Prevalence and risk factors of early fecal carriage of *Enterococcus faecalis* and *Staphylococcus spp* and their antimicrobial resistant patterns among healthy neonates born in a hospital setting in central Saudi Arabia

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ABSTRACT

الأهداف: للتحقق من مدى انتشار ومقاومة المضادات الحيوية وعوامل الخطر في المكورات المعوية البرازية (انتيروكوكس فيكالس) والمكورات العنقودية (ستافيلوكوكس اسبيشز) في براز سعودي حديثي الولادة 150 الأصحاء المولودين في مستشفى الولادة والأطفال في وسط المملكة العربية السعودية.

الطريقة: أجريت هذه الدراسة العملية في مستشفى البكيرية العام و مستشفى الولادة والأطفال في منطقة القصيم، المملكة العربية السعودية في الفترة من يونيو 2012 م حتى يناير 2013م. وتم تحديد عزلات المكورات المعوية البرازية و المكورات العنقودية يدوياً وكذلك باستخدام نظام فايتاك للتأكيد الهوية على مستوى الأنواع و اختبار حساسية المضادات الحيوية Vitek2 system.

النتائج: تم عزل انتيروكوكس فيكالس (n=73)؛ استافيلوكوكس سبيشز (n=18). على عكس المكورات العنقودية، فإن انتشار انتيروكوكس فيكالس لا تختلف كثيراً عن اليوم الأول من العمر حتى اليوم السابع ولا لنوع التغذية كذلك. ولكنها كانت أعلى نسبياً بين الولادة الطبيعية، مقارنة بالولادة القيصرية. نسبة انتشار كل من المكورات العنقودية و استافيلوكوكس ابيديرميدس يزيد مع زيادة وزن الجسم (p=0.025) و هذا الفارق كان واضحاً معنوياً للاستافيلوكوس ابيديرميدس. شكلت مقاومة انتيروكوكس فيكالس عالية المستوى للجنتاميسين أو ستربتومايسين بنسبة 25% و 11% على التوالي . 30% من استافيلوكوكس ابيديرميدس مقاومة لل أوكساسيلين و أظهرت النتائج نمط متعدد المقاومة(5) ولوحظ هذا النمط المتعدد في المكورات العنقودية الذهبيةMRSA.

الخاتمة: على عكس المكورات العنقودية، فإن فارق الانتيروكوكس فيكالس لحديثي الولادة لا تختلف كثيراً فيما يتعلق في نوع الولاده، و العمرحتى سبع أيام وكذلك لنوعية التغذية. اعتبار وجود الجينات المتحكمة في تعدد أنماط المقاومة للمضادات الحيوية، في براز حديثي الولادة على أنه مستودع ومصدر خطير لانتشارها في المستشفيات، والعدوى المتقاطعة بين المرضى وكذلك انتشارها في المجتمع عموما.

Objectives: To investigate the prevalence, antibiotic resistant profiles, and risk factors of early fecal carriage of *Enterococcus faecalis (E. faecalis)* and staphylococci among 150 healthy Saudi neonates born in a hospital setting in central Saudi Arabia.

Methods. This prospective study was conducted in Al-Bukayriyah General Hospital, Qassim, Saudi Arabia, between June 2012 and January 2013. The *E. faecalis* and *Staphylococcus spp.* isolates were identified manually, and Vitek2 system was used for identity confirmation at the species level and minimum inhibitory concentration-susceptibility testing.

Results: *Enterococcus faecalis* (n=73) and *Staphylococcus spp.* (n=18) were recovered. Unlike staphylococci, *E. faecalis* colonization did not significantly vary from day one up to 7 days of life, regardless of the type of feeding, but it was relatively higher among vaginally versus cesarean delivery. Both *Staphylococcus epidermidis* (*S. epidermidis)* and *Staphylococcus aureus* carriage increase as the body weight increases, and this difference was significant (p=0.025) for *S. epidermidis*. High-level resistance in Gentamycin among *E. faecalis* isolates was 25% and 11% to Streptomycin. Thirty percent of *S. epidermidis* were resistant to oxacillin and exhibited multidrug-resistant (MDR) patterns of 5 resistant markers, which were also observed among 2/5 (40%) of *Methicillin-resistant Staphylococcus aureus* isolates.

