirabilis* have occurred sporadically (6)

Setting and study design. We made a retrospective, observational study of the outbreak of infections caused by

P. mirabilis-producing MBL. The study involved patients hospitalized in a 12-bed surgical intensive care unit (ICU) of the University Clinical Center of Serbia, Belgrade. Our study was conducted during the period of June–November, 2008. An independent physician reviewed the medical records of all infected patients. Data collected included the age, sex, and underlying disease for each individual, as well as the source and date of MBL producing *P. mirabilis* isolates. The patients were followed until they were either discharged from the hospital or died. Rectal colonization with MBL-producing *P. mirabilis* isolates was determined twice, in July and again in November from cultures of rectal swabs.

Microbiologic studies. All enterobacterial isolates were tested for MBL production by a disk-diffusion test based on the synergy between imipenem and EDTA, an MBL inhibitor (7,8). Only isolates from clinical specimens (one per patient) were studied. Species identification of isolated bacteria was done with API 20E strips (bioMerieux, France). Antimicrobial susceptibilities were determined by disc diffusion, in accordance with recommendations of Clinical and Laboratory Standards Institute. Minimal inhibitory concentrations (MICs) of imipenem and meropenem were evaluated with Etest (AB Biodisk). All isolates were screened for extended-spectrum beta-lactamases (ESBLs) activity; this involved the double-disc synergy test, which is based on synergy between clavulanate and ceftriaxone, ceftazidime and cefepime. The addition of EDTA to the clavulanate disc enabled us to show the production of ESBL in previously ESBL negative strains as a result of the synergy between EDTA-clavulanate and cefotaxime and/or cefepime disk (9). Disk diffusion based on synergy between imipenem and the MBL inhibitor EDTA was then used to detect MBL production (9). We used a multiplex PCR assay to detect and differentiate each of five families of MBL genes: IPM, VIM, SPM, GIM and SIM (10).

In July and in November, 2008, we recorded the point prevalence of rectal colonization by MBL-producing *P. mirabilis* in our ICU. Samples were obtained by rubbing pre-moistened swabs over the rectal area. Swab samples were then plated on MacConkey agar plates. Procedures of identification and phenotypic and susceptibility testing were as described above.

Results

We identified MBL-producing isolates of *P. mirabilis* that were not susceptible to carbapenems in patients in a surgical ICU. During the five month study period, nine patients were infected with a *P. mirabilis* strains that produced MBL (Tables 1 and 2). Early in the study, we found that 10 out of 12 patients (83%) had rectal colonization with MBL-producing *P. mirabilis* strains. In the last month of the study (November) none had the MBL-producing bacteria. Six of nine MBL-positive *P. mirabilis* strains belonged to the IPM family, and three other strains belonged to the VIM family (Table 1). All VIM postive isolates were collected in the third month (September). The last IPM-positive isolate was collected in late July. These strains were multiresitant in antibiotic disk diffusion tests; the three VIM isolates were susceptible to amikacin and ciprofloxacin (Table 1). Two of the nine isolates with molecular markers for MBL were negative in the imipenem-EDTA synergy test (Table 1). All of the isolates produced ESBL.

We found no MBL-producing *P. mirabilis* strains in patients out of the ICU. All patients who had MBL-producing *P. mirabilis* isolates were hospitalized in the same surgical ICU. Table 2 shows the clinical characteristics and outcomes of 9 patients who were diagnosed with the infection. The mean age was 48.5 years (range 38-73 years): six patients were male. The mean length of stay in hospital before infection was 21 days (range 6-43 days). Bacteremia was diagnosed in 7 patients with 3 intravascular catheter-related cases, a surgical site infection was diagnosed in one, and a urinary tract infection was diagnosed in one patient. All infected patients were treated previously with carbapenems (6 to 20 days), and all had central venous and urinary catheters in place. Overall mortality among the cohort of infected patients was 56%.

