Acta Microbiologica et Immunologica Hungarica, 57 (1), pp. 43–53 (2010)
DOI: 10.1556/AMicr.57.2010.1.4

INVESTIGATION OF EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* OUTBREAKS IN HUNGARY BETWEEN 2005 AND 2008

EMESE SZILÁGYI[1]*, M. FÜZI[2], IVELINA DAMJANOVA[3], KAROLINA BÖRÖCZ[1], KATALIN SZŐNYI[1], Á. TÓTH[4] and K. NAGY[2]

[1]Department of Hospital Epidemiology and Hygiene, National Center for Epidemiology, Budapest, Hungary
[2]Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary
[3]Department of Phage Typing and Molecular Epidemiology, National Center for Epidemiology, Budapest, Hungary
[4]Department of Bacteriology, National Center for Epidemiology, Budapest, Hungary

(Received: 3 December 2009; accepted: 15 January 2010)

Fourteen outbreaks in Hungary between 2005 and 2008 caused by extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* (ESBL-KP) were epidemiologically investigated and the isolated pathogens were characterized by molecular techniques. Ten of the fourteen outbreaks occurred in adult wards and four in neonatal units affecting a total number of 73 patients. 54% [40] of the patients developed bloodstream infections and 21.9%–21.9% [16] pneumonia and surgical site infections, respectively. The overall rate of mortality proved high: 36.9% [27]. Outbreaks in adults affected more patients, had higher attack rates, were more prolonged in duration and had a 6.9-fold higher mortality rate than outbreaks observed in neonates. The outbreaks in neonates were caused by SHV-type ESBL-producing klebsiellae, while in the „adult outbreaks" exclusively CTX-M-type ESBL-KP strains were involved. While the outbreak strains isolated from neonatal units could be assigned to a variety of pulsotypes, the previously described *K. pneumoniae* epidemic clones, ST15 and ST147, could be identified among the pathogens causing outbreaks in adult units.

Keywords: ESBL outbreak, PFGE, MLST

* Corresponding author; E-mail: szilagyi.emese@oek.antsz.hu; Miklós Füzi: fuzmik@net.sote.hu; Ivelina Damjanova: damjanova.ivelina@oek.antsz.hu; Karolina Böröcz: borocz.karolina@oek.antsz.hu; Katalin Szőnyi: szonyi.andrasne@oek.antsz.hu; Ákos Tóth: toth.akos@oek.antsz.hu; Károly Nagy: nagykar@net.sote.hu

1217-8950/$20.00 © 2010 Akadémiai Kiadó, Budapest

Introduction

Though outbreak cases account for only five percent of nosocomial infections worldwide, outbreaks are important because they result in significant morbidity and mortality in a short period of time, may cause disruption of services by ward closures and may be costly to investigate and control [1].

Third generation cephalosporins are often used to treat infections caused by Gram-negative pathogens. Only a few years subsequent to their introduction, in 1983, enzymes that hydrolise third-generation cephalosporins and other β-lactam antibiotics except for cephamycins and carbapenems were reported [2]. Since then, extended-spectrum beta-lactamase (ESBL)-producing pathogens have been established as important causes of sporadic nosocomial infections and outbreaks all over the world [3, 4].

In Hungary *K. pneumoniae* has been the most frequently isolated ESBL-producing pathogen accounting for 65% to 75% of all isolated ESBL-producing bacteria [5]. Data obtained by the National Nosocomial Surveillance System, launched at the National Center for Epidemiology in 2004, showed that ESBL-KP is the second most frequent pathogen among multidrug resistant organisms (MDRO) after methicillin-resistant *Staphylococcus aureus* (MRSA). In addition, ESBL-KP proved the most common pathogen among patients in neonatal intensive care units (NICUs) in Hungary [6]. This is in agreement with figures released by the "Outbreak Database" (the worldwide database for nosocomial outbreaks: http://www.outbreak-database.com), showing that *K. pneumoniae* is the most frequent causative agent in NICUs, representing 20.3% of all pathogens, 35% of which are ESBL-producers [7].