Conclusion: *Enterococcus faecalis* did not significantly vary in relation to type of delivery, age up to 7 days, and type of feeding. The neonatal fecal carriage of MDR isolates should be considered as a crucial reservoir to the further spread of antimicrobial resistance genes among hospitals, cross infections, and the community.

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The gastrointestinal microbiota plays a crucial role in health and disease of the host through its impact on nutrition, pathogenesis, and immunology.[1] Unlike the adult human gut microbiota, the infant gut microbiota possesses a relatively simple structure, but is rather unstable overtime.[2] *Enterococci* are among the first bacteria to colonize the neonatal gastrointestinal tract, and are also recognized as the leading and most common nosocomial pathogens worldwide.[3] The genus *Enterococcus* is a Gram-positive, fermentative facultative aerobic cocci that is ubiquitous and highly adapted to hospital environments and others.[4-7] Consequently, enterococci have become recognized as serious nosocomial pathogens causing urinary tract infections, biliary tract infections, wound infections, intra-abdominal abscesses, endocarditis, bacteremia, as well as neonatal septicemia, and more than 85-90% of these infections are due to *Enterococcus faecalis* (*E. faecalis).*[3,4,8] Additionally, there are at least 3 major reasons for the emergence of multidrug-resistant (MDR) enterococci: a) baseline intrinsic resistance to several antimicrobial agent, b) acquired resistance via mobility of the resistance genes on plasmids and transposons, and the open chromosomal exchange, and c) the inter and intra homologous transferability of resistance among related bacteria.[3] Apart from the multi-resistant nature of enterococci, which indeed facilitate their initial intestinal colonization and competition with other intestinal flora, several putative virulence factors may also contribute to the pathogenicity of enterococci converting them from just mere colonization strains to nosocomial pathogens.[3,8] Thus, enterococcus is currently recognized as a major reservoir in the dissemination of resistant genes worldwide, including travelling to endemic countries.[9] Consequently, the evolution of antimicrobial resistance in enterococci has posed enormous challenges for clinicians[10] because of its inherent resistance to several commonly used antibiotics such as cephalosporins, low level aminoglycosides, and low level clindamycin. Perhaps more importantly, because of their acquired resistance, sometimes, to all currently available antibiotics, which results in the selection and spreading of MDR strains in hospitals and community.[11] Risk factors including indiscriminate use of antibiotics, prolonged hospital stay, severity of illness, and immune-suppression are mainly responsible for nosocomial acquisition of drug resistant enteroccoci. This ultimately leads to environmental contamination and cross infections.[8] Furthermore, recent data also suggest that the human gastrointestinal tract may be an important reservoir of MDR-*Staphylococcus spp*. strains, and there is considerable evidence that the gastrointestinal tract also provides an important source for transmission and dissemination of these organisms.[12] While several Saudi researchers have studied the adult fecal carriage of resistant bacterial strains,[13-18] in this first report, we describe the prevalence and antibiotic resistant profiles of early fecal carriage of *E. faecalis* and *Staphylococcus spp.* among healthy 150 Saudi neonates born in hospital setting in central Saudi Arabia, and their risk factors for the prevalence of colonization, in relation to age (≤7 days), type of feeding, mode of delivery, and body weights.

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Methods. Fecal samples were obtained from neonates aged 1-7 days (Ds) at Al-Bukayriyah General Hospital (BGH) and the Maternity and Children Hospital (MCH) Qassim region, central Saudi Arabia between June 2012 and January 2013. The study protocol was approved by the hospitals and the Collage of Applied Medical Sciences, King Saud University, Saudi Arabia, Research Ethical Committees, and written informed consent and questionaire for different characteristics was completed and taken from both parents of all of the newborns who agree to participate in the study. Epidemiological data were recorded for each neonate in respect to type of delivery, age, weight, and type of feeding. Mothers who had taken antibiotics 2 weeks prior to the delivery were excluded from the study. While several Gram negative and Gram positive bacterial isolates were isolated from the 150 examined neonates fecal specimens (**Table 1**), this study deals only with the Gram positive cocci, *E. faecalis* and *Staphylococcus spp.,* The other recovered neonatal fecal Gram negative enteric bacteria were excluded from the current study, and are reported separately.[19]

