The evaluation of clusters of hospital infections due to multidrug-resistant *Salmonella enterica* serovar typhimurium in the neonatal unit: a two-year experience

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Seven clusters of hospital infection due to *Salmonella enterica* serovar typhimurium were documented in the neonatology clinic of a children’s hospital between April 2002 and March 2004. Eighty-one neonates were infected. Three cases were asymptomatic, 73 cases had gastroenteritis as the only clinical condition, and 5 cases had bacteremia associated with gastroenteritis. All isolates from stool and blood samples (n=86) were identified as *Salmonella enterica* serovar typhimurium.

Extended-spectrum beta-lactamase (ESBL) production was determined by clavulanate disk potentiation assay in all isolates. Enterobacterial Repetitive Intergenic Consensus polymerase chain reaction (ERIC-PCR) was performed in 26 selected isolates, which were chosen as being representative of different clusters, to determine the clonal relationship. PCR, isoelectric focusing and sequence analysis revealed the production of CTX-M-3, TEM-1 and SHV-12 by these isolates in 23%, 76.9% and 100%, respectively. None of the isolates had PER β-lactamase production.

Standard infection control measures such as handwashing and disinfection procedures were implemented in initial clusters. During the two-year period, the infection control policy of the hospital was improved with appropriate actions such as assignment of an infection control nurse and increasing the number of staff of the clinic, and finally, with the establishment of an active surveillance program, the clusters were stopped.

Key words: *Salmonella enterica* serovar typhimurium, neonatal unit.

As an agent for healthcare salmonella outbreaks, *Salmonella* is one of the most serious problems for neonatology clinics due to the long incubation period, high proportion of asymptomatic infections and prolonged carriage of *Salmonella* species in infants. Sporadic infections in newborns can evolve easily to outbreaks, and due to its persistence in the environment, clearance of the agent from the unit would be complicated. In addition, as a member of the family of *Enterobactericeae*, the increased antimicrobial resistance of *Salmonella* is another important problem in these cases1,2.

In this report, clusters of hospital infections due to extended-spectrum beta-lactamase (ESBL) producing *Salmonella enterica* serovar typhimurium (*S. typhimurium*) in a neonatology clinic are evaluated, and the infection control management and microbiological data of the infection clusters are discussed.

Material and Methods

Between April 2002 and March 2004, several clusters of hospital infection in a neonatal unit of a children’s hospital were investigated.
Eighty-one affected neonates presenting gastroenteritis were included into the study.

**Isolates:** Eighty-six Salmonella isolates (81 feces, 5 blood isolates) from 81 newborns with ESBL activity and which were recovered from newborn patients during a Salmonella outbreak in the neonatal and premature intensive care units in Behçet Uz Children’s Hospital, Izmir, Turkey were included in the study. All isolates were identified by conventional techniques, confirmed by the API 20E system (bioMérieux, France) and serotyped with respect to somatic (O) and flagellar (H) antigens using commercial antisera (Refik Saydam Hygiene Center, Turkey). As all isolates were identical in serotyping and had the same antimicrobial resistance profile, 26 Salmonella isolates that were considered as representative of the different clusters were selected randomly for advanced molecular microbiological analysis.

**Antibiotic Susceptibility Tests:** Susceptibility patterns of all the isolates and their transconjugants to ampicillin (AM), amoxicillin-clavulanate (AMC), ceftazidime (CAZ), cefotaxime (CTX), aztreonam (ATM), imipenem (IPM), cefoxitin (FOX), ciprofloxacin (CIP), gentamicin (G), amikacin (AK), tetracycline (TE), and chloramphenicol (C) were determined by Clinical and Laboratory Standards Institute (CLSI) disk diffusion method. Minimal inhibitory concentration (MIC) of CTX and CAZ either alone or with clavulanate (4 mg/L) was evaluated by CLSI microdilution method. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

**Detection of ESBL Activity:** Extended-spectrum beta-lactamase (ESBL) activity was screened by CLSI disk diffusion breakpoints for ESBLs (ATM ≤27 mm, CAZ ≤22 mm, CTX ≤27 mm), and confirmed by clavulanate disk potentiation assay.

