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Prevalence of fecal carriage of extended-spectrum- and metallo-β-lactamase-producing gram-negative bacteria among neonates born in a hospital setting in central Saudi Arabia

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BACKGROUND AND OBJECTIVE: Commensal neonatal fecal flora constitute a reservoir of antibiotic resistance. The aim of this study was to characterize the prevalence of fecal carriage of extended spectrum beta-lactamases (ESBLs) and carbapenemase producing gram-negative bacteria among 150 neonates who were born in two hospitals in central Saudi Arabia.

PATIENTS AND METHODS: From June 2012 to Janauary 2013, 150 healthy neonates ≤7 days of age were screened for intestinal enteric bacteria colonization and the influence of age, mode of delivery, body weight and breast feeding of neonates on colonzation rates. The isolates were identified manually and by VITEK 2 and antibiotic susceptibility was tested. Phenotypic tests were performed to seek ESBL and metallo-β-lactamase (MBL) producers in recovered isolates.

RESULTS: Among 150 fecal specimens, 188 gram-negative rods were recovered. *E coli* (n=130; 69.1%) was the commonest organism followed by *Klebsiella pneumoniae* (n=23; 12.2%), *Pseudomonas aeuroginosa* (n=14; 7.5%), *Acinetobacter baumannii* (n=9; 4.8%), Enterobacter spp. (n=9; 4.8%) and *Morganella morganii* (n=3; 1.6%). *E coli* colonization during the first day of life was 95.5% (n=43/45), which was relatively higher than in subsequent days. The colonization rate of *E coli* among low weight babies (≤3 kg) was significantly higher than fully grown neonates (≥4 kg)(*P*=.03). The prevalence of ESBL and MBL was 15.0% (n=28/188) and 1.6% (n=3/188). The prevalence rates of ESBL in *E coli* , *K pneumoniae*, *E cloacae*, *M morganii* and *E aerogenes* were 16.9% (n=22/130), 4.3 % (n=1/23), 60% (n=3/5), 33.3% (n=1/3), and 25% (n=1/4), respectively. All *E cloacae* with ESBLs and MBLs were resistant to all tested antibiotics including carbapenems.

CONCLUSION: Within the first week of life, the most important determinants of gut enteric bacteria composition in neonates were age in days, body weight, mode of delivery, and type of neonatal feeding. The findings of ESBLs- and MBLs-producing enteric bacteria as neonate-gut colonizers may have wider implication or linkage to prevention of nosocomial and community-acquired infections by these strains.

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.[1,2] The gastrointestinal microbiota play an essential role in the health and disease of the host through its impact on nutrition, pathogenesis and immunol-ogy. The gastrointestinal tract is the main reservoir and source for transmission of nosocomial pathogens, including Enterobacteriaceae.[3] Previous studies have demonstrated fecal colonization by extended-spectrum-β-lactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae.[4-6] The first fecal colonization with ESBL-producing *Enterobacteriaceae* were identified in Spain in 2003.[7] Since then, rates of colonization by ESBL-producing *Enterobacteriaceae* have increased

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 orgaisms were counted (colony forming units/gram of stool) and then identfied 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), ceftazidime (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 ceftazidime (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 ceftazidime (30

μg) and cefotaxime (30 μg) with and without clavulanate (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 clavulanate compared to the zone of corresponding discs without clavulanate.