Collection of samples and microbiological methods. Fresh neonatal faeces were aseptically collected in sterile containers from diapers of 150 neonates (1-7 days old) and immediately transported to the microbiology laboratory and processed for bacterial isolate isolation on relevant media within 4 hours of collection. According to Jost et al,[20] media targeting the facultative anaerobic Gram positive cocci include MacConkey agar (Saudi

Prepared Media Laboratory, Riyadh, KSA), Bile esculin agar (Saudi Prepared Media Laboratory, Riyadh, KSA), Mannitol salt agar (Oxoid), Nutrient agar (Oxoid), and Blood agar. One gram of stool specimen was suspended in equal volume of sterile phosphate buffered saline (PBS), pH 7.0, and gently homogenized. Ten-fold serial dilutions were carried out in PBS. Aliquot (100 µL) of each dilution were directly inoculated onto MacConkey agar, Bile esculin agar, Mannitol salt agar, Nutrient agar, and Blood agar and incubated aerobically for 48-72 hours at 37°C. In this study, presumptive separated colonies of only enterococci, and staphylococci were picked up and purified by subculture streak on the same primary medium of isolation and subjected to Gram staining, manual biochemical reactions,[4,5] and identity at the species level, which was also confirmed by automated Vitek2 (BioMe´rieux, Marcy-l'Étoile, France) identification systems and used for minimum inhibitory concentration (MIC)-susceptibility testing. The identified isolates were stored in brain heart infusion broth containing 20% glycerol at -70^{0}C. Antimicrobial susceptibility for *E. faecalis* testing, Vitek2-card (AST-P586), and for *Staphylococcus spp.* Vitek2-card (AST-P580) were inoculated according to manufacture instructions with a bacterial suspension prepared in 0.45% saline equal to the turbidity of a 0.5 McFarland standard with the Densi-Chek 2 system (BioMe´rieux, Marcy-l'Étoile, France). The results were interpreted according to the current Clinical and Laboratory Standards Institute (CLSI) Guidelines.[27] Each of the 73 *E. faecalis* specimens was tested against: penicillin (PEN), ampicillin (AMP), GEN/SYN, gentamycin high level (GHL), STR/SYN, streptomycin high level (SHL), levofloxacin (LVX), erythromycin (ERY), linezolid (LZD), teicoplanin (TEC), vancomycin (VAN), tetracycline (TET), tigecycline (TGC), nitrofurantoin (NIT), and trimethoprim-sulfamethoxazole (SXT).

While each of 18 *Staphylococcus spp* was tested against PEN, LVX, ERY, LZD, TEC, VAN, TET, TGC, NIT, SXT. GEN, oxacillin (OXA), tobramycin (TOB), rifampicin (RFB), moxifloxacin (MXL), and CLI, clindamycin (CLI)

Statistical analysis. Data was stored and analyzed using the Statistical Package for Social Sciences version 19.0 (SPSS Inc., Chicago, IL, USA). Fishers exact test one way analysis of variance (ANOVA), Kruskal-walis test (non-parametric test), Chi-square liner trend, and Chi-square test were used, and a *p*-value <0.05 was considered as significant.[21]

Results. In the present study, a total of 91 (61%) facultative Gram positive coccal bacterial isolate were isolated (**Table 1**). *Enterococcus faecalis* were the most predominant isolates representing 80% (n=73/91), followed by *S.epidermidis* (n=13/91, 14%), and the less commonly encountered, *S. aureus* (n=5/91, 6%). Only 11% (8/73) of *E. faecalis* isolates were isolated solely, while 67% (49/73) were isolated in association with *E.coli* isolates (**Table 2**). The recovery of ≥2 organisms in association with *E. faecalis* was less frequently encountered and varied from 1-6%. The data of the clinical characteristics of 150 examined neonates (**Table 3**) showed that unlike staphylococci, the prevalence of *E. faecalis* colonization did not significantly (*p*=0.555) vary from day one up to 7 days of life, regardless of the type of feeding (*p*=0.318); but it was relatively higher among vaginally delivered neonates (*p*=0.505), as compared with those delivered by cesarean (51% versus 46%). Maximum fecal carriage of *E. faecalis* occurred with neonates of low body weight (group 1, <2 kg, 75%). Whereas, the prevalence of *S.epidermidis* or *S.aureus* fecal carriage was comparatively higher among neonates delivered by cesarean section. Meanwhile, their prevalence-colonization increased as the body weight increases and ranged from (0 to 29%), and this difference was significant (*p*=0.025) for *S. epidermidis*. However, the type of feeding did not significantly affect neonate-prevalence-colonization by *E. faecalis* (**Table 3**), presumably due its early colonization during delivery and/or immediately after birth. While breast feeding neonates showed almost zero prevalence-colonization for *Staphylococcus spp*. during the first 2 days of life, but considerable prevalence carriages for these isolates were observed in those neonates with bottle and/or mixed feeding within the range of 4-12%.

