Epidemiology and molecular analysis of MDR Enterobacteriaceae isolated from bacteraemias in a Greek hospital in a three-year period (2009-2011)

P 1369

I. Galani, K. Orlandou, F. Panagea, M. Drogari-Apiranthitou, G. Petrikkos, M. Souli*

4th Dept Internal Medicine, Athens Medical School, Attikon University General Hospital

Abstract

Objectives: The aim of this study was to retrospectively analyze the epidemiology of MDR enterobacteriaceae isolated from patients with bacteraemia in University Hospital ATTIKON from 2009 to 2011.

Methods: 247 single-patient blood isolates were collected. Susceptibility testing was performed according to CLSI guidelines. All strains were phenotypically evaluated for the production of β-lactamases and carbapenemases. Isoelectric focusing was performed in all ESBL and/or carbapenemase producing isolates and the respective genes were detected by PCR. Clonal relationship was assessed by REP-PCR.

Results: Among *E.coli* 5.1% were ESBL and 1.2% were carbapenemase producers while 64.5% of *K.pneumoniae* (Kp), 10.3% of *Enterobacter sp* (Ent) and *P.mirabilis* (Pm) were ESBL and/or carbapenemase producers. A total of 51 isolates (10 *E.coli*, 36 Kp, 3 Ent and 2 Pm) were studied further. *Bla*$_{CTX-M}$ was the predominant (50%) ESBL among *E.coli* isolates. KPC producing *E.coli* isolates were rare (1.2%) while *bla*$_{KPC}$ was the predominant mechanism among Kp (73%). Co-existence of *bla*$_{KPC}$ and *bla*$_{VIM}$ occurred in 14.1%, with one isolate (2.9%) harboring also *bla*$_{OXA-48}$. *Bla*$_{VIM}$ was the sole resistance mechanism in 5.7% of Kp, SHV-12 was the predominant ESBL (40%) followed by CTX-M- type (5.7%). Among Ent, *bla*$_{KPC}$ and *bla*$_{VIM}$ occurred in two and one isolates, respectively, one Pm harbored a *bla*$_{VIM}$ gene and OXA-10/17 was the only ESBL found in both species. No clonal spread of resistant *E.coli*, Ent and Pm strains was observed. Kp isolates belonged to 13 different clonal types with C type the predominant one (48.6%). In 2009, clonal type C represented the 62.5 % of the isolates declining to 46.7% in 2010 and disappearing in 2011. New clonal types were detected in 2009 (n=3), 2010 (n=7) and 2011 (n=3).

Conclusions: When KPC Kp emerged in Greece in 2008, they dominated and rapidly disseminated making KPC production the most common mechanism of resistance in Kp the last years. Apart from the epidemic clone C (first detected in 2008), diffusion of *bla*$_{KPC}$ to at least 11 additional clones has taken place, reducing the incidence of epidemic clone C. Notably, isolates carrying both *bla*$_{KPC}$ and *bla*$_{VIM}$ belonged to 4 different clonal types including clone C while co-production of VIM, KPC and OXA-48 was also described by a clone C Kp strain. The predominant ESBL among nosocomial *E.coli* were CTX-M-type enzymes while the dissemination of carbapenemases to other species was documented in the present study.

Objectives

Bacteraemia represent a major cause of death in industrialized countries such as Europe and the USA, with large increases in incidence and mortality being seen over the past 20 years. According to the Greek System for the Surveillance of Antimicrobial Resistance (WHONET) (www.mednet.gr/whonet/), resistance to third generation cephalosporins of *E. coli* strains isolated from bacteraemias is ranging from 7.3 to 15.9% . Much higher is the resistance of other Gram-negative bacteria such as *Klebsiella pneumoniae* (44.5-70.2%) and *Proteus mirabilis* (8.7-38.9%). In particular strains isolated from ICU patients the rates of resistance to third generation cephalosporins accounts to 17.5-27% for *E. coli*, 76.5-92.2% for *K. pneumoniae* and 19.2-64% for *P. mirabilis* isolates.

Extended-spectrum β-lactamases (ESBLs) have emerged gradually since the mid 1980s in members of the Enterobacteriaceae family. They hydrolyse extended-spectrum cephalosporins but are not active against cephamycins and carbapenems and yet are inhibited by β-lactamase inhibitors. There are various groups of ESBL types, of which the most common in Enterobacteriaceae species are the CTX-M, SHV and TEM types. ESBL-producers are associated with increasing prevalence rates worldwide as well as increased morbidity and mortality, especially amongst patients in intensive care and high dependency units.

Until 2001, the majority of multidrug-resistant *K. pneumoniae* isolated in Greek hospitals produced ESBLs, with SHV-5 being the most prevalent type, followed by CTX-M and GES type enzymes. During the period 2002–2007, *K. pneumoniae* producing the metallo-β-lactamase (MBL) VIM-1 were isolated at increasing frequencies. In late 2007, the first *K. pneumoniae* producing KPC-2 β-lactamase was isolated and until the end of 2008 producers of the latter carbapenemase became predominant in the major hospitals throughout the country. Thus, findings during the last decade show a continuously evolving situation requiring regular monitoring. In this study we retrospectively analyze the epidemiology of MDR enterobacteriaceae isolated from patients with bacteraemia in University Hospital ATTIKON from 2009 to 2011.

