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Nosocomial outbreak of *Enterobacter gergoviae* bacteraemia in a neonatal intensive care unit

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Summary: A nosocomial outbreak of bacteraemia, caused by *Enterobacter gergoviae* infected 11 babies, nine of whom were premature, and was investigated in the neonatal intensive care unit (NICU) of a general hospital in Johor Bahru, Malaysia. The strain that was isolated from the babies was also isolated from the dextrose saline used for the dilution of parenteral antibiotics and from the hands of a healthcare worker on duty in the nursery. Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested chromosomal DNA confirmed a possible cross-contamination of parenteral dextrose saline and the healthcare worker. Prompt and effective control measures were initiated within NICU and the nosocomial infection of *E. gergoviae* was brought to an abrupt end. To the best of our knowledge, this is the first documented outbreak of *E. gergoviae* in the NICU in a hospital in the state of Johor, Malaysia.

Keywords: Nosocomial outbreak; neonatal intensive care unit; *Enterobacter gergoviae*.

Introduction

Enterobacter species have emerged as important nosocomial pathogens and frequently colonize hospitalized patients.[1] Common reservoirs for these organisms include wounds and the gastrointestinal, urinary and respiratory tracts.[2] *Enterobacter aerogenes* and *Enterobacter cloacae* are commonly encountered as nosocomial pathogens but *Enterobacter gergoviae* is a rare human pathogen. *E. gergoviae* was reported to represent 0.4% of all *Enterobacter* isolates in a hospital in Spain.[3] Infections with these species are usually associated with the presence of risk factors such as prolonged hospital stay especially if part of the stay was spent in an intensive care unit.[4] Other risk factors include immunosuppression, the presence of a foreign device, prior use of antimicrobial agents in the patient involved and extremes of age. Children in paediatric hospitals often have an enhanced susceptibility to infection by *Enterobacter* species.

In addition, the frequent use of extended-spectrum cephalosporins and other new β-lactam agents exert a selective pressure resulting in the emergence of resistant strains. The capacity of *Enterobacter* species spontaneously to derepress antibiotic resistance genes and the resulting resistance toward many β-lactam antibiotics complicates the treatment of such infections.[5] Within the hospital setting, there is a need to prevent colonization with these potentially pathogenic bacteria.

We describe here a nosocomial outbreak of *E. gergoviae* bacteraemia in a neonatal intensive care unit (NICU), affecting 11 babies, nine of whom were premature. Newer molecular techniques for establishing the presence or absence of clonality can be effective in tracking the spread of nosocomial infections from genetically related pathogens. Demonstration of epidemiological relationships

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between different clinical isolates demand differentiation of these isolates below species level. Pulsed-field gel electrophoresis (PFGE) is one of the most discriminatory DNA-based typing methods which is widely used to characterize epidemiologically related isolates. In this study, we established a PFGE protocol for differentiating *E. gergoviae* strains, and provide evidence to link the outbreak strains to the parenteral dextrose saline used in the NICU. To the best of our knowledge, this is the first documented major outbreak of *E. gergoviae* in the NICU of a large general hospital in the state of Johor in West Malaysia.

Patients and methods

Clinical setting

The NICU of the Sultanah Aminah Hospital, Johor Baru can accommodate a maximum of 57 babies. Approximately 1300 live babies are delivered each month, and premature babies and those from complicated and septic deliveries are admitted to the NICU.

During a 12 day period from the 10–22 November 2000, *E. gergoviae* was isolated from the blood cultures of 11 babies, all born in the hospital. The sudden increase in the number of cases of *E. gergoviae* bacteraemia within a short period of time in the NICU indicated an outbreak. Therefore, an epidemiological study was carried out to determine the source of the organism in order to control and prevent the spread of the infection.

Bacteriology

Blood cultures were tested using the Bactec method. In order to detect the source of the outbreak, a large number of samples were collected from the following: 30 samples from various parentral solutions such as heparin, dextrose saline, salbutamol, frusemide and gentamicin, as well as from povidone, iodine, water for humidifiers, water for oxygen and ventilation tubing, 50 handprints of healthcare workers, two detergent samples and two incubator filters. Swabs moistened with sterile water were used for environmental sampling, and liquids such as dextrose saline were aspirated aseptically and all were inoculated directly onto ox-blood agar plates. *Enterobacter* organisms were isolated by standard procedures and identified to the species level and then confirmed as *E. gergoviae* by the API 20E system (bioMérieux). Antimicrobial susceptibilities were determined by the disc diffusion method according to NCCLS standards.[6] In our laboratory, the presence of chromosomal β-lactamase is routinely tested in strains of *Enterobacter* species and *Pseudomonas aeruginosa* using the direct induction method by placing a disk containing a labile weak inducer such as cefotaxime close to one containing a strong inducer such as cefoxitin. Induction is then detected from the blunting of the cefotaxime zone adjacent to the cefoxitin disc.[7]