Results of the MIC (50/90) susceptibility testing (**Table 4**) revealed that all *E. faecalis* isolates were completely sensitive (100%) to Teicoplanin,

Table 1 - Distribution number of recovered positive and negative bacterial isolates from 150 examined neonate fecal specimens.

Type of bacterial isolate	n (%)
Escherichia coli	130 (35.2)
Enterococcus faecalis	73 (19.8)
Lactobacillus spp.	70 (19.0)
Klebsiella pneumoniae	23 (6.2)
Clostridium spp.	20 (5.4)
Pseudomonas aeruginosa	14 (3.8)
Staphylococcus epidermidis	13 (3.5)
Acinetobacter bumannii	9 (2.4)
Enterobacter cloacae	5 (1.6)
Staphylococcus aureus	5 (1.6)
Enterobacter aerogenes	4 (1.1)
Morganella morganii	3 (0.8)
Total	**369 (100)**

Vancomycin, or Tigecyclin, but these isolates were resistant to Trimethoprim/Sulfamethoxazole (97%), Erythromycin (49%), Tetracycline (38%), and Levofloxacin (18%). Meanwhile, high level resistance towards the amino glycoside Gentamycin accounted for 25% and Streptomycin for 11% of the isolates.

Table 4 shows that all *Staphylococcus spp*. isolates exhibited full susceptibility (100%) to Linezolid, Teicoplanin, Vancomycin, Tigecyclin, Nitrofurantion, Moxifloxacin or Clindamycin. However, as expected, none of *S. aureus* isolates, and only 53.8% of *S. epidermidis* that were susceptible to penicillin. The *S. epidermidis* resistance rates against Erythromycin was 46.2% and Trimethoprim/Sulfamethoxazole were 30.8%, while 40% (n=2/5) of *S. aureus* isolates were resistant to Oxacillin, Gentamycin, or Tobramycin. Results also showed that 30% (n=3/13) and 40% (n=2/5) of *S. epidermidis* and *S. aureus* isolates were resistant to Oxacillin, and exhibited MDR patterns of 5 R markers (OXA-PEN-TET-GEN-TOB), which was also observed among 2 strains of *S.aureus*. Therefore, it is concluded that these *S. aureus* strains are MRSA, and Oxacillin resistant *S. epidermidis* isolates.

Table 2 - Distribution of *Enterococcus faecalis (E. faecalis)* to number of its associated organisms (n=73).

E. faecalis and associated isolates	Number	%
E. faecalis with no associate	8	11
E. faecalis + *E. coli*	49	67
E. faecalis + *Pseudomonas aeroginosa*	1	1
E. faecalis + *Staphylococcus epidermidis*	3	4
E. faecalis + *Eterobactercloace*	1	1
E. faecalis + *Enterobacter aerogenes*	1	1
E. faecalis + *E. coli* + *Klebsiella pneumoniae*	4	6
E. faecalis + *E. coli* + *Pseudomonas aeruginosa*	4	6
E. faecalis + *E. coli* + *Staphylococcus aureus*	2	3
Total	**73**	**100**

