Antimicrobial susceptibility and virulence factors of enterococci colonizing intestinal tract of Jordanian infants

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Abstract

Objective: This study investigated distribution of *enterococci* colonizing intestinal tract of infants, their putative virulence factors and antimicrobial susceptibility patterns.

Methods: A total of 82/186 (44.1%) fecal enterococcal isolates were recovered from infants. All enterococci isolates were identified either *E. faecalis* or *E. faecium* using culture and PCR.

Results: A significant higher intestinal colonization of enterococci was detected among non-hospitalized compared to hospitalized patients with a percentage of (72 % vs. 28%), respectively. *E. faecalis* was the predominant species isolated from both groups (75.6%). It had also significantly higher virulence factors genes than *E. faecium*, while *E. faecium* had higher rates of antimicrobial resistance than *E. faecalis*. There was no significant factor related to intestinal colonization of enterococci with birth weight, gestational age of mother, gender, type of feeding, present of disease or administration of antibiotics to mothers or infants.

Conclusion: This study presents important epidemiological data on the intestinal colonization of enterococci in both hospitalized and non-hospitalized Jordanian infants.

Key word: Fecal enterococci, Infants, Antimicrobial resistance, Virulence factors

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Introduction

In recent years, enterococci have emerged as important pathogens, particularly in immunocompromised patients and intensive care units. They cause frequently more infections in patients hospitalized for a long time or who have received multiple antibiotic therapy [1,2]. *E. faecalis* and

E. faecium are the most prevalent species cultured from human infections and are frequently associated with nosocomial infections worldwide [3,4,5].

The emergence of vancomycin-resistance enterococci (VRE) during recent years has seriously affected the treatment and infection control of these bacteria especially in hospitalized patients. *E. faecium* strains are frequently more resistant than *E. faecalis* to all antibiotics that are effective in the treatment of vancomycin-susceptible enterococci. This new development leaves clinicians treating VRE infections with limited therapeutic options [6-7].

Enterococci are among the first organisms to colonize the intestinal tract of infants. These microorganisms originate mostly from the intestinal and vaginal flora of the mother. They can be also acquired during ingestion of breast milk, formula milk or from environmental sources. An increase in the prevalence of *E. faecium* has been recently observed to occur among neonatal patients in ICUs in many countries, and this shift is likely to be explained in part by the rapid emergence of VRE [6-7]. In addition, it has been suggested that certain virulence factors of enterococci may play a role in intestinal colonization and later infection [8-10].

This study aimed to investigate the important epidemiological features, including antimicrobial susceptibility and virulence factors of common encountered *Enterococcus* species in the intestine of Jordanian infants.

Material and Methods

Study design and patients

The present study included two examined groups; hospitalized and non-hospitalized infants from the Jordan University Hospital, aged from one day to one year. A total of 93 fecal swab specimens were obtained from hospitalized babies admitted to the neonatal intensive care unit (NICU) of the Jordan University Hospital (JUH) for various diseases, from October 2013 through September 2014. At the same period another 93 of fecal specimens obtained from babies attending for routine clinical examination to the outpatient clinic of the Pediatric Department, JUH. A special information sheet was designed to record demographic and clinical data about the patients including infant's name, age, birth weight, gestational age of mother, gender, type of feeding, type of disease if present and administration of antibiotics at time of fecal sampling or two weeks prior to sampling. This study was approved by the Faculty of Graduate Studies, The Jordan University. Permission was also obtained from the Institutional Ethical Review Board (IERB) at the Jordan University Hospital in Amman, Jordan. An approval has been obtained from all parents of the infants which were informed about the nature of the research.

Culture specimen, identification and susceptibility test

Fecal swab specimens were collected from babies using sterile cotton swabs pre-moistened with 0.9% saline. All fecal specimens were transferred immediately to the Microbiology Laboratory/ Faculty of Medicine/ Jordan University, and were inoculated directly onto Bile-Esculin agar plates and incubated for 24-48 hours at 37°C. Culture plates were examined to detect the presence of gray to black colonies of enterococci. Initial identification of enterococci to genus level was based on Gram-staining properties,

catalase negative test, and growth in the presence of 6.5% NaCl. All *Enterococcus spp.* isolates were stored in Brain Heart Infusion broth with (15% glycerol) at -70 °C for further analysis.

All *Enterococcus* species isolates were examined for antimicrobial susceptibility using disc diffusion method according to guidelines of Clinical and Laboratory Standards Institute [11]. Vancomycin, linezolid and teicoplanin susceptibility were determined using Etest (BioMerieux, France) and was interpreted according to the CLSI guidelines. *E. faecalis* ATCC 29212 was included as a positive control.

