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

Talat A. El-Kersh, MSc, PhD, Mohammed A. Marie, MSc, PhD, Yazeed A. Al-Sheikh, MSc, PhD, Mohamed H. Al-Agamy, MSc, PhD, Ahmad A. Al Bloushy, BSc, MSc.

ABSTRACT

The 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.

Saudi Med J 2016; Vol. 37 (3): 280-287
doi: 10.15537/smj.2016.3.13871

From the Department of Clinical Laboratory Sciences (El-Kersh, Marie, Al-Sheikh), the Department of Clinical Laboratory Sciences (Al-Agamy), College of Applied Medical Sciences, and the Department of Pharmaceutics (Al Bloushy), Microbiology Division, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia.

Received 25th October 2015. Accepted 14th January 2016.

Address correspondence and reprint request to: Mr. Ahmad A. Al Bloushy, Al Bukayriyah General Hospital, Qassim, Kingdom of Saudi Arabia.
E-mail: prince_a0996@hotmail.com

ABSTRACT

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

الطريقة: أجريت هذه الدراسة العملية في مستشفى البكيرية العام، المستشفى للأطفال والنساء في منطقة القصيم، المملكة العربية السعودية، وتم تحديد عزلات المكورات بين يونيو 2012 حتى يناير 2013. وتم تأكيد الهوية على مستوى الأنواع واختبار حساسية المضادات الحيوية باستخدام نظام فايتاك (Vitek2 system).

النتائج: تم عزل انتيروكوكس فيكالس (n=73) واستافيلوكوكس اسبيشز (n=18). غير أن انتشار انتيروكوكس فيكالس لم يختلف كثيراً من اليوم الأول حتى اليوم السابع، ولا تختلف النسب بين النوعين، ولكنها كانت أعلى في ولادة الطبيعية. بالنسبة للسماكة أو سماح، نسبة انتشار كل من المكورات المعوية والانسيفوايرسية كانت أعلى عند زيادة وزن الجسم.

 McGregor, B. (2016). The Effect of Maternal Nutrition on Newborn’s Microbiome. Nutrients, 8(10), 721. doi: 10.3390/nu8100721

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.
The gastrointestinal microbiota plays a crucial role in health and disease of the host through its impact on nutrition, pathogenesis, and immunology. Unlike the adult human gut microbiota, the infant gut microbiota possesses a relatively simple structure, but is rather unstable overtime. 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. The genus Enterococcus is a Gram-positive, fermentative facultative aerobic cocci that is ubiquitous and highly adapted to hospital environments and others. 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). 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. 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. Thus, enterococcus is currently recognized as a major reservoir in the dissemination of resistant genes worldwide, including travelling to endemic countries. Consequently, the evolution of antimicrobial resistance in enterococci has posed enormous challenges for clinicians 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. 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 enterococci. This ultimately leads to environmental contamination and cross infections. 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. While several Saudi researchers have studied the adult fecal carriage of resistant bacterial strains, 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.

**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 questionnaire 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.

**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., media targeting the facultative anaerobic Gram positive cocci include MacConkey agar (Saudi

**Disclosure.** Authors declare no conflict of interests, and the work was not supported or funded by any drug company. This study was funded by the King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia (Project A-S-12-1015).
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, 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°C. Antimicrobial susceptibility for E. faecalis testing, Vitek2-card (AST-P586), and for Staphylococcus spp. Vitek2-card (AST-P580) were inoculated according to manufacturer 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, gentamicin 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, 79%). 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 carries 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 baumannii   | 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 Moxifloxacin or Clindamycin. However, as expected, was 46.2% and Trimethoprim/Sulfamethoxazole were resistance rates against Erythromycin S. epidermidis S. epidermidis none of 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.

