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Field Epidemiology Training Program, Bureau of Epidemiology
Department of Disease Control, Ministry of Public Health, Thailand
Tel: +6625901734-5, Fax: +6625918581, Email: osireditor@osirjournal.net, http://www.osirjournal.net

An Outbreak of Multi-drug Resistant Enterotoxigenic *Escherichia coli* (ETEC) Infection among Infants in a City of Southwest China, 2012

Xi Chen[1,2], Yuan W[1,3], Zhang LJ[1,*], Li Q[2], Zhang ZD[2], Wang H[2], Sun YJ[1,4]

1 Chinese Field Epidemiology Training Program, Chinese Center for Disease Control, Beijing, China

2 Zigong Center of Disease Control, Sichuan Province, China

3 Sichuan Center of Disease Control, Sichuan Province, China

4 Zhuhai Center of Disease Control, Guangdong Province, China

*Corresponding author, email address: cfetpzlj@126.com

Abstract

Multi-drug resistant (MDR) diarrheagenic *Escherichia coli* has rapidly spread worldwide and represents the most serious threat to management of diarrhea in developing countries. We investigated an outbreak of severe diarrhea in a neonatal ward during 1 Apr-30 Jul 2012, where 60 suspected cases were found. To identify possible sources of infection, we conducted a case-control study through which we identified the increased infection risk of 4.6 fold for each liter of bottle milk feeding (OR = 4.6, 95%CI = 1.50-14.70). *E. coli* serotype O128:H45 with virulence gene stIb was found in 14 out of 18 stool or diaper swab samples and were MDR. This was a neonatal diarrhea outbreak caused by multi-drug resistant *E. coli* while bottle milk feeding was the possible vehicle in facilitating the transmission. This outbreak reinforces that enterotoxigenic *Escherichia coli* (ETEC) should be considered when the clinical picture is consistent and common gastrointestinal pathogens are not found.

Keywords: multi-drug resistant, ETEC, hospital-associated infections

Introduction

Enterotoxigenic *Escherichia coli* (ETEC) is responsible for large proportion of infant diarrhea, especially in developing countries.[1,2]ETEC produces one or both of two enterotoxins: heat-labile enterotoxin and heat-stable enterotoxin. Diseases caused by ETEC may range from mild diarrhea to severe diarrhea similar to cholera, with profuse watery diarrhea, abdominal cramp, fever, nausea, chills, loss of appetite, headache, muscle aches and, finally, dehydration from loss of fluid.[3]

ETEC produces an immunologic protective response, reflecting the observation that attack rates are higher in children and decrease with age.[4,5] People are usually infected by ETEC through contaminated food and water consumption, and breastfeeding provides some protection in infants.[6] Researches on ETEC that target diarrhea patients suggested 4-6% prevalence in China.[7,8]

On 24 Jun 2012, Center of Disease Control (CDC) in Zigong City received a report from Hospital A, indicating that its neonatal ward received three patients with severe diarrhea. Symptoms of the patients were cholera-like and two had developed severe dehydration. Bacteria culture of stool specimens and rotavirus tests were negative and empiric antimicrobial treatment (amoxicillin, cefotaxime and piperacillin) failed. Two out of the three patients were hospitalized in Hospital B before onset of illness. The local health bureau conducted an investigation to determine whether this event was an outbreak, identify the pathogen and infection sources, and implement control measures.

Methods

Initial Investigation and Case Definition

Zigong is an industrialized city in the southwest of China (Figure 1), with a population of about three million. There were four hospitals that had neonatal wards in Zigong. The investigation team included experts in epidemiology, pediatrics, hospital infection control and laboratory testing. The team searched for cases by probing medical records in all neonatal wards of Zigong from 1 Apr 2012.

A suspected case was defined as a hospitalized neonate aged less than 28 days with loose stool seven or more times, or watery stool one or more times