Outbreaks of ESBL-producing bacteria have been controlled by the restricted use of 3rd generation cephalosporins and the implementation of infection control measures including screening of contacts, intensified surveillance cultures and isolation- and barrier precautions for infected and colonized patients [8–10].

The objectives of this study were the review of outbreaks caused by ESBL-KP in Hungary between 2005 and 2008 and the genetic characterization of the isolated pathogens.

Materials and Methods

Reporting of nosocomial outbreaks to the public health authorities is mandatory in Hungary. Data have to be provided electronically to NCE. The electronic

questionnaire includes the following inquiries: name of pathogen, type(s) of infections, beginning and end of outbreak, identified source of infection, number of patients and health care workers exposed, number of infected and colonized patients and health care workers, risk factors for patients, number of deaths, number of ward(s) affected, mode of transmission, failures in infection control system, newly introduced infection control measures and laboratory results. In addition, contacts of infected patients are actively screened for colonization with the outbreak strain. Environmental samples are taken to detect any reservoirs. Health care workers involved in the care of infected or colonized patients are also screened by rectal swabs. Isolated relevant pathogens are sent to the NCE ESBL Reference Laboratory for further investigation with molecular techniques.

Laboratory methods

Identification of the isolates was carried out by standard procedures and/or by automatic identification systems (VITEK – bioMérieux, Marcy l'Etoile, France or Micronaut E system – Genzyme Virotech GmbH, Ruesselsheim, Germany). Initial antibiotic susceptibility tests were performed locally by the Kirby–Bauer disc diffusion method in line with CLSI guidelines [11] or by one of the above automatic systems. The putative production of an ESBL was tested in the NCE ESBL Reference Laboratory with the ESBL Etest (AB Biodisk, Solna, Sweden) and/or with the ESBL combined disk test (MAST Diagnostics, Merseyside, UK) according to manufacturers' instruction. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were included as quality control strains in all sessions.

The pulsed-field gel electrophoresis assay (PFGE) was performed in line with the standardized CDC protocol [12]. Gels were interpreted with Fingerprinting II Informatix Software (Bio-Rad). Levels of similarity were calculated with the Dice coefficient, and the UPGMA (unweighted pair group method with arithmetic averages) was used for the cluster analysis of the PFGE patterns. Pulsotypes (PTs) were defined at 85% similarity between macrorestriction patterns and marked by letters according to the criteria established by Tenover et al. [13].

Multilocus sequence typing (MLST) was performed on ten selected isolates according to Diancourt et al. [14]. Allele sequences and sequence types (STs) were verified at the http://pubmlst.org/kpneumoniae website.

Results

Ten of the fourteen outbreaks investigated occurred in adult wards and four in neonatal units. Eight of the adult outbreaks and three of the neonatal outbreaks affected patients in intensive care units. The median duration of the outbreaks was 46 days (range 22–116 days). The baseline epidemiological data collected about the outbreaks are summarized in Table I.

The total number of infected patients was 73. In addition, 53 patients and 5 health care workers proved colonized with ESBL-KP.

Twenty-seven patients died, causing a crude mortality of 36.9%. In three outbreaks the outbreak strain was identified in environmental samples as well. Attack rates varied between 9.6% to 50% when considering only infections and 11.8% to 87.3% when colonized patients were included. In 5 outbreaks 2 wards were affected due to patient transfer to another ward. In 7 outbreaks (50%) an index patient could be identified as the source of infection. In one outbreak infected haemodialysis fluid was microbiologically confirmed as the source of infection. In 6 outbreaks (43%) the source remained unknown.

Modes of transmission were direct and indirect contact in all outbreaks, except in one, where the first 3 patients were infected by contaminated haemodialysis fluid. Additional patients acquired infection by contact transmission also in this outbreak.

Environmental samples were taken in each outbreak. ESBL-KP with identical pulsotype to that of the outbreak strain could be identified in the environment, confirming indirect transmission of the pathogen. Screening of health care workers (HCW) was performed in 13 outbreaks. In three outbreaks one HCW in each, in one outbreak two HCWs showed intestinal colonization with ESBL-KP with the same pulsotype as of the outbreak strain.

