was distinctly shorter and consisted of 232 amino acids [5], of which 224 were identical to the Rep protein of plasmid pSCS34.

Acknowledgment

The authors wish to thank Vera Nöding for excellent technical assistance.

Funding: The work of SS, DV and ID was supported by project grant no. 145069B from the Ministry of Science, Republic of Serbia.

Competing interests: None declared.

Ethical approval: Not required.

References

[1] Werckenthin C, Cardoso M, Martel JL, Schwarz S. Antimicrobial resistance in staphylococci from animals with particular reference to bovine *Staphylococcus aureus*, porcine *Staphylococcus hyicus*, and canine *Staphylococcus intermedius*. Vet Res 2001;32:341–62.

[2] Stepanović S, Ježek P, Vuković D, Dakić I, Petras P. Isolation of members of the *Staphylococcus sciuri* group from urine and their relationship to urinary tract infections. J Clin Microbiol 2003;41:5262–4.

[3] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Sixteenth informational supplement. M100-A16. Wayne, PA: CLSI; 2006.

[4] Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiol Rev 2004;28:519–42.

[5] Horinouchi S, Weisblum B. Nucleotide sequence and functional map of pC194, a plasmid that specifies inducible chloramphenicol resistance. J Bacteriol 1982;150:815–25.

[6] Werner G, Hildebrandt B, Klare I, Witte W. Linkage of determinants for streptogramin A, macrolide-lincosamide-streptogramin B, and chloramphenicol resistance on a conjugative plasmid in *Enterococcus faecium* and dissemination of this cluster among streptogramin-resistant enterococci. Int J Med Microbiol 2000;290:543–8.

[7] Takamatsu D, Osaki M, Sekizaki T. Chloramphenicol resistance transposable element TnSs1 of *Streptococcus suis*, a transposon flanked by IS*6*-family elements. Plasmid 2003;49:143–51.

[8] Lüthje P, von Köckritz-Blickwede M, Schwarz S. Identification and characterization of nine novel types of small staphylococcal plasmids carrying the lincosamide nucleotidyltransferase gene *lnu*(A). J Antimicrob Chemother 2007;59:600–6.

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1 September 2008

doi:10.1016/j.ijantimicag.2008.09.020

Failure of cefepime therapy in neutropenic patients with extended-spectrum β-lactamase-producing Gram-negative bacteraemia

Sir,

The marked increase in the incidence of infections due to extended-spectrum β-lactamase (ESBL)-producing organisms in recent years is of great concern as these organisms are resistant to a broad range of β-lactams [1]. A major problem with ESBLs is their ability to cause therapeutic failure with cephalosporins even when the host organism appears to be susceptible to these agents in laboratory tests [2].

Cefepime has been recommended as an empirical regimen for patients with febrile neutropenia according to the 2002 clinical practice guidelines of the Infectious Diseases Society of America (IDSA) [3]. On the basis of antimicrobial susceptibility data, cefepime appears to be a therapeutic option for the treatment of infections caused by ESBL-producing organisms. However, there are scanty published data regarding the clinical outcome of empirical cefepime therapy in febrile neutropenia caused by ESBL-producing *Escherichia coli* or *Klebsiella pneumoniae* (ESBL-EK) [4,5]. Therefore, in the current study we evaluated the clinical outcome of patients with neutropenic fever who received cefepime therapy for ESBL-EK bacteraemia.

Data from the Clinical Microbiological Laboratory and the medical records of individuals admitted to Samsung Medical Center, Seoul, South Korea, a 1300-bed tertiary-care university hospital, from January 2005 to December 2006 were reviewed retrospectively to identify patients with *E. coli* or *K. pneumoniae* bacteraemia. In this institute, cefepime (2 g every 12 h) has been the most commonly used empirical agent to treat cancer patients with febrile neutropenia. Febrile neutropenic patients with bacteraemia due to ESBL-EK and who were treated with cefepime were selected.

Species identification was carried out using VITEK II (bioMérieux, Hazelwood, MO) using a standard identification card. Antibiotic susceptibility testing was performed using the microdilution method. Minimum inhibitory concentration (MIC) breakpoints and quality control protocols were according to standards established by the Clinical and Laboratory Standards Institute. For each isolate, MICs were determined for inoculum sizes of 10^5 colony-forming units (CFU)/mL and 10^7 CFU/mL. Polymerase chain reaction (PCR) and sequence analysis were used to identify the gene responsible for the ESBL phenotype in ESBL-producers. PCR for bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ was performed as per a previously described protocol [6].

Fifteen febrile neutropenic patients with ESBL-EK bacteraemia received cefepime during the study period. One patient with mixed bacteraemia, seven patients with cefepime-resistant ESBL-EK bacteraemia and one patient who died of sepsis within 48 h of receiving cefepime were excluded. Thus, six patients were included in the study.