Molecular typing

Genomic DNA from *E. gergoviae* was prepared in agarose plugs by modification of a previously described method.[8] Initial preparation of the chromosomal DNA showed a lot of degradation and was thought to be due to a higher production of nucleases from these strains. Hence, the cells were pretreated with 1% formaldehyde before being mixed with molten agarose. The plugs were digested with 10 U of *Xba*I, and PFGE of the digested DNA plugs was then performed using a CHEF DRII system (Bio-Rad, CA USA) for 24 h at 6 V/cm with pulse times ramped linearly from 5 to 50 s. After electrophoresis, the gel was stained in 0.5 μg/mL ethidium bromide for 20 min before being photographed using an ultraviolet transilluminator.

Results

E. gergoviae was isolated from the blood cultures of 11 babies but epidemiological data and viable cultures were only available for nine. Their ages ranged from nine days to two months and their body weights from 0.92 to 2.07 kg. There were three positive environmental isolates out of 90 samples processed. Two of these were from the handprints of healthcare workers and one from the multidose in-use dextrose parenteral solution for the dilution of antimicrobials. The nine clinical isolates and three environmental isolates had identical antimicrobial profiles by the disc diffusion method, i.e., sensitive to ampicillin, ampicillin–sulbactam, imipenem, netilmicin, cefoperazone, aztreonam, ceftazidime, cefotaxime, ceftriaxone, co-trimoxazole and ciprofloxacin. There was intermediate sensitivity to cefuroxime and amoxycillin clavulanate. But at induction all nine clinical and three environmental strains were found to produce β-lactamase. In view

of this, the babies were successfully treated with imipenem.

Molecular typing

PFGE was performed on the eight bacteraemic isolates, two from handprints of healthcare workers and one environmental isolate from the bottle of dextrose saline. The *Xba*I–PFGE profiles for 10 out of the 11 isolates were similar indicating that the strains probably originated from the same source (Table I, Figure 1). Isolates EG1–EG8 were from bacteraemic patients while isolates EG9 and EG11 were from the handprints of a healthcare worker and a bottle of dextrose saline respectively (Table I). PFGE of *Xba*I-digested chromosomal DNA from these 10 isolates gave similar banding patterns consisting of about 19 DNA fragments ranging in size from 30 kb to about 500 kb. Isolate EG10, which was from another healthcare worker, was distinctly different from the rest with seven DNA fragment difference (Table I, Figure 1, lane 10) showing that this particular isolate was unrelated to the outbreak strains.

Table I Xba*l profiles of* Enterobacter gergoviae *from neonatal intensive care unit*

Date of isolation	Patient	Strain no.	*Xba*l-profile
13/11/00	4	EG1	EGX1
16/11/00	5	EG2	EGX1
20/11/00	6	EG3	EGX1
21/11/00	7	EG4	EGX1
22/11/00	8	EG5	EGX1
22/11/00	9	EG6	EGX1
22/11/00	10	EG7	EGX1
22/11/00	11	EG8	EGX1
18/11/00	Handprint HCW 1	EG9	EGX1
18/11/00	Handprint HCW 2	EG10	ECX2
18/11/00	Dextrose saline	EG11	EGX1

HCW, healthcare worker.

It became apparent during the investigation that there was a breakdown in certain infection control practices in the NICU, such as inadequate handwashing techniques and incorrect handling of multidose vials and parenteral solutions with no records of when these were opened. In addition, there was an influx of student nurses during this outbreak.

Control measures

All infected babies were cohorted and strict barrier nursing was implemented. Handwashing techniques were strictly reinforced for all categories of staff. Handwashing, particularly before handling multidose vials was emphasized, as well as documentation of date and time that the vials were opened. Additional measures included the cleaning and disinfection of the NICU, use of separate stethoscopes for cohorted babies and disinfection of the diaphragms of stethoscopes between babies in the nursery, wearing of gowns and aprons before entering the NICU, and thorough cleaning of suction tubes before autoclaving or disinfection. Other measure were thorough scrubbing of the washbasins in the NICU, stopping the practice of topping up