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 without and with 4 μL (of 750 μg) 0.5 M EDTA (ethylenediaminetetraacetic acid, Sigma, USA) solution were placed on the plates and the plates were incubated at 35 C0 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 iso-lated, 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 aeuroginosa* (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 aeuroginosa* (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 aeuroginosa* (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-

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

Characteristics		Number of neonates	Recovered gram negative bacteria (n=188)							
			E coli (n=130)		K pneumoniae (n=23)		P aeruginosa (n=14)		Others[a] (n=21)	
			n/%	P	n/%	P	n/%	P	n/%	P
Age (days)	1	45	43/95.6		6/13.3		1/2.2		8/17.8	
	2	47	39/83	.03	11/23.4	.554	5/10.6	.021	8/17	.450
	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	.249	10/12.8	.374	5/6.4	.200	9/11.5	.366
	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	.0001	11/16.9	.848	3/4.6	.002	8/12.3	.917
	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	.239	10/12.1	.075	7/8.4	.904	17/20.5	.02
	Mixed	40	33/82.5		5/12.5		4/10		4/10	

[a]*A baumannii* (n=9), *E cloacae* (n=5), *E aerogenes* (n=4) and *M morganii* (n=3). [1]Chi - Square Linear trend

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*.[6] In Saudi Arabia, to our knowledge, there is not a single report on the fecal colonization of ESBL-, MBLs- 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.[15-18] In Turkey and India, however, *K pneumoniae* predominates over *E coli* in neonatal fecal specimens.[19,20] 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.[15,21] 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

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		MIC 50/90	R		MIC 50/90	R		MIC 50/90	R		MIC 50/90
	No	%		No	%		No	%		No	%	
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/≥64	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/≥2
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; FEP, cefepime; CXM, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; GEN, gentamicin; AMK, amikacin; 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		MIC 50/90	R		MIC 50/90	R		MIC 50/90
	No	%		No	%		No	%	
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	16/≥64	9	100	32/≥64
FOX	1	33.3	16/≥16	14	100	64/≥64	9	100	64/≥64
CTX	1	33.3	16/≥16	14	100	16/≥64	9	100	32/≥64
CAZ	1	33.3	16/≥16	1	7	16/≥16	0	0	16/≥16
FEP	0	0	2/≥2	0	0	2/≥16	0	0	1/≥16
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; FEP, cefepime; CXM, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; MEM, meropenem; NIT, nitrofurantoin; SXT, sulfamethoxazole- trimethoprim; R=Resistant.

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.[22] Our study found that the neonatal microbiota of the gut differ between those delivered by caesarian 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.[1] 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).[23] 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.[24] 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 (<2kg, 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

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%)
	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)
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)	0 (0)	1(100)	0 (0)	0/0	0 (0)
CXM	22(100)	17(15.7)	1 (100)	0 (0)	3 (100)	1 (50)	1(100)	0.0(0)	1(100)	2 (100)
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)	0 (0)	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 (36)	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)	7.0(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, cefoxitin; CTX, cefotaxime; GEN, gentamicin; AMK, Amikacin; CIP, ciprofloxacin; MEM, meropenem; NIT, nitrofurantoin; SXT, 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.[18,25] 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. bumannii 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.[29,30] 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.[4,5,31-34] 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 precaustions, primarly 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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REFERENCES

1. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 2010; 107(26):11971-5.

2. Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr 1999; 69:1035S–45S

3. Donskey CJ. The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. Clin Infect Dis 2004; 39(2):219-26.

4. Al-Agamy MHM, Ali MS, Salem MM, Mohamed TR. Faecal Colonization by Extended-Spectrum Beta-Lactamase-Producing Escherichia coli from Hospitalized Patients. 2009

5. Garrido A, Seral C, Gude MJ, Casado C, González-Domínguez M, Sáenz Y, Castillo FJ. Characterization of plasmid-mediated ?-lactamases in fecal colonizing patients in the hospital and community setting in Spain. Microb Drug Resist 2014;20(4):301-4.

6. Zhao ZC, Xu XH, Liu MB, Wu J, Lin J, Li B. Fecal carriage of carbapenem-resistant Enterobacteriaceae in a Chinese university hospital. Am J Infect Control 2014;42(5):e61-4.

7. Mirelis B, Navarro F, Miró E, Mesa RJ, Coll P, Prats G.Community transmission of extended-spectrum beta-lactamase. Emerg Infect Dis 2003;9(8):1024-5.