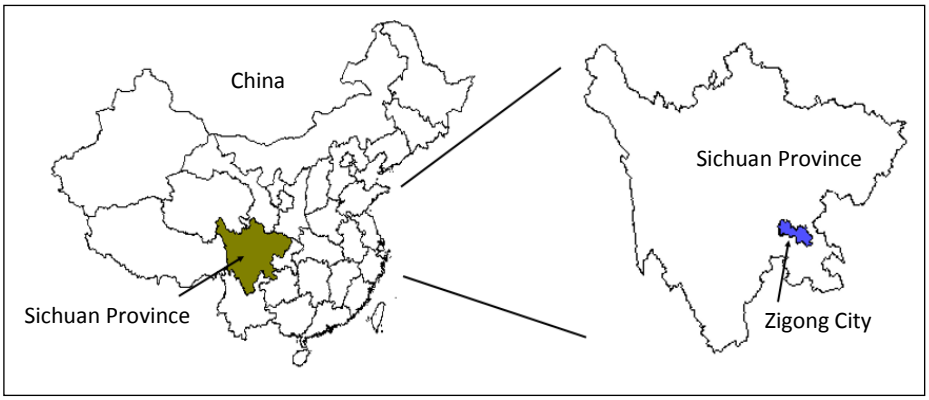

Figure 1. Location of Zigong City, Sichuan Province, China

within 24 hours during 1 Apr-30 Jul 2012 in Zigong City. A confirmed case was defined as a suspected case with stool specimen tested positive for *Escherichia coli* (*E. coli*) O128:H45. As the patients might have been hospitalized more than once, we defined 12 to 48 hours before onset of illness as the exposure period to identify the exposed hospital. In this way, all patients could have only one exposed hospital under this definition.

Environmental and Epidemiological Investigation

The investigation team reviewed the supply records of milk powder and water, and collected (both opened and sealed) milk powder samples for microbial testing. The team checked the layout of neonatal ward and observed milk preparing process, bottle feeding and hand hygiene behavior of the nurses in neonatal ward of Hospital B.

Case-control Study

The investigation team matched medical records of Hospital B by hospitalization date in a case-control study. A control was defined as a patient without diarrhea who was hospitalized in the neonatal ward during exposed period of a corresponding infected cased. Each case was matched with two controls. Variables collected were gender, age, birth weight and volume of bottle milk feeding.

Microbiological Testing

The investigation team also collected clinical samples from patients, nurses and doctors, and environmental samples from the neonatal ward of Hospital B. The collected samples were transported immediately to the laboratory in the municipal CDC for testing.

Stool and diaper swab samples were tested for pathogenic *E. coli*, *Listeria monocytogenes*, *Enterobacter sakazakii*, *Bacillus cereus*, *Salmonella*, *Shigella*, *Vibrio cholera* and *Vibrio parahaemolyticus* according to Chinese testing standards.[9] Environmental swabs from neonatal wards of Hospitals A, B, C and D were tested for bacterial count and pathogenic *E. coli*. In addition, milk powder from the hospitals were collected and tested for bacteria culture using tryptic soytone broth produced by China Beijing Luqiao Tech Co. Limited.

Pathogenic *E. coli* was isolated by CHROMagar ECC medium (produced by China Zhenzhou Biocell Biotech Co. Limited) and pathogenicity of isolated clones was identified by API 20E biochemical identification system (produced by France bioMérieux). Then, isolated clones were typed by O and H diagnostic serum (first typed with the serum produced by China Tianrun Biomedical Co. Limited and then confirmed by serum produced by Denmark Serological Research Institute). Finally, polymerase chain reaction (PCR) testing was done using *E. coli* virulence genes stx1, stx2, eaeA, lt, stIb, aggR, ipaH, rrs sequence probes.[10] In addition, drug resistance was tested by Etest method, according to Clinical and Laboratory Standards Institute standard 2009[11].

Data Analysis

The data was analyzed by Epi Info7[12]. The analytical methods were Fisher's exact test and matched logistic regression (Binary logistic regression, forward and stratified by match group).

Results

The investigation team identified a total of 532 patients who were admitted in four hospitals during 1 Apr-30 Jul 2012, with 60 suspected cases and 13 confirmed cases. Among the suspected cases, 38 (63.3%) had diarrhea seven times or more within 24 hours while 48 (80.0%) had watery stool and 10 (1.7%) had axillary temperature more than 38°C. However, none of them had mucus stool. There were 44 (73.3%) suspected cases hospitalized in Hospital B. Attack rate of the neonatal ward of Hospital B was significantly higher than the baseline of Zigong and the other three hospitals (Table 1). Geographic distribution of cases' residence scattered throughout Zigong and two nearby cities (Figure 2).