The clinical details of the six patients are summarised in Table 1. All of the six patients were admitted in a general ward at the time of the initiation of cefepime therapy. All patients were categorised as high-risk as defined by the IDSA guidelines [3]. Among the six patients, there was only one clinical cure in a patient with urinary tract infection. After 72 h of treatment, the infecting organisms persisted in four patients and were eradicated in two patients. In standard inoculum tests, all isolates were susceptible to cefepime. However, the MICs of all isolates were ≥128 mg/L in higher inoculum tests.

Although ESBLs hydrolyse cephalosporins, many ESBL-producing organisms are not resistant to all cephalosporins, including cefepime, when tested in vitro. Ambrose et al. [7] have suggested that cefepime at 2 g every 12 h has a high probability of achieving pharmacokinetic/pharmacodynamic targets that have previously been correlated with clinical success. However, several anecdotal reports have been published in which patients have had treatment failure when receiving cefepime as treatment for infections by ESBL-producing organisms in non-urinary infections [4,5]. An organism may appear to be susceptible to a drug when tested in vitro using a standard inoculum but the drug may be ineffective

Table 1
Clinical outcome of six febrile neutropenia patients with extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* and *Escherichia coli* bacteraemia treated empirically with cefepime, to which the causative organisms were susceptible in vitro.

Age (years)/ sex	Underlying disease	Site of infection	Organism	Characteristics of ESBL	Initial antibiotic regimen	Cefepime MIC (μg/mL)		Treatment outcome
						10^5 CFU/mL	10^7 CFU/mL	
52/F	Multiple myeloma	Urinary tract	*E. coli*	TEM	FEP	8	>256	Cure: complete response to initial antimicrobial therapy
70/F	Lymphoma	Unknown	*K. pneumoniae*	TEM	FEP, VAN	8	>256	Failure: persistent fever and bacteraemia after 3 days; changed to imipenem, but infected by MRSA on Day 9 and died on Day 30 of treatment
40/M	Aplastic anaemia	Biliary tract	*E. coli*	TEM	FEP	8	>256	Failure: persistent fever and bacteraemia after 3 days; changed to meropenem without biliary intervention and died on Day 20 of treatment
41/M	Aplastic anaemia	Lung	*K. pneumoniae*	TEM	FEP	2	128	Failure: persistent fever and bacteraemia after 3 days; changed to imipenem and transferred to a hospice hospital on Day 15
45/F	Lymphoma	Perianal abscess	*E. coli*	TEM	FEP, VAN, MTZ	2	128	Delayed response: persistent fever after 3 days; changed to imipenem and vancomycin, and 1 day later fever subsided but died on Day 20 of treatment
70/M	Lymphoma	Unknown	*K. pneumoniae*	CTX-M	FEP	2	128	Failure: persistent fever and bacteraemia after 3 days; changed to imipenem, but infected by *Candida* on Day 5 and died on Day 24 of treatment

MIC, minimum inhibitory concentration; CFU, colony-forming units; FEP, cefepime; VAN, vancomycin; MRSA, meticillin-resistant *Staphylococcus aureus*; MTZ, metronidazole.

in vivo owing to a high inoculum. A high-inoculum effect was recently reported with cefepime for ESBL-EK [8]. However, few clinical studies have been conducted deliberately to confirm the findings of in vitro and animal model studies.

In this report, a suboptimal clinical outcome was observed when cefepime treatment was used for bacteraemia caused by ESBL-EK, which may not appear to be resistant on the basis of cefepime MICs of 8 μg/mL (the conventional MIC cut-off value). Furthermore, the 'inoculum effect' with cefepime was also demonstrated in these isolates. Thus, our findings strongly support the concept that cefepime should not be used as first-line therapy against ESBL-producers.

Acknowledgments

The authors thank Ji Yoeun Suh and Mi Young Lee for technical support.

Funding: No funding sources.

Competing interests: None declared.

Ethical approval: Not required.

References

[1] Schwaber MJ, Navon-Venezia S, Kaye KS, Ben-Ami R, Schwartz D, Carmeli Y. Clinical and economic impact of bacteremia with extended-spectrum-beta-lactamase-producing Enterobacteriaceae. Antimicrob Agents Chemother 2006;50:1257–62.

[2] Paterson DL, Ko WC, Von Gottberg A, Casellas JM, Mulazimoglu L, Klugman KP, et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory. J Clin Microbiol 2001;39:2206–12.

[3] Hughes WT, Armstrong D, Bodey GP, Bow EJ, Brown AE, Calandra T, et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Infect Dis 2002;34:730–51.