Table II shows the number and types of infections, the duration of individual outbreaks and the pulsotypes and sequence types of outbreak strains.

Bloodstream infections proved the most frequent type of infection. Patients with ESBL-KP bloodstream infections were diagnosed in nine outbreaks; bloodstream infection proved the only type of infection in three outbreaks. Additional types of infections were also detected, surgical site infections and cases of pneumonia in 7-7 outbreaks, respectively, and a single urinary tract infection. There were three outbreaks consisting entirely of cases of pneumonia.

Common risk factors for outbreaks affecting adults were severe underlying diseases, invasive procedures (insertion of central venous catheter, mechanical ventilation and/or urinary catheter) and recent surgery. In NICUs and in high risk

Table I

Baseline characteristics ESBL-KP outbreaks, 2005–2008

Hospital	Affected wards	Year	No. of patients exposed	No. of patients infected	No. of patients colonized	Attack rate 1 (%)	Attack rate 2 (%)	No of deaths	Rate of mortality (%)
A	ICU Traumatology	2005	94	9	14	9.6	24.5	4	44.4
A	Traumatology	2005	15	4	0	26.7	26.7	0	0
B	ICU	2005	41	11	2	26.8	61.4	6	54.5
C	ICU, Surgery	2005	18	5	6	27.8	31.7	3	60
D	ICU, Neurology	2006	45	9	3	20.0	66.7	3	33.3
E	NICU Nursery	2006	55	6	4	10.9	18.0	0	0
F	ICU, Neurosurgery	2006	43	6	5	14.0	25.6	5	83.3
F	Nursery	2006	8	4	3	50.0	87.3	0	0
G	ICU	2007	8	3	0	37.5	37.5	0	0
C	NICU	2007	22	2	12	9.1	29.2	0	0
H	ICU	2007	8	3	0	37.5	37.5	0	0
I	ICU	2008	6	2	3	33.3	83.3	3	100*
J	Nephrology	2008	54	6	0	11.1	11.8	2	33.3
K	NICU	2008	14	3	1	21.4	28.6	1	33.3

*All infected patients and one colonized patient died
ICU: intensive care unit
NICU: neonatal intensive care unit
Attack rate 1: no. of infected patients/no of exposed patients × 100
Attack rate 2: no. of infected and colonized patients/no of exposed patients x 100
Mortality rate: no of deaths/no of infected patients × 100

Table II

Type of infections, duration of ESBL-KP outbreaks and genotypes of pathogens

Hospital code Year	Affected wards	Clinical infections patients infected	No of of outbreak (days)	Duration	Pulsotype	MLST
A 2005	Traumatology,ICU	5 BSI, 4 SSI	9	116	R	ST 147
A 2005	Traumatology	3 SSI,1UTI	4	36	R	ST 147
B 2005	ICU	8 BSI, 2 PN, 1 SSI	11	46	N	ST 15
C 2005	ICU, Surgical	3 SSI, 1BSI, 1PN	5	72	N	ST 15
D 2006	ICU-Neurology	8 BSI,1 PN	9	51	Z	
E 2006	NICU nursery	6 PN	6	105	KP032	
F 2006	ICU, Neurosurgery	3 BSI, 2 SSI,1 PN	6	50	L	
F 2006	Nursery	4 BSI	4	22	X	
G 2007	ICU	3 PN	3	46	Z	
C 2007	NICU	2 PN	2	62	Q	
H 2007	ICU	6 BSI, 2 SSI	3	43	Z	
I 2008	ICU	2 BSI	2	30	K	
J 2008	Nephrology	6 BSI	6	32	N	ST 15
K 2008	NICU	2 BSI, 1 SSI	3	24	KP016	

MLST: multilocus sequence typing
ICU: intensive care unit
NICU: neonatal intensive care unit
BSI: bloodstream infection
SSI: surgical site infection

nursery prematurity, low birth weight, prolonged hospital stay, previous antibiotic therapy, invasive procedures, inability of breast feeding were identified risk factors.

