The neonatal gastrointestinal tract is considered sterile, but at birth the neonate is exposed for the first time to a wide array of bacteria from a variety of sources: from the birth canal via maternal vaginal and fecal microbiota, from the hospital environment and from handling by parents and nursing staff. The gastrointestinal microbiota play an essential role in the health and disease of the host through its impact on nutrition, pathogenesis and immunology. The gastrointestinal tract is the main reservoir and source for transmission of nosocomial pathogens, including Enterobacteriaceae. Previous studies have demonstrated fecal colonization by extended-spectrum -β-lactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae. The first fecal colonization with ESBL-producing Enterobacteriaceae were identified in Spain in 2003. Since then, rates of colonization by ESBL-producing Enterobacteriaceae have increased...
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dramatically worldwide.\(^5\)\(^6\) CTX-M-15 is the most common ESBL gene worldwide including Saudi Arabia.\(^8\) Carbapenem resistance in *Enterobacteriaceae* has been largely due to acquisition of carbapenemase genes, which lead to international dissemination of carbapenemase-producing Enterobacteriaceae. The most common carbapenemases are KPC, NDM, VIM, IMP and OXA-48.\(^9\)\(^11\) Previous studies reported fecal carriage of carbapenemase-producing Enterobacteriaceae.\(^6\) To the best of our knowledge there are no reports in Saudi Arabia on the fecal colonization of ESBL- and carbapenemase-producing isolates and multidrug resistant (MDR) isolates from neonates. Therefore, we conducted the present study to investigate the prevalence of fecal carriage of ESBLs and carbapenemase-producing gram-negative bacteria among 150 neonates who were born in two hospitals in central Saudi Arabia. None of the enrolled subjects nor their mothers were seriously ill and/or hospitalized in intensive care units. Nevertheless, antibiotic use before delivery cannot be ruled out. We also assessed the risk factors (age in days, mode of delivery, body weight and type of neonatal feeding) on the prevalence of gram-negative bacteria fecal carriage.

**PATIENTS AND METHODS**

*Bacterial strains*

Stool samples were collected from 150 neonates aged from 1-7 days at two maternity and children hospitals, Bukyriah, Qassim region, Saudi Arabia between June 2012 and January 2013. The local medical ethical committee approved the study protocol. Each parent who agreed to participate provided written informed consent and completed a questionnaire on neonatal characteristics. Epidemiological data were recorded for each neonate included type of delivery, age, weight and type of feeding. Fresh neonatal stool specimens were aseptically collected in sterile containers and immediately transported to the microbiology laboratory. Stool specimens were suspended in equal volumes of sterile phosphate buffered saline, pH 7 (PBS) and gently homogenized. Ten-fold serial dilutions were done in PBS. Aliquots (100 µL) of each dilution were directly inoculated onto blood agar and MacConkey agar (Oxoid Microbiology Products, UK). After 48 h incubation at 37°C, the recovered organisms were counted (colony forming units/gram of stool) and then identified manually and by using automated identification systems of API or VITEK 2 (Biomerieux, SA, Marcy, l’Etoile, France).\(^{12}\) The isolates were stored in brain heart infusion broth containing 20% glycerol at -70°C. The laboratory strains, *E coli* ATCC 35218, *K pneumoniae* ATCC 700603, and *P aeruginosa* ATCC 27853, were used as quality control strains in antibiotic sensitivity testing. The laboratory strains, *K pneumoniae* ATCC700603 (positive control) and *E coli* ATCC25922 (negative control) were used as quality control strains in ESBL production. The laboratory strains, *K pneumoniae* ATCC1705 (positive control) and *K pneumoniae* ATCC1706 (negative control) were used as quality control strains in the Modified Hodge Test (MHT).