**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 establish 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 neonates, while there was a tendency for enterococci, staphylococci, and enterobacteria. As expected.24 In Ireland, Cooke et al25 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 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 aeruginosa | 1   | 1  |
| E. faecalis + Staphylococcus epidermidis | 3  | 4  |
| E. faecalis + Enterobacteriaceae  | 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

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

*significant difference with p≤0.05
Fecal carriage of MDR of *E. faecalis* in neonates ... El-Kersh et al

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) | MIC 50/90 (µg/ml) | Staphylococcus epidermidis (n=13) | MIC 50/90 (µg/ml) | Staphylococcus aureus (n=5) | MIC 50/90 (µg/ml) |
|----------------------|-----------------------------|------------------|-----------------------------------|------------------|-----------------------------|------------------|
|                      |                            | Sensitive (%)     | Sensitive n (%)                   | Sensitive (%)     | Sensitive n (%)             | Sensitive (%)     |
| 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±0.2        | 11 (84.6)                         | 0.25±1           | 5 (100)                     | 0.25±1           |
| Erythromycin         | 37 (50.7)                  | 0.25±0.2         | 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/Sulphamethoxazole | 3 (4.1) | 10/≥32          | 9 (69.2)                          | 10/≥320          | 5 (100)                     | 10/≥30           |
| 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         |
| Mosiloxacin          | 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                          | --               |
| Gentamycin high 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., 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 resistant to Erythromycin or Trimethoprim/sulfamethoxazole, apparently due to their frequent antecedent use among pregnant women. Furthermore, taking in consideration, the MIC for oxacillin-CLSI12 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 β-lactam antibiotics but also to other categories of other antibiotics as confirmed in current study and others.5,28 In a study conducted in India 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, 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. 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 Levofloxacine (82%). In a study from India, 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 Levofloxacine (18%).

In a study from Saudi Arabia, 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 aminoglycoside antibiotics accounted for 25% and 11% of the tested normal neonatal fecal E. faecalis isolates. These findings are consistent with previous study from Greece 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 β-lactams or glycopeptides and virtually all commercially available aminoglycosides, including Gentamicin, Tobramycin, Netilmicin, Kanamycin, and Amikacin.

This study also revealed that 12% of the 73 E. faecalis isolates exhibited resistance against Nitrofurantion, and 18% against Levofloxacine. Even with Linezolid, newly introduced drug into clinic use, 6% of tested E. faecalis isolates were resistant to this drug. In contrast, studies from India (0.5% [n=204]) and Iran 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. Furthermore, it is well recognized by Arias & Murray and Garrido et al 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 (acylase) and ii-2’ Phosphotransferase, which also inactivate in all other amino-glycosides antibiotics except Streptomycin. Whereas HLG resistance may be ribosomal-mediated or due to the production of Streptomycin adenylytransferase, which inactivates Streptomycin, but none of the other amino-glycosides antibiotics.

The present study possesses some limitations, such as absence of molecular characterization of Enterococci resistant genes and/or MRSA genotypic characteristics, and tracing their source of dissemination. Likewise, the recently introduced approach by Piras et al 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 Enterococci 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. faecalis 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.

Acknowledgment. The authors would like to express their thanks and gratitude to King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia for funding this project.

References
1. Young VB. The intestinal microbiota in health and disease. *Carr Opin Gastroenterol* 2012; 28: 63-69.
2. Turroni F, Peano C, Pass DA, Foroni E, Severgnini M, Claesson MJ, et al. Diversity of Bifidobacteria within the Infant Gut Microbiota. *PLoS One* 2012; 7: e36957.
3. Banerjee T, Anupurba S. Prevalence of virulence factors and drug resistance in clinical isolates of Enterococci: A study from North India. *J Pathog* 2015; (2015): 1-7.
4. Salem-Bekhit MM, Moussa IM, Muharram MM, Alanazy FK, Hefni HM. Prevalence and antimicrobial resistance pattern of multidrug-resistant enterococci isolated from clinical specimens. Indian J Med Microbiol 2012; 30: 44-51.

5. Aamodt H, Mohn SC, Maselle S, Manji KP, Willems R, Jureen R, et al. Genetic relatedness and risk factor analysis of ampicillin-resistant and high-level gentamicin-resistant enterococci causing bloodstream infections in Tanzanian children. BMC Infect Dis 2015; 15: 107.

6. Hammada AM, Hassanb HA, Shimamotod T. Prevalence, antibiotic resistance and virulence of Enterococcus spp. in Egyptian fresh raw milk cheese. Food Control 2015; 50: 815-820.

