

## Antimicrobial Susceptibility of Metallo-beta-Lactamase Positive and Negative *Klebsiella pneumoniae* Strains Isolated from Intensive Care Unit Patients

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### Abstract

Carbapenem resistance in Gram-negative bacteria is a worldwide increasing and one of the most disturbing problems, given these antibiotics are drugs of choice in the treatment of infections caused by extended-spectrum-beta-lactamase producing strains. In this study the antibiotic susceptibility of metallo-beta-lactamase-positive and negative *Klebsiella pneumoniae* strains isolated from intensive care unit (ICU) patients was evaluated. The presence of genes encoding MBLs was determined with a commercial kit hyplex® MBL ID (BAG HEALTH CARE). The MBL-producing isolates were the first *K. pneumoniae* isolates of this kind identified in Poland. It seems that methods for detecting MBLs in *Enterobacteriaceae* should be included in contemporary standards of microbiological diagnostics in the country.

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**Key words:** *Klebsiella pneumoniae*, hospital acquired infection, Hyplex MBL-ID, metallo-beta-lactamase

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Carbapenems are the drugs of choice in the treatment of infections caused by extended-spectrum beta-lactamase (ESBL)-positive strains which are resistant to all penicillins, cephalosporins and monobactams (Livermore, 2008). ESBL-producing strains causing hospital-acquired infections are a serious therapeutic and epidemiological problem, especially in ICU patients who are of high risk for infections caused by multidrug-resistant bacterial strains. *Klebsiella pneumoniae* are one of the most frequently isolated pathogens from ICU patients (Rudnicka *et al.*, 2005), and one of the most frequent ESBL producers, including Poland (Empel *et al.*, 2008). Recently, an even more disturbing problem has emerged, which is the growing number of reports on metallo-beta-lactamase (MBL)-positive *Enterobacteriaceae* strains, which follow the Gram-negative non-fermenters (Watanabe *et al.*, 1991; Giakkoupi *et al.*, 2003, Luzzaro *et al.*, 2004, Pournaras *et al.*, 2005). MBLs hydrolyze penicillins, cephalosporins and carbapenems and are not inhibited by beta-lactamase inhibitors (Walsh *et al.*, 2005; Queenan and Bush, 2007).

The aim of this study was to evaluate the antimicrobial susceptibility of 25 *K. pneumoniae* strains isolated in 2006–2007 from 22 ICU patients in the Dr A. Jurasz University Hospital in Bydgoszcz. The group included the first *K. pneumoniae* isolates with MBL identified in Poland.

The study included 25 *K. pneumoniae* strains from 22 ICU patients. Except for four MBLs-positive, the strains were isolated from different patients and various samples: eight (32,0%) isolates were from blood and BAL (bronchoalveolar lavage), three were from wound swabs, two were from fistulae swab and urine samples, and single isolates were from the abdominal and peritoneal fluid. All isolates except those from wound and fistulae swabs were recovered as monocultures.

The isolates were identified with commercial ID32E tests (bioMérieux). The antimicrobial susceptibility tests were performed by the disk-diffusion method according to the recommendations of the Polish National Reference Centre for Antimicrobial Susceptibility Testing (Hryniewicz *et al.*, 2006). The