**Distribution of gram-negative bacteria by clinical characteristics of subjects**

To assess the effect of age on enteric bacteria colonization, neonates were categorized into four groups according to age. Group 1 comprised 45 neonates (1 day/old); group 2, 47 neonates (2 days/old); group 3, 41 neonates (3 days/old); and group 4, 17 neonates (4-7 days/old). To evaluate the influence of mode of delivery on colonization rates, the neonates were classified by vaginal (78 neonates) or cesarean (72 neonates) delivery. To assess the effect of the body weight on colonization rates, the neonates were categorized in to four groups according to weight. Group 1 comprised 4 neonates (<2 kg); group 2, 65 neonates (2-3 kg); group 3, 74 neonates (3-4 kg), and group 4, 7 neonates (>4 kg). Furthermore, to determine the rate of colonization according to the type of neonatal feeding, the neonates were classified into three groups: group 1 was breastfeeding, group 2 was bottle feeding and group 3 were mixed (bottle and breast), which included 27, 83, and 40 neonates, respectively.

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing of 188 gram-negative rods from fecal isolates was done using the VITEK 2 system according to the manufacturer’s instructions. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for the following antibiotics:\(^{13}\) amikacin (AMK), ampicillin (AMP), amoxicillin/clavulanic acid (AMC), cefepime (FEP), cefotaxime (CTX), cefazidime (CAZ), cefuroxime (CXM), ciprofloxacin (CIP), gentamicin (GEN), meropenem (MEM), nitrofurantoin (NIT), piperacillin/tazobactam (TPZ), and sulfamethoxazole-trimethoprim (SXT).

**Phenotypic detection of ESBLs**

ESBL screening was carried out by disk diffusion using cefazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg) or aztreonam (30 µg), in accordance with the recommendations of the CLSI guidelines.\(^{13}\) A combined disk test (CDT) was performed to seek ESBL producers as per CLSI guidelines.\(^{13}\) Discs of cefazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg) or aztreonam (30 µg) were placed together as a single unit with a 5 mm distance in between. The presence of ESBL production was confirmed by inhibition zone diameter of ≥16 mm.
μg) and cefotaxime (30 μg) with and without clavulante (Becton Dickinson, USA) were placed on Mueller-Hinton agar plate (Saudi Prepared Media Laboratory, Riyadh, Saudi Arabia). ESBL phenotype was defined as an increase of ≥5 mm in the zone around the disc containing clavulante compared to the zone of corresponding discs without clavulante.

Phenotypic detection of carbapenemases
The MHT was used to detect carbapenemase production. This test was conducted according to CLSI guidelines.13 A 0.5 McFarland’s suspension of E. coli ATCC 25922 was diluted 1:10 in sterile saline. This was inoculated on a Mueller-Hinton agar plate. An ertapenem disc was placed in the center of the agar plate; 3–5 colonies of the test organism were inoculated in a straight line, from the edge of the disc, up to a distance of at least 20 mm. The plates were incubated at 37°C overnight and examined the next day. Isolates with the cloverleaf images of inhibition were considered carbapenemase-positive isolates.

Phenotypic detection of MBLs
The isolates that showed reduced susceptibility to imipenem (<27 mm zone size) were selected to screen metallo-β-lactamase (MBL) phenotypically according to CDT method.14 A 0.5 McFarland turbidity standard of overnight culture of the test strain was swabbed on Mueller-Hinton agar plates. Two disks of imipenem containing EDTA solution were placed on the plates and the plates were incubated at 35°C for 24 hrs. An increase of at least 5 mm around the disc of imipenem containing EDTA than the sizes of the inhibition zones produced by imipenem was recorded as a positive result.

Statistical methods
Data were stored and analyzed using SPSS 19 (Statistical Package for Social Science; release 19.0). Fishers exact test one way analysis of variance (ANOVA), Kruskal-Wallis test (non-parametric test), chi-square linear trend, chi-square test were used and a P value <.05 was considered as significant.

