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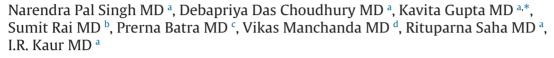
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Major Article

Predictors for gut colonization of carbapenem-resistant Enterobacteriaceae in neonates in a neonatal intensive care unit



^a Department of Microbiology, UCMS & GTB Hospital, New Delhi, India

^b Department of Microbiology, VMMC & Safdarjang Hospital, New Delhi, India

^c Department of Paediatrics, UCMS & GTB Hospital, New Delhi, India

^d Maulana Azad Medical College, New Delhi, India

Key Words: Carbapenem-resistant Enterobacteriaceae neonatal intensive care unit risk factors colonization **Background:** With the emergence of carbapenem-resistant isolates, the therapeutic alternatives have become limited. Various factors are responsible for carbapenem-resistant *Enterobacteriaceae* (CRE) gut colonization. This study was conducted to determine predictors for CRE gut colonization in neonates who were hospital delivered and admitted in a neonatal intensive care unit (NICU).

Methods: Three rectal swabs were collected from 300 hospital-delivered and NICU-admitted neonates (likely to stay for >3 days). The data collected for the possible risk factors for CRE gut colonization were namely mode of delivery, prolonged rupture of membrane >18 hours, period of gestation, birth weight, meconium-stained liquor, ventilation, intravenous catheter, nasogastric (NG) tube, NG feeding, breastfeeding, katori spoon feeding, top feeding, expressed breastmilk, antibiotics administration, and duration of hospitalization. *P* < .05 was considered as statistically significant.

Results: A total of 26 cases of CRE were isolated from 300 neonates. Statistically significant risk factors were found to be NG tube, breastfeeding, NG feeding, top feeding, expressed breastmilk, ventilation, antibiotic administration, and duration of hospitalization. Top feeding and antibiotics administration were identified as 2 independent risk factors by multiple logistic regression.

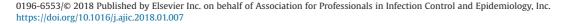
Conclusions: Active surveillance of cultures from hospitalized patients and implementation of preventive efforts can reduce the risk of CRE.

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Carbapenem-resistant *Enterobacteriaceae* (CRE) has emerged as a major cause of nosocomial infection in several regions around the world, including India. *Enterobacteriaceae* strains resistant to \geq 3 classes of antibiotics are endemic in many hospitals and account for a significant proportion of hospital-acquired bloodstream infections.¹ Carbapenems are usually used as a last resort of treatment for severe infections caused by the multidrug-resistant gramnegative bacteria. However, the emergence of carbapenem-resistant isolates and their progressive geographic dissemination has limited therapeutic alternatives with potential threat of CRE outbreaks. Out-

E-mail address: drkavitagupta2010@gmail.com (K. Gupta). Conflicts of interest: None to report.

breaks of infection have been described for many different groups of patients; among them, infants are associated with worse outcomes with the suboptimal empirical antibiotic treatment.²⁻⁴ Persistent colonization with CRE strains in neonates has potential implications not only for themselves but also for their contacts.⁵ This colonization may be associated with diseases in the neonatal period such as late-onset sepsis, necrotizing enterocolitis, and postneonatal diseases (eg, atopy, inflammatory bowel disease, irritable bowel syndrome).^{5,6} It increases the likelihood of appearance of CRE infections and eventually evolution of carbapenemase genes in the community. Colonization of the gastrointestinal tract by CRE may be asymptomatic, and this constitutes a reservoir for transmission that may remain unidentified in hospitals that do not implement active surveillance testing. Fecal carriage of CRE strains is particularly problematic because Enterobacteriaceae are common causes of both health care- and community-acquired infections, raising the possibility of spread of CRE into the community.⁶ These issues,





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^{*} Address correspondence to Kavita Gupta, MD, Dept of Microbiology, UCMS & GTB Hospital, H-204, DDA Flats, Pocket-C, Molarband, Mohan Estate, New Delhi 110044, India.

combined with the limited therapeutic options available to treat patients infected with these organisms, have made CRE of epidemiologic importance globally. Factors associated with fecal carriage of CRE strains include antibiotic exposure, indwelling devices, parental nutrition, malignancy, nonsurgical invasive procedures, prolonged hospital stay, admission to intensive care units, and so forth.⁷ The underlying chronic diseases, carbapenem usage, and nasogastric (NG) tube placement also have a role as risk factors for acquisition of CRE gut colonization.⁸ There is paucity of data for possible risk factors of CRE gut colonization in hospitalized neonates in India and worldwide. Therefore, this study was undertaken to determine predictors for CRE carriage in neonates who were delivered in hospital and admitted in a neonatal intensive care unit (NICU).