7. Comerlato CB, Carvalho de Resende MC, Caierão J, d’Azevedo AM. Presence of virulence factors in Enterococcus faecalis and Enterococcus faecium susceptible and resistant to vancomycin. Mem Inst Oswaldo Cruz 2013; 108: 590-595.

8. Bhardwaj S, Dhawale KBJ, Patil M, Divase S. Enterococcus faecium and Enterococcus faecalis, the nosocomial pathogens with special reference to multi-drug resistance and phenotypic characterization. International Journal of Pharmaceutical Science and Practice 2013; 2: 1-10.

9. Wernera G, Coque MT, Franze CM, Grohmann E, Hegstade K, Jenseng L, et al. Antibiotic resistant enterococci Tales of a drug resistance gene trafficker. Int J Med Microbiol 2013; 303: 360-379.

10. Kirtzalidou EI, Mitsou EK, Pramateftaki P, Kyriacou A. Screening fecal enterococci from Greek healthy infants for susceptibility to antimicrobial agents. Microb Drug Resis 2012; 18: 578-585.

11. Deshpande VR, Karmarkar MG, Mehta PR. Prevalence of multi-drug-resistant enterococci in a tertiary care hospital in Mumbai, India. J Infect Dev Ctries 2013; 7: 155-158.

12. Kernbauer E, Maureta K, Torres V, Shopsin B, Cadvella K. Gastrointestinal dissemination and transmission of Staphylococcus aureus following Bacteremia. Infect Immun 2015; 83: 372-378.

13. Dilip M, Anil KV, Paul Breezy, Madhan S, Russel JK, Anand M, et al. Fecal carriage rates of extended-spectrum β-lactamase-producing Escherichia coli among antibiotic naive healthy human volunteers. Microb Drug Resist 2015; 21: 59-64.

14. Fam NS, Defasque S, Bert F, Leflon-Guibout V, El-Ray A, El-Ghannam M, et al. Faecal carriage of extended-spectrum ß-lactamase (ESBL)-producing enterobacteria in liver disease patients from two hospitals in Egypt and France: a comparative epidemiological study. Epidemiol Infect 2015; 143: 1247-1255.

15. El-Mahdy TS, El-Ahmady M, Goering RV. Molecular characterization of methicillin-resistant Staphylococcus aureus isolated over a 2-year period in a Qatari hospital from multinational patients. Clin Microbiol Infect 2014; 20: 169-173.

16. Halawani EM. β-lactam antibiotic resistance in Escherichia coli commensal facial flora of healthy population in Taif, Saudi Arabia. African Journal of Microbiology Research 2011; 5: 73-78.

17. El-kersh TA, Neyazi SM, Al-shiekh YA, Niazy AA. Phenotypic traits and comparative detection methods of vaginal carriage of Group B streptococci and its associated micro-biota in term pregnant Saudi women. African Journal of Microbiology Research 2012; 6: 403-413.

18. Al-Agamy MH, Shibi 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: 214-216.

19. Elkersh TA, Marie MA, Al-Sheikh YA, Al Blousyha AA, Al-Agamy MH. 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. Ann Saudi Med 2015; 35: 240-247.

20. Jost T, Lacroix C, Braegger CP, Chassard C. New insights in gut microbiota establishment in healthy breast fed neonates. Plos One 2012; 7: e44595.

21. Ludbrook J. Analysis of 2 x 2 tables of frequencies: matching test to experimental design. Int J Epidemiol 2008; 37: 1430-1435.

22. Bergström A, Skov TH, Bahl MI, Roager HM, Christensen LB, Ejlerskov KT, et al. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. Appl Environ Microbiol 2014; 8: 2889-2900.

23. Sharma P, Kaur P, Aggarwal A. Staphylococcus aureus. The predominant pathogen in the neonatal ICU of a tertiary care hospital in amritsar, India. J Clin Diagn Res 2013; 7: 66-69.

24. Pandey PK, Verma P, Kumar H, Bavdekar A, Patole MS, Shouche S. Comparative analysis of fecal microflora of healthy full-term Indian infants born with different methods of delivery (vaginal vs cesarean): Acinetobacter spp. prevalence in vaginally born infants. J Biotics 2012; 37: 989-998.