RESULTS

Distribution of gram-negative bacteria from the neonatal fecal specimens
One hundred fifty fecal specimens from Saudi neonates aged from one day to 7 days were examined for intestinal microbiota. Of 188 gram-negative rods isolated, 165 (87.7%) were oxidase-negative fermentative gram-negative rods, including Enterobacteriaceae: E. coli (n=130); K pneumoniae (n=23); E cloacae (n=5); E aerogenes (n=4); M morganii (n=3). Twenty-three (12.2%) of the 188 specimens were non-glucose fermenting gram-negative rods: P. aeruginosa (n=14) and Acinetobacter baumannii (n=9). E. coli strains were the most common isolates in this study and represent 69.1% (n=130/188) of recovered gram-negative rods. From 150 fecal specimens, 20 (15.4%) of 130 E. coli strains were recovered as the sole organism. The following organisms were also found: Enterococcus faecalis (n=68/130, 52.3%), K pneumoniae (n=13/130, 10%), A. baumannii (n=8/130, 6.1%), P. aeruginosa (n=7/130, 5.4%), E. aerogenes (n=4/130, 3.1%). Additionally, E. coli was found in association with two other organisms: both E. faecalis and K pneumoniae (n=4/130, 3.1%); both E. faecalis and P. aeruginosa (n=4/130, 3.1%); both Enterococcus faecalis and Staphylococcus aureus (n=2/130, 1.5%).

Distribution of gram-negative bacteria by clinical characteristics of neonates

Table 1 shows the effect of the age, mode of delivery, body weights and breastfeeding on the intestinal microbiota. Among the four age groups, the frequency of E. coli colonization in group 1 was significantly higher than observed in groups 2, 3, and 4 (P<.03). Among the four age groups, the frequency of K pneumoniae among the neonates increased from 13.3% on day 1 to 23.4% on day 2 and then decreased to 11% in the following days (9.76% in group 3 and 11.77% group 4). The frequency of P. aeruginosa among the neonates increased from 2.2% on day 1 to 10.6% on day 2 then slightly decreased to 9.7% on day 3 and then markedly increased to 23.5% in group 4. Frequency of E. coli colonization rate among vaginal delivery of neonates (70/78, 89.7%) appeared slightly higher than colonization among those delivered by cesarean (60/72, 83.3%) (P=.249), while the colonization rate of K pneumoniae among virginal delivery of neonates (10/78, 12.8%) appeared slightly lower than its colonization among those delivered by cesarean (13/72, 18.1%) (P=.374). On the other hand, there was a difference between colonization rates of P aeruginosa in vaginal (5/78; 6.4%) and cesarean (9/72; 12.5%) delivery (P=0.2). The results showed that the prevalence of E. coli colonization in group 1 (<2 kg) and in group 2 (2-3 kg) were 100% (n=6/6) and 88.9% (n=56/63), respectively which are significantly higher than its colonization (P=.0001) in other body weight groups with frequencies of 63.26% (n=62/98) and 60% (n=6/10) for groups 3 and 4, re-
spectively. Whereas the influence of feeding type on colonization indicates that the prevalence of *E. coli*, *K. pneumoniae* and *P. aeruginosa* appears slightly higher among neonates with breast feeding as compared with bottle feeding alone or mixed with breastfeeding.

**Antibiotic susceptibility**

The resistance patterns for *E. coli* (n=130), *K. pneumoniae* (n=23), *E. cloacae* (n=5), *E. aerogenes* (n=4), *M. morganii* (n=3), *P. aeruginosa* (n=14) and *A. baumannii* (n=9) strains are shown in Table 2. All isolates were susceptible to meropenem and amikacin with the only exception observed in two isolates of *E. cloacae*; 98%, 94%, and 90% of isolates were sensitive to ciprofloxacin, cefepime, and piperacillin/tazobactam, respectively.

**Prevalence of ESBLs and carbapenemases**

In the CDT for ESBLs for the 188 gram-negative isolates, 28 (14.9%) had a positive result while the MHT and imipenem/ imipenem-EDTA test were positive MBL for 3/188 (1.6%). Three isolates were positive for both the ESBL and metallo-carbapenemases tests (Table 3). ESBL and MBL were not detected in *P. aeruginosa* or *A. baumannii*. Table 3 demonstrates the resistance patterns of 28 ESBL-positive and 160 ESBL-negative strains.

