Extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* infection in a neonatal intensive care unit

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Brief report

Background: **A molecular epidemiological survey was conducted on an extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* (ESBLKp) infection in our neonatal intensive care unit (NICU) from February to June 2008.**

Methods: **Cultures of clinical samples from neonates in the NICU, the hands of healthcare workers and the environment of the NICU were subjected to ESBLKp isolation. Pulsed-field gel electrophoresis was performed to determine *Klebsiella pneumoniae* strains (type A-D).**

Results: **In 1439 neonates, 38 (2.6%) had infections and 65 (4.5%) had colonizations with ESBLKp. Microbiological sampling of the NICU environment yielded 33 (14.9%) ESBLKp isolates from 222 samples. Clone A was found in 88.2% of the infected neonates, 66.7% of the colonized neonates, 69.7% of the environmental samples, and the hands of a healthcare worker.**

Conclusions: **The detection rate of ESBLKp is high in environmental samples, especially those from frequently touched surfaces. Since ESBLKp was identified on the hands of a healthcare worker in the present study, hand and environmental hygiene is mandatory for infection control in neonatal intensive care units.**

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Key words: **infection;**
***Klebsiella Pneumoniae*;**
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Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is an important cause of nosocomial infections in neonatal intensive care units (NICU).[1,2] During the past decade, infection outbreaks of extended-spectrum-beta-lactamase-producing *K. pneumoniae* (ESBLKp) have been frequently seen in pediatric hospitals and NICUs, although the therapeutic options are limited because of their resistance to multi-antibiotics.[1-4]

We investigated an outbreak of ESBLKp infection in our NICU from February 2008 to June 2008. In this article, we report a molecular investigation of this nosocomial outbreak and its epidemiologic characteristics.

Methods

Women's Hospital, Zhejiang University School of Medicine is a teaching hospital in Zhejiang Province, China, which has 28 654 births per year, of whom 3770 are admitted to the NICU. The NICU has three rooms which has a capacity of 30, 15 and 3 patients, respectively. It has 43 physicians and 29 nurses, with a patient to nurse ratio of 50:29. In February 2008, routine surveillance revealed increased ESBLKp infection in neonates in the NICU. ESBLKp infection was observed in NICU patients with symptoms or signs of infection along with ESBLKp isolated from the blood, urine, cerebrospinal fluid, endotracheal aspirate or other aseptically obtained fluid. General hygienic measures were then strengthened to improve hand-washing practice and to isolate or cohort ESBLKp-infected and colonized patients. Double disk synergy tests were used to examine the neonates by body surface culture for ESBLKp carriage and to perform specific and routine environmental surveys. A total of 1255 samples were collected from all hospitalized neonates and cultured thereafter.

Environmental cultures were performed using a swab moistened with sterile saline and included work surfaces, sinks, incubators, solutions, tubes, respiratory equipment, suction catheters, medications and fluids,

heaters, scales, feeding bottles and formulas in the NICU. All healthcare workers (HCWs) who had direct contact with neonates hospitalized in the NICU had samples taken for cultures of ESBL-producing *K. pneumoniae* on the second Wednesday each month. Hand impressions were collected with a swab moistened with sterile saline.

The cultures were performed with McConkey agar containing ceftazidime to facilitate isolation of ESBLKp. Microorganisms were identified using the API 20E system (Montalieu Vercieu, France). Isolates were screened for ESBL production by testing for resistance to cefpodoxime (1 μg/mL). If resistance was detected, ESBL production was confirmed further using E-test strips (AB Biodisk, Solna, Sweden) of cefotaxime and ceftazidime alone or in combination with clavulanic acid. It was confirmed if the minimal inhibitory concentration of either cefotaxime or ceftazidime was decreased by two or more folds dilution with the addition of clavulanic acid. All strains were subjected to antibiotic susceptibility testing using ampicillin, ampicillin/sulbactam, cephazoline, ceftazidime, cefotaxime, ceftriaxone, ciprofloxacin, gentamicin, ciprofloxacin, imipenem, levofloxacin, tobramycin, nitrofurantoin and piperacillin/tazobactam.

Molecular typing was performed using pulsed-field gel electrophoresis (PFGE). Genomic DNA was prepared as reported previously.[5,6] After XbaI digestion (TAKARA biotechnology CO., LTD, Japan), the DNA was electrophorased through pre-melted 1% Seakem Gold agarose (containing 1% SDS) solution in a CHEF-DRIII apparatus (Bio-Rad Laboratories, Hercules, CA, USA) under the following conditions: an initial pulse of 4 seconds and a final pulse of 40 seconds at 200 V/cm for 19 hours at 14ºC. The gels were photographed and digitalized with a GelDoc (Bio-Rad Laboratories, Inc., USA). The images were analyzed with Quantity One (Bio-Rad Laboratories, Inc., USA).