Table 1. Distribution of cases in Zigong City, Sichuan Province, China, 1 Apr-30 Jul 2012

Hospital	Number of neonate	Number of case	Attack rate (%)	Relative risk (95%CI)
B	285	44	15.4	228.5 (110.3-473.3)
A	57	2	3.5	45.5 (9.6-215.5)
C	161	1	0.6	7.8 (1.0-61.7)
D	29	0	0	-
Not hospitalized	11272*	9[#]	0.2	reference

** Average population growth in Zigong City was 2,818 per month in 2010.*

[#]Four cases had no hospitalization history during exposure period and lived outside Zigong City.

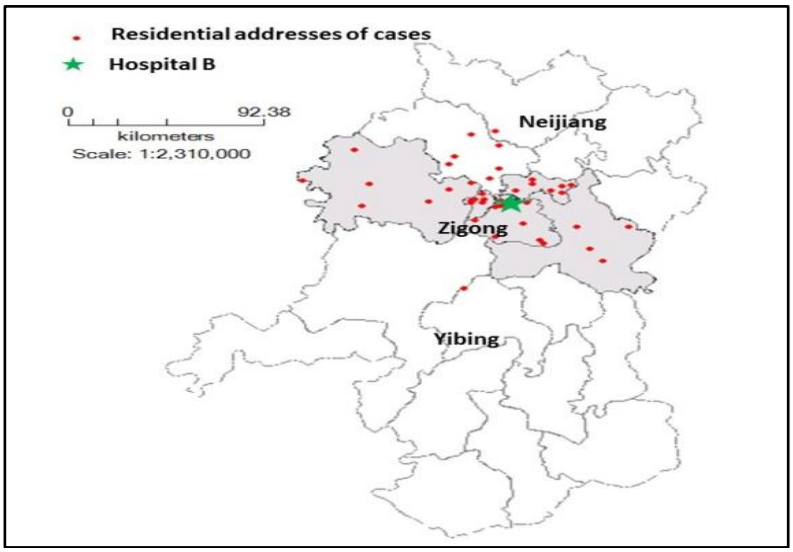

Figure 2. Distribution of cases by residential address in Zigong City, Sichuan Province, China, 1 Apr-30 Jul 2012

Out of 44 cases in Hospital B, 23 (52.3%) were males, with average birth weight 3.2 kilogram (median 3.1 kg, range 2.1-4.3 kg),and average age at the onset of symptoms was 10.4 days (median 9 days, range 3-28 days).

Environmental and Epidemiological Investigation

The investigation team identified that bacterial contamination of baby incubator button, sink edge and bed label, was higher than the acceptable standard levels (less than 5 CFU/m^2)[13] in the neonatal ward. The milk preparation room, neonatal bathroom and toilet were located nearby and used the same water supply and sewer pipes (Figure 3). Nurses prepared milk by mixing boiling water with pre-cooled boiled water in a measuring cup before adding milk powder. Nurses cared for neonates with bare hands and washed hands with cleaning solution before changing to care another neonate. One nurse might perform various duties of patient care, such as milk preparation, manual feeding and bathing during one shift. A neonate with diarrhea was not isolated because the isolation room was occupied by other patients.

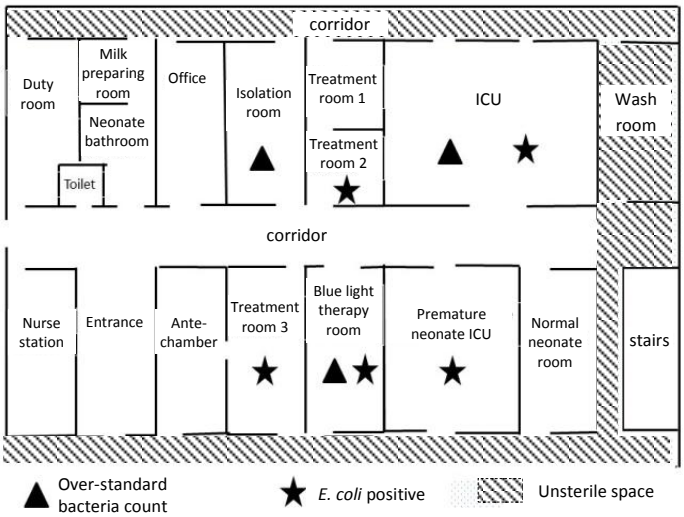

Figure 3. Ward layout and distribution of bacterial contamination in neonatal ward of Hospital B, Zigong City, Sichuan Province, China, 1 Apr-30 Jul 2012

Case-control Study

In the case-control study, 44 cases were matched to 82 controls. The study revealed that risk of infection increased 4.6 fold for each liter of bottle milk feeding (OR=4.6, 95% CI=1.50-14.70) (Table 2). The transmission chain revealed the expose period of most cases (93.2%, 41/44) overlapped with at least one case's diarrhea period (Figure 4).