8. Al-Agamy MH, Shibl AM, Hafez MM, Al-Ahdal MN, Memish ZA, Khubnani H. Molecular characteristics of extended-spectrum ?-lactamase-producing Escherichia coli in Riyadh: emergence of CTX-M-15-producing E coli ST131. Ann Clin Microbiol Antimicrob 2014; 7:13:4.

9. Chen L, Mathema B, Chavda KD, DeLeo FR, Bonomo RA, Kreiswirth BN. Carbapenemase-producing Klebsiella pneumoniae: molecular and genetic decoding. Trends Microbiol 2014;22(12):686-96.

10. Al-Agamy MH, Shibl AM, Elkhizzi NA, Meunier D, Turton JF, Livermore DM. Persistence of Klebsiella pneumoniae clones with OXA-48 or NDM carbapenemases causing bacteraemias in a Riyadh hospital. Diagn Microbiol Infect Dis 2013;76(2):214-6.

11. Poirel, L., Al-Agamy, M.H., Shibl, A.M., Memish, Z. Couperey F., Nordmann P: VIM-1 and NDM-1-producing enterobacterial isolates, Saudi Arabia. 23rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), which held on Berlin from 26th to 28th April 2013. P 1291.

12. Collado MC,Calabuig M, Sanz Y. Differences between the Fecal Microbiota of Coeliac Infants and Healthy Controls. Curr Issue intestinal micrbiol 2006; 8: 9–14.

13. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing:24th informational supplement, CLSI document M100-S24, Wayne, PA, CLSI, 2014.

14. Bashir D, Thokar MA, Fomda BA, Bashir G, Zahoor D, Ahmad S and Toboli AS. Detection of metallo-beta-lactamase (MBL) producing Pseudomonas aeruginosa at a tertiary care hospital in Kashmir. Afr J Microbiol Res. 2011; 5(2): 164-72.

15. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA, Stobberingh EE.Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics 2006;118(2):511-21.

16. Calva JJ, Sifuentes-Osornio J, Cerón C. Antimicrobial resistance in fecal flora:longitudinal community-based surveillance of children from urban Mexico. Antimicrob Agents Chemother 1996;40(7):1699-702.

17. Jost T, Lacroix C, Braegger CP, Chassard C. New Insights in Gut Microbiota Establishment in Healthy Breast Fed Neonates. PloS One 2012; 7(8):e44595.

18. Sharma N, Chaudhry R, Panigrahi P. Quantitative and qualitative study of intestinal flora in neonates. J Glob Infect Dis. 2012;4(4):188-92.

19. Duman M, Abacioglu H, Karaman M, Duman N, Özkan H.?-lactam antibiotic resistance in aerobic commensal fecal flora of newborns. Pediatrics International; 2005;47:(3) 267–73.

20. Das P, Singh AK, Pal T, Dasgupta S, Ramamurthy T, Basu S. Colonization of the gut with Gram-negative bacilli, its association with neonatal sepsis and its clinical relevance in a developing country. J Med Microbiol 2011;60(11);1651–60.

21. Huurre A, Kalliomäki M, Rautava S, Rinne M, Salminen S, Isolauri E. Mode of Delivery-effects on gut microbiota and humoral immunity. Neonatol. 2008;93:236–40.

22. Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Chari RS, Sears MR, Becker AB, Scott JA, Kozyrskyj AL (2013). Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. CMAJ. Mar; 185(5):385-94.

23. Goldani H AS, Bettiol H, Barbieri M A, Silva A AM, Agranonik M, Morais M B, Goldani M Z (2011). Cesarean delivery is associated with an increased risk of obesity in adulthood in a Brazilian birth cohort study; Am J Clin Nutr; 93(6):1344-1347.

24. Tschudin-Sutter S, Frei R(2010). Battegay M, Hoesli I, Widmer AF. Extended spectrum ß-lactamase-producing Escherichia coli in neonatal care unit. Emerg Infect Dis; 16(11):1758-60.

