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CARBAPENEMASE-PRODUCING ENTEROBACTERIACEAE, ISOLATED IN UNIVERSITY HOSPITAL - STARA ZAGORA

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ABSTRACT

There are a large number of β-lactamases in the Enterobacteriaceae. These are enzymes that inactivate β-lactam antibiotics and differ in their spectrum of activity against penicillins, cephalosporins, carbapenems and aztreonam. The carbapenemases are broad spectrum β-lactamases, that inactivate carbapenems and other β-lactams. Their determination requires tests not included in routine practices and is the basis of a successful antibiotic therapy. We report on five strains of the Enterobacteriaceae, isolated from clinical specimens in the University Hospital - Stara Zagora. Hodge test for detection of carbapenemase activity was carried out. The antibiotic sensitivity of microorganisms to antimicrobials commonly used in practice is described.

Key words: K.pneumoniae, E. cloacae, C. freundii, Hodge test

INTRODUCTION

Carbapenemases are bacterial enzymes from the group of β-lactamases that hydrolyze penicillins, cephalosporins, carbapenems and aztreonam. In most cases they are not inactivated by β-lactamase inhibitors such as clavulanic acid and tazobactam (1). Bacteria that produce these enzymes cause dangerous infections and carbapenems are often the first choice for treatment. Carbapenemase - producing bacteria are generally resistant to other groups of antimicrobials (3, 14).

Carbapenemases belong to Ambler classes A, B and D. Classes A and D have serine-related activity. Class B are metallo- β -lactamases and contain zinc in their active area (6).

Class A includes chromosome or plasmid determined β-lactamases that confer resistance to carbapenems, penicillins and aztreonam and are susceptible to β-lactamase inhibitors. From this class most often occurs KPC: plasmid-mediated β-lactamase, first observed in K.pneumoniae, and later also found in other Gram-negative bacteria (9, 11). KPC-producing bacteria are usually resistant to fluoroquinolones and aminoglycosides but sensitive to colistin and tigecyclin. Class A carbapenemases result in reduced susceptibility or complete resistance to carbapenems and can sometimes go unrecognized by laboratory detection (6, 8, 10).

Class B carbapenemases (metallo-β-lactamases) are sensitive to EDTA. They are usually found in P.aeruginosa and other non-fermentative Gram-negative bacteria, but can be observed in Enterobacteriaceae too. The most common among enterobacteria is carbapenemase NDM-1 wich spreads through plasmid. Class B confer resistance to all β-lactams except aztreonam. Bacteria carrying the metallo-β-lactamases are usually resistant to other classes of antibiotics - aminoglycosides, fluoroquinolones and are susceptible only to colistin, fosfomycin and tigecyclin. Organisms carrying these enzymes cause serious diseases, but most often they are isolated as a result of colonization rather than infection (6, 8, 10).

Class D carbapenemases include OXA-type β-lactamases. They are found in A. baumannii and P.aeruginosa and rarely in Enterovacteriaceae. Their genes can be found

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in plasmids or in the chromosome. They cause resistance to penicillins, cephalosporins and aztreonam. They are less active to carbapenems, but easily mutate, increasing their range of action (6, 8, 10).

Multiple antibiotic resistance is often due to a combination of extended-spectrum β-lactamases, carbapenemases and non-enzymatic mechanisms of resistance, some of which may remain undetected routinely by disk-diffusion method of Bauer-Kirby (2, 13, 15). This requires the use of complementary methods. There are both phenotypic and genetic methods for detection of carbapenemase production. Phenotypic methods include double - disk synergy and modified Hodge test (4, 7, 12). The genetic methods (PCR) are more accurate.

The purpose of this study is to draw an attention to the presence of carbapenems-resistant Enterobacteriaceae and the ensuing problems in our hospital.

MATERIALS AND METHODS

For the period from May 2011 to April 2012 five strains of carbapenemase-producing enterobacteria were found. The organisms were isolated during routine microbiological diagnostics and were identified by an automated system Sceptor, BBL. The antibiotic sensitivity was determined using DDM (disk diffusion method of Bauer-Kirby, CLSI 2010)(2). Modified Hodge test was used to determine the carbapenemase production. The ESBL (broad spectrum β-lactamase) production was demonstrated with DDS (method of double-disk synergy) (2, 5).

RESULTS

Five bacterial strains from 4 patients of surgical and intensive care clinics were isolated: 3 strains Klebsiella pneumoniae, 1 - Enterobacter cloacae and 1 - Citrobacter freundii.

Refers to clinically significant microorganisms isolated from wounds, urine, tracheobronchial secret. The bacteria were isolated 2 to 3 times from different materials of each patient. All isolates were resistant to cephalosporins 1 to 4 generation, penicillins and Aztreonam. They showed moderate sensitivity to β-lactamase inhibitors. ESBLs were detected in all of them. All isolates of K. pneumoniae were resistant to Ciprofloxacin and Levofloxacin and showed variable sensitivity to aminoglycoside Amikacin and Tobramycin: some of the isolates were resistant even at initial isolation, the rest after subcultivation and further testing. The strains E. cloacae and C. freundii were susceptible to fluoroquinolones and resistant to aminoglycosides.

When using DDM only 2 isolates of K. pneumoniae showed resistance to carbapenems in primary culture. The other 3 isolates showed zones corresponding to the resistance after subcultivation. All strains when tested by the Hodge test showed positive results. This gives us grounds to assume the existence of a carbapenemase production.

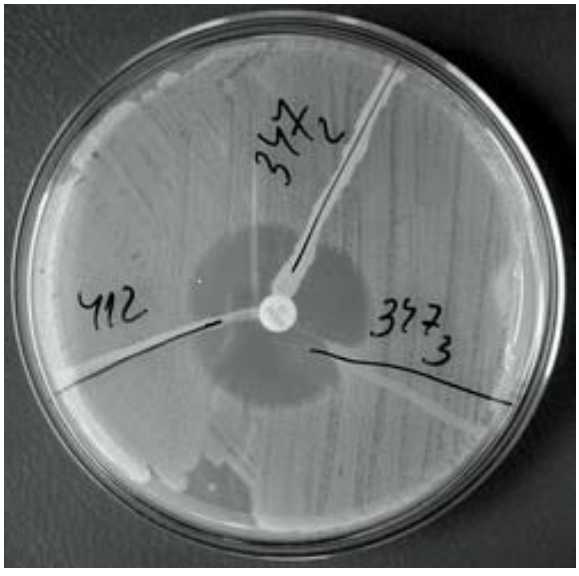

Figure 1. Hodge test - positive result for isolate № 347_3

The simultaneously isolation of K. pneumoniae and E.cloacae from one patient does not exclude the possibility of plasmid transfer of enzyme carbapenemase.

All strains except C. freundii showed a sensitivity to tigecyclin as an alternative drug of choice.

CONCLUSIONS

Carbapenems resistant enterobacteria are spreading increasingly. They are present in our hospital too. Due to their multiple resistance they are a serious therapeutic problem. This requires their correct determination and interpretation, which is the basis of any effective antibiotic therapy. Enzymes carbapenemases do not occur constantly and visibly in the bacterial population in routine testing. This requires additional in vitro tests. With proven or expected carbapenemase production, Enterobacteriaceae isolates should be tested with additional antibiotics as a possible alternative treatment.

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