EMERGENCE OF MULTIDRUG-RESISTANT PROTEUS MIRABILIS IN A NURSING HOME IN CROATIA

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The rapid emergence of antibiotic resistance among Gram-negative bacteria is serious threat to the management of infectious diseases. β-lactam antibiotics are the most frequently used antimicrobials for empirical therapy. Production of β-lactamases is one of the strategies adopted by bacteria to develop resistance to β-lactam class of antibiotics. The development of highly stable expanded-spectrum cephalosporins at the beginning of 1980s was quickly followed by the emergence of extended-spectrum β-lactamases (ESBLs) in Klebsiella pneumoniae and other Enterobacteriaceae. These enzymes are usually plasmid-mediated and most frequently derived from parental TEM-1, TEM-2 and SHV-1 β-lactamases by point mutation that alter the configuration of active site to expand their spectrum of activity. AmpC enzymes hydrolyze first and second and third generation cephalosporins and cephamycins and are resistant to β-lactamase inhibitors. Unlike ESBLs they are not inhibited by clavulanic acid, sulbactam or tazobactam. Proteus mirabilis is a multidrug-resistant pathogen in long-term care facilities. The aim of this study was the identification of ESBL production in some isolates of P. mirabilis collected in University Hospital Center Zagreb and to determine the susceptibility to piperacillin/tazobactam.

**RESULTS**

The study demonstrated predominance of plasmid-mediated AmpC β-lactamase CMY-16 among tested strains. AmpC β-lactamase detection is not routinely carried out in many microbiology laboratories. This could be attributed to lack of awareness or lack of resources for detection. AmpC β-lactamases are able to hydrolyze both cephalosporins and carbapenems. There are several inhibitor-based tests for identification of AmpC β-lactamases including disk test and E-test. The production of CMY-16 β-lactamase was associated with resistance or reduced susceptibility to 3rd generation cephalosporins and combination of amoxicillin with clavulanic acid. From the therapeutic point of view, it is important to distinguish between ESBLs and AmpC β-lactamases in University Hospital Center Zagreb.

**CONCLUSIONS**

The acquired blaCMY genes have escaped from the chromosome of Clinical and Molecular Microbiology, University of Zagreb, Split. The aim of this study was the molecular characterization of cephalosporin-resistant Proteus mirabilis strains from a nursing home in Zagreb.

**Material and Methods**

**Bacteria**

Twenty consecutive non-duplicate P. mirabilis strains with reduced susceptibility to cefazidime (zone diameter of < 22 mm) were isolated from urine samples collected from patients between October 2011 and October 2012 at nursing home Godan in Zagreb, Croatia. The isolates were identified by conventional biochemical tests using standard recommended techniques.

**Susceptibility testing**

The susceptibility testing to amoxicillin, cefuroxime, cefazidime, gentamicin, ciprofloxacin, tetracycline, chloramphenicol, and amoxicillin and combined disk testing using disks of cefazidime, cefotaxime, ceftaxime and cefepime with and without clavulanate (10 µg) were performed to detect ESBLs. Deformation of the inhibition zone around cephalosporin disks towards central disk with amoxicillin/clavulanic acid in DST or augmentation of inhibition zone around cephalosporin disks for at least 5 mm in the presence of clavulanic acid compared to control disks without clavulanic acid in combined disk test indicated production of ESBL. E. coli ATCC 25922 and K. pneumoniae ATCC 70063 were used as quality control strain. Detection of extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases was performed using double-disc-synergy test (DDST) using the combined plates containing ceftazidime (1 mg/L) and rifampicin (256 µg/L) or piperacillin/tazobactam (100 µg/L) and clavulanic acid compared to control disks without clavulanic acid in combined disk test.

**Conjugation**

P. mirabilis strains were investigated for the transferability of their resistance determinants. Conjugation experiments were set up employing plasmid-free and rifampicin-resistant E. coli A15 R− recipient strain. Transconjugants were selected on the combined plates containing cefazidime (1 mg/L) and rifampicin (256 mg/L). The frequency of conjugation was expressed relatively to the number of donor cells.

**Characterization of β-lactamases**

The presence of blacm, blabla, blactx, bladha, and bladha genes was investigated by polymerase chain reaction (PCR) using primers and conditions as described previously. Amplicons were column-purified (Qiagen DNA purification kit) and sequenced directly using ABI primers (Applied Biosystems). Sequences were analyzed using BioEdit v.7.0.9. (Ibis Biosciences) program. Designation of bla genes based on identified mutations was done according to Bush and Jacoby's classification scheme.

Detection of quinolone resistance determinants

Plasmid borne quinolone resistance genes qnrA, qnrB and qnrS were determined by PCR as described previously.

**Phenotypic detection of β-lactamases and susceptibility testing**

All strains tested phenotypically positive for AmpC but negative for ESBLs. An augmentation of the inhibition zone around cefazidime disks of at least 5 mm was seen with PBA with no clavulanic acid. The strains were resistant to amoxicillin alone and combined with clavulanic acid, piperacillin, cefotaxime, cefoxitin, gentamicin, ciprofloxacin, tetracycline, chloramphenicol, sulfaemetoxazol, trimethoprim and ciprofloxacin, but susceptible to cefoxitin, trimethoprim and meropenem with MIC of imipenem being slightly higher than those of meropenem (Table 1). There was variable level of susceptibility resistance to cefazidime, cefotaxime and trimethoprim of piperacillin with tazobactam in disk-diffusion test all strains except one were resistant to sulfaemtazol oxalazol (cotrimoxazol). The strains did not transfer resistance to E. coli recipient strain.

**Characterization of β-lactamases**

All twenty P. mirabilis strains yielded an amplification of 631 bp with primers specific for CMY-16 β-lactamase genes. Sequencing of amplicons revealed the blaCMY-16 β-lactamase allele in all strains. The strains cohaboured TEM-1 β-lactamase.

Detection of quinolone resistance determinants

qnrA, qnrB and qnrS genes were not found.

**Characterization of plasmids**

Plasmid encoding CMY-16 were not belong to any known PBRT.