25. Kaczmarek A, Budzy?ska A, Gospodarek E. The occurrence of Escherichia coli with K1 surface antigen in pregnant women and in newborns. Med Dosw Mikrobiol 2010;62(1):59-65.

26. Thompson-Chagoyan OC, Maldonado J, Gil A. Colonization and impact of disease and other factors on intestinal microbiota. Dig Dis Sci 2007;52:2069–77.

27. Nelson RK, Poroyko V, Morowitz MJ, Liu D, Alverdy JC. Effect of dietary monosaccharides on Pseudomonas aeruginosa virulence. Surg Infect 2012;14(1):35-42.

28. Yang CM, Lin MF, Lin CH, Huang YT, Hsu CT, Liou ML. Characterization of antimicrobial resistance patterns and integrons in human fecal Escherichia coli in Taiwan. Jpn J Infect Dis 2009;62(3):177-81.

29. Curello J, MacDougall C Beyond Susceptible and Resistant, Part II: Treatment of Infections Due to Gram-Negative Organisms ProducingExtended-Spectrum ?-Lactamases. J Pediatr Pharmacol Ther. 2014;19(3):156-64.

30. Bjorn B, Jureen R, Manji KP, Tamim BS, Mwakagile DSM, Urassa WK, Fataki M, Msangi V, Tellevik MG, Maselle SY, Langeland N. High Rate of Fatal Cases of Pediatric Septicemia Caused by Gram- Negative Bacteria with Extended-Spectrum Beta-Lactamases in Dar es Salaam, Tanzania. J Clin Microbiol 2005; 43:745-9.

31. Singh NK, Patel M, Leger MM. Risk of resistant infections with Enterobacteriaceae in hospitalized neonates. Pediatr Infect Dis J 2002;21: 1029-33.

32. Kader AA, Kumar A, Kamath KA. Fecal carriage of extended spectrum b-lactamase-producing Escherichia coli and Klebsiella pneumoniae in patients and asymptomatic healthy individuals. Infect Control Hosp Epidemiol 2007;28:1114-6.

33. Kiremitci A, Dinleyici EC, Yargic ZA, Durmaz G, Tekin N, Aybey AD, Aksit MA. Prevalence and Risk Factors of Fecal Carriage of Extended-Spectrum ?-lactamase (ESBL) -Producing Enterobacteriaceae in Hospitalized and Ambulatory Children. J Pediatr Inf 2011;5: 54-8.

34. Desimoni MC, Esquivel GP, Merino LA.Fecal colonization by extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in a neonatal intensive care unit. Enferm Infecc Microbiol Clin 2004;22(9):507-11.

35. Moyo S, Aboud S, Kasubi M, Lyamuya E, Maselle S. Antimicrobial resistance among producers and non-producers of extended spectrum beta-lactamases in urinary isolates at a tertiary Hospital in Tanzania. BMC Res Notes 2010; 3:348.

36. Yu WL, Cheng HS, Lin HC, Peng CT, Tsai CH. Outbreak inves-tigation of nosocomial Enterobacter cloacae bacteraemia in a neonatal intensive care unit. Scand J Infect Dis 2000;32:293?8.

37. Fok TF, Lee CH, Wong EM. Risk factors for Enterobacter septicemia in a neonatal unit: case-control study. Clin Infect Dis 1998; 27:1204?9.

38. Harbarth S, Sudre P, Dharan S, Cadenas M, Pittet D. Outbreak of Enterobacter cloacaerelated to understaffing, overcrowding, and poor hygiene practices. Infect Control Hosp Epidemiol 1999; 20:598?603.

39. Chen HN, Lee ML, Yu WK, Lin YW, Tsao LY. Late-onset Enterobacter cloacae sepsis in very-low-birth-weight neonates: experience in a medical center. Pediatr Neonatol 2009; 50(1):3-7.