Contributors to outbreak were insufficiencies in hand hygiene, incorrect practice in handling invasive devices, low nurse/patient ratio and deficiencies in using barriers (gloves, gowns).

Implemented infection control measures were: cohort isolation of patients, contact isolation of infected and colonized patients (use of gloves, gowns, dedicated devices), active screening for contacts by rectal swabs and any relevant clinical specimen and appropriate treatment of infected patients.

The number of infected and colonized patients per outbreak and the average attack rate were higher and the average duration was longer in adults than in neonates. The average proportion of patients affected was 5.9% versus 3.5%; the average attack rate was 34.6% versus 29% and the average duration of outbreak was 48 versus 43 days in adults and neonates, respectively. The average rate of colonization was higher in neonates than in adults (6% versus 2.9%). The overall rate of mortality was 46.5% among adults and 6.7% in neonates.

The outbreaks in neonates were caused exclusively by SHV-type ESBL-producing klebsiellae, while in the „adult outbreaks" exclusively CTX-M-type ESBL-KP epidemic and endemic clones were involved.

All of the outbreaks reported from neonatal units were caused by distinct genetic clones (Q, KP032, X and KP016), while in adults half of the outbreaks were caused by epidemic clones, and belonged to pulsotype N/ST15 and pulsotype R/ST147. The multilocus sequence typing of strains with additional pulsotypes is in progress.

Discussion

Prior to 2005 ESBL-KP outbreaks occurred exclusively in neonates in Hungary. Between 1998 and 2004 five SHV-type ESBL-producing *K. pneumoniae* outbreaks were reported from neonatal intensive care units throughout the country [6]. In 2005 a sudden expansion and countrywide dissemination of CTX-M-15 type beta-lactamase-producing *K. pneumoniae* epidemic clones was observed causing both outbreaks and sporadic infections in adult patients [5, 15].

The results show that outbreaks caused by ESBL-KP strains were predominant in the adult patient population (71.4%) between 2005 and 2008. However, outbreaks affecting neonates have not ceased in the investigated time period.

Outbreaks in adults affected more patients, had higher attack rates, were more prolonged in duration and had a 6.9-fold higher mortality rate than outbreaks observed in neonates.

In 43% of the outbreaks the source of infection could not be identified. This proportion is similar to that reported by other authors [7]. Contaminated haemodialysis solution as source of one of the outbreaks is evidence of deficient hygienic practice. In addition, common sources of infections with ESBL-KP have been described: contaminated aspiration tubes, roll board in operating rooms, ultrasonographic coupling gel, infusions used for more patients [16–19]. The fact that contact transmission was observed in all investigated outbreaks warrants the strengthening of hand hygiene measures in Hungary. The contamination of the environment detected in three of the outbreaks emphasizes the need for better disinfection practices for both equipment and surfaces. Screening of contacts, performed by routine (weekly) screening during an outbreak is crucial in the arrest of transmission of the infection. Unfortunately, there is no way to effectively decolonize patients or staff carrying ESBL-KP enterically, thus, patients harboring the outbreak strain can serve as sources of infection and often contribute to the prolongation of outbreaks.

Strict infection control measures, including active surveillance cultures, contact precautions for all ESBL-KP infected or colonized patients, good hand hygiene compliance and antimicrobial stewardship, as recommended by the International Infection Control Council, can significantly reduce nosocomial infection rates and may prevent outbreaks [20].

It remains obscure why nosocomial infections in NICUs are caused by SHV-producing klebsiellae while adult ICUs are exclusively affected by CTX-M-producing isolates. Diverse habits of antibiotic use and the lack of patient transfer between the two types of departments could account for the difference. In contrast to SHV-producing klebsiellae, CTX-M-producing strains are almost always resistant to fluoroquinolones, thus, the use of fluoroquinolone type antibiotics in adult ICUs could facilitate the dissemination of CTX-M-producing pathogens. Moreover, it is well-established that SHV-producing klebsiellae had been prevalent in NICUs and caused sporadic infections in adult ICUs prior to the widespread dissemination of CTX-M-producing pathogens in Hungary in 2005 [5, 6, 21]. It is conceivable that the use of fluoroquinolones in adult ICUs contributed to the elimination of fluoroquinolone susceptible SHV-producing isolates and rendered the colonization/infection of patients with CTX-M-producing klebsiellae possible, while SHV-producing pathogens continued to affect patients in NICUs.

