INTESTINAL COLONIZATION BY EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING ENTEROBACTERIACEAE IN INFANTS

Gloria Concepción Huerta-García, Guadalupe Miranda-Novales, Rita Díaz-Ramos, Guillermo Vázquez-Rosales and Fortino Solórzano-Santos

1 Department of Infectious Diseases, Hospital de Pediatría, Centro Medico Nacional Siglo XXI, IMSS, México D.F, México; 2 Medical Research Unit in Hospital Epidemiology, Coordinación de Investigación en Salud, IMSS, México D.F, México; 3 Coordinación de Unidades Medicas de Alta Especialidad, IMSS, México D.F, México

ABSTRACT

Background: Members of the Enterobacteriaceae family are common agents of nosocomial infections. Intestinal colonization by these microorganisms represents a major step in the development of systemic infection. Extended-spectrum β-lactamase-producing bacteria are usually associated with outbreaks, but endemic infections are common in intensive care units. Objective: To determine the frequency of intestinal colonization with extended-spectrum β-lactamase-producing Enterobacteriaceae in newborns. Patients and Methods: This was a descriptive cohort study. Newborns from two general hospitals (A and B) in Mexico City were included during a five-month period; those with a hospital stay > 7 days were selected. Fecal samples were obtained by rectal swab on day 7 and every week until discharge. Extended-spectrum β-lactamase production was confirmed in enterobacteria by the Etest. Clonal relatedness was established by pulsed-field gel electrophoresis. Results: 102 newborns were included; 63/102 (61.7%) were colonized by extended-spectrum β-lactamase-producing Enterobacteriaceae on day 7, 17/21 (81%) on day 14, and 6/8 (75%) on day 21 of hospitalization. Klebsiella pneumoniae was recovered most frequently (75.4%). A predominant clone (95%) was found in hospital B, and a major clone (75%) in Hospital A. Other extended-spectrum β-lactamase-producing Enterobacteriaceae isolates were Enterobacter spp. (16%) and Escherichia coli (7.6%). Conclusions: High rates of colonization and horizontal transmission of extended-spectrum β-lactamase-producing Enterobacteriaceae were found in the newborn care units of two general hospitals. Clonal relatedness was identified. Lack of adherence to standard precautions and hand hygiene were determining factors. (REV INVES CLIN. 2015;67:313-7)

INTRODUCTION

Members of the family Enterobacteriaceae are common agents of nosocomial infections. Most of these bacteria are extended-spectrum β-lactamase (ESBL) producers\(^1\)\(^,\)\(^2\). The ESBL can cleave the β-lactam ring and confer increased resistance to commonly used and newer β-lactam antibiotics, including third- and fourth-generation cephalosporins and monobactams. Klebsiella pneumoniae and Escherichia coli are the most frequent ESBL-producing enterobacteria (ESBL-PE) among isolates from patients in pediatric hospitals. These microorganisms can also acquire resistance to other antimicrobial classes such as fluoroquinolones, tetracyclines, and aminoglycosides, which further limits the therapeutic options\(^3\)\(^,\)\(^4\).

The gastrointestinal tract of the newborn can be easily colonized by ESBL-PE\(^5\). A high fecal carriage of ESBL-producing strains is a risk factor for systemic infection in neonatal intensive care units (NICU) and nurseries, and can cause endemic and epidemic infections\(^6\)\(^-\)\(^9\). Molecular epidemiology studies have been used to describe community and hospital dissemination, risk factors for infection, and transmission during outbreaks of ESBL-PE\(^10\)\(^-\)\(^12\).

The aim of this study was to determine the frequency of ESBL-PE colonization in newborns with a hospital stay longer than seven days, in two general hospitals.

PATIENTS AND METHODS

This was a descriptive cohort study. Newborns born in two general hospitals (A and B) were included during a five-month period. Hospital A attends 650 births per month and Hospital B, 1,000 births. There are three main care areas for newborns: rooming-in units for healthy newborns, a semi-intensive care unit (SICU), and an NICU for those patients who require special treatment and care during their recovery.

The SICU in Hospital A has 35 incubators, with a separation of 15 cm between each, in a large room with two hand-washing sinks. The NICU in this hospital has a 50 m\(^2\) area with six radiant warmers 50 cm apart. An alcohol-based hand sanitizer is available for hand hygiene.

Hospital B has a general care unit with 30 incubators and two SICUs with 30 incubators each; the space between incubators is 20 cm. There is one hand-washing sink for every 10 incubators. The NICU has two rooms with three radiant warmers, 50 cm apart.