MATERIALS AND METHODS

The study was conducted in the Departments of Microbiology and Paediatrics, of the same tertiary hospital, in East Delhi, India, for over a period of 1 year. The study was approved by the institutional ethical committee, and written informed consent was taken from either of the parents. A total of 300 hospital-delivered neonates who were admitted in the NICU and likely to stay for ≥ 3 days in the NICU were included in the study. Three rectal swabs (sterile cotton swab) were taken: (1) first sample, within 24 hours of birth; (2) second sample, on day 3; and (3) last sample, collected before discharge or up to day 10, whichever was earlier. Swabs from all neonates were screened for CRE according to Centers for Disease Control and Prevention (CDC) criteria.9 Rectal swabs were inoculated in trypticase soy broth containing ertapenem disk $(10 \,\mu g)$ within 15-20 minutes of collection, and they were further subcultured on MacConkey agar after overnight incubation. Lactosefermenting colonies grown on MacConkey agar plates after 18-24 hours of incubation were further identified using standard protocol up to species level.¹⁰ Susceptibility testing was performed by the disk diffusion (Kirby-Bauer) method following Clinical and Laboratory Standards Institute guidelines.¹¹ Isolates showing positive disk screen test with ertapenem $(10 \,\mu g)$ or meropenem (10 µg) were suspected as possible CRE, and Modified Hodge Test (MHT) was performed on all possible CRE.¹¹

Procedure of the MHT¹¹

For the MHT, 0.5 McFarland standard suspension (using either direct colony suspension or growth method) of *E. coli* ATCC 25922 (the indicator organism) (American Type Culture Collection, Rockville, MD) was prepared in saline and was further diluted to 1:10 to perform MHT. A Mueller-Hinton Agar plate was inoculated with *E. coli* ATCC 25922 as per the routine disk diffusion procedure. The plates were allowed to dry for 3-10 minutes, after which an ertapenem disk was placed in the center of the plate. Using a 10- μ L loop, 3-5 colonies of test as well as quality control organisms (grown overnight on a blood agar plate) inoculated in a straight line out from the edge of the disk. Plates were incubated overnight at 35°C. Plates were examined the next day for enhanced growth around the test or quality control organism streak at the intersection of the streak and the zone of inhibition.

Interpretations

Enhanced growth was considered positive for carbapenemase production, and no enhanced growth was considered negative for carbapenemase production.

Quality control strains for the MHT

The MHT-positive strain was *K. pneumoniae* ATCC BAA-1705 (American Type Culture Collection), and the MHT-negative strain was *K. pneumoniae* ATCC BAA-1706 (American Type Culture Collection).

Final confirmation of phenotypically confirmed CRE isolates was done by real-time polymerase chain reaction (PCR) with cyber green chemistry to identify the presence of *NDM-1*, *IMP*, *VIM*, and *KPC* genes.

Data collection

The clinical information collected as possible risk factors for CRE gut colonization were prolonged rupture of membrane >18 hours, mode of delivery of lower segment caesarean section, birth weight, period of gestation, mean duration of hospital stay, NG tube, mode of feeding (NG feeding, breastfeeding, or katori spoon feeding), type of feeding (top feeding or expressed breastfeeding), ventilation, antibiotics administration, and duration of hospitalization. Expressed breastfeeding and top feeding were done either by NG tube or katori spoon.

Statistical methods

Univariable analysis of risk factors was done using χ^2 /Fisher and unpaired Student *t* test to identify the risk factors for CRE colonization. Statistically significant risk factors for CRE colonization in the study group were considered where *P* < .05. Multivariable logistic regression analysis was performed on statistically significant risk factors for CRE gut colonization to identify independent risk factors.

Ethical considerations

The study protocol was approved by the ethical committee (letter no. UCMS/M-1251/2013) for research projects of the hospital. A written informed consent was also taken from either of the parents after explaining the importance of the study. Finally, patient's findings were reported to the attending physicians to manage and treat patients accordingly.

RESULTS

A total of 26 of 300 (8.7%) neonates had possible CRE (positive with ertapenem and or meropenem disk screen test) according to CDC criteria.¹¹ Out of the 26 isolates, 25 isolates were resistant to both ertapenem and meropenem, 1 isolate was ertapenem resistant, and 1 isolate was meropenem sensitive. All 26 isolates with positive disk screen test were also MHT positive. Because of financial constraints, only *NDM*, *KPC*, *IMP*, and *VIM* were identified because they are the common ones. Out of the 26 phenotypically detected CRE isolates, 19 were identified as having a resistance gene in PCR and 7 isolates were negative for PCR. Because of financial constraints, PCR was not performed for OXA-48 and OXA-181. Distribution of isolates harboring carbapenemase is shown in Figure 1.

A total of 26 isolates were recovered using CDC methods; among them, 15 were term and 11 were preterm neonates. Table 1 depicts the distribution of CRE in term and preterm neonates. None of the neonates had CRE gut colonization in the first sample, the second sample showed 6 CRE isolates, and 20 new CRE isolates were identified in the third sample. All the neonates were born with meconium-stained liquor. Mean duration of hospital stay was found to be statistically significant among CRE carriers. Other statistically Download English Version:

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