In addition, it is not clear why ESBL-KP infections proved more serious in adult ICUs than in NICUs. There are no data suggesting that SHV-producing klebsiellae could be less virulent than CTX-M-producing strains. The most probable explanation is that adult ICU patients are more prone to develop serious generalized infections, which are difficult to treat. Adult ICU patients are more frequently ventilated and carry invasive devices than neonates in NICUs. Furthermore, attending neonates is far easier than looking after adults.

International evidence based guidelines for preventing healthcare associated infections are available, and strict adherence to these guidelines can dramatically decrease nosocomial infection rates [22–30]. Development of evidence based recommendations for prevention of nosocomial infections is under way in Hungary. Domestic guidelines and high compliance to them should improve patient care by significant reduction in occurrence of nosocomial infections.

References

1. Beck-Sauge, C., Jarvis, W., Martone, W. J.: Outbreak investigations. Infect Control Hosp. Epidemiol. **18,** 138–145 (1997).
2. Knothe, H., Sarah, P., Antal, M., Mitsuhashi, S.: Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection **11,** 315–317 (1983).
3. Bush, K.: New beta-lactamases in Gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. Clin. Infect. Dis. **32,** 1085–1089 (2001).
4. Paterson, D. L., Bonomo, R. A.: Extended-spectrum beta-lactamases: a clinical update. Clin. Microbiol. Rev. **18,** 657–686 (2005).
5. Damjanova, I., Tóth, Á., Pászti, J., Hajbel-Vékony, G., Jakab, M., Betra, J., Füzi, M.: Expansion and countrywide dissemination of ST11, ST 15 and ST147 ciprofloxacin-resistant CTX-M-15 type β-lactamase-producing *Klebsiella pneumoniae* epidemic clones in Hungary in 2005-the new 'MRSA'? J. Antimicrob. Chemother. **62,** 978–985 (2008).
6. Damjanova, I., Tóth, Á., Pászti, J., Füzi, M.: Epidemiology of SHV-type beta-lactamase-producing *Klebsiella* spp. from outbreaks in five geographically distant Hungarian neonatal intensive care unit: widespread dissemination of epidemic R-plasmides. Int. J. Antimicrob. Agents **29,** 665–671 (2007)
7. Gastmeier, P., Loui, A., Stamm-Balderjahn, S., Hansen, S., Zuschneid, I., Soh,r D.: Outbreaks in neonatal intensive care units – They are not like others. Am. J. Infect. Control **35,** 172–176 (2007).
8. Meyer, K. S., Urban, C., Eagan, J. A., Berger, B. J., Rahal, J. J.: Nosocomial outbreak of *Klebsiellae* infection resistant to late-generation of chephalosporins. Ann. Intern. Med. **119,** 353–358 (1993).
9. Lucet, J. C., Decre, D., Fichelle, A.: Control of prolonged potbreak extended-spectrum beta-lactamase-producing enterobactericeae in a university hospital. Clin. Infect. Dis. **29,** 1411–1418 (1999).