E. coli - Escherichia coli

Discussion. In agreement with Bergstrom et al,[22] the first bacteria to establish in the neonatal gut are usually aerobic or facultative anaerobic bacteria, such as enterococci, staphylococci, and enterobacteria. As these bacteria establishe their gut niches and grow, this lead to oxygen-depletion, and thereby permits further colonization by lactobacilli and various obligate anaerobes later on. In India, a study on the quantitative and qualitative spectrum of intestinal flora in neonates, revealed that mean log CFU (colony forming unit) of Gram positive bacteria and Gram negative bacteria were statistically insignificant from D3 to D14. Although statistically insignificant, the present study revealed that the carriage rate of *S. aureus* was higher among neonates delivered by cesarean than those vaginally delivered (p=0.159). However, a study in India,[23] showed that *S. aureus* was the most abundant bacterial species present in vaginal birth infants. This trend also holds true among formula fed as compared with breast fed neonates, but the situation is reversed with *S. epidermidis* as expected.[24] In Ireland, Cooke et al[25] investigated the gut flora of Irish breastfed and formula-fed neonates aged between birth and 6 weeks old, and found that *E. coli* was more dominant (p=0.042) in the gut flora of 6-week-old formula-fed neonates, while there was a tendency for *Bifidobacterium spp.* (beneficially obligate Gram positive

Table 3 - Distribution of different Gram positive bacteria in relation to clinical characteristics of enrolled neonate-subjects.

Characteristic	N	*Enterococcus faecalis* n (%)	*P*-value	*Staphylococcus epidermidis* n (%)	*P*-value	*Staphylococcus aureus* n (%)	*P*-value
Age			0.555		0.485		0.068
1 days	45	21 (47)		5 (11)			
2 days	47	26 (55)		-		1 (2)	
3 days	41	20 (49)		6 (15)		3 (7)	
4-7 days	17	6 (35)		2 (12)		1 (6)	
Mode of delivery			0.505		0.307		0.159
Vaginal	78	40 (51)		5 (6)		1 (1)	
Cesarean	72	33 (46)		8 (11)		4 (6)	
Body-weight			0.279		0.029*		0.112
<2 kg	4	3 (75)		0.0		0.0	
2-3 kg	65	30 (46)		3 (5)		1 (2)	
3.1-4 kg	74	36 (49)		8 (11)		3 (4)	
>4 kg	7	4 (57)		2 (29)		1 (14)	
Feeding-type					0.147		0.523
Breast	27	14 (52)		6 (22)		0.0	
Bottle	83	36 (43)	0.318	10 (12)		3 (4)	
Mixed	40	23 (56)		3 (8)		2 (5)	

*significant difference with $p \leq 0.05$

Table 4 - Antibiotic susceptibility patterns (MIC50/90) of neonate-fecal (n=73) *Enterococcus faecalis (E. faecalis)*, and *Staphylococcus spp*. (n=18) isolates.

Antimicrobial	*Enterococcus faecalis* n=73 Sensitive (%)	MIC 50/90 (µg/ml)	*Staphylococcus epidermidis* (n=13) Sensitive n (%)	MIC 50/90 (µg/ml)	*Staphylococcus aureus* (n=5) Sensitive n (%)	MIC 50/90 (µg/ml)
Penicillin	67 (91.8)	0.12/≥0.5	7 (53.8)	0.25/≥0.25	0 (00)	0.25/≥0.25
Levofloxacin	60 (82.2)	0.025/≥8	11 (84.6)	0.25/≥1	5 (100)	0.25/≥1
Erythromycin	37 (50.7)	0.25/≥2	7 (53.8)	0.25/≥1	5 (100)	0.25/≥0.25
Linezolid	68 (93.2)	1/≥2	13 (100)	0.25/≥1	5 (100)	0.25/≥1
Teicoplanin	73 (100)	0.5/≥0.5	13 (100)	2/≥8	5 (100)	0.5/≥0.5
Vancomycin	73 (100)	0.5/≥4	13 (100)	1/≥4	5 (100)	0.5/≥1
Tetracycline	41 (56.1)	1/≥1	9 (69.2)	0.25/≥16	3 (60)	1/≥16
Tigecycline	73 (100)	0.12//≥0.25	13 (100)	0.12//≥0.25	5 (100)	0.12/≥0.12
Nitrofurantoin	64 (87.6)	16/≥32	13 (100)	16/≥32	5 (100)	32/≥32
Trimethoprim/ Sulfamethoxazole	3 (4.1)	10/≥32	9 (69.2)	10/≥320	5 (100)	10/≥10
Gentamycin	N/D	-	11 (85.0)	0.5/≥16	3 (60)	0.5/≥16
Oxacillin	N/D	-	9 (69.2)	0.12//≥0.5	3 (60)	0.12//≥4
Tobramycin	N/D	-	11 (85.0)	1/≥16	3 (60)	1/≥16
Rifampicin	N/D	-	11 (85.0)	0.5/≥0.5	5 (100)	0.5/≥0.5
Moxifloxacin	N/D	-	13 (100)	0.25/≥1	5 (100)	0.25/≥0.25
Clindamycin	N/D	-	13 (100)	0.25/≥1	5 (100)	0.25/≥0.25
Ampicillin	68 (93.2)	2/≥2	N/D	--	N/D	--
Gentamycinhigh level	55 (75.3)	>2000	N/D	--	N/D	--
Streptomycin high level	65 (89.0)	>500	N/D	--	N/D	--