Discussion

We described an outbreak of MBL-producing *P. mirabilis* infection. The infection occurred after prolonged hospitalization (mean duration 21 days). At the beginning of the outbreak, rectal colonization was identified in almost all patients in the ICU. Like in Greek hospitals, bacteremia was the most common result of infection. (5,11)

A study from a Greek hospital reported that mortality with MBL-producing enterobacteria was 68.8% (11). We were unable to establish mortality attributable indirectly to the infection, otherwise our mortality figures might have been higher. According to the Greek authors, patients infected with an organism, for which the MIC-s of both imipenem and meropenem were >4 μg/mL, were more likely to die than those infected with VIM-positive carbapenem-susceptible or VIM-negative organisms (11). Their molecular epidemiology studies confirmed that the outbreak of MBL-producing *Klebsiella pneumoniae* infection was polyclonal.

In the outbreak that we studied, there were at least two clones of *P. mirabilis* resistant to carbapenems (VIM and IMP), suggesting the possibility of two separate outbreaks caused by IPM and VIM strains (Table 1 and 2). We were unable to do epidemiological typing, which could have confirmed this possibility. Furthermore, the observational design of our study did not allow for any conclusions as to the potential risk for acquiring MBL- producing *P. mirabilis* infection in our ICU.

Infection control measures were intensified throughout the ICU, beginning in June, to contain the outbreak. Dedi-

cated infection control personnel ensured compliance with all hygiene and contact isolations measures and with adherence of personnel to meticulous environmental cleaning. This stringent regime was successful, because by the end of the year, we found no infected or colonized patients in this ICU.

The susceptibility profile of our *P. mirabilis* isolates (Table 1) underscores the limited therapeutic choices available for the treatment of infected patients. The isolated strains also accumulated other beta-lactam resistance mechanisms, *e.g,* all strain produced ESBL. Tygecyclin and colistin are possible options for the treatment of these infections (5,11).

In contrast to our findings, the Greek authors reported that *P. mirabilis* clinical isolates carrying VIM-1 MBL had MICs for imipenem of 32 to >128 mg/L, and MICs for meropenem ranged from 1 to 8 mg/L (12). Possibly the strains that we isolated had acquired additional mechanisms of resistance (ESBL).

MBL detection by clinical laboratories is hindered by the fact that the presence of the MBL gene does not always confer resistance (1). Our isolates expressed high level of resistance to carbapenems, making it easy to detect them by disk diffusion. We noted that the imipenem-EDTA synergy test does not include all MBL positive isolates (Table 1). The multiplex PCR assay is rapid and simple, making it suitable for monitoring MBL-producing enterobacteria, but many laboratories do not have access to this technology.

Our observations provide some insight into the epidemiology and the clinical importance of this new threat, MBL-producing *P. mirabilis*. As MBL-producing *P. mirabilis* emerges as a life-threatening pathogen, it is critical to ensure timely and effective treatment of infections caused by this agent. More importantly, there is an urgent need to implement guidelines for the detection of MBL-producing bacteria and enhanced infection control measures to contain their dissemination.

No potential conflict of interest relevant to this article was reported.

Table 1. Microbiological characteristics of 9 *Proteus mirabilis* isolates exhibiting in vitro carbapenem resistance

Case/isolate	Imipenem MIC, μg/mL	Meropenem MIC, μg/mL	Antimicrobial susceptibility phenotype*	EDTA synergy test	Double disc synergy test for ESBL detection	MBL family
1	>32	>32	-	-	+	IPM
2	>32	>32	-	-	+	IPM
3	>32	>32	-	+	+	IPM
4	>32	>32	-	+	+	IPM
5	>32	>32	-	+	+	IPM
6	>32	>32	-	+	+	IPM
7	>32	>32	AMK, CIP	+	+	VIM
8	>32	>16	AMK, CIP	+	+	VIM
9	>32	>32	AMK, CIP	+	+	VIM

*, Phenotype was determined by disc diffusion test. All isolates were resistant to ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, ceftriaxone, ceftazidime, cefotaxime, cefepime, gentamicin, and trimethoprim-sulfamethoxasole; all but three were resistant to amikacin and ciprofloxacin.

