Nosocomial *Citrobacter* Infection in Neonatal Intensive Care Unit in a Hospital of Nepal

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Abstract

Introduction: Neonatal *Citrobacter* infection is either acquired horizontally or vertically as a nosocomial infection. The source of nosocomial *Citrobacter* is either hands of medical staff or the innate objects. **Objective:** The aim of this study was to study nosocomial *Citrobacter* infection in neonates admitted in Neonatal Intensive Care Unit (NICU) and trace the source of infection. **Methods:** The study was conducted in NICU in a hospital in Kathmandu, Nepal during a period of January to March 2010. Specimens were collected from neonates, hands of medical staff and innate objects and were processed using a standard microbiological method. **Results:** The prevalence of neonatal nosocomial infection was 32.6% (29/89). *Citrobacter* spp. was isolated in 11 neonates admitted in NICU with the prevalence rate of 37.9% (11/29) among other pathogens. Umbilical cord infection was most common (n=8). These isolates were grouped into five antibiotypes (I, 4; II, 3; III, 2; IV, I; V, 1). All of these isolates were multi-drug resistant showing susceptibility towards quinolones. The isolate of *Citrobacter* spp. was also recovered from a nasal prong which was grouped with 4 other clinical strains. **Conclusion:** Multi-drug resistant nosocomial *Citrobacter* spp. was inflicting neonates in NICU and the source of this pathogen was traced to nasal prong. Nosocomial *Citrobacter* infection is a common problem of neonates in NICU. This will lead to increase neonatal mortality if infection prevention and control practices are not initiated.

Key words: Neonates, Citrobacter spp., nasal prong, infection control, Nepal

Introduction

The bacteria of the genus *Citrobacter* are occasional inhabitants of soil, sewage, water, and food and also an infrequent colonizer of human and animal gastrointestinal tract[1]. In most occasions these *Citrobacter* spp. are of low virulence and do not cause disease but they can be a source to multiple infections, like respiratory tract, urinary tract, intra-peritoneal, wound, sepsis, meningitis, and brain abscess[2]. This pathogen inflicted neonates in health care settings in 1978 and since then this has emerged as a successful nosocomial pathogen of neonates[1-3]. The surveillance of *Citrobacter* infection in American medical centers showed the prevalence rate of 0.8% among Gram negative infections while this accounted for 3-6% among *Enterobacteriaceae* in a hospital setting[4,5]. This pathogen is an emerging nosocomial pathogen across the globe[4,6,7] and has also been recovered from sputum, surgical wound infection and otitis media in Nepal[8-11].

Although, *Citrobacter* infection has been described in adults, this is also a major problem in neonates admitted in Neonatal Intensive Care Units (NICU)[12,13]. They develop sepsis and meningitis and are at higher risk of developing cerebral abscess. Neonates and infants are also at risk of developing osteomyelitis, septic arthritis, lung abscess, skin infection and urinary tract infection[14-18].

The infection in neonates are either horizontally transferred as a nosocomial infection or vertically

transferred from mother during delivery[18,19]. The source of nosocomial *Citrobacter* infection is attributed to various nate and innate objects (hand of medical staff, water for injection, face mask, nasal prongs, stethoscopes etc.) in NICU[20]. Septicemia is one of the major killers of neonates, but *Citrobacter* spp. sepsis has rarely been described in Nepal[21,22]. *Citrobacters* along with other pathogens from various specimens were frequently isolated from neonates admitted in NICU of the study site during January to March 2010. Hence, we studied the nosocomial *Citrobacter* infections in neonates in NICU in a hospital in Kathmandu, Nepal and traced the source of this pathogen.

Material and Method

Study settings, samples and bacterial identification

This cross-sectional study was conducted in Department of Microbiology, Kathmandu Medical College, Kathmandu, Nepal during January to March 2010. Eighty nine clinical samples were collected from 45 nosocomial infected neonates. Neonatal nosocomial and/or NICU acquired infection was defined as infection in new born babies developed after 48 hours of admission in NICU[23]. Sterile cotton swabs were soaked in sterile normal saline and swabs were taken from hands nursing staff (n=3) and innate objects like, baby cots (n=11), incubator (n=1), face masks (n=3), nasal prong (n=1), stethoscopes (n=6), water for injection (n=1), and intravenous infusion stands (n=10). The samples and swabs collected from neonates were inoculated into Blood agar and MacConkey agar. Swabs taken from hands of nursing staff and innate objects were left into a tube containing Brain Heart Infusion Broth. The inoculated media were incubated at 37^0C for 24 hours. The blood was inoculated in Brain Heart Infusion broth, incubated at 37^0C for 24 hours and sub-cultured every 24 hours for 48 hours on Blood agar and MacConkey agar. The organisms grown were identified based on standard phenotypic methods[24]. All media were purchased from Hi-Media Laboratories Pvt. Ltd., India.