**Conjugation Experiments:** *E. coli* K-12 strain J53-2, which had been mutated to rifampicin resistance, was used as the recipient. The selected isolates were investigated for their ability to transfer their resistance determinants to the recipient strain as described previously. The transconjugants were selected on Mueller-Hinton agar plates containing CTX (2 mg/L) and rifampicin (50 mg/L).

**Plasmid Analysis:** Plasmid DNA from the selected isolates and transconjugants was isolated by the method of Kado and Liu and separated by electrophoresis in a 0.6% gel with Hind III digests of bacteriophage lambda DNA as molecular size markers.

**Beta-Lactamase Extraction and Isoelectric Focusing (IEF):** Crude beta-lactamas were prepared as described previously. The enzymes were identified by analytical IEF by comparison with known beta-lactamas (TEM-1 pl: 5.4 and SHV-2 pl: 7.6). Beta-lactamase bands were visualized by staining with nitrocefin (500 mg/L) (Fig. 1).

**Molecular Fingerprinting:** The clonal relationship of the selected isolates was determined by Enterobacterial Repetitive Intergenic Consensus polymerase chain reaction (ERIC-PCR) by using the ERIC-2 primer (5’AAG TAA GTG ACT GGG GTG AGC G 3’). Detection of blaCTX-M: A 550 bp segment of blaCTX-M was amplified using primers (5’ CGC TTT GCG ATG TGT GCA G 3’ and 5’ ACC GCG ATA TCG TTG GT 3’) (6). The amplification program consisted of 30 cycles of denaturation at 94°C for 1 minute (min), annealing at 40°C for 1 min, and extension at 72°C for 2 min. Amplicons were visualized after electrophoresis on 1% agarose gels containing ethidium bromide (EtBr). Plasmid DNA, which had been eluted from 54 of the selected transconjugants, was also used in blaCTX-M PCR.

**DNA Sequencing:** Direct sequencing of the complete blaCTX-M gene was done on both strands as described by Macrogen Inc (Korea) using an automated DNA sequencer (ABI Prism 310) according to the method described by Gniadkowski et al.

**Results**

**Clusters of Hospital Infection**

Seven clusters of hospital infection due to *S. typhimurium* were observed in 81 neonates between April 2002 and March 2004 (Fig. 1). Thirty-seven (45.7%) were premature while 44 (54.3%) were mature neonates. Forty-one (50.6%) cases were female and 40 (49.4%) were male.
In four clusters, the index cases could be determined, but the source of the first cluster was not clearly described. All index cases were admitted to the hospital for the first time except for one neonate who was referred from another hospital. Three cases were asymptomatic, 73 cases had gastroenteritis as the only clinical picture, and 5 cases had bacteremia associated with gastroenteritis. In 3 of them, death resulted from serious underlying diseases, such as neural tube defect (n=2) and hepatic coma (n=1), but in 1 case, admitted with hyperbilirubinemia, septic shock was determined as the cause of death.

**Infection Control Management**

Standard infection control measures such as handwashing and disinfection procedures were implemented in initial clusters. After the fourth cluster, an infection control nurse was assigned and education programs for infection control were updated. The staff of the neonatology unit and associated units plus the mothers of neonates were screened for Salmonella carriage. Microbial contamination was searched in the water supply system of the hospital. In the fifth cluster, a technical breakdown was detected in the sterilizer for feeding bottles. Following the fifth cluster of infection, the neonatal unit was closed completely after the discharge of all patients. The clinic was reopened with overstaffed and improved clinical conditions. Nevertheless, a new outbreak developed due to a neonate previously treated and followed in the unit. Then, an active surveillance program was planned and began to be performed constantly.

Although a seventh cluster developed, additional clusters of infection were prevented. No further case or cluster of Salmonella infection has been observed to date.