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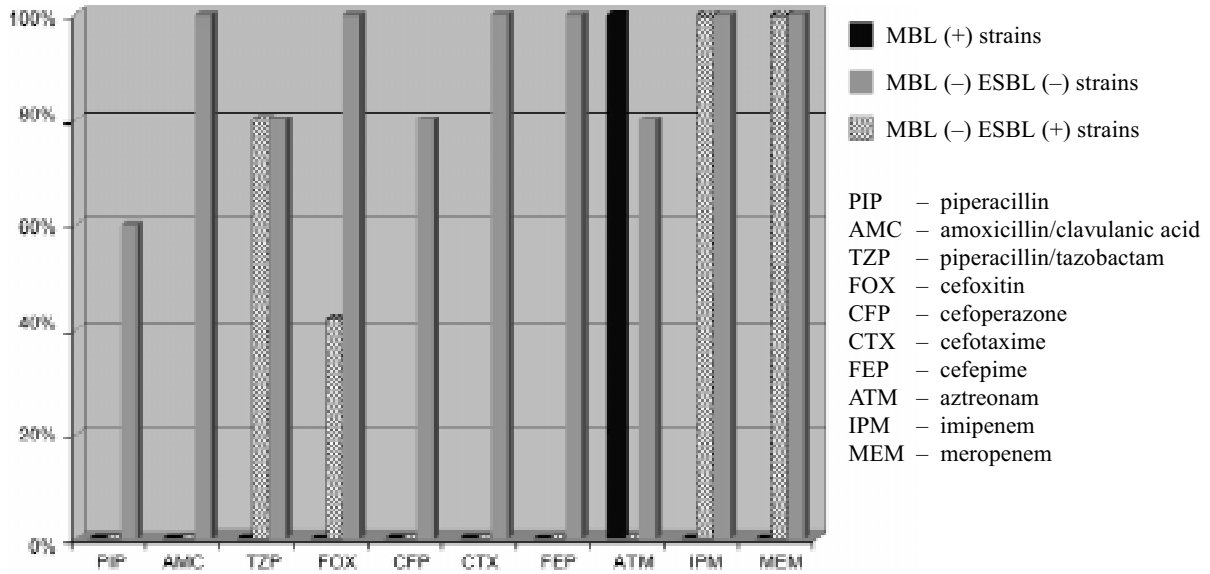


Fig. 1. Sensivity of *K. pneumoniae* strains to selected beta-lactam antibiotics

presence of ESBLs and MBLs was detected with respective double-disk synergy tests (Arakawa *et al.*, 2000; Jarlier *et al.*, 1988). For all MBL-positive isolates MIC (minimal inhibitory concentration) values of imipenem, meropenem, aztreonam, gentamicin, amikacin, tetracycline and ciprofloxacin were determined with Etest (AB Biodisk) and interpreted due to Clinical Laboratory Standards Institute (CLSI, 2007) recommendations. The presence of MBL genes was tested in an isolate from blood with a commercial kit Hyplex<sup>®</sup> MBL ID (BAG HEALTH CARE).

According to the results of MBL testing, the study strains were divided into two subgroups: MBL-positive – four strains and MBL-negative – 21 isolates. Among MBL-negative 16 strains were ESBL-producing. The results of antimicrobial susceptibility

testing are presented in Fig. 1 and Fig. 2. All strains were resistant to ampicillin. The MBL-producers showed a high-level beta-lactam resistance pattern with resistance to all compounds except for aztreonam. Of the remaining antimicrobials tested, only gentamicin and tetracycline showed *in vitro* activity against these isolates.

The MBL-positive *K. pneumoniae* isolates were obtained from one patient and were recovered from a wound swab, urine, blood and abdominal fluid. MICs against the MBL-positive isolates were: >32 µg/ml for imipenem and meropenem, 0.25–0.5 µg/ml for aztreonam, 2–3 µg/ml for gentamicin, 32–64 mg/ml for amikacin. Tetracycline MICs varied from 0.5 µg/ml to 32 µg/ml. Ciprofloxacin MICs were 4–32 µg/ml for isolates from wound swab and abdominal fluid.