PCR Molecular tests

Multiplex PCR reaction and primers were used for identification of *E. faecalis*, *E. faecium* isolates by detection of genes encoding D-alanine-D-alanine ligases specific for *E. faecalis* and *E. faecium* as reported by Kariyama *et al.* [12]. The glycopeptide resistance genotypes (*vanA*, *vanB*, *vanC1* and *vanC2/C3*) were also determined by use of primers and multiplex PCR as described by Kariyama *et al.* [12]. Uniplex PCR and primers were used to detect the following virulence factor genes (*efaA fs*, *efaA fm*, *gel E*) as reported by Cariolato *et al.* [13], and (*ace*, *esp*, *cylA* genes) as performed by Creti *et al.* [14]. *E. faecalis* ATCC 29212 was used as a positive control for *ace*, *cylA*, *efaA* genes and *E. faecalis* ATCC 51299 was used as a positive control for *esp*, *gel E*, *cylA*, *ace* and *efaA*.

Statistical analysis

Data generated from the study was tabulated as Microsoft Excel sheets and Uploaded to Statistical Package for Social Sciences (SPSS version 19). Frequency and percentage were calculated for the categorical data and Pearson's chi-squared test or Fisher's exact test were applied to determine potential factors associated with *E. faecalis* and *E. faecium* and to determine whether there are any statistical differences between groups. The level of significance was set at a *p* value of ≤ 0.05 to test the hypothesis of no association

Results

A total of 186 hospitalized and non-hospitalized infants aged between one day and one year were included in this study. Of these 107 (57.5%) were males and 79 (42.5%) were females. A total of 82/196 (44.1%) isolates of *Enterococcus spp.* were recovered from fecal samples, of these 62 *E. faecalis* (19 inpatients and 43 from outpatients) and 20 *E. faecium* (4 from inpatients and 16 from outpatients) isolates were identified (**Table 1**). Both *E. faecalis* and *E. faecium* were significantly more found in outpatient infants than in hospitalized infants (72 vs. 28%) as shown in **Table 1**.

Antimicrobial susceptibility patterns of 20 *E. faecium* and 62 *E. faecalis* using disc diffusion test and

Table 1. *Enterococcus* species detected by PCR from 186 fecal specimens of each 93 hospitalized and non-hospitalized infants*

Species	Inpatients No. (%)	Outpatients No. (%)	Total No. (%)	P-Value
<i>E. faecium</i>	4 (17.4)	16 (27.1)	20 (24.4)	0.007
<i>E. faecalis</i>	19 (82.6)	43 (72.9)	62 (75.6)	0.002
Total	23 (28.0)	59 (72.0)	82/186 (44)	0.001

*Including 57% males and 43 % females; and there was no significant factor related to occurrence of enterococci with birth weight, gestational age of mother, gender, type of feeding, present of disease or administration of antibiotics to mothers or infants.

Table 2. Antibiotic susceptibility patterns of 62 *E. faecalis* and 20 *E. faecium* isolates

Antibiotics	<i>E. faecalis</i> *			<i>E. faecium</i> *		
	Susceptible no. (%)	MIC90 (µg/ml)	Range µg/ml	Susceptible No.(%)	MIC90 (µg/ml)	Range (µg/ml)
Ampicillin	62 (100)	-	-	14 (70.0)	-	-
Chloramphenicol	24 (38.7)	-	-	13 (65.0)	-	-
Gentamicin**	38 (61.3)	-	-	14 (70.0)	-	-
Levofloxacin	35 (56.5)	-	-	4 (20.0)	-	-
Tigecycline	62 (100)			20(100)		
Teicoplanin	62 (100)	0.44	0.016-2	20(100)	0.65	0.16-2
Linezolid	62(100)	1.48	1-2	20(100)	1.63	1-2
Vancomycin	62(100)	2.4	2-4	20(100)	2.7	2-4

*All isolates of *E. faecalis* and *E. faecium* isolates from inpatients showed much similar higher resistant rates in the range to chloramphenicol, levofloxacin gentamicin, respectively ** high level gentamicin (120µg/ml)

E-test are shown in **Table 2**. *E. faecium* strains were resistant to ampicillin, chloramphenicol, and levofloxacin with percentages of 30, 20, 30, respectively, while *E. faecalis* strains were resistant to the same antibiotics with percentages of 6.5, 25.8, 9.7, respectively. No resistance to vancomycin, teicoplanin and linezolid was detected in both species using Etest.