**Microbiological Data**

All isolates (n=86) were identified as *S. enterica* serovar typhimurium. ESBL production was detected in all isolates. While all were resistant to CAZ (MIC >256 mgL⁻¹), CTX (MIC 128->256 mgL⁻¹), chloramphenicol and gentamicin, no resistance to fluoroquinolones or meropenem was detected. ERIC-PCR patterns of the selected isolates (n=26) were identical (Fig. 2). PCR, IEF and sequence analysis revealed the production of CTX-M-3, TEM-1 and SHV-12 by these isolates in 23%, 76.9% and 100%, respectively. None of the isolates had PER β-lactamase production.

**Discussion**

Newborns and prematures are at increased risk of infection because of many factors, such as immaturity of the immune system, fragility of the cutaneous barrier and lack of endogenous microflora. It was reported that Salmonella infections are relatively rare in newborns, but because of the high potential of spread of these microorganisms, nosocomial outbreaks due to enteric pathogens can occur. While the mothers were generally the suspected index cases, subsequent cases are the result of contaminated objects in the nursery environment serving as a reservoir coming into contact with the hands of attending personnel. Feeding formulas and medical equipment were reported as the sources for Salmonella outbreaks in neonatal units. Even though...
index cases were found in four clusters, the index case for the first cluster could not be determined in our study. One of the neonates with bacteremia was suggested as the index case but no confirmation could be done. Hands of healthcare personnel were reported to serve as the main contamination source as a consequence of several manipulations in the neonatal clinic11. Although many precautions, such as patient isolation, strict handwashing and disinfection of the clinic, were conducted, overcrowding and understaffing of the clinic caused the subsequent clusters. Determination of the index cases in the following clusters as mostly newly admitted neonates and no detection of Salmonella carriage and absence of any iatrogenic infection source suggested the probability of community-acquired multidrug–resistant (MDR) *S. typhimurium* infections.

It was stated that the early introduction of infection control measures in neonatal clinics can prevent the outbreaks12. During the two-year period of this study, the infection control policy of the hospital was improved with appropriate actions such as assignment of an infection control nurse and overstaffing of the clinic, and finally, with the establishment of an active surveillance program, the clusters were stopped.

As the incidence of infections had increased substantially in many countries in recent years, MDR *S. typhimurium* has been recognized as a serious public health problem since 1988, when the resistance to extended-spectrum cephalosporins was first recognized13-15. MDR *S. typhimurium* strains have been reported since the 1970’s in our country16. In 2001, ESBL production of Salmonella isolates from Turkey was determined in 15%17. In a recent report from Turkey, the high incidence of multidrug resistance among *S. typhimurium* isolates, at a rate of 76%, was emphasized18. Microbiological studies revealed that in our isolates, the clusters have originated from the identical strain of MDR *S. enterica* serovar Typhimurium. Although CTX-M-3 and TEM-1 production was detected partially, the production of SHV-12, a novel ESBL first described in 199719, was revealed in all isolates. Another study from Turkey also reported SHV-2a and SHV-5a production in Salmonella species isolated in children20. SHV-12 production in nontyphoid Salmonella was reported in different studies from different countries, and it was pointed out that nontyphoid Salmonella producing TEM or SHV ESBLs may have changed bla genes with other Enterobacteriaceae frequently encountered in hospitals21,22.

Although it was stated that age younger than 3 months is no longer a single indication for antibiotherapy23, the evidence regarding the increased risk of developing bacteremia in patients infected with multiple resistant Salmonella strains24 prompted us to administer antibiotherapy in all the patients. The relatively lower incidence of bacteremia (6.1%) in our cases, in contrast to previously reported rates of developing bacteremia in infants and young children with Salmonella gastroenteritis (6.5%-24.3%)25, may be considered as the result of the antibiotherapy. Due to the limited experience with the usage of fluoroquinolones in neonates, meropenem was considered as the single choice for our patients.

As emphasized by reporting the results of the first cluster, we believe this to be the first report of SHV-12 producing Salmonella from Turkey. However, dissemination of new MDR Salmonella strains must be expected in the future, which will reduce the therapeutic options.

REFERENCES