10. Pena, C., Pujol, M., Ardanuy, C.: Epidemiology and succesful control of a large outbreak due to *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases. Antimicrob. Agents Chemother. **42,** 53–58 (1998).
11. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth International Supplement M100-S15. CLSI, Wayne, PA, USA, 2005.
12. Center for Disease Control and Prevention. Standardized Molecular Subtyping of Foodborne Pathogens by Pulsed-Field Gel Electrophoresis: Training Manual. Atlanta, GA: Center for Disease Control and Prevention, 2000.
13. Tenover, F. C., Arbeit, R. D., Goering, R. V. et al.: Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strai typing. J. Clin. Microbiol. **33,** 2233–2239 (1995).
14. Diancourt, L., Passet, V., Vehoef, J.: Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J. Clin. Microbiol. **43,** 4178–4182 (2005).
15. Damjanova, I., Tóth, Á., Pászti, J., Füzi, M.: Nationwide spread of clonally related CTX-M15-producing multidrug resistant *Klebsiella pneumoniae* strains in Hungary. Eur. J. Clin. Microbiol. Infect. Dis. **25,** 275–278 (2006).
16. Randrianirina, F., Vedy, S., Rakotovao, D.: Role of contaminated aspiration tubes in nosocomial outbreak of *Klebsiella pneumoniae* producing SHV-2 and CTX-M-15 extended-spectrum beta lactamases. J. Hosp. Infect. **72,** 23–29 (2009).
17. van't Veen, A., van der Zee, A, Nelson, J.: Outbreak of infection with multiresistant *Klebsiella pneumoniae* strain associated with contaminated roll boards in operating rooms. J. Clin. Microbiol. **43,** 4961–4967 (2005).
18. Gaillot, J., Maruejouls, C., Abachin, E.: Nosocomial outbreak of *Klebsiella pneumoniae* producing SHV-5 extended spectrum-lactamase, originated from a contaminated ultrasonographie coupling gel. J. Clin. Microbiol. **36,** 1357–1360 (1998).
19. Moodley, P., Coovadia, Y. M., Strum, A. W.: Intravenous glucose preparation as the source of an outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* infections in the neonatal unit of a regional hospital in KwaZulu-Natal. S. Afr. Med. J. **95,** 861–864 (2005).
20. Friedman, C., Callery, S., Jeanes, A.: Best Practices for Patients with Extended Spectrum Beta-lactamase *E. coli* and *Klebsiella*. Washington DC: Association for Professionals in Infection Control and Epidemiolgy; 2006.
21. Tóth, Á., Gacs, M., Márialigeti, Á., Pászti, J., Füzi, M.: Occurence and regional distribution of SHV-type extended-spectrum beta-lactamases in Hungary. Eur. J. Clin. Microbiol. Infect. Dis. **24,** 284–287 (2005).
22. Marschall, J., Mermel, L. A., Classen, D.: Strategies to prevent central line-associated bloodstream infections in acute care hospitals. Infect. Control Hosp. Epidemiol. **29** Suplement1: S 22–S30 (2008).
23. Gastmeier, P., Geffers, C.: Prevention of catheter-related bloodstream infections: Analysis of studies published between 2002 ad 2005. J. Hosp. Infect. **64,** 326–335 (2006).
24. Pratt, R. J., Pellowe, C. M., Wilson, J. A.: Epic2: National evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England. J. Hosp. Infect. **65** (Suppl 1): S1–S64 (2007).
25. O'Grady, N. P., Alexander, M., Dellinger, E. P.: Guidelines for the prevention of intravascular catheter-related infections. MMWR Recomm. Rep. 51(RR-10), 1–29 (2002).

26. Young, E. M., Commiskey, M. L., Wilson, S. J.: Translating evidence into practice to prevent central venous catheter-associated bloodstream infections: A system-based intervention. Am. J. Infect. Control **34,** 503–506 (2006).
27. Coffin, S. A., Klompas, M., Classen, D.: Strategies to prevent ventilator-associated pneumonia in acute care hospitals. Infect. Control Hosp. Epidemiol. **29** Suplement1, S 31–S40 (2008).
28. Gastmeier, P., Geffers, C.: Prevention of ventilator-associated pneumonia: Analyssis of studies published since 2004. J. Hosp. Infect. **67,** 1–8 (2007).
29. Tablan, O. C., Anderson, L. J., Besser, R.: Guidelines for preventing health-care-associated pneumonia, 2003: Recommendations of CDC ad the Healthcare Infection Control Preactices Advisory Commitee. MMWR RecommRep 53, 1–36 (2004).
30. Lai, K., Baker, S., Fontecchio, S.: Impact of program of intensive surveillance ad intervention targeting ventilated patients in the reduction of ventilator-associated pneumonia ad its cost-effectiveness. Infect .Control Hosp. Epidemiol. **24,** 859–863 (2003).