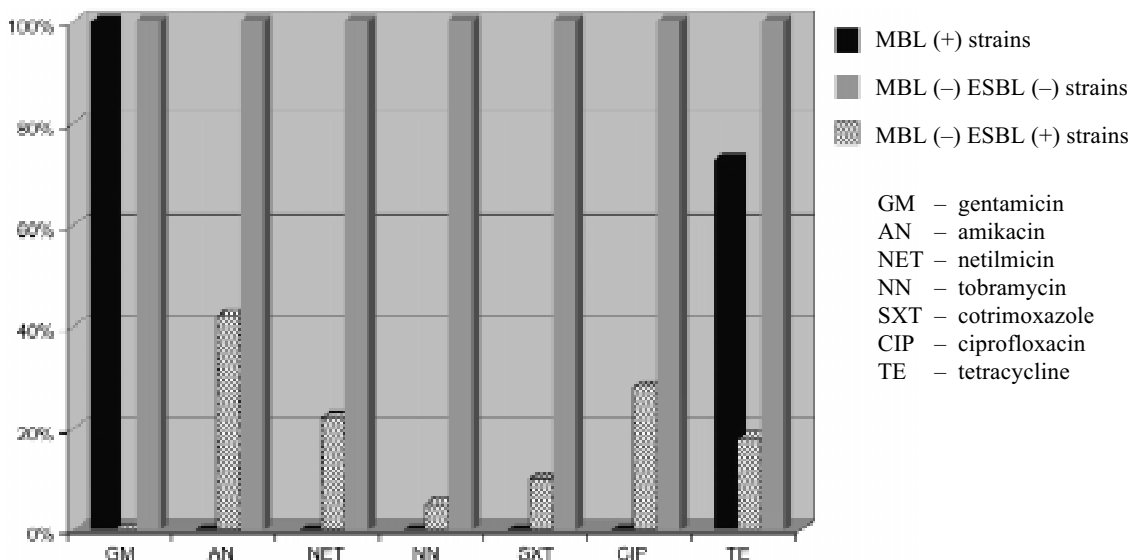


Fig. 2. Sensivity of *K. pneumoniae* strains to selected non-beta-lactam antimicrobials

The isolate from blood was found to carry a *bla*<sub>VIM-1</sub>-like family gene, located in a conjugative plasmid (data not shown).

MBL-producing *Enterobacteriaceae* have been increasingly isolated in recent years (Luzzaro *et al.*, 2004; Toraman *et al.*, 2004; Tórtola *et al.*, 2005; Kassis-Chikhani *et al.*, 2006) especially rapidly disseminating in hospitals in Greece (Giakkoupi *et al.*, 2003; Pournaras *et al.*, 2005). Although MBLs hydrolyze all beta-lactams except for monobactams, enterobacterial isolates with these enzymes relatively frequently appear to be susceptible *in vitro* to various beta-lactams, including carbapenems (Walsh *et al.*, 2005; Queenan and Bush, 2007). The possibility of their use in such cases, however, has been a controversial issue (Souli *et al.*, 2006). Of note is the high-level resistance phenotype observed with the MBL producers in our study.

A VIM-type MBL (VIM-1) was first described in a *Pseudomonas aeruginosa* strain from Verona, Italy, from 1996 (Cornaglia *et al.*, 2000). Currently 12 variants of these enzymes are known and they are divided into three groups: VIM-1-like, VIM-2-like and VIM-7. The VIM-1-like and VIM-2-like enzymes show around 90% identity at the amino-acid sequence level, and whereas VIM-2-like MBLs have been spreading mostly in *P. aeruginosa*, the VIM-1-like  $\beta$ -lactamases have been more characteristic for *Enterobacteriaceae* (Giakkoupi *et al.*, 2003; Pournaras *et al.*, 2005, Walsh *et al.*, 2005). A preliminary further analysis suggested that the enzyme present in our *K. pneumoniae* isolates belonged to the VIM-1 group as well (data not shown).

To our knowledge, the four MBL producers from a single patient reported here have been the first MBL-positive *K. pneumoniae* isolates in Poland. Owing to their multi-drug and high-level resistance there have been almost no therapeutic options left. Identification of such organisms in a hospital, even for carriage should be a signal for implementation of the most stringent infection control procedures. Polish clinical microbiology laboratories should start routine testing for MBLs in *Enterobacteriaceae*.

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