[4] Zanetti G, Bally F, Greub G, Garbino J, Kinge T, Lew D, et al. Cefepime versus imipenem–cilastatin for treatment of nosocomial pneumonia in intensive care unit patients: a multicenter, evaluator-blind, prospective, randomized study. Antimicrob Agents Chemother 2003;47:3442–7.

[5] Song W, Moland ES, Hanson ND, Lewis JS, Jorgensen JH, Thomson KS. Failure of cefepime therapy in treatment of *Klebsiella pneumoniae* bacteremia. J Clin Microbiol 2005;43:4891–4.

[6] Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, et al. Bloodstream infections due to extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. Antimicrob Agents Chemother 2004;48:4574–81.

[7] Ambrose PG, Bhavnani SM, Jones RN. Pharmacokinetics–pharmacodynamics of cefepime and piperacillin–tazobactam against *Escherichia coli* and *Klebsiella pneumoniae* strains producing extended-spectrum beta-lactamases: report from the ARREST program. Antimicrob Agents Chemother 2003;47:1643–6.

[8] Thomson KS, Moland ES. Cefepime, piperacillin–tazobactam, and the inoculum effect in tests with extended-spectrum beta-lactamase-producing Enterobacteriaceae. Antimicrob Agents Chemother 2001;45:3548–54.

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27 March 2008

doi:10.1016/j.ijantimicag.2008.10.003

Extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in a Neonatal Intensive Care Unit in León, Nicaragua

Sir,

The spread of multiresistant extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in hospital settings has become a global health problem [1]. The aim of this study was to assess the prevalence of ESBLs among *Klebsiella pneumoniae* strains isolated from neonates with septicaemia and from the environment of the Neonatal Intensive Care Unit (NICU) as a source of infection at a hospital in Nicaragua.

Blood samples for bacterial isolation and clinical information were obtained from 135 neonates admitted to the NICU of the Hospital Escuela Dr. Oscar Danilo Rosales Arguello (HEODRA) in León, Nicaragua, between August and October 2005 as prospective surveillance. Ninety-eight samples were collected from environmental screening of the NICU carried out once during the same period. Swabs moistened with sterile saline solution were used for sampling of work surfaces, sinks, incubators, solutions and equipment for intubation and enteral feeding, and the hands and noses of nurses and physicians.

Identification of Gram-negative species was done using the API 20E identification system (bioMérieux, Marcy-l'Etoile, France). Minimal inhibitory concentrations (Table 1) for seven antibiotics were determined by the agar dilution method according to the Clinical and Laboratory Standards Institute [2,3]. Data were analysed using WHONET 5.4 and SPSS 15.0 software. Kruskal–Wallis *H*-test for multiple comparison and Mann–Whitney *U*-test for comparing two groups of *K. pneumoniae* (strains from neonates with septicaemia and strains from the environment) in terms of antibiotic resistance to each antibiotic were used. A *P*-value of <0.05 was considered significant. ESBL production was detected using the Etest system (AB BIODISK, Solna, Sweden) with cefotaxime/cefotaxime + clavulanic acid, ceftazidime/ceftazidime + clavulanic acid, and cefepime/cefepime + clavulanic acid. All isolates were screened for resistance genes encoding SHV-, TEM-, CTX-M- and OXA-type enzymes following the procedure described by Fang et al. [4]. Polymerase chain reaction (PCR) amplification was carried out on a DNA Thermal Cycler GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA). Epidemiological relationships between the 26 *K. pneumoniae* isolates were analysed by random amplified polymorphic DNA (RAPD) as described by Touati et al. [5] with some modifications. RAPD typing was performed using PuReTaq Ready-To-Go™ PCR Beads (GE Healthcare UK Ltd., Little Chalfont, UK) with the following primers: primer 4 (5′-AAGAGCCCGT-3′); and primer 5 (5′-AACGCGCAAC-3′) (Thermo Fisher Scientific, Ulm, Germany). PCR amplification was carried out as follows: 1 cycle at 94 °C for 5 min, 35 (primer 4) and 31 (primer 5) cycles at 94 °C for 5 s, 42 °C for 30 s and 72 °C for 1 min, with a final extension period at 72 °C for 5 min. Following amplification, the banding pattern of randomly amplified DNA was visualised and analysed on 1.5% agarose gel in Tris–acetate buffer 1×. A negative control with no target DNA was included in each PCR. Reproducibility of the amplification results was evaluated in parallel experiments by repetition of the PCR reactions three times. Electrophoresed agarose gels were analysed using the program Molecular Analyst/PC Fingerprint Version 1.12 (Bio-Rad,

Table 1
Characteristics and susceptibilities of *Klebsiella pneumoniae* isolates from neonates with sepsis and from the Neonatal Intensive Care Unit environment.