ND - not determined, MIC - minimum inhibitory concentration

anaerobes) to be more prevalent in the gut flora of breastfed neonates at 2-5 days (p=0.108). The present study revealed that unlike staphylococci, the prevalence of *E. faecalis* colonization did not significantly vary from day one up to 7 days of life, regardless of the type of feeding, but it was relatively higher among vaginally delivered neonates, compared with those delivered by cesarean (51% versus 46%), presumably because of its early colonization and/or trans-localization during and/or immediately after birth.

Regarding our data on antimicrobial susceptibility testing, in agreement with Powera et al,[26] only ~50% of the neonates recovered fecal *S.epidermidis* and 0% of the *S. aureus* isolates were susceptible to penicillin. approximately 25% of the isolates were resistance to Erythromycin or Trimethoprim/sulfamethoxazole, apparently due to their frequent antecedent use among pregnant women. Furthermore, taking in consideration, the MIC for oxacillin-CLSI[27] breakpoints for *S. aureus* (sensitive [S]: ≤2 µg/ml and resistant [R]: ≥4 µg/ml) and those for *S.epidermidis* (S= ≤0.25 µg/ml and R= ≥0.5 µg/ml), our results showed that 40% (2/5) and approximately 31% (4/13) of these isolates were resistant to oxacillin. As expected, these oxacillin resistant staphylococcal isolates, exhibited MDR patterns mainly of 5R markers (OXA-PEN-TET-GEN-TOB). The demonstration of these strains in normal fecal neonate's specimens should be considered as an alarming signal to the spread of resistant gene markers among our hospitals and community. Methicillin-resistant Staphylococcus aureus (MRSA) strains are not only resistant to all ß-lactam antibiotics but also to other categories of other antibiotics as confirmed in current study and others.[15,28] In a study conducted in India[20] on neonatal sepsis, *S. aureus* followed by Coagulase Negative Staphylococci (CoNS) was the most frequently detected as the etiological agent. The authors added that *S. aureus* was the main pathogen in both early and late-onset sepsis, and 57.4% of the *S. aureus* isolates were found to be methicillin resistant. A similar study from Riyadh, Saudi Arabia, also revealed that the single most frequent organism was *S. epidermidis* accounting for 36% (58/190) of all proven cases.[29] Therefore, *S. epidermidis*, though it is a normal flora, under certain circumstances especially MDR strains, may cause fatal neonatal diseases. Also from Saudi Arabia,[30] an investigation on the correlation of neonatal sepsis and the extremely low-birth-weight, showed that *E. coli* (29%) was the most common causing early onset sepsis, whereas, CoNS (50%) was the most common infecting organism causing late onset sepsis.[30] The importance of *Enterococci* (primarily, *E. faecalis* and *E. faecium*)

as a leading cause of nosocomial infections in several countries is well recognized.[31] In this study, all neonatal fecal *E. faecalis* isolates (n=73) were sensitive (100%) to Teicoplanin, Vancomycin or Tigecyclin followed by Ampicillin (94%), Linezolid (94%), Penicillin (93%), Streptomycin (89%), Nitrofurantion (88%), and Levofloxacin (82%). In a study from India,[11] a total of 100 hospital clinical *E. faecalis* isolates showed full sensitivity (100%) to Teicoplanin, Vancomycin, or Ampicillin, but these isolates were resistant to Trimethoprim/Sulfamethoxazole (97%), Erythromycin (49%), Tetracycline (38%), and Levofloxacin (18%).