25. Cooke G, Behan J, Clarke N, Gorman W, Costello M. Comparing the gut flora of Irish breastfed and formula-fed neonates aged between birth and 6 weeks old. Microbial Ecology in Health and Disease 2005; 17: 163-168.

26. Powera SE, O’Toolea PW, Santonata C, Rossa RP, Fitzgeralda GF. Intestinal microbiota, diet and health. Br J Nutr 2014; 111: 387-402.

27. Clinical and Laboratory Standards Institute. CLSI Publishes New Antimicrobial Susceptibility Testing Standards. Wayne (PA): Clinical and Laboratory Standards Institute; 2010.

28. Benito D, Lozano C, Jiménez A, Albüjar M, Gómez A, Rodríguez J, et al. Characterization of Staphylococcus aureus strains isolated from faeces of healthy neonates and potential mother-to-infant microbial transmission through breastfeeding. FEMS Microb Ecol 2015; 91: pii: fiv007.

29. Al-Humaidan OS, El-Kersh TA, Al-Akeel RA. Risk factors of methicillin-resistant Staphylococcus aureus among health care staff in a teaching hospital in central Saudi Arabia. Saudi Med J 2015; 36: 1084-1090.

30. Haque KN, Chagia AH, Shaheed MM. Half a decade of neonatal sepsis, Riyadh, Saudi Arabia. J Trop Pediatr 1990; 36: 20-23.

31. Gopinath R, Prakash M. Antibacterial activity of three medicinal plants against clinically isolated Multidrug Resistant Enterococcus faecalis (MDRE). Int J Curr Microbiol App Sci 2013; 2: 6-14.

32. Salem-Bekhit MM, Moussa IM, Muharram MM, Elsheerbini AM, AlRejaie S. Increasing prevalence of high-level gentamicin resistant enterococci: An emerging clinical problem. African Journal of Microbiology Research 2011; 5: 5713-5720.

33. Pourakbari B, Aghdam MK, Mahmoudi S, Ashtiani MT, Sabouni F, Movahedi Z, et al. High frequency of Vancomycin-Resistant Enterococcus faecalis in an Iranian referral children medical hospital. Maedica (Buchar) 2012; 7: 201-204.
Fecal carriage of MDR of \textit{E. faecalis} in neonates ... El-Kersh et al

34. Haug M. Construction and application of a novel pRE25-derived plasmid to monitor horizontal transfer of antibiotic resistance genes from \textit{Enterococcus faecalis} to food and gut associated microbes in a colonic fermentation model. Zurich: ETH Institutional Repository; 2010.

35. Arias CA, Murray BE. The rise of the Enterococcus: beyond vancomycin resistance. \textit{Nat Rev Microbiol} 2012; 10: 266-276.

36. Garrido AM, Gálvez A, Pulido RP. Antimicrobial resistance in Enterococci. \textit{J Infect Dis Ther} 2014; 2: 1 50.

37. Piras C, Soggiu A, Bonizzi L, Gaviraghi A, Deriu F, De Martino L, et al. Comparative proteomics to evaluate multi drug resistance in \textit{Escherichia coli} \textit{Mol Biosyst} 2012; 8: 1060-1067.

38. Piras C, Soggiu A, Greco V, Martino PA, Del Chierico F, Putignani L, et al. Mechanisms of antibiotic resistance to enrofloxacin in \textit{Uropathogenic Escherichia coli} in dog. \textit{Mol Biosyst} 2015; 127: 365-376.

Related Articles

Balto HA, Shakoor ZA, Kanfar MA. Combined effect of a mixture of tetracycline, acid, and detergent, and nisin against \textit{Enterococcus faecalis} and \textit{Actinomyces viscosus} biofilms. \textit{Saudi Med J} 2015; 36: 211-215.

Li JM, Zhao RH, Li ST, Xie CX, Jiang HH, Ding WJ, et al. Down-regulation of fecal miR-143 and miR-145 as potential markers for colorectal cancer. \textit{Saudi Med J} 2012; 33: 24-29.

El-Amin NM, Faidah HS. Vancomycin-resistant Enterococci. Prevalence and risk factors for fecal carriage in patients at tertiary care hospitals. \textit{Saudi Med J} 2011; 32: 966-967.