Microbiological Testing

The clinical samples were classified by the exposed hospitals. Since Hospital B stopped receiving diarrhea patients on 19 Jun 2012, many patients went to Hospitals A and C. Thus, samples from exposed patients admitted in Hospitals A and C were collected as well. The investigation team isolated 14 pathogenic *E. coli* clones from 18 stool or anal swab samples (15 from Hospital B, 2 from Hospital A and 1 from Hospital C). Among which, 13 clones were serotype O128:H45, including 11 exposed in Hospital B and the other two in Hospitals A and C. We found heat-stable enterotoxin gene stIb in all 13 clones. These clones exhibited full resistance to amoxicillin, piperacillin, cefalotin, cefotaxime and sulfamethoxazole; intermediate resistance to ceftazidime; and sensitive to amoxicillin-clavulanic acid, piperacillin-tazobactam, cephalosporin thiophene, cefepime, meropenem, imipenem, gentamicin, kanamycin, amikacin, tobramycin, norfloxacin, levofloxacin, tetracycline and chloramphenicol. Physical features of milk powder met the related Chinese regulation standards and were negative for bacteria culture. Among 11 stool or anal swab samples from doctors and nurses working in the neonatal ward of Hospital B, all were pathogenic *E. coli* negative.

Table 2. Case-control study in neonatal ward of Hospital B, Zigong City, Sichuan Province, China, 1 Apr-30 Jul 2012

	Average		Odds ratio	95% CI	P-value
	Case	Control			
Body weight (kg)	3.2	2.8	2.9	1.30-6.20	<0.01
Milk feeding (liter/24 hours)	0.6	0.4	4.6	1.50-14.70	<0.01
Age (day)	10	9	1	0.98-1.11	>0.05
Gender ratio (male)	1.3	1.1	1.2	0.60-2.40	>0.05