Results

During the study period, 65 (4.5%) of 1439 neonates admitted to the NICU became colonized by ESBLKp. Infection by ESBLKp occurred in 38 neonates (2.6%). The first case was a premature infant (30 weeks of gestation) with pneumonia caused by an ESBLKp who was identified in February 2008.

All the 38 patients were preterm, and 53% of them showed respiratory distress syndrome with feeding intolerance (Table). All babies were admitted on the first day after birth. Among them, 14 patients were diagnosed with sepsis, and ESBLKp samples were isolated from the blood; the other 24 patients were diagnosed with pneumonia, and ESBLKp samples were isolated from the nasopharyngeal secretions and sputum (Table). The percentage of *K. pneumoniae* isolates found to be ESBL producers is 95.06%. All the isolates were 100% sensitive to imipenem, and imipenem was used for infection treatment. Only one patient infected with pneumonia died (Table).

The five environmental surveys including 222 samples yielded 33 ESBLKp isolates. One isolate was obtained from an incubator, the other 32 were obtained from gastric tubes. Of the 32 HCWs for whom cultures were performed, 1 (3.1%) had a positive culture for ESBLKp. Of the 15 pharyngeal swab cultures from the NICU staffs, 2 (13.3%) were positive. In total, 76 of 81 (93.8%) *K. pneumoniae* isolates were ESBL producers. All strains isolated were susceptible to imipenem, nitrofurantoin and piperacillin/azobactam. There was resistance to multiple antibiotics including ampicillin/sulbactam (52.5%) and gentamicin (85%), and 100% resistance was seen for ampicillin, ceftazidime, cefotaxime, ceftriaxone, and cephazoline.

Molecular analysis of all isolated ESBLKp strains identified four PFGE patterns, which were designated as type A-D. PFGE type A was the epidemic clone and responsible for 28 (73.7%) infections and 43 (66.2%) colonizations. PFGE clone B was responsible for 7 infections and 22 colonizations. Of the 33 stains isolated from environmental samples, 23 (69.7%) belonged to type A and 8 (25%) to type B. The strains isolated from HCW cultures and pharyngeal swab cultures were PFGE type A.

Discussion

K. pneumoniae is an important hospital acquired pathogen with the potential of causing severe morbidity and mortality in pediatric patients. Outbreaks of infections due to ESBLKp have been reported in ICUs including NICU.[7,8] The most common reservoir for this pathogen seems to be the gastrointestinal tract of colonized patients, and patient-to-patient transmission is facilitated by transient or persistent hand carriage of healthcare workers.[9,10] In the present study, ESBLKp was found to be colonized in NICU patients before the infection.

In the study, we found that possible sources of ESBLKp included gastric tubes, incubators and HCWs. Moreover, the hands and throat swabs of medical staff carried the type A clone, which was not only found throughout the entire study (16 weeks), but also was the dominant strain for infected people (88.2%) and colonized patients (66.7%).

Gupta et al[11] found a dominant strain of *K. pneumoniae* on the hands of two medical staff in their