Specimen code	Antimicrobial resistance profile	MIC (mg/L)							β-lactamase gene profile				ESBL Etest	RAPD type
		AMC	CRO	CAZ	CIP	GEN	IPM	SXT	SHV	TEM	CTX-M	OXA		
100997[n]	CRO, GEN, SXT	16	128	16	0.032	64	0.19	4	+	–	–	–	+	Clone 1
150905[n]	CRO, CAZ, GEN, SXT	16	128	64	0.064	64	0.19	4	+	+	+	–	+	Clone 3
513830[n]	CRO, CAZ, GEN, SXT	16	128	128	0.064	64	0.25	4	+	+	+	–	+	Clone 3
514903[n]	AMC, CRO, CAZ, GEN, SXT	32	128	128	0.064	64	0.19	4	+	+	+	–	+	Clone 3
517296[n]	CRO, CAZ, GEN, SXT	16	128	128	0.064	64	0.25	4	+	+	+	–	+	Clone 3
517530[n]	CRO, CAZ, GEN, SXT	16	128	128	0.064	64	0.19	4	+	+	+	–	+	Clone 3
517645[n]	AMC, CRO, CAZ, GEN, SXT	32	128	128	0.064	64	0.19	4	+	+	+	–	+	Clone 3
518990(ii)[n]	CRO, CAZ, GEN, SXT	16	128	128	0.064	64	0.19	4	+	+	+	–	+	Clone 3
519213[n]	CRO, GEN, SXT	16	128	4	0.064	64	0.25	4	+	+	+	–	+	Clone 3
520067[n]	CRO, CAZ, GEN, SXT	16	128	128	0.032	64	0.19	4	+	+	+	–	+	Clone 3
520098[n]	AMC, CRO, CAZ, GEN, SXT	32	128	128	0.064	64	0.25	4	+	+	+	–	+	Clone 3
520360[n]	CRO, CAZ, GEN, SXT	16	128	128	0.032	64	0.25	4	–	+	–	–	+	Clone 3
520665[n]	AMC, CRO, CAZ, GEN, SXT	32	128	128	0.032	64	0.19	4	+	+	+	–	+	Clone 3
569230[n]	CRO, CAZ, GEN, SXT	16	128	64	0.032	64	0.19	4	+	+	+	–	+	Clone 3
camam	CRO, CAZ, GEN, SXT	16	128	64	0.032	64	0.25	4	+	+	+	–	+	Clone 3
catem4	AMC, CRO, CAZ, CIP, GEN, SXT	32	128	64	32	32	0.25	4	+	+	+	–	+	Clone 3
emcuba	CRO, CAZ, GEN, SXT	16	128	32	0.032	32	0.25	4	+	+	+	–	+	Clone 4
este2	CRO, CAZ, GEN, SXT	16	128	32	0.032	32	0.38	4	+	+	+	–	+	Clone 3
ma76b	CIP	4	0.064	0.25	4	0.5	0.19	0.25	–	–	–	–	–	Clone 2
soliv4	AMC, CRO, CAZ, GEN, SXT	32	128	64	0.016	32	0.25	4	+	+	+	–	+	Clone 3
soliv5	CRO, CAZ, GEN, SXT	16	128	64	0.125	32	0.25	4	+	+	+	–	+	Clone 3
soliv6	CRO, CAZ, GEN, SXT	16	128	64	0.032	32	0.25	4	+	+	+	–	+	Clone 3
soliv8	CRO, CAZ, GEN, SXT	16	128	64	0.032	32	0.19	4	+	+	+	–	+	Clone 3
solm1	CRO, CAZ, CIP, GEN, SXT	16	128	64	4	32	0.25	4	+	+	+	–	+	Clone 3
term8	CRO, CAZ, GEN, IPM, SXT	16	128	32	0.032	32	32	4	–	+	–	–	+	Clone 5
vent6	CRO, CAZ, GEN, SXT	16	128	128	0.064	32	0.25	4	+	+	+	–	+	Clone 3

[n], *K. pneumoniae* isolated from a neonate with sepsis; camam, *K. pneumoniae* isolated from a bed; catem, *K. pneumoniae* isolated from a catheter; emcuba, *K. pneumoniae* isolated from a incubator; este, *K. pneumoniae* isolated from a stethoscope; ma, *K. pneumoniae* isolated from a medical doctor's hand; sol, *K. pneumoniae* isolated from parenteral solutions; term, *K. pneumoniae* isolated from a thermometer; vent, *K. pneumoniae* isolated from a ventilator; AMC, amoxicillin/clavulanic acid; CRO, ceftriaxone; CAZ, ceftazidime; CIP, ciprofloxacin; GEN, gentamicin; IPM, imipenem; STX, trimethoprim/sulfamethoxazole; MIC, minimum inhibitory concentration; ESBL, extended-spectrum β-lactamase; RAPD, random amplified polymorphic DNA.