Antibiotic susceptibility test: Kirby-Bauer disk diffusion test was performed to assess the in-vitro activity of different antibiotics to the isolated *Citrobacter* spp. as described before[8]. Briefly, colonies from a grown media were suspended in peptone water to reach the turbidity of 0.5 McFarland standard ($BaSO_4$ turbidity standard) and was uniformly streaked on Muller Hinton agar plate. The antibiotic disks were placed over the media. The plate was incubated at 37C for 18 hours and the zone of inhibition (mm) was measured. The zone size was interpreted based on the guidelines of manufacturer which is based on CLSI guidelines[25]. Muller Hinton agar and antibiotic disks were purchased from Hi-Media Laboratories Pvt. Ltd., India. Multi-drug resistant (MDR) isolates were defined as the isolates that were resistant to two or more than two classes out of three groups of antibiotics; β-lactams (eg. Piperacillin, ticarcillin, ceftazidime, cefepime, imipenem), amino glycosides (eg. gentamicin, tobramicin) and fluroquinoloones (eg. ciprofloxacin, norfloxacin)[25].

Results

Neonatal *Citrobacter* infections

Among 89 samples received for bacterial culture, microorganisms were isolated from 29 (32.6%) samples. Among the culture isolated specimens, 11 (37.9%) were *Citrobacter* spp. (Table 1). They were isolated from umbilical swab (n=8), urine (n=1), blood (n=1), and eye swab (n=1). The neonates infected with *Citrobacter* spp. were all during their first week of life. *Klebsiella* spp. (n=5), *Escherichia coli* (n=4), *Acinetobacter* spp. (n=3), *Proteus* spp. (n=2), *Enterobacter* spp. (n=1) and *Staphyloccous aureus* (n=3) were identified by culture and using biochemical properties.

Citrobacter spp. from nate and innate objects

Most *Citrobacter* infections in NICU were noscomial borne hence to detect the source of *Citrobacter* spp. several samples from nate and innate objects were collected and processed. Among several innate objects studied *Citrobacter* spp. was recovered from a nasal prong. *Staphylococcus aureus* was also isolated from baby cots (n=2) and incubator. *Acinetobacter* spp. was isolated from water for injection. Hands of nursing staff and other innate objects were free from pathogenic organisms.

Antibiotic Susceptibility pattern and Antibiotypes

All of the isolates isolated from neonates and nasal prong showed MDR phenotype (Table 1). Most of these isolates were sensitive to quinolones tested (n=11). Among the β-lactam antibiotics tested, Ceftriaxone and Amoxycillin and Clavulenic Acid combination was sensitive to 5 and 1 isolate respectively. Based on the antibiotic sensitivity pattern these *Citrobacte* spp. were grouped into 5 antibiotypes (I to V). *Citrobacter* spp. isolated from a nasal prong (C109) had a similar antibiotype with 4 other *Citrobacter* spp. isolated from neonates. Antibiotype type IV (C3409) was resistant to all the antibiotics tested.

Table 1. **Antibiotic susceptibility patterns of *Citrobacter* spp. from various specimens in neonates.**

Identification no.	Age (days)	Specimen	Antibiotypes	Sensitive to
C11709	5	US	I	OF, CF, NX
C52809	NA	US		
C54109	4	U		
C316709	NA	US		
C109	NA	NP		
C12309	NA	US	II	CI,AK,NT,CF, OF, NX
C27309	5	US		
C40809	6	US		
C24309	4	ES	III	CI, OF, CF, NX
C32209	4	US		
C3409	NA	US	IV	None
C21809	NA	BL	V	CA, OF, AK, NT

Eleven antibiotics (µg/disk) tested were, Ampicillin (A) (10), Piperacillin (PC) (100), Amoxy-Clavulenic Acid (CA) (20/10), Ceftriaxone (CI) (30), Cephalexin (CP) (30), Amikacin (AK) (30), Netilmicin (NT) (30), Ciprofloxacin (CF) (5), Ofloxacin (OF) (5), Norfloxacin (NX) (10), and Sulpha/Trimithorpim (CO) (23.75+1.25), U, Urine; US, Umbilical Swab; ES, Eye Swab; BL, Blood; NP, Nasal Prong; "NA", age not available.