Overall, *E. faecalis* has significantly more virulence determinant genes (*efaA fs*, *ace*, *esp*, *gel E*, *cylA*) than the *E. faecium* isolates. The virulence gene (*efaA fm*) was detected only in 65% of *E. faecium* isolates (**Table 3**).

Discussion

This study demonstrates that intestines of Jordanian infants aged less than one year admitted to outpatients clinics or neonatal intensive care unit (NICU) at JUH, were frequently colonized with enterococci (44 %). The highest carriage rate of en-

terococci (72%) was significantly ($P\text{-value} = 0.001$) observed among the non-hospitalized infants (aged > one month), while carriage rate in hospitalized infants accounted only for 28% (**Table 1**). This

Table 3. Distribution of virulence genes among 62 *E. faecalis* and 20 *E. faecium* isolates.

Virulence gene	<i>E. faecalis</i> No. (%)	<i>E. faecium</i> No. (%)	P-Value*
efaA fs	62 (100)	10 (50)	0.001
ace	59 (95.2)	12 (60)	0.001
esp	59 (95.2)	12 (60)	0.001
gelE	50 (80.6)	9 (45)	0.001
cylA	46 (74.2)	10 (50)	0.001
efaA fm	0	13 (65)	0.001

*Significant incidence of all virulence genes in *E. faecalis* compared to *E. faecium* isolates, except *efaA fm* was significantly associated with *E. faecium*

result may due to the fact that most hospitalized infants were on antibiotic treatment which has reduced the colonization with enterococci, especially the susceptible strains. The majority of enterococci isolates were *E. faecalis* (75.6%) as confirmed by PCR method. The results of this study are generally in agreement with other recently published studies from various countries including Jordan [6,7,15,16].

A previous Jordanian study has reported that 83% of nosocomial isolates from patients including infants at JUH, over a five-year period were *E. faecalis*, followed by 16% *E. faecium* using PCR detection method [16].

A recent study from Greece, showed prevalence of various species of enterococci isolates from fecal samples of 97 healthy infants after their delivery on day, 4, 30, and 90, and the most frequently identified species was *E. faecalis* (54.6%) followed by *E. faecium* (12%) [17]. A study from Germany over a 12-month period, has also found that 23% of the 274 infants in a neonatal intensive care unit, were mostly colonized with *E. faecalis* [18]. The reason for different distribution rates of enterococci species recovered from fecal specimens of infants and others in various countries may be related to the types and amount of antibiotics usage in these countries which are known to contribute rapidly for change of the gut microbiota [3].

The present study demonstrates that most *E. faecalis* and *E. faecium* isolates were highly susceptible to most tested antibiotics, and *E. faecalis* isolates showed more high rates of susceptibility than *E. faecium* to ampicillin, chloramphenicol, gentamicin and levofloxacin in the range (93.5-65.5%). In addition, all *E. faecalis* and *E. faecium* isolates from inpatients showed higher resistant rates to chloramphenicol than those isolates from outpatients (**Table 3**). Additionally, similar results has been re-

ported by a previous Jordanian study where all *E. faecalis* isolated from patients with dental diseases and healthy controls were 100% susceptible to ampicillin, ciprofloxacin, chloramphenicol, vancomycin, and teicoplanin, but to less extent to erythromycin 62.5% [19]. However, numerous recent studies from various continents have reported an increasing occurrence of infection due to multidrug resistance of *E. faecalis* and *E. faecium*, and some of these studies documented increasing incidence of vancomycin-resistant in *Enterococcus spp.* isolated from serious human infections including infants [4,7, 9,20].

This study shows that *E. faecalis* isolates carried statistically significant more virulence determinants than the *E. faecium* isolates (**Table 3**). The present study has also demonstrated that both *Enterococcus* species isolates from hospitalized infants harbored more virulence genes than isolates from non-hospitalized. This result is different from other published study which has indicated that intestinal enterococci isolates from outpatients carry slightly more virulence genes than isolates from hospitalized patients [10].

However, The overall results of this study are comparable to the results represented by other worldwide studies which showed that *E. faecalis* carried higher rates and more multiple virulence determinates as compared with *E. faecium* [21-24]. Additionally, this study has detected high occurrence rates of most important putative virulence factors in enterococci isolates from infants, including collagen-binding protein (Ace). However, it has been shown that enterococcal virulence determinates are still considered a complex issue in causing infection because the essential factors for their pathogenic potential have not yet been well described [22].

In conclusion, this study shows important epidemiological features of fecal enterococci isolates

from Jordanian infants, especially their susceptibility to frequently used antimicrobials and association with potential virulence determinates.

There is no conflicts of interest related to this study.

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