Table 2. Clinical characteristics of 9 patients infected by metallo-beta-lactamase- producing *Proteus mirabilis**

Case	Date of sampling in 2008	Gender/age	Underlying disease	Source of infection	Previous carbapenem therapy (duration, days)	CVK	Urinary catheter	Outcome
1	9 June	m/38	Acute pancreatitis	CVK	14	+	+	Death
2	16 June	m/40	Acute pancreatitis	Blood	19	+	+	Death
3	17 June	m/72	Ileus	Wound	20	+	+	Death
4	29 July	m/60	Multiple trauma	Urine	13	+	+	Death
5	10 July	m/19	Multiple trauma	CVK	6	+	+	Discharge
6	23 June	f/73	Acute cholecystitis	Blood	12	+	+	Discharge
7	19 September	f/34	Multiple trauma	Blood	11	+	+	Death
8	23 September	m/51	Ileus	Blood	6	+	+	Discharge
9	30 September	f/50	Multiple trauma	CVK	10	+	+	Discharge

* The order of cases corresponds to the order on the table 1.

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Epidemija infekcije u hirurškoj jedinici intenzivne nege izazvane bakterijom Proteus mirabilis koja stvara metalo-beta-laktamazu

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APSTRAKT

Uvod. U radu je opisana epidemija infekcije u hirurškoj jedinici intenzivne nege (JIN) izazvane izolatima *P. mirabilis* koji stvaraju metalo-beta-laktamaze (MBL).

Metode. Ovo je opservaciona retrospektivna studija. Podaci o bolesnicima, od kojih su poticali izolati *P. mirabilis* koji stvaraju MBL, prikupljeni su i analizirani retrospektivno. Svi izolati enterobakterija u posmatranom periodu su ispitani na stvaranje MBL. Izolati iz kliničkih uzoraka koji stvaraju MBL (samo jedan izolat od jednog bolesnika) ispitani su primenom *multiplex* lančane reakcije polimeraze kojom su detektovane i diferencirane familije gena za stvaranje MBL: IPM, VIM, SPM, GIM i SIM. Jula i novembra 2008. godine u ovoj jedinici intenzivne nege, uzeti su brisevi rektuma od svih bolesnika i ispitani na prisustvo izolata *P. mirabilis* koji stvaraju MBL.

Rezultati. U periodu juni - novembar 2008. godine, ukupno devet pacijenata je bilo inficirano sa *P. mirabilis* koji stvara MBL. Epidemijski izolati prvi put su otkriveni u junu te godine i brzo su se proširili na odeljenju pa je sledećeg meseca, u studiji preseka, kod 10 od ukupno 12 pacijenata (83,3%) nađena kolonizacija rektuma sa *P. mirabilis* koji stvara MBL. Sa druge strane, posle primene epidemioloških mera kontrole i nadzora, novembra 2008. godine, u posmatranoj jedinici intenzivne nege nije bilo bolesnika s kolonizacijom rektuma ili infekcijom bakterijama *P. mirabilis* koje stvaraju MBL. Utvrđeno je sedam slučajeva bakterijemije uključujući tri slučaja povezana sa primenom intravenskog katetera, jedna infekcija rane hirurškog mesta i jedna infekcija urinarnog trakta. Šest od devet MBL pozitivnih izolata *P. mirabilis* pripadalo je IPM familiji, dok su ostala tri pripadala VIM familiji. Stopa smrtnosti inficiranih pacijenata iznosila je 56%, ali se nije mogla utvrditi smtrnost koja bi se mogla pripisati samoj infekciji.

Zaključak. Pojava *P. mirabilis* koji stvara MBL u našoj bolnici predstavlja važan izazov za kliničke lekare, mikrobiologe i bolničke epidemiologe jer uz postojeću visoku učestalost rezistencije ograničava se mogućnost efikasne antibiotske terapije. Epidemiološke mere kontrole i nadzora nad ovom epidemijom bile su uspešne.

KLJUČNE REČI

karbapenemi, rezistencija na antibiotike, bolničke infekcije, enterobakterije