Characterization of ertapenem resistance in Klebsiella pneumoniae from Croatia

B. Bedenic[1,*], J. Vranes[2], Z. Bosnjak[3], A. Budimir[4], S. Kalenic[3]

[1] *School of Medicine, University of Zagreb, Clinical Hospital Center Zagreb, Zagreb, Croatia*
[2] *Zagreb Institue of Public Health ''Andrija Stampar'', Zagreb, Croatia*
[3] *Clinical Hospital Center Zagreb, Zagreb, Croatia*
[4] *School of Medicine University of Zagreb, Clinical Hospital Center Zagreb, Zagreb, Croatia*

Background: *Klebsiella pneumoniae* isolates with reduced susceptibility to carbapenems were recently reported in USA, UK, Turkey and some other countries of the world. Recently eight Klebsiella pneumoniae strains with reduced susceptibility to carbapenems were isolates in four different hospitals in Croatia. The aim of the study was to determine the mechanisms of ertapenem resistance in these strains.

Methods: Antibiotic susceptibilities were determined by broth microdilution method according to CLSI. Transferability of ertapenem resistance was determined by conjugation (broth mating method) using E. coli A15 R- as recipient. Production of metallo β-lactamases was detected by double-disk synergy test and E test. B-lactamases were characterized by PCR with primers specific for extended-spectrum β-lactamases, plasmid-mediated ampC β-lactamases, metallo β-lactamases of VIM and IMP series, KPC and OXA-48 β-lactamases. Genotyping of the strains was performed by PFGE.

Results: All strains were resistant to ceftazidime, cefotaxime, ceftriaxone, piperacillin alone and combined with tazobactam, amoxycillin/clavulanate, gentamicin and ciprofloxacin. All except one strains showed resistance to ertapenem, intermediate susceptibility or resistance to meropenem and intermediate susceptibility or full susceptiblity to imipenem. One strain was resistant to all three carbapenems. Ertapenem resistance was not transferable by conjugation to E. coli recipient in neither of our strains. PCR revealed the presence of blaSHV and blaCTX-M genes. Multiplex PCR was positive for group 1 CTX-M β-lactamases. Sequencing of representative blaCTX-M genes revealed the presence of CTX-M-15 betalactamase. The strain resistant to all three carbapenem was positive by E test for MBLs. However, PCR was negative for VIM and IMP β-lactamases. No KPC or plasmid-mediated ampC β-lactamases were found. The strains were not clonally related as shown by PFGE and displayed distinct PFGE fingerprints.

Conclusion: This is the first report of carbapenem resistant Klebsiellae in Croatia. Ertapenem resistance in Klebsiella was previously reported in UK, Turkey and Israel mainly due to the production of CTX-M $-lactamases of group 1 combined with porin loss (OmpK36 or OmpK35). The characterization of outer membrane porins needs to be done to clarify the mechanisms of ertapenem resistance in our strains. Further testing is necessary to determine the mech-

doi:10.1016/j.ijid.2010.02.1561

23.006

Changing trends in antimicrobial resistance among salmonella serotypes in Southern India

S. rao[1,*], B. Kabir[2]

[1] *kasturba Medical College & Hospital,Manipal, Karnataka, India*
[2] *Kasturba Medical College and Hospital, Madhav Nagar, Manipal, India*

Background: Enteric fever caused by drug resistant *Salmonella enterica* serotype Typhi and *Salmonella enterica* serotype Paratyphi A has been the major public health concern in the Indian Subcontinent.

Methods: A Retrospective analysis of antibiogram and resistance pattern to Ciprofloxacin, Nalidixic acid, Ceftriaxone Azithromicin and other routine antibiotics to *Salmonella* isolates from PUO cases from blood cultures during 2005-2006 combined with a follow up during 2007-2008 represents the data presented in this study.

Results: Of the 2247 from PUO cases 198 salmonella species (65 were *Salmonella typhi*, 132 *Salmonella paratyphi* A and 1 *Salmonella enteritidis*. *Salmonella typhi* and *Salmonella paratyphi* A serotypes were sensitive to Chloramphenicol, Ampicillin, Cotrimoxazole, and Cefriaxone and sensitive /intermediate to Ciprofloxacin and resistant to Nalidixic acid (except one). MIC for Ciprofloxacin and Nalidixic acid resistance strains was 0.5/1 μg/ml except for 3 MDR salmonella strains which had MIC value of 16 μg/ml. This was reflected on disc diffusion test as intermediate zone of inhibition. Retrospective blood culture analysis of 2005-2006 has shown that MDR *Salmonella typhi* was common isolate then and the strains were sensitive for Ciprofloxacin (MIC being 0.125μg/ml). No antibiotic resistant *Salmonella paratyphi* A was isolated during this period.

Conclusion: Salmonella strains with Nalidixic acid resistance and reduced susceptibility and MIC to Ciprofloxacin have emerged as major cause of enteric fever in Indian Subcontinent. Nalidixic acid susceptibility (30μg disc) can be reliably used to monitor Ciprofloxacin resistance.

doi:10.1016/j.ijid.2010.02.1562

23.007

Molecular epidemiology of aminoglycosides resistance in Acinetobacter spp. with emergence of multidrug-resistant strains in hospitalized patients in Iran

R. Moniri*, R. Kheltabadi Farahani

Kashan University of Medical Sciences, Kashan, Iran, Islamic Republic of

Background: Acinetobacter spp. is emerging as an important nosocomial pathogen and is characterized by increasing antimicrobial resistance. Our aim was to evaluate antimicrobial susceptibility and aminoglycosides resistance genes of Acinetobacter spp. isolated from hospitalized patients.

