

doi:10.1016/j.ijid.2008.05.1195

69.015

Emergence of Clonally Related SHV-5 ESBL-producing *Escherichia coli* Strains in the Community

J. Vranes^{1,*}, B. Bedenic², A. Bauernfeind³

¹ Zagreb Institute of Public Health, Zagreb, Croatia

² Zagreb University Medical School, Zagreb, Croatia

³ MICOER, Munich, Germany

Background: Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* is an emergent cause of urinary tract infections in nonhospitalized patients in different countries. The aim of this study was the molecular characterization of ESBL-producing *E. coli* strains isolated from urine of outpatients in Zagreb region.

Materials and Methods: During the five-month study period a total of 2, 451 *E. coli* strains were isolated from urine of nonhospitalized patients with significant bacteriuria. ESBL production was detected by double-disk diffusion technique and by < 3-dilution reduction in the minimal inhibitory concentration of ceftazidime in the presence of clavulanate. A total of 39 ESBL-producing *E. coli* strains (1.59%) were collected and characterised. Molecular relatedness between the strains was analyzed by pulsed-field gel electrophoresis (PFGE) and isolates were considered to be genetically related if the Dice coefficient correlation was > 80%. Beta-lactamases were characterized by isoelectric focusing, substrate profile determination, polymerase chain reaction and sequencing of blaSHV genes. PCR products were subjected to PCR Nhe test to distinguish between SHV-1 and SHV-ESBL. Plasmids were extracted by alkaline lysis method and digested with EcoR1 enzyme.

Results: Chromosomal DNA analysis by PFGE exhibited a great genomic similarity among ESBL strains. The Dice coefficient of similarity for 25 strains was equal or more than 83.49%. All strains produced a beta-lactamase with the isoelectric point of 8.2. Enzymes produced by the strains antagonized the activity of the disks containing ceftazidime, cefotaxime, ceftriaxone and aztreonam but not cefoxitin and imipenem. All strains yielded an amplicon with primers specific for SHV beta-lactamases. PCR Nhe test demonstrated SHV-ESBL in all tested strains. Fifteen strains possessed an additional TEM beta-lactamase. No CTX-M beta-lactamases was found. Based on sequencing of blaSHV genes enzymes of five strains were identified as SHV-5 beta-lactamase which conferred on the producing isolates high level of ceftazidime and aztreonam resistance. Mutations at the Ambler positions 238 and 240 were found in all gene sequences. The blaSHV genes were encoded on related plasmids which also carried resistance genes for aminoglycosides. Plasmids were assigned to nine fingerprinting patterns (A-F).

Conclusion: The study demonstrated community-associated emergence of clonally related SHV-5 beta-lactamase-producing *E. coli* strains. Other studies on ESBLs in the outpatient populations reported CTX-M ESBLs to be most prevalent in the community setting. The

strains were resistant to aminoglycosides which are not prescribed outside of hospitals, could indicate the possible food origin of ESBL *E. coli* strains.

doi:10.1016/j.ijid.2008.05.1196

69.016

Botulism - A Risk of Traditionalist or Underdeveloped Countries?

S. Draghici^{1,*}, V. Coldea², I. Lenard¹

¹ University of Oradea, Oradea, Romania

² Emergency Clinical County Hospital Oradea, Oradea, Romania

Background: In the past 4 years the number of patients diagnosed with botulism has increased in Bihor county, situated in the western region of Romania. The hospitalized cases presented medium and severe forms, fortunately not leading to death due to timely administration of antitoxic serum. Meat and vegetables products (cans) are incriminated as a source of infection, being manipulated in conditions of deficient hygiene.

Methods: We conducted a retrospective clinico-epidemiological study of botulism cases hospitalized in Bihor county during 2004–2007. Diagnosis was based on clinical signs, epidemiological data and in a small number of cases (6) on identifying botulinum toxin through biological tests.

Results: In the period 2004–2007, 29 persons were diagnosed with botulism in Bihor county (4.83% inhabitants), with an almost equal distribution on sexes, mostly adults - 22 cases (75%). Clinical forms of disease were: mild - 7 cases (24%), moderate - 13 cases (44.5%) and severe - 9 cases (31.5%). The frequently encountered symptoms were: mydriasis, xerostomia, diplopia, palpebral ptosis, dysphonia, digestive disorders, in severe forms respiratory muscles paralysis with respiratory failure. Administration of bivalent antitoxin A+B immediately after admission and supportive care stopped paralysees' progression and prevented death. The source of infection was identified in 93% of consumers, consisting in smoked ham, other home-made meat dishes and vegetable cans. In the close vicinity of those households or rural areas, bovines and equines are reared, which are intestinal carriers of *Clostridium botulinum*.

Conclusion: The preparation of vegetable cans and keeping of smoked meat products in unhygienic conditions in the presence of vectors (flies, cockroaches) is the cause of botulism cases in Transylvania. This traditional practice is interwoven with the economical rationale of impoverished Romanian peasants, who should, however, respect alimentary hygiene.

doi:10.1016/j.ijid.2008.05.1197