Table. Neonates infected with ESBLKp in the neonatal intensive care unit

Patient no.	Gestational age (wk)	Reason for admission	Admission date	Duration of hospitalization (d)	Infections	Isolation date	Sample positive for ESBLKp	PFGE type	Outcome
1	33	TTN	2008.2.18	45	Pneumonia	2008.2.26	Nasopharyngeal secretions	A	Cured
2	33	TTN	2008.1.31	38	Pneumonia	2008.2.26	Nasopharyngeal secretions	B	Cured
3	27	NRDS	2008.2.20	30	Pneumonia	2008.2.28	Nasopharyngeal secretions	D	Cured
4	30	NRDS	2008.1.31	42	Pneumonia	2008.2.29	Nasopharyngeal secretions	D	Cured
5	29	NRDS	2008.2.18	52	Pneumonia	2008.3.13	Nasopharyngeal secretions	A	Cured
6	29	NRDS	2008.1.20	21	Pneumonia	2008.3.14	Nasopharyngeal secretions	A	Cured
7	30	TTN	2008.2.18	67	Pneumonia	2008.3.17	Nasopharyngeal secretions	A	Cured
8	35	NRDS	2008.3.11	45	Sepsis	2008.3.17	Blood, nasopharyngeal secretions	A	Cured
9	30	Asphyxia	2008.2.21	50	Pneumonia	2008.3.17	Sputum	A	Cured
10	31	TTN	2008.3.5	42	Sepsis	2008.3.7	Blood, nasopharyngeal secretions	A	Cured
11	30	NRDS	2008.3.12	54	Sepsis	2008.4.9	Blood, nasopharyngeal secretions	A	Cured
12	29	NRDS	2008.3.18	63	Sepsis	2008.4.9	Blood, nasopharyngeal secretions	A	Cured
13	27	Asphyxia	2008.3.6	66	Pneumonia	2008.4.9	Nasopharyngeal secretions	A	Cured
14	30	NRDS	2008.3.27	42	Pneumonia	2008.4.9	Nasopharyngeal secretions	A	Cured
15	30	NRDS	2008.3.19	28	Pneumonia	2008.4.9	Nasopharyngeal secretions	A	Cured
16	30	NRDS	2008.3.16	40	Pneumonia	2008.4.9	Nasopharyngeal secretions	A	Cured
17	32	TTN	2008.3.31	15	Pneumonia	2008.4.9	Nasopharyngeal secretions	A	Cured
18	30	Asphyxia	2008.3.31	40	Pneumonia	2008.4.9	Nasopharyngeal secretions	A	Cured
19	28	NRDS	2008.3.3	103	Pneumonia	2008.4.9	Nasopharyngeal secretions	B	Cured
20	29	NRDS	2008.3.18	38	Pneumonia	2008.4.9	Nasopharyngeal secretions	A, B	Cured
21	29	Asphyxia	2008.3.5	62	Pneumonia	2008.4.9	Nasopharyngeal secretions	B	Cured
22	30	Prematurity	2008.3.16	45	Sepsis	2008.4.9	Blood, nasopharyngeal secretions	A	Cured
23	29	Asphyxia	2008.5.6	45	Pneumonia	2008.5.14	Nasopharyngeal secretions	A	Cured
24	31	Prematurity	2008.4.23	24	Sepsis	2008.5.14	Blood, nasopharyngeal secretions	A	Cured
25	33	Asphyxia	2008.4.29	21	Sepsis	2008.5.14	Blood	B	Cured
26	31	Asphyxia	2008.5.6	19	Pneumonia	2008.5.14	Nasopharyngeal secretions	A	Cured
27	31	NRDS	2008.4.15	29	Sepsis	2008.5.14	Blood, nasopharyngeal secretions	A	Cured
28	29	NRDS	2008.5.16	39	Pneumonia	2008.5.27	Nasopharyngeal secretions	B	Cured
29	30	Prematurity	2008.5.12	50	Sepsis	2008.5.29	Blood, nasopharyngeal secretions	A	Cured
30	31	NRDS	2008.5.9	39	Sepsis	2008.5.29	Blood, nasopharyngeal secretions	A	Cured
31	28	Pneumonia	2008.5.18	100	Pneumonia	2008.6.4	Nasopharyngeal secretions	A	Cured
32	30	NRDS	2008.5.18	72	Pneumonia	2008.6.4	Nasopharyngeal secretions	B	Cured
33	28	NRDS	2008.5.18	153	Pneumonia	2008.6.5	Sputum	A	Died
34	32	Asphyxia	2008.5.21	32	Sepsis	2008.6.9	Blood, nasopharyngeal secretions	A	Cured
35	28	NRDS	2008.6.5	32	Sepsis	2008.6.11	Blood, nasopharyngeal secretions	A	Cured
36	30	NRDS	2008.6.2	37	Sepsis	2008.6.11	Blood	D	Cured
37	34	Prematurity	2008.6.1	30	Sepsis	2008.6.11	Blood	B	Cured
38	32	NRDS	2008.6.6	33	Pneumonia	2008.6.14	Nasopharyngeal secretions	A	Cured

ESBLKp: extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*; NRDS: neonatal respiratory distress syndrome; TTN: transient tachypnea of newborn; PFGE: pulsed-field gel electrophoresis.

investigations into the outbreak of *K. pneumoniae* in an NICU. Cassettari et al[12] considered the prevalence of *K. pneumoniae* for 6 months in an NICU was due to a staff member suffering from onychomycosis. Research into the frequency of contact of ICU patients with the medical staffs revealed that the medical staffs were in direct contact with patients 159 times per day (95% CI: 144-178/day) and experienced indirect contact with patients 191 times per day (95% CI: 174-210/day).[13] Therefore, the hands of the medical staffs play an important role and function in preventing and controlling healthcare associated infections. Mechanical ventilation, total parenteral nutrition administrations, duration of hospitalization, and central venous catheter use may also serve as risk factors in this outbreak in NICU.

The PFGE typing for the 33 strains of *K. pneumoniae* from environmental samples showed that type A clone still took the dominant place (69.7%) among the four genotypes from A to D. Type C and D clones only occupied a small proportion (12.5%) and appeared at the end of the outbreak, which may be attributed to incomplete cleaning and sterilization of the instruments used in the NICU. Strategies required to control outbreaks of infections include strict use of antibiotics for the medical staff, application of sensitive antibiotics

according to the antibiogram results, isolation and cohort of infected and colonized patients, transmission precautions, and surveillance of patients, environment and HCWs. In addition, hand hygiene must be stressed before and after handling with each patient, before and after use of any invasive device, and before entering and upon leaving isolation areas.

In conclusion, ESBLKp can leads to serious outbreaks of infections in neonates. Strict measures for infection control of ESBLKp should be taken in hospitals. More importantly, preventive measures are necessary to control nosocomial infections in hospitals.

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