Microbiological procedures

Fecal samples were obtained by rectal swabs from all patients on day 7 of hospitalization and every seven days until discharge, regardless of whether colonization was detected in previous swabs. Stuart medium was used for sample transport. Samples were plated rolling the swab over a small area of the agar surface, streaking for isolation with a sterile loop onto MacConkey agar plates containing 1 mg/l of ceftazidime, and were incubated at 37°C for 18-24 hours. Colony types of gram-negative bacteria that differed in morphology (size, shape, consistency, and color) samples were counted separately and identified using the API\(^®\)-20E system (bioMérieux, Clinical Diagnostics). Members of the family Enterobacteriaceae were stored in tryptic soy broth with 20% glycerol at –20°C. The ESBL phenotypic production was performed by the disk diffusion method on Müller-Hinton agar plates using the method of Cormican, et al.\(^13\), with Etest strips with ceftazidime/ceftazidime plus clavulanic acid (AB Biodisk, Solna, Sweden) method as described before\(^14\). Control strains used were E. coli ATCC 25922 (negative control) and E. coli J53-2, ATCC BAA-199 (positive control).

Pulsed-field gel electrophoresis

Bacterial DNA was prepared as described previously\(^15\). XbaI and SpeI were used for restriction analysis. Pulsed-field gel electrophoresis (PFGE) was performed using the CHEF-DR III Gene Path System (Bio-Rad Laboratories). Clonal relatedness was established according to the criteria of Tenover, et al., as indistinguishable, closely related (three or fewer band differences), possibly related (four to six band differences), and unrelated (seven or more band differences)\(^16\).

Statistical analysis

Data were analyzed using descriptive statistics and are presented as simple frequencies and percentages. Chi-square statistical analyses were performed using
SPSS version 20. P values ≤ 0.05 were interpreted as statistically significant.

The Ethical Review Board approved the study, and verbal consent to participate in the study was obtained from parents or guardians.

RESULTS

One hundred and two newborns were included in the study: 55 from Hospital A and 47 from Hospital B. Eighty-four newborns were hospitalized in the SICU and 18 in the NICU; 53% were girls, and 47% were boys. The most frequent causes of hospitalization were low birth weight, asphyxia, and respiratory distress syndrome (33.3, 23.5, and 10.8%, respectively). In both hospitals, the SICU occupancy rate was 80%, and the patient:nurse ratio was 8:1. In the NICUs, the occupancy rate was 50%, and the patient:nurse ratio was 2:1. Compliance in the use of alcohol gel for hand hygiene before touching infants was 18% in Hospital A and 21% in Hospital B (p > 0.05).

The first sample was taken with a rectal swab on day 7 in 102 newborns; colonization with ESBL-PE was identified in 61.7% (63/102): 58% (32/55) in Hospital A and 66% (31/47) in Hospital B (p > 0.05) (Table 1). Only 21 patients were available for a second sample: in Hospital A, 10/12 (83%) and in Hospital B, 7/9 (78%) were colonized by ESBL-PE. From eight patients available for a third sample, 4/5 (80%) in Hospital A and 2/3 (66%) in Hospital B were colonized by ESBL-PE.

A total of 102 ESBL-PE isolates were obtained from 74 patients, resulting in an average of 1.4 isolates per patient. K. pneumoniae was recovered most frequently in 77 isolates (75.4%), followed by Enterobacter spp. in 16% (n = 17) and E. coli in 7.6% (n = 8).

One isolate of the same genus and species from each patient was selected for genotyping by PFGE. Genomic DNA was obtained from 55 ESBL-PE: 41 were K. pneumoniae (20 from Hospital A, 21 from Hospital B), eight were E. cloacae (six from Hospital A, two from Hospital B), and six were E. coli (three from each hospital).

Among K. pneumoniae strains from Hospital A digested with XbaI, one major identical profile was identified in 15/20 isolates. A second profile was identified in two isolates, and there were three unrelated isolates. Among strains obtained from Hospital B, a predominance of one profile was evident (20/21) (Fig. 1 A and B). Of E. cloacae isolates from Hospital A digested with SpeI, 3/6 had an identical profile. From the E. coli isolates digested with SpeI from Hospital A, 2/3 were identical. E. cloacae and E. coli

<table>
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<tr>
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<th>Hospital B</th>
<th>p value</th>
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<td>n = 47</td>
<td>0.56</td>
<td>n =102</td>
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<tr>
<td>– First sample</td>
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<td>31 (66%)</td>
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<td>0.33</td>
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<tr>
<td>– First sample</td>
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<tr>
<td>– n (%)</td>
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<td>31 (77%)</td>
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<td>– First sample</td>
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<td>– n (%)</td>
<td>2 (18%)</td>
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isolates obtained from Hospital B were not related (data not shown).

