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An outbreak of neonatal septicaemia by *Enterobacter cloacae*

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ABSTRACT

Objective: To report an outbreak of *Enterobacter cloacae* (*E. cloacae*) in a neonatal intensive care unit of a rural hospital in Karnataka and conduct a prospective study on neonatal septicaemia. **Methods:** The diagnosis was based on culture, buffy coat smear examination and C-reactive protien test. **Results:** *E. cloacae* was isolated from 18 neonates with septicaemic syndrome, of which 8 babies died. Source of infection was found to be the water used to bathe the babies. The strain was multidrug resistant, extended-spectrum beta-lactamase producer and was sensitive only to amikacin and flouroquinolones. **Conclusions:** Clinical significance of *E. cloacae* as neonatal pathogen warrants attention of clinicians as well as microbiologists. Breaches in infection control measures should be avoided to prevent such outbreaks.

1. Introduction

Neonatal septicaemia (NS) is a haunting problem for neonatologists all over the world with considerable morbidity and mortality. The bacteriological profile of NS is constantly changing along with the source of infection. This clinical entity is more commonly associated with preterm and low birthweight babies. In the Indian scenario, members of Enterobacteriaceae continue to be a challenging problem for the sick and debilitated neonates. The recent literature review has revealed the significant role of species of the genus *Enterobacter* such as *Enterobacter cloacae* (*E. cloacae*), *Enterobacter sakazaki*, *Enterobacter aerogenes* in causing infections, especially in neonatal intensive care units (NICU)[1-7].

During a prospective study on NS for a period of one year, conducted in the Department of Microbiology in our institute, which is located in a rural area in Karnataka State, South India, an outbreak of septicaemia caused by *E. cloacae* was encountered in the NICU. The causative agents were found to be multipledrug resistant, extended-spectrum beta-lactamase (ESBL) producer.

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2. Materials and methods

The usual protocol for the processing of NICU samples was followed in all the cases. Briefly, 3 mL of blood was collected from the clinically suspected cases of NS with aseptic precautions. 1 mL was inoculated into 5 mL brain heart infusion (BHI) broth with 0.025% sodium polyanethol sulfonate (Hi Media Laboratories, Pvt. Ltd. Mumbai), another 1 mL into a sterile container to separate serum for C-reactive protien (CRP) test and the remaining 1 mL into a bottle containing ethylene diamine tetra acetic acid (EDTA) for buffy coat smear examination. A second sample of 1 mL blood was collected from another site and inoculated into BHI broth, to rule out the contamination.

Blood culture bottles were incubated at 37 ℃ and subcultured onto blood agar, Mac Conkey agar and chocolate agar for 1, 2, 3, 5 and 7 days or till the growth appears. Identification of the isolates was performed according to the standard procedures. Antimicrobial susceptibility testing was done following Clinical Laboratories Standard Institute (CLSI) recommendations[8], by using commercial discs from Hi Media Pvt. Ltd. Mumbai.

CRP test was done by latex agglutination method using kit supplied by Human Diagnostic Company. Sera showing distinct agglutination indicated CRP content of more

than 6 mg/L in the undiluted serum and were subjected to semiquantitative test. Buffy coat smear examination was conducted by the technique of Brook *et al*[9]. The blood sample with EDTA was transferred into sterile Wintrobe tube and centrifuged at 2 500 rpm for 15 minutes. The buffy coat layer was separated using a sterile syringe and needle and subjected to gram stain. The stained smears were examined under oil immersion objective.

A striking experience encountered during the study was an outbreak caused by *E. cloacae* for a span of about one month affecting 18 neonates. With the isolation of *E. cloacae* from 3 consecutive samples, an outbreak was suspected in the NICU and infection control measures were implemented. Environmental samples were collected from baby cots, trays, baby linen, instruments like baby incubators, suction apparatus and articles like soap, water, milk, feeding bottles, floor and wall of NICU for microbiological sampling. Staff handling the neonates and mothers of all babies were screened to rule out the possibility of carrier status.

The strains were also tested for ESBL by using ceftazidime (30 μg) and ceftazidime-clavulanic acid (30/10 μg) according to the procedures of CLSI and found to be ESBL producers.

3. Results

All the 18 neonates from which *E. cloacae* isolated, were less than 8 days old. 12 were male and 6 were female babies. In the present outbreak, 8 out of 18 babies died within 1 week of admission and 10 were improved by treatment, showing mortality rate of 44.4%. The birth weight of these neonates varied from 1.3-2.8 kg. 12 babies (66.7%) had low birthweight and 13 (72.2%) were preterm babies. Among the 8 babies died, we could demonstrate gram negative bacilli in the buffy coat smears and had a CRP titre of more than 48 mg/L.