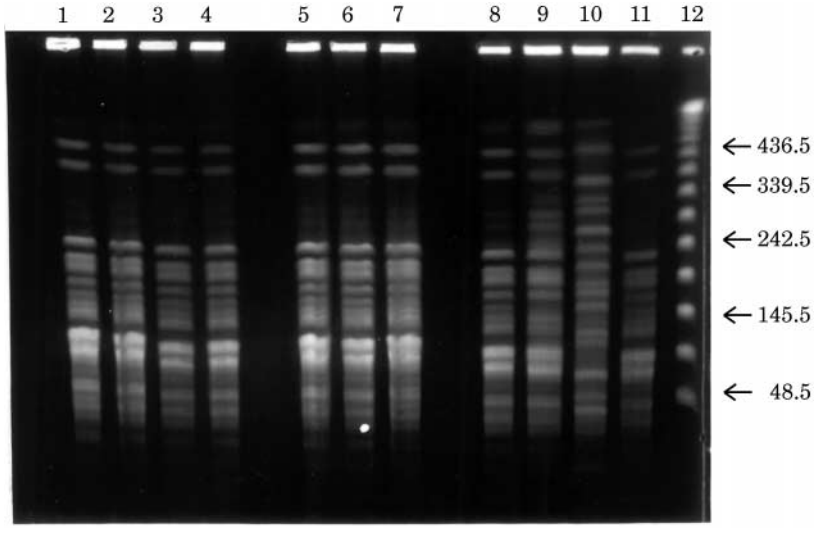

Figure 1 PFGE patterns of *Xba*l-digested DNA of *E. gergoviae*. Lanes 1–11: isolates EGX1 to EGX11; lane 12: bacteriophage Lambda ladder PFG marker. Numbers at side indicate molecular weights in kilobase pairs.

handwashing solutions and the provision of sufficient paper towels. Daily visits by the infection control nurse and microbiological surveillance of all samples from the nursery was carried out until the outbreak was brought under control, and no further cases were detected within a week of implementing the above measures.

Discussion

An outbreak is defined as an infection caused by the same organism occurring in two or more patients within a defined time and space.[9] Outbreaks have resulted in prolonged hospital stay, escalating costs in therapy, with an increase in mortality.[10,11] Here, we describe an outbreak of bacteraemia with a potentially multidrug-resistant strain of *E. gergoviae* involving 11 babies, nine of whom were premature. The same strain was isolated from the babies involved in the outbreak, the dextrose saline used for the dilution of parenteral antibiotics and from the hands of a healthcare worker on duty in the NICU. All the strains produced extended spectrum β-lactamases (ESBL) at induction. We did not further characterize the ESBLs, but the most frequently characterized ESBL of *E. gergoviae* was reported to be CTX-M-10 ESBL.[3]

The microbiology laboratory plays an important role in providing rapid and accurate information as to the offending organism involved, as well as detecting resistant mechanisms of these organisms. In vitro detection of resistant phenotypes is useful in guiding therapy and in the elucidation of cross-resistance to antimicrobials. Many laboratories do not routinely detect resistance mechanisms. A recent survey in the US showed only 32% of the laboratories did so, and of this subset only 17% used adequate methods.[12] In our study, the provision of such laboratory information and the use of appropriate antibiotic therapy may have contributed to a favourable clinical outcome.

Evidence for the involvement of an epidemic strain in this particular outbreak was based on the identical DNA fingerprints obtained by PFGE. Studies at this molecular level strongly indicated that the source of *E. gergoviae* bacteraemia could have been the parenteral dextrose saline bottle, and cross-contamination was further facilitated by carriage in the hands of the healthcare workers concerned. As a result of prompt and effective measures instituted by the infection control unit, the outbreak was brought to an abrupt end.

The present study reiterates the importance and relevance of molecular-based technique such as PFGE to rapidly trace the source of nosocomial infections. To facilitate rapid and appropriate intervention, a typing system has to be highly reproducible, provide rapid, reliable information, easy to perform, discriminative and provide additional data not otherwise easily available. In this study, PFGE protocol was established to determine rapidly and accurately the clonality of the *E. gergoviae* strains within three days. The data obtained indicated a possible cross-contamination of the parenteral dextrose saline solution and a hospital healthcare worker.

In conclusion, it must be emphasized that control of cross-infection is not about one strategy alone but several strategies working together. The first being implementation of infection control policies that is understood and practiced, without exception, by all category of healthcare workers and long-staying visitors. The second strategy concerns the ability to make a diagnosis of nosocomial infection and subsequently be able to predict an impending outbreak and to determine the source of the outbreak. The third would be to achieve a reduction in antimicrobial resistance in the hospital setting through rational and careful antibiotic prescribing habits.[13] It is hoped that reports like ours will help in preventing devastating outcomes such as cross-infection and outbreak in hospitals, particularly in intensive care units.

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