Methods

- A total of 247 isolates were collected from blood cultures of hospitalized patients of the University General Hospital “Attikon”, between January 2009 - December 2011.
- Only one isolate per species per patient was permitted .
- MIC determinations were performed with the BD Phoenix automated system. Results were interpreted in accordance with CLSI criteria (CLSI Document, M100-S22, 2012).
- All strains were phenotypically evaluated for the production of MBL and KPC production with EDTA–imipenem and imipenem–boronic acid disk synergy tests, respectively (Tsakris et al, 2010).
- ESBL production was evaluated using the CLSI confirmatory test with and without carbapenemase inhibitors (EDTA, Boronic acid).
- Isoelectric focusing was performed in all ESBL and/or carbapenemase producing isolates and the respective genes were detected by PCR with specific primers.
- Clonal relationship was assessed by REP-PCR (Versalovic et al, 1991).

Results

- Among *E.coli* 5.1% were ESBL and 1.2% were carbapenemase producers.
  - *bla*$_{CTX-M}$ was the predominant (50%) ESBL among *E.coli* isolates.
  - KPC producing *E.coli* isolates were rare (1.2%).
- Among *K.pneumoniae* 64.5% were ESBL and/or carbapenemase producers.
  - *bla*$_{KPC}$ was the predominant mechanism among *K. pneumoniae* (73%).
  - Co-existence of *bla*$_{KPC}$ and *bla*$_{VIM}$ occurred in 14.1%, with one isolate (2.9%) harboring also *bla*$_{OXA-48}$.
  - *bla*$_{VIM}$ was the sole resistance mechanism in 5.7% of *K. pneumoniae*, SHV-12 was the predominant ESBL (40%) followed by CTX-M- type (5.7%).
- Among *Enterobacter sp* and *P. mirabilis* 10.3% were ESBL and/or carbapenemase producers.
  - *bla*$_{KPC}$ and *bla*$_{VIM}$ occurred in two and one *Enterobacter sp* isolates, respectively.
  - One *P. mirabilis* isolate harbored a *bla*$_{VIM}$ gene.
  - OXA-10/17 was the only ESBL found in both species.

Figure 1. Prevalence of Enterobacterial isolates

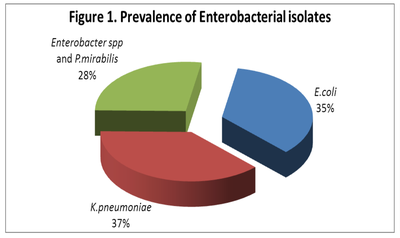
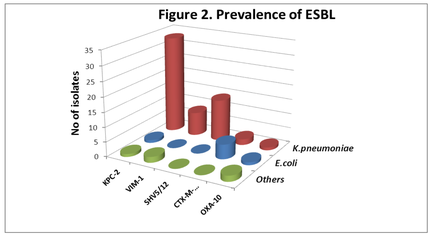


Figure 2. Prevalence of ESBL



Results

- No clonal spread of resistant *E.coli*, *Enterobacter sp* and *P. mirabilis* strains was observed.
- *K. pneumoniae* isolates belonged to 13 different clonal types with C type the predominant one (48.6%). In 2009, clonal type C represented the 62.5 % of the isolates declining to 46.7% in 2010 and disappearing in 2011. New clonal types were detected in 2009 (n=3), 2010 (n=7) and 2011 (n=3).

Figure 3. Prevalence of *K. pneumoniae* clones

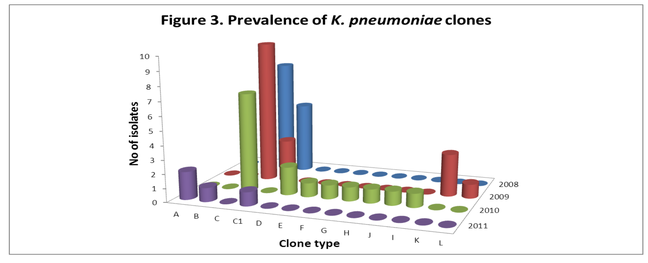


Photo 1. Rep-PCR of *E.coli* isolates

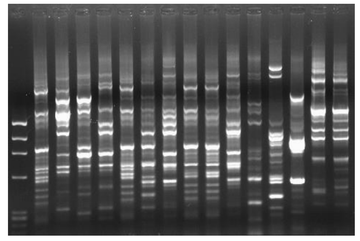
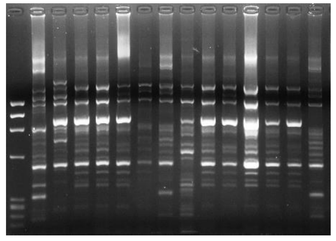


Photo 2. Rep-PCR of *K.pneumoniae* isolates



Conclusions

- When KPC *K. pneumoniae* emerged in Greece in 2008, they dominated and rapidly disseminated making KPC production the most common mechanism of resistance in *K. pneumoniae* the last years.
- Apart from the epidemic clone C (first detected in 2008), diffusion of *bla*KPC to at least 11 additional clones has taken place, reducing the incidence of epidemic clone C.
- Notably, isolates carrying both *bla*$_{KPC}$ and *bla*$_{VIM}$ belonged to 4 different clonal types including clone C while co-production of VIM, KPC and OXA-48 was also described by a clone C *K. pneumoniae* strain.
- The predominant ESBL among nosocomial *E.coli* were CTX-M-type enzymes while the dissemination of carbapenemases to other species was documented in the present study.

Contact Information: Irene Galani, University General Hospital “ATTIKON”, Rimini 1, 124 62 Chaidari, Greece.
Tel : ++321055831984, Fax : ++32105326446, E mail: egalani@med.uoa.gr