During the study, none of the colonized patients developed systemic infections caused by ESBL-PE.

DISCUSSION

In this study, a high fecal colonization rate by ESBL-PE was found in newborns on day 7 of admittance to intermediate care units. As reported by Boo, et al., there is a 1.3-times greater risk of colonization for each additional hospitalization day\(^9\). In a study of healthy, low birth weight, exclusively breast-fed neonates, Kothari, et al., found that 65% of the neonates were colonized by enterobacteria immediately after birth, and that 14.3% were colonized by at least one ESBL-PE isolate on day 21\(^17\). The frequency of colonization with ESBL-PE increased to 27.1%, predominantly with *E. coli* isolates. This contrasts with the high frequency (75%) of ESBL-PE colonization found in our study on day 21 of hospitalization, mainly by *K. pneumoniae*, with similar findings observed by Nordberg, et al., where intestinal colonization was 56% after seven days of hospitalization\(^18\). Intestinal colonization by ESBL-PE in newborns is related to long hospital stay, hospital overcrowding\(^19,20\), low rates of compliance with hand hygiene and standard precautions, and poor availability of alcohol-based and other hand hygiene products. Environmental contamination is also a potential source of resistant pathogens\(^5,8,21\).

Genotyping of ESBL-producing *K. pneumoniae* demonstrated horizontal spread (the spread of an infectious agent from one individual to another, usually through contact with bodily excretions or fluids that contain the agent) in these intermediate care units. Hospital overcrowding, nurse understaffing, and the limited availability of sinks, facilitated dissemination. In contrast, the frequency of fecal colonization was low in the NICU patients. Unlike the SICU, the number of newborns in the NICU was limited, the length of stay was 14-21 days, and the nurse:newborn ratio was better.

Even though the association between colonization with ESBL-producing strains and clinically significant infections is well described, none of the patients included developed systemic infections during the study period. However, as a limitation in our study, we did not analyze the incidence or prevalence of infections caused by these microorganisms during the study. Reddy, et al., found a frequency of 8.5% of subsequent infection in previously colonized high-risk patients\(^22\). It is unclear whether surveillance strategies can decrease dissemination since infection also occurs in low-risk patients. Endemic ESBL-PE poses a threat in the NICU\(^23\) because parenteral antibiotics do not prevent or eradicate intestinal colonization\(^24\) and outbreaks are common, have particular characteristics, and involve more patients with a high mortality risk\(^25,26\). The recommended treatment for these infections is carbapenems, although extended use might lead to the selection of multiresistant organisms. Thus, every effort should be made to reduce bacterial selection and horizontal transmission.

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Figure 1. Pulsed-field gel electrophoresis analysis of XbaI-digested genomic DNA from representative extended-spectrum \(\beta\)-lactamases-producing *Klebsiella pneumoniae*.

Panel A. Lane LL, Lambda ladder pulsed-field gel electrophoresis marker; lanes 1-7, major clone in Hospital A.

Panel B. Lanes 1-9, predominant clone. Lane 10, unrelated strain.
Because there are few active antimicrobials available, it is imperative to employ simple nonpharmaceutical measures to control endemic clones, which may be able to survive in natural and artificial reservoirs. Adequate infection control practice, to limit horizontal spread of bacteria, should be enforced. Recommendations for adequate control of nosocomial infections were strengthened at the two hospitals after results were analyzed.

Another limitation of this study was the screening method to detect ESBL-PE isolates. Even though it is well known that ceftazidime is usually the best substrate for TEM and SHV ESBLs, when used alone it could fail to recognize CTX-M-producing strains susceptible to ceftazidime, and these types of ESBL could have escaped our initial detection.

In summary, high rates of colonization and horizontal transmission of ESBL-PE were found in the newborn care units of two general hospitals. Intestinal colonization by ESBL-PE in newborns was related with hospital overcrowding, low rates of compliance with hand hygiene and standard precautions, and poor availability of alcohol-based and other hand hygiene products. The dynamics of transmission seemed to depend more on the environmental factors.

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REFERENCES