**DISCUSSION**

Previous studies have reported fecal carriage of carbapenemase-producing *Enterobacteriaceae*. In Saudi Arabia, to our knowledge, there is not a single report on the fecal colonization of ESBL-, MBL- and multidrug resistant (MDR) isolates from neonates. Accordingly, we conducted the present study to investigate the prevalence of fecal carriage of ESBLs and carbapenemase-producing gram-negative bacteria among 150 neonates born in two Qassim hospitals, in central Saudi Arabia. We also assessed the risk factors (age in days, mode of delivery, body weight and type of neonatal feeding) on the prevalence of fecal carriage of gram-negative bacteria. In this study, *E. coli* was the most prevalent isolate followed by *K. pneumonia*, which is similar to previous findings in the United States and Mexico. In Turkey and India, however, *K. pneumoniae* predominates over *E. coli* in neonatal fecal specimens. The bacterial colonization pattern in the neonatal gut depends on various factors: gestational age, mode of delivery, type of feeding, bacterial interactions, antimicrobial therapy and other environmental factors. Our results showed that the frequency of *E. coli* colonization in the first day of life was 95%, which was relatively higher than colonization in subsequent days of life. This result demonstrates that *E. coli* colonization during the first days

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**Table 1. Distribution of different gram-negative bacteria by clinical characteristics of subjects.**

| Characteristics | Number of neonates | *E. coli* (n=130) | *K. pneumoniae* (n=23) | *P. aeruginosa* (n=14) | Others* (n=21) |
|-----------------|-------------------|-------------------|-------------------|-------------------|----------------|
| **Age (days)**  |                   |                   |                   |                   |                |
| 1               | 45                | 43/95.6           | 6/13.3            | 1/2.2             | 8/17.8         |
| 2               | 47                | 39/83             | 11/23.4           | 5/10.6            | 8/17           |
| 3               | 41                | 36/87.8           | 4/9.7             | 4/9.7             | 1/2.4          |
| 4-7             | 17                | 12/70.6           | 2/11.7            | 4/23.5            | 4/23.5         |
| **Mode of delivery** |               |                   |                   |                   |                |
| Vaginal         | 78                | 70/89.74          | 10/12.8           | 5/6.4             | 9/11.5         |
| Cesarean        | 72                | 60/83.3           | 13/18.1           | 9/12.5            | 12/16.6        |
| **Body-weight/kg** |               |                   |                   |                   |                |
| <2              | 4                 | 4/100             | 1/25              | 0/0               | 0/0            |
| 2-3             | 65                | 58/89.2           | 11/16.9           | 3/4.6             | 8/12.3         |
| 3-4             | 72                | 62/86.1           | 7/9.7             | 6/8.3             | 13/18.1        |
| >4              | 9                 | 6/66.6            | 4/44.4            | 5/55.5            | 0/0            |
| **Feeding-type** |                   |                   |                   |                   |                |
| Breast          | 27                | 26/96.3           | 8/29.6            | 3/11.1            | 0/0            |
| Bottle          | 83                | 71/85.5           | 10/12.1           | 7/8.4             | 17/20.5        |
| Mixed           | 40                | 33/82.5           | 5/12.5            | 4/10              | 4/10           |

*Chi - Square linear trend

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### Table 2. Resistance patterns and MIC50/90 of 188 gram negative rods isolated from neonate’s stool.