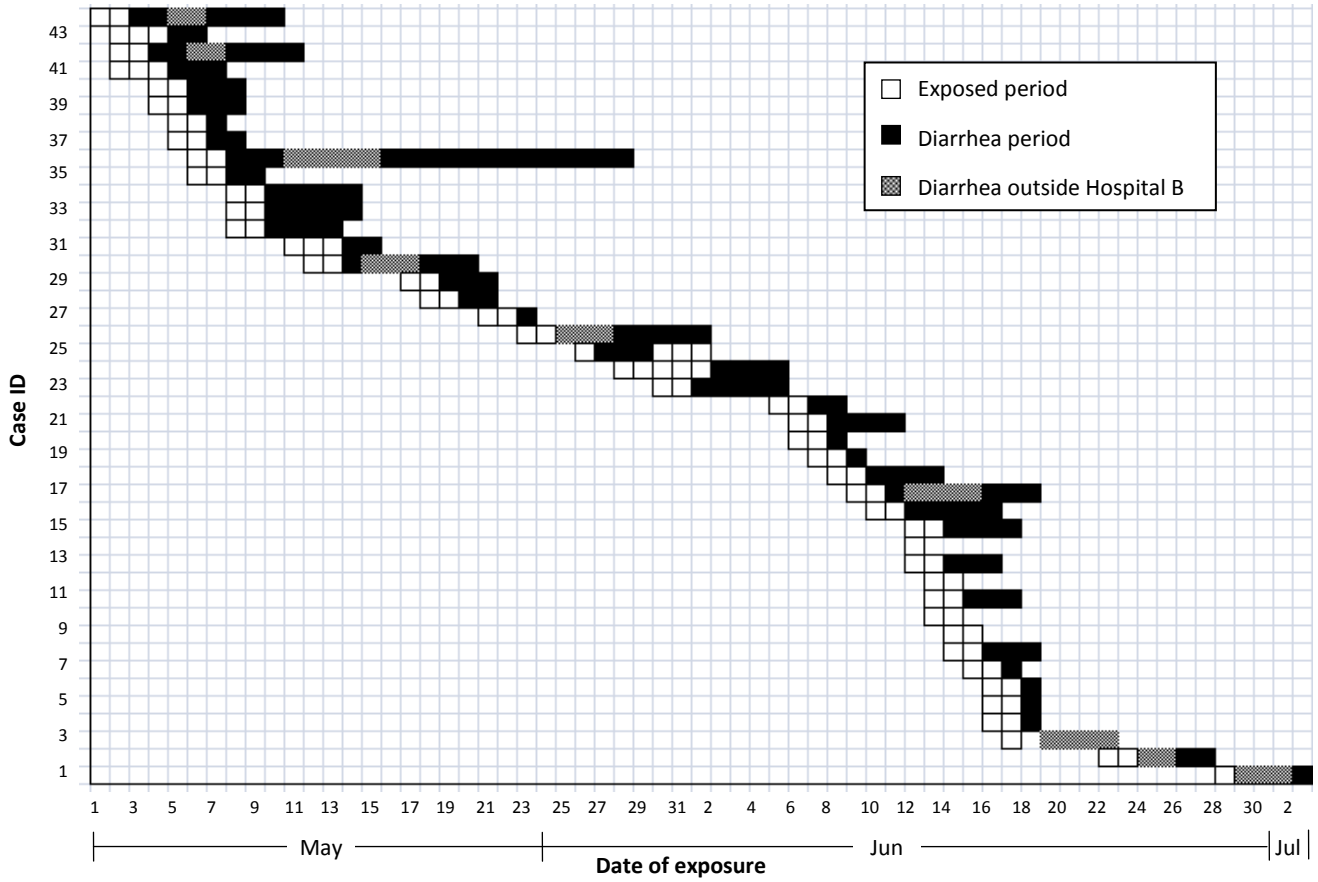

Figure 4. Exposure and diarrhea period of cases in neonatal ward of Hospital B, Zigong City, Sichuan Province, China, 1 May-2 Jul 2012

Outbreak Management

Hospital B noticed an unusual rise of diarrhea patients in the neonatal ward in early May 2012. Therefore, they reinforced hand hygiene management, and changed the brand of milk powder and water source from tap water to bottled water. Nevertheless, new cases were still increasing. In addition to fluid infusion, Hospital B administered antibiotics and antiviral drugs, which had little effect. Hospital B later reduced frequency of milk feeding to patients in early June 2012, which led to a drop of patients with diarrhea. The number of cases reached its peak on 15 Jun 2012 and the neonatal ward in Hospital B stopped admitting new diarrhea patients on 19 Jun 2012. The number of new cases dropped sharply thereafter (Figure 5).

Epidemiological investigation began on 25 Jun 2012 and control measures were implemented by local health bureau according to the recommendations of the investigation team on 26 Jun 2012. Control measures included treating cases with ceftazidime (50-100 mcg/kg) and placing in isolated rooms, dividing nurses in each neonatal ward into two teams with one team provided care specifically for patients with diarrhea, and redesigning and sanitizing the milk preparation room in neonatal ward of Hospital B. All cases recovered soon after and the last case was reported on 1 Jul 2012.

Discussion

Acute infectious diarrhea is the second most common cause of death in children living in developing countries.[3] Major etiologic agents that account for the estimated 1.5 million deaths per year include ETEC, rotavirus, *Vibrio cholerae* and *Shigella spp.*[14,15]Diagnosis of ETEC depends upon identification of enterotoxin genes, which is fastidious and expensive. None of laboratories in four hospitals in Zigong City included ETEC identification in their routine laboratory tests, resulting in late diagnosis of the pathogen. In this outbreak, although symptoms of cases were distinguishable from the usual neonatal diarrhea, case records were not systematically collected and analyzed in Hospital B until the investigation. Doctors responsible for hospital infection control should include ETEC infection as part of their differential diagnoses when facing clusters of patients with cholera-like diarrhea.

Drug resistance was widely reported and discussed for ETEC infection control.[16,17] In this outbreak, multi-drug resistance of the responsible ETEC clone not only hampered the treatment of patients, but also misled the investigation to consider viral infections as the cause of diarrhea since there was little effect following antibiotic treatment.