Methods: Sixty isolates were identified as Acinetobacter species. The isolates were tested for antibiotic resistance by disc diffusion method for 12 antimicrobials. The presence of *aphA6, aacC1 aadA1,* and *aadB* genes were detected using PCR.

Results: From the isolated Acinetobacter spp. the highest resistance rate showed against amikacin, tobramycin, and ceftazidim, respectively; while isolated bacteria were more sensitive to ampicillic/subactam. More than 66% of the isolates were resistant to at least three classes of antibiotics, and 27.5% of MDR strains were resistant to all seven tested classes of antimicrobials. The higher MDR rate presented in bacteria isolated from the ICU and blood samples. More than 60% of the MDR bacteria were resistance to amikacin, ceftazidim, ciprofloxacin, piperacillin/tazobactam, doxycycline, tobramycin and levofloxacin. Also, more than 60% of the isolates contained phosphotransferase *aphA6, and* acetyltransferase genes *aacC1,* but adenylyltransferase genes *aadA1* (41.7%), and *aadB* (3.3%) were less prominent. In this study 21.7% of the strains contain three aminoglycoside resistance genes (*aphA6*, *aacC1* and *aadA1)*.

Conclusion: The rising trend of resistance to aminoglycosides poses an alarming threat to treatment of such infections. The findings showed that clinical isolates of Acinetobacter spp. in our hospital carrying various kinds of aminoglycoside resistance genes.

doi:10.1016/j.ijid.2010.02.1563

23.008

Incidence of Carbapanemase Resistance Gene (KPC) among *Klebsiella pneumoniae* isolates and its Clinical Implications

B. Yegneswaran*, W. Numsuwan, D. Alcid

Drexel University College of Medicine / St Peter's University Hospital, New Brunswick, NJ, USA

Background: Carbapenem antibiotics (Imipenem, Ertapenem, and Meropenem) idicated for infections caused by extended-spectrum {beta}-lactamase (ESBL) carrying pathogens. Carbapenem resistance has been unusual in isolates of *Klebsiella pneumoniae*. The aim of this study is to identify the prevalence of KPC positive *Klebsiella pneumoniae*, and it's clinical significance.

Methods: All isolates of *Klebsiella pneumoniae* species from October 1, 2007 to September 30, 2009 were tested for the presence of KPC gene using the modified Hodge test. Medical records of patients with KPC were studied.

Results: Over the period of two-years 40,309 samples were submitted for culture and sensitivity, out of which 7,836 were positive. Of the positives, there were 106 isolates of *K pneumoniae* and 11 were ESBL positive. Of the ESBL producing isolates, 8 carried the Carbapenem-hydrolyzing β-lactamase. Of the eight, three isolates were reported as being susceptible to Imipenem. Although all the eight isolates were resistant using the Hodge test. Piperacillin/Tazobactam (PT) and Vancomycin were the antibiotic used 7 of the 8 patients prior to isolation of *Klebsiella pneumoniae* resistant to Carbapenems. One patient was excluded in outcome as one patient's sample was clinically thought to be a contaminant was not treated. 3 patients in whom resistance to carbapenem was reported had their antibiotic was changed to Tigecycline and Polymyxin B resulting in clinical improvement. Of the remaining 4 patients who were reported as sensitive to carbapenem three patients had to undergo a repeat surgery due to clinical deterioration and one patient clinically died.

Conclusion: The incidence of KPC gene at our hospital was 7.5%. KPC positive isolatesare rapidly emerging pathogens. It is very important to keep this organism in mind as if not treated there is a 100% probability of having a poor outcome. There is a complete cross resistance to all Carbapenems containing KPC, therefore if KPC is present, the *K pneumoniae* will be resistant to all Carbapenem regardless of the routine susceptibility testing as shown in three isolates that are KPC positive but susceptible to Imipenem. Current automated systems used for susceptibility testing may not accurately identify all these isolates. We must also control the use of antibiotics specially PT to prevent emergence of KPC positive organisms.

doi:10.1016/j.ijid.2010.02.1564

23.009

In vitro activity of Tigecycline against molecularly defined Carbapenemase producing *Acinetobacter baumannii*

M. Hackel[1,*], P. Higgins[2], H. Seifert[2], S. Bouchillon[1], B. Johnson[1], R. Badal[1], J. johnson[1], D. Hoban[1], S. Hawser[3], M. Dowzicky[4]

[1] *International Health Management Associates, Inc., Schaumburg, IL, USA*
[2] *Institute for Medical Microbiology, Cologne, Germany*
[3] *IHMA Europe Sàrl, Epalinges, Switzerland*
[4] *Pfizer Inc, Collegeville, PA, USA*

Background: Acinetobacter baumannii are important opportunistic pathogens with increasing rates of multi-antibiotic resistance due to both intrinsic and acquired mechanisms. Carbapenems are often used to treat these infections, however carbapenem resistance is increasingly reported, leaving few therapeutic options. This resistance is most often associated with acquired or intrinsic OXA-group carbapenemase production. While *A. baumannii* carry the intrinsic OXA-51-like carbapenemase gene, carbapenem resistance has only been associated with these genes when the insertion sequence IS*Aba1* is upstream. In this study, we evaluated the *in vitro* activity of tigecycline against genetically defined *A. baumannii* from the Tigecycline Evaluation Surveillance Trial.

Methods: A total 352 imipenem resistant *Acinetobacter baumanii* from 35 countries (2004 to 2006) were evaluated. MICs were determined by broth microdilution and interpreted according to CLSI guidelines. Carbapenemase genes were detected by multiplex PCR.