| Antibiotic | Antibiotic susceptibility pattern |
|------------|----------------------------------|
|            | *E coli* (n=130)                 | *K pneumoniae* (n=23) | *E cloacae* (n=5) | *E aerogenes* (n=4) |
|            | R | No | % | MIC 50/90 | R | No | % | MIC 50/90 | R | No | % | MIC 50/90 | R | No | % | MIC 50/90 |
| AMP        | 73 | 56.1 | 16/≥32 | 23 | 100 | 32/≥32 | 3 | 60 | 32/≥32 | 3 | 75 | 8/≥32 |
| AMC        | 53 | 40.7 | 16/≥32 | 1 | 5 | 16/≥32 | 3 | 60 | 16/≥32 | 3 | 75 | 16/≥32 |
| TZP        | 14 | 1 | 32/≥128 | 1 | 5 | 128/≥128 | 3 | 60 | 128/≥128 | 1 | 25 | 128/≥128 |
| CXM        | 39 | 3 | 16/≥64 | 1 | 5 | 64/≥64 | 4 | 80 | 64/≥64 | 1 | 25 | 64/≥64 |
| FOX        | 55 | 4 | 32/≥64 | 0 | 0 | 4/≥4 | 4 | 80 | 4/≥4 | 1 | 25 | 64/≥64 |
| CTX        | 18 | 1 | 16/≥64 | 1 | 5 | 64/≥64 | 3 | 60 | 64/≥64 | 1 | 25 | 64/≥64 |
| CAZ        | 17 | 13 | 16/≥64 | 1 | 5 | 16/≥16 | 3 | 60 | 16/≥16 | 1 | 25 | 64/≥64 |
| FEP        | 8 | 6 | 8/≥4 | 1 | 5 | 4/≥4 | 3 | 60 | 4/≥4 | 0 | 0 | 1/≥2 |
| MEM        | 0 | 0 | 0.25/≥0.25 | 0 | 0 | 0.25/≥0.25 | 3 | 60 | 2/≥2 | 0 | 0 | 0.25/≥0.25 |
| AMK        | 0 | 0 | 2/≥4 | 0 | 0 | 2/≥4 | 3 | 60 | 2/≥4 | 0 | 0 | 2/≥4 |
| GEN        | 8 | 6 | 16/≥16 | 1 | 5 | 16/≥16 | 3 | 60 | 16/≥16 | 0 | 0 | 1/≥1 |
| CIP        | 2 | 1.5 | 4/≥4 | 0 | 0 | 0.25/≥0.25 | 3 | 60 | 0.25/≥0.25 | 0 | 0 | 0.25/≥0.25 |
| NIT        | 65 | 50 | 64/≥512 | 20 | 87 | 64/≥128 | 5 | 100 | 64/≥128 | 4 | 100 | 64/≥128 |
| SXT        | 14 | 11 | 80/≥320 | 1 | 5 | 320/≥320 | 5 | 100 | 320/≥320 | 0 | 0 | 20/≥20 |

AMP, ampicillin; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; CAZ, ceftazidime; CIP, ciprofloxacin; MEM, meropenem; NIT, nitrofurantoin; SXT, sulfamethoxazole-trimethoprim; R=Resistant.

### Table 2. Resistance patterns and MIC50/90 of 188 gram negative rods isolated from neonate’s stool.

| Antibiotic | Antibiotic susceptibility pattern |
|------------|----------------------------------|
|            | *M morganii* (n=3)               | *P aeruginosa* (n=14) | *A baumannii* (n=9) |
|            | R | No | % | MIC 50/90 | R | No | % | MIC 50/90 | R | No | % | MIC 50/90 |
| AMP        | 3 | 100 | 32/≥32 | 14 | 100 | 32/≥32 | 9 | 100 | 16/≥32 |
| AMC        | 3 | 100 | 32/≥32 | 14 | 100 | 16/≥32 | 7 | 77.7 | 16/≥32 |
| TZP        | 0 | 0 | 4/≥4 | 2 | 14 | 128/≥128 | 6 | 66.7 | 64/≥128 |
| CXM        | 3 | 100 | 64/≥64 | 14 | 100 | 64/≥64 | 9 | 100 | 32/≥64 |
| FOX        | 1 | 33.3 | 16/≥16 | 14 | 100 | 16/≥64 | 9 | 100 | 64/≥64 |
| CTX        | 1 | 33.3 | 16/≥16 | 1 | 7 | 16/≥16 | 0 | 0 | 16/≥16 |
| CAZ        | 1 | 33.3 | 16/≥16 | 1 | 7 | 16/≥16 | 0 | 0 | 16/≥16 |
| FEP        | 0 | 0 | 2/≥2 | 0 | 0 | 2/≥2 | 0 | 0 | 1/≥1 |
| MEM        | 0 | 0 | 0.25/≥0.25 | 0 | 0 | 0.25/≥0.25 | 0 | 0 | 0.25/≥0.25 |
| AMK        | 0 | 0 | 2/≥2 | 0 | 0 | 2/≥2 | 0 | 0 | 2/≥2 |
| GEN        | 0 | 0 | 1/≥1 | 1 | 7 | 16/≥16 | 0 | 0 | 16/≥16 |
| CIP        | 0 | 0 | 0.25/≥0.25 | 0 | 0 | 0.25/≥0.25 | 0 | 0 | 0.25/≥0.25 |
| NIT        | 3 | 100 | 128/≥128 | 14 | 100 | 16/≥512 | 9 | 100 | 16/≥512 |
| SXT        | 0 | 0 | 20/≥20 | 12 | 86 | 80/≥160 | 3 | 33.3 | 80/≥160 |