Discussion

Hospital acquired infection is a major threat to hospitalized neonates causing high morbidity and mortality in developing countries[26]. During the period of study, the rate of nosocomial neonatal infection was 32.6% and *Citrobacter* spp. was most frequently isolated (n=11). The prevalence rate was 37.9% among the pathogens infecting neonates. *Staphylococcus aureus* (38%), coagulase negative *Staphyloccous* spp. (21%), *E. coli* and *Enterobacter* spp. were isolated as nosocomial pathogens in sepsis in a hospital in Nepal[21]. High prevalence of *Citrobacter* spp. in this study highlights the emergence of this pathogen as a successful nosocomial pathogen. *Acinetobacter* spp., *E. coli*, *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., and *Staphylococcus aureus* were also isolated from neonates albeit at low prevalence. Umbilical cord infection was most common (n=8) while each of three neonates had sepsis, urinary tract infection and ophthalmitis. Umbilical cord infection was also most common in newborns in a community based study on Southern Nepal and this infection could be more in neonates born in the hospital where infection control is compromised[27]. *Citrobacter* meningitis leading to brain abscess is commonly encountered in NICU but none of the neonates had this disease[12,13].

Citrobacter spp. is carried in the hands of medical staff and in innate objects present in the hospital ward[20]. When babies are handled by the colonized medical staffs or procedures (catheterization, opening intravenous lines, giving intravenous fluids and antibiotics, oxygen delivery through face mask and nasal prongs etc.) are carried on these neonates, bacteria are introduced inside the body leading to multiple diseases. To investigate the source of *Citrobacter* spp. in NICU, various samples from the hands of medical staff and innate objects were colleted. *Citrobacter* spp. was recovered from a nasal prong which had similar antibiotype to 4 other *Citrobacters* belonging to antibiotype I. This pathogen was the source of infection to these neonates admitted to NICU based on antibiotyping. However, antibiotyping is not a reliable tool to investigate the clonal relationship and robust molecular typing tools are needed[28]. *Acinetobacter* spp. was also isolated from water for injection and *Staphylococcus aureus* was isolated from two baby cots but the antibiotic resistance pattern did not match with the clinical isolates. The duty doctor and nursing staffs were notified on time to prevent the further spread of these pathogens in NICU.

The *Citrobacters* isolated were MDR and antibiotype IV was resistant to all antibiotics tested. Most of these isolates were resistant to common antibiotics used for treatment like, β-lactams and aminoglycosides. However, *Citrobacter* spp. isolated from neonates were sensitive to quinolones. The present study was supported by the documentation of MDR strains of *Citrobacter* spp. from sputum from a hospital in Kathmandu but these strains were susceptible to cefoperazone and sulbactam combination[8]. This combination was not tested in the present study so we could not know the sensitivity of this combination. *Citrobacters* isolated from children in Manipal College of Medical Science, Nepal also showed resistance to β-lactams, amino glycosides and also to quinolones[29]. The strains of *Citrobacter* spp. resistant to these antibiotics and even to carbapenems have been noticed outside Nepal[7,30]. If newer generation

antibiotics like, carbapenems, colistin and tigecycline are prescribed without antibiotic prescribing policies the resistant *Citrobater* may emerge leading to therapeutic deadlock. This study highlights the need for nosocomial infection prevention and control to decrease *Citrobacter* morbidity and mortality among neonates.

Conclusion

Nosocomial *Citrobacter* infection was common among neonates admitted in NICU and the source of this infection was nasal prong. The frequent isolation of this pathogen in NICU with growing antibiotic resistance raises a threat to this present antibiotic era and is a concern for all physicians involved in the treatment. If infection prevention and control practices are not initiated nosocomial *Citrobacter* could be the major cause of neonatal mortality in NICU in this hospital.

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