E. cloacae was isolated from the water used to bathe the neonates, but not from any of the environmental samples from NICU or from the staff and the mothers of the sick babies. All the isolates of *E. cloacae* from the neonates as well as the isolate from water exhibited same biochemical reactions and antibiogram. All these strains were sensitive to amikacin, ciprofloxacin, ofloxacin, sparfloxacin and were resistant to ampicillin, cefotaxime, ceftriaxone, ceftazidime, cephalexin, cefuroxime and gentamicin. The other organisms isolated during this one month duration from the NICU were one strain of *Klebsiella pneumoniae* and one strain of *Pseudomonas aeruginosa*.

4. Discussion

Neonatal sepsis is an invasive clinical entity characterized by diminished spontaneous activity, respiratory distress, apnea, bradycardia associated with bacteraemia. It has got a predilection to low birth weight babies, those with depressed respiratory function at birth, male babies as well as those with congenital anomalies. Based on the duration of occurrence, it may be an early onset one (within 7 days of birth) or a late onset one (after 7 days)[10]. According to the data from the National Neonatal Perinatal Database (NNPD) 2002-2003, neonatal sepsis is the commonest cause of neonatal mortality in India and the incidence is 30/100 live births[11].

E. cloacae is emerging as a neonatal pathogen, responsible for various invasive infections including bacteraemia, respiratory tract infections, endocarditis which were associated with severe mortality[1-4]. This organism is reported to cause many outbreaks in NICU as documented in the previous literatures[12-16]. Infection management of these 'ICU bugs' is complicated by resistance to multiple antibiotics[4]. In the present report, all the strains isolated were resistant to penicillins, cephalosporins and gentamicin. As ciprofloxacin was not used for the treatment of infants, amikacin presented as a ray of hope to clinicians to silence this deadly menace. In addition, these strains were ESBL producers. Outbreaks due to ESBL producing *E. cloacae* are available in the published literature from various geographical regions[17-22].

The source of infection in *E. cloacae* outbreaks varied from milk powder[4], thermometers[14], transducer head used in ICU[15] as well as colonized patients[2]. In the present outbreak, the source of infection was found to be water which was used to bathe the babies. Investigation into the matter revealed that obvious negligence from the staff nurse to use inadequately heated lukewarm water instead of boiled and cooled water predisposed to infection. All the isolates from the babies and the water were of the same biotype and exhibited the same antibiogram.

E. cloacae may be overlooked due to close morphological resemblance to *Klebsiella* species on Mac conkey agar. In contrast with *Klebsiella*, *E. cloacae* are motile, usually decarboxylates ornithine and do not hydrolyse urease[4,23].

As reported by other authors[24,25], male preponderance is also found in the present study (66.7%). The fact that preterm and low birth weight babies are more vulnerable to infection, is proved beyond doubt[25,26]. The present report, is also in accordance with this finding. 72.2% were preterm and 66.7% were low birth weight babies.

Among the rapid diagnostic tests for NS, buffy coat smear examination and CRP tests were evaluated by various workers and shown to have variable results[24,27,28]. In this outbreak, 7 out of 8 neonates who succumbed to infection could demonstrate gram negative bacilli in the buffy coat smear and had a CRP titre of more than 48 mg/L. Hence, positive blood culture with these two tests can serve as markers associated with mortality.

It is a well known fact that no health care facility is free of problem of nosocomial outbreaks, though the source of infection and the causative agent may be

varying. Microbiologists can play a key role in identifying the aetiological agent and its antibiogram for accurate management of patients with infection and instituting surveillance programme. The report of such outbreaks helps in making the hospital personnel aware of the possible source of infection, its management and surveillance.

The outbreak in the present study was controlled by the implementation of infection control measures such as fumigation of NICU, thorough hand washing techniques, screening the staff in NICU, disinfecting thermometers, cohorting of infected children, proper sterilization of water and restriction of visitors. The present report emphasizes the significant role of *E. cloacae* as a neonatal pathogen which warrants attention of the clinicians as well as microbiologists. A cautious approach to avoid the breaches in infection control measures with utmost care and dedication by the medical personnels, can prevent such outbreaks.

Conflict of interest statement

We declare that we have no conflict of interest.

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