AMP, ampicillin; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; CAZ, ceftazidime; CIP, ciprofloxacin; MEM, meropenem; NIT, nitrofurantoin; SXT, sulfamethoxazole-trimethoprim; R=Resistant.
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of life is transient. The *K. pneumoniae* colonization rate during the first days of life is apparently different from that of *E. coli*. The frequency of *K. pneumoniae* among the neonates 1 day old was 13% and it increased to 23% on day 2, and then decreased, reaching a plateau of about 11% in the following days for group 3 and group 4. This suggests acquisition later on from the environment rather than acquisition during delivery, whereas the *P. aeruginosa* colonization rate increased in parallel with neonatal age, reaching its maximum colonization by day 4-7. Although other gram-negative rods were limited in number, the trend of colonization behaved more or less similar to that of *P. aeruginosa*. The frequency of *E. coli* colonization with vaginal delivery appears slightly higher than its colonization among those delivered by cesarean delivery, but the frequencies of *K. pneumoniae* (13/72, 18%) and *P. aeruginosa* (9/72, 12.5%) colonization appeared slightly higher among neonates delivered by cesarean than by vaginal delivery (13/72, 18%, 9/72, 12.5%), respectively, suggesting the lack of efficient application of infection control measures and environmental acquisition despite cesarean delivery.

Our results are similar to a previous study. Our study found that the neonatal microbiota of the gut differ between those delivered by cesarian section and those delivered vaginally. According to Dominguez-Bello et al. (2010), upon delivery the neonate is exposed for the first time to a wide array of microbes from a variety of sources, including maternal bacteria, which shapes establishment of the microbiota, and subsequently, its role in child health. Thus Goldani et al. (2011) found that the obesity rate in adults born by cesarean delivery was 15.2% while in those born by vaginal delivery it was 10.4% (*P* = .002). In a previous study in Switzerland, it was found that an outbreak of ESBL–producing *E. coli* in a neonatal care unit began with transmission from a mother to her newborn twins during vaginal delivery. Subsequently, infection spread by healthcare worker contact with other neonates; a healthcare worker also was infected. The present study demonstrates that the prevalence of *E. coli* predominates significantly among neonates with low body weight (<2 kg, 6/6, 100%) as compared to higher body weight (>2 kg up to 4 kg, 60% to 89%). Results suggest that neonates with low body

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### Table 3. Resistance patterns of ESBL-positive and ESBL-negative strains isolated from neonates.