Based on the medical records and before identification of pathogens by laboratory, the board of pediatricians determined to use high doses of ceftazidime to treat patients and found instant effect in spite of the fact that this ETEC clone exhibited intermediate resistance to ceftazidime. Antibiotic may stimulate further verotoxin production by *Shigella*-like toxin-producing bacteria such as enterohemorrhagic *Escherichia coli* (EHEC) and thereby increasing the risk of hemolytic uremic syndrome (HUS). It was worth to mention that the pediatricians excluded the risk of inducing HUS before using antibiotic treatment because low fever and absence of mucus stool strongly suggested that the symptoms could be caused by exotoxin. High doses of new generation antimicrobials might be a substitutive option to treat diarrhea in children with cholera-like symptom where ETEC test methods yet to be established.[3]

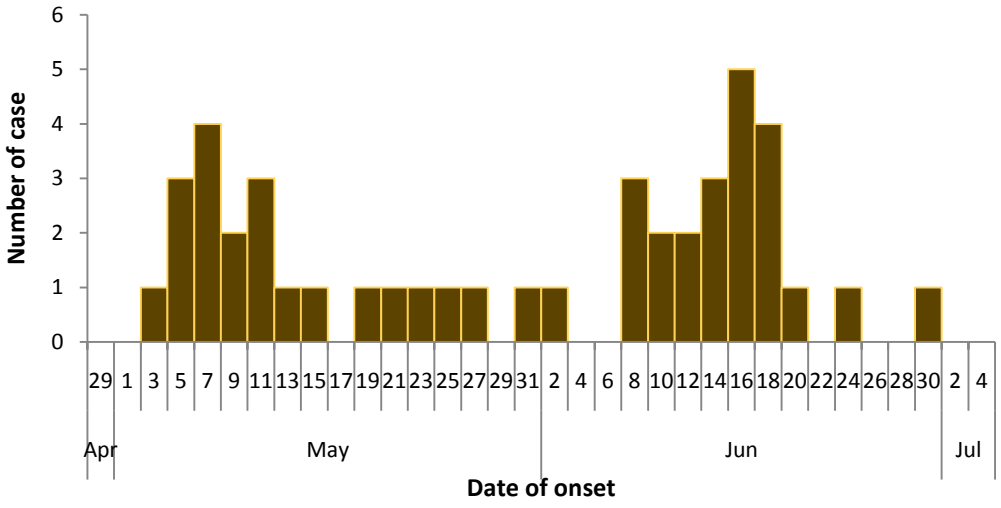

Figure 5. Number of patients by date of onset in Hospital B, Zigong City, Sichuan Province, China, 29 Apr-4 Jul 2012

ETEC usually infects patients through consumption of contaminated food or water.[3] Changing water and milk powder did not control the outbreak, which indicated that the water and milk powder were not the source of infection. ETEC infecting dose is high (106-1010 CFU), with lower doses being less pathogenic.[18] Though the definition of controls could not exclude patients who might be infected with ETEC, the case-control study provided strong evidence that the diarrhea cases were most likely associated with bottle milk feeding as attack rate of the patients with more body weight (often need more milk feeds) was significantly higher than that of the patients with lower body weight. Considering the milk preparation room of this neonatal ward was placed next to the bathroom and the nurse prepared milk at appropriate feeding temperature by mixing pre-cooled boiled water with boiling water, the main contamination source of ETEC might be the measuring cup used to measure the pre-cooled water and mixed milk powder at temperature not high enough to kill bacteria.

Bottle milk feeding alone could not explain failure in hygiene enforcement of Hospital B and the confirmed cases who were exposed in Hospitals A and C. There were also reports stating that ETEC could be transmitted by contacts.[19] Though the neonates were not isolated in separate rooms, they were prevented from direct contact by being kept in baby incubators. After we ruled out the water and milk powder contamination, nurses and health care set-up were the remaining possible vehicles to facilitate *E. coli* transmission. Isolation of not only patients, but also nurses who took care of diarrhea patients might be a practical supplement to prevent *E. coli* hospital infection. Although reducing diarrhea patient density might be another option to interrupt the transmission chain as Hospital B did, that might drive patients to go to other hospitals and increase the risk for a larger outbreak and social problems.

The biggest challenge of this outbreak investigation was to identify cases and exposed hospitals because neonates often have physiological diarrhea and go to hospitals very often. It was a successful experience that the epidemiologists consulted pediatricians from either the investigation team or staff from the involved hospitals when defining case and exposed period. Main limitation of this investigation was that although this ETEC clone was multi-drug resistant which implied that it probably evolved in Hospital B or another antimicrobial-rich environment, the investigation did not identify the clone as having evolved in Hospital B or brought in by a patient. The investigation team did not find direct evidence of milk contamination because disinfection and changing of milk preparing instruments were already conducted in Hospital B after mid-June 2012.

Neonatal wards are confined environment with vulnerable patients, where ETEC outbreaks may cause severe consequences. Access to ETEC identification methods should be established to provide laboratory testing in hospitals with neonatal ward.

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Suggested Citation

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