In a study from Saudi Arabia,[32] out of 96 *E. faecalis* clinical hospital isolates, 21% exhibited high level resistance against Gentamycin (HLG) and 23% against Streptomycin (HLS). In comparison, this study revealed that high level resistance against these amino-glycoside antibiotics accounted for 25% and 11% of the tested normal neonatal fecal *E. faecalis* isolates. These findings are consistent with previous study from Greek[10] where antibiotic-resistant enterococci proved to be already established in the fecal microbiota of neonates, from the first day of an infant's life. Thus, MDR strains of 6 R markers (GEN/SYN-AMP-PEN-TET-ERY-SXT) or GEN/SYN-STR/SYN-AMP-TET-ERY-SXT) were observed among *E. faecalis* isolates. Hence, these isolates precluded the synergistic bactericidal effect of combined exposure to antibiotic-targeting cell wall synthesis inhibition such as ß-lactams or glycopeptides and virtually all commercially available aminoglycosides, including Gentamicin, Tobramycin, Netilmicin, Kanamycin, and Amikacin.[32]

This study also revealed that 12% of the 73 *E. faecalis* isolates exhibited resistance against Nitrofurantion, and 18% against Levofloxacin. Even with Linezolid, newly introduced drug into clinic use, 6% of tested *E. fecalis* isolates were resistant to this drug. In contrast, studies from India[11] (0.5% [n=204]) and Iran[33] (0% [n=91]) showed resistance against Linezolid. In contrast, none of our MDR staphylococci, including MRSA isolates was resistant to this drug. Hence, it is concluded that *E. faecalis* is highly efficient to rapidly acquire and maintained newly introduced antimicrobial resistant genes.[34] Furthermore, it is well recognized by Arias & Murray[35] and Garrido et al[36] that the 3 types of resistance of most significance in enterococci are high-level resistance to the amino-glycoside-antibiotics, Ampicillin resistance and glycopeptide (Vancomycin) resistance. In this study, Ampicillin resistance (Amp-R) was associated with both HLS (>2000 µg/ml) and HLG (>500 µg/ml) in 4 isolates as well as several other resistance markers (GEN/SYN-STR/SYN-AMP-TET-ERY-SXT) and only with one strain. The Amp-R marker was associated with HLG (>500 µg/ml) resistance, again with several other resistance markers (GEN/SYN-AMP-PEN-TET-ERY-SXT). These findings emphasize that in vitro susceptibility testing must be performed to both Gentamycin and Streptomycin because of differences in the mechanisms of resistance. High level resistance against Gentamycin resistance is associated with 2 different enzymatic inactivations; i-6'acetyltransferase (acetylase) and ii-2' Phosphotransferase, which also inactivate in all other amino-glycosides antibiotics except Streptomycin. Whereas HLS resistance may be ribosomal-mediated or due to the production of Streptomycin adenyltransferase, which inactivates Streptomycin, but none of the other amino-glycosides antibiotics.[31,36]

The present study possesses some limitations, such as absence of molecular characterization of Enterocooci resistant genes and/or MRSA genotypic characteristics, and tracing their source of dissemination. Likewise, the recently introduced approach by Piras et al[37,38] in determining the differential proteomic profiling between sensitive and drug resistant bacterial strains was not attempted. Also, this study was performed only in 150 neonate fecal specimens from 2 hospitals in the Qassim region. Accordingly, larger numbers of neonates and a multi hospital-setting are recommended to discourse the prevalence of MDR among Enterocooci and MRSA in different hospitals and community infections.

In conclusion, the demonstration of HLG and HLS and other antimicrobial R markers among Saudi neonatal *E.faecails* isolates as well as the MRSA strains, is alarming, and suggests a wide dissemination of resistance genes in our society. Thus, obligates physicians to follow the terms of the infection-control policies including patient-isolation, surveillance programs, and should use antibiotics appropriately, in an effort to prevent further spread of these MDR strains among hospitals and later on, among the community at large.

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