| Antibiotics | *E. coli* (n=130) | *K. pneumoniae* (n=23%) | *E. cloacae* (n=5) | *E. aerogenes* (n=4) | *M. morganii* (n=1/3; 33%) |
|-------------|------------------|------------------------|-------------------|---------------------|------------------------|
|             | ESBL positive (n=22/130; 17%) | ESBL negative (n=108/130; 83%) | ESBL positive (n=1/23; 4%) | ESBL negative (n=22/23; 95.6%) | ESBL positive (n=3/5; 60%) | ESBL negative (n=2/5; 40%) | ESBL positive (n=1/4; 25%) | ESBL negative (n=3/4; 75%) | ESBL positive (n=1/3; 33.3%) | ESBL negative (n=2/3; 66.7%) |
| AMP         | 22 (100)         | 69 (63.9)              | 1 (100)           | 22 (100)            | 3 (100)                | 0 (0)                   | 1 (100)            | 2 (67.7)              | 1 (100)            | 2 (100)          |
| AMC         | 21 (95.5)        | 32 (29.6)              | 1 (100)           | 0 (0)               | 3 (100)                | 0 (0)                   | 1 (100)            | 2 (67.7)              | 1 (100)            | 2 (100)          |
| TZP         | 10 (45)          | 4 (3.7)                | 1 (100)           | 0 (0)               | 3 (100)                | 1 (50)                  | 1 (100)            | 0 (0)                 | 1 (100)            | 2 (100)          |
| CXM         | 22 (100)         | 17 (15.7)              | 1 (100)           | 0 (0)               | 3 (100)                | 0 (0)                   | 1 (100)            | 1 (100)              | 1 (100)            | 0 (0)            |
| FOX         | 21 (95.5)        | 34 (31.5)              | 0 (0)             | 0 (0)               | 3.0 (100)              | 1 (50)                  | 1 (100)            | 3 (100)              | 1 (100)            | 0 (0)            |
| CTX         | 18 (82)          | 0 (0)                  | 1 (100)           | 0 (0)               | 3 (100)                | 0 (0)                   | 1 (100)            | 1 (100)              | 1 (100)            | 0 (0)            |
| CAZ         | 17 (72)          | 0 (0)                  | 1 (100)           | 0 (0)               | 3 (100)                | 0 (0)                   | 1 (100)            | 0 (0)                 | 1 (100)            | 0 (0)            |
| FEP         | 8 (38)           | 0 (0)                  | 1 (100)           | 0 (0)               | 3 (100)                | 0 (0)                   | 0 (0)              | 0 (0)                 | 0 (0)              | 0 (0)            |
| MEM         | 0 (0)            | 0 (0)                  | 0 (0)             | 0 (0)               | 3 (100)                | 0 (0)                   | 0 (0)              | 0 (0)                 | 0 (0)              | 0 (0)            |
| AMK         | 0 (0)            | 0 (0)                  | 1 (100)           | 0 (0)               | 3 (100)                | 0 (0)                   | 0 (0)              | 0 (0)                 | 0 (0)              | 0 (0)            |
| GEN         | 3 (13.6)         | 5 (5)                  | 1 (100)           | 0 (0)               | 3 (100)                | 0 (0)                   | 0 (0)              | 0 (0)                 | 0 (0)              | 0 (0)            |
| CIP         | 2 (9.1)          | 0 (0)                  | 0 (0)             | 0 (0)               | 3 (100)                | 0 (0)                   | 0 (0)              | 0 (0)                 | 0 (0)              | 0 (0)            |
| NIT         | 9 (40.9)         | 56 (51.8)              | 1 (100)           | 8 (36.4)            | 3 (100)                | 2 (100)                 | 1 (100)            | 3 (100)              | 1 (100)            | 2 (100)          |
| SXT         | 7 (31.8)         | 7 (6.5)                | 1 (100)           | 0 (0)               | 3 (100)                | 0 (0)                   | 0 (0)              | 0 (0)                 | 0 (0)              | 0 (0)            |

AMP: ampicillin; AMC: amoxicillin-clavulanic acid; TZP: piperacillin-tazobactam; CAZ: ceftazidime; FEP: cefepime; CXM: cefuroxime; FOX: cefotaxime; CTX: cefotaxime; GEN: gentamicin; AMK: amikacin; CIP: ciprofloxacin; MDA: meropenem; NIT: nitrofurantoin; SX, sulfamethoxazole-trimethoprim.
weight are highly vulnerable to colonization with E coli as the predominate enteric bacteria. This may explain the frequent implication of E coli neonatal sepsis and/or meningitis in premature babies who inherently have a lower body weight.16,25 In current study, the influence of feeding type on the neonate’s microbiota seems to indicate that the prevalence of E coli, K pneumoniae and P aeruginosa appears slightly higher among neonates who undergo breastfeeding as compared with bottle feeding alone or mixed with breastfeeding. It has been reported that colonization in breastfed neonates differs from formula fed neonates.15,21 On other hand, some authors have found that the fecal microflora of breastfed and formula fed infants are essentially the same.26 In the present study, the prevalence of P aeruginosa and A. buumannii significantly increased among formula feeding as compared to breast feeding neonates. These findings are compatible with those of a previous study.27 Hence our results would seem to encourage breastfeeding as a major protecting factor for the colonization of such hazardous pathogens.

Most of the 188 isolates were sensitive to meropenem (98%), ciprofloxacin (98%), amikacin (98%) and gentamicin (94%). All gram-negative bacterial isolates exhibited high susceptibility against amoxicillin-clavulanate than ampicillin. This finding is consistent with previous studies.19,28 ESBLs are class A enzymes that are inhibited in vitro by β-lactamase inhibitors, whereas those belonging to class B, C and D are not affected.29 ESBL-producing bacteria have spread widely and have become a major cause of nosocomial infections associated with high mortality rates.26,30 A PubMed search found no reports on the prevalence of fecal carriage of ESBL- and MBL-producing strains recovered from neonates in Saudi Arabia. The data presented here provide the first insight into the fecal colonization of ESBL- and carbapenemase-producing strains in Qassim, Saudi Arabia. According to the phenotypic test, the overall prevalence of ESBLs was 15%. This percentage is considered very high in neonates. For comparison, the prevalence of ESBL fecal carriage in the present study was, more or less, in agreement with previous studies.5,31-34 The prevalence of ESBLs was different according to the type of organism. In the current study, the prevalences of ESBL among E coli, K pneumoniae, M morganii and E aerogenes were 17%, 4%, 33%, and 25%, respectively. On the other hand, 60% (3/5) of E cloacae harbored both ESBL and MBL. Meropenem exhibited full activity against all tested strains, with the exception of three E cloacae strains, presumably due to the influence of carbapenemase among these intermediate resistant strains. Fecal colonization in neonatal intensive care units by ESBL and/or MDR bacteria can be a source of nosocomial infections.35 Gram-negative bacteria that exhibited no ESBLs showed fewer MDR patterns in general as compared to positive ESBLs strains, as expected. The risk factors for E cloacae infection in neonates include being small for gestational age, having low birth weight, having ongoing parenteral nutrition, understaffing, over-crowding, poor hygienic practices, contaminated thermometers, presence of E cloacae in the stool and exposure to personnel with contaminated hands.36-38 E cloacae was responsible for several episodes of late neonatal sepsis in very-low-birth-weight neonates in Taiwan.39 The occurrence and detection of MDR strains underline the extraordinary spread of responsible genes among our local enterobacterial strains, and indeed a great deal of these MDR strains can be avoided by the more efficient application of infection control measures, and efficient hand washing.

The present study has some limitations, which include absence of molecular characterization of ESBL, and MBL expressed by the bacteria as well as their dissemination sources. Additionally, this study was conducted at only two medical centers in the Qassim area. Therefore, comprehensive multi-center studies in Saudi Arabia are recommended to address the emerging problem of ESBLs and/or MBL-associated infections in order to preserve the continued usefulness of most antimicrobial drugs; and to define the ESBL- and MBL-genotypes as well as epidemiological studies to trace the source of infection.

In summary, within the first week of life, the most important determinants of gut enteric bacteria composition in neonates were age-day, body weight, mode of delivery, and type of neonatal feeding. The findings of ESBLs- and MBLs-producing enteric bacteria as neonate-gut colonizers confirms previous studies and may have wider implication or linkage to prevention of nosocomial and community-acquired infections by these strains. Targeted surveillance of high-risk patients and screening is essential to prevent outbreaks; meanwhile in a hospital setting the standard precautions, primarily hand washing, must be reinforced and rational use of antibiotics should be an obligation.

Conflict of interest
The authors declared no competing interests.

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