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## Original article

# Multidrug resistant *Enterococci* as a community acquired infection in diarrheal cases of children

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## ABSTRACT

**Background and Objectives:** *Enterococci* causes many serious and life-threatening infections. *Enterococci* may be the cause of diarrhea in children. Multidrug-resistant (MDR) *Enterococci* limits treatment options. So, the aim of our study was to detect the frequency and the antibiotic resistance profile, both phenotypically and genotypically, of MDR *Enterococci* isolated from the stools of children suffering from gastroenteritis who needed hospitalization. **Materials and Methods:** *Enterococci* were isolated from stool samples from children. One hundred ten (110) infants (in patients) with typical signs of gastroenteritis (nausea, vomiting, abdominal pain, diarrhea). The Vitek 2 Compact System is used for identification and antimicrobial susceptibility testing. Drug resistance genes (ermB, aph(3')IIIa, and tetM) and virulence genes (esp) were identified using molecular techniques. **Results:** Thirty-six isolates of *Enterococci* were diagnosed phenotypically by routine lab examination and Vitek2 followed by genotypic characterization by multiplex PCR for three resistant genes: ermB (100%), aph(3')IIIa (63.9%) and tetM (91.7%) and one virulence gene (esp (25%)). The frequency of *Enterococci* among studied patients was 32.7% (36/110). Most of the *Enterococci* isolated from stool were *Enterococcus faecium* (86.1%). MDR *Enterococci* was found to be 94.4% (34/36) in total isolates. **Conclusion:** MDR *Enterococci* was isolated in community acquired diarrhea in young aged (less than 24 months) children. More than one resistance gene: ermB, aph(3')IIIa, tetM and virulence gene (esp) were detected in isolates. The presence of MDR strains is risky at a young age as it limits treatment options. Drug-resistant genes may be transmitted to a child through a carrier mother or cross infection from the hospital.

## Introduction

*Enterococci* are Gram-positive, catalase-negative, non-spore-forming, facultative anaerobic bacteria that live in the human digestive tract [1]. After birth, *Enterococci* are the first bacteria to enter the neonate's gastrointestinal tract. They can also

enter through a mother's breast milk or intimate touch [2]. Even though *Enterococcus* spp. cohabit in the intestinal tract, it can still cause diarrhea in elderly people, kids, and people with impaired immune systems [3].

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*Enterococci*, one of the most common nosocomial infections, is now well-established and is growing more resistant to antibiotics. All nosocomial enterococcal infections are currently caused by either *Enterococcus (E.) faecalis* or *E. faecium* [4]. Despite the fact that *E. faecalis* is the most harmful of the group, *E. faecium* is growing in importance as a result of its greater antibiotic resistance [5]. Infections that arise in hospitals are frequently brought on by these bacteria, including meningitis, endocarditis, bacteremia, infant sepsis, surgical and burn wound infections, and urinary tract infections (UTIs) associated with catheters [5].

*Enterococci* are typically harmless in healthy people. They become opportunistic pathogens by infecting people in intensive care units, with severe underlying conditions, or who are immunocompromised. As a result, extended hospitalization and/or indiscriminate antibiotic use can directly correlate with disease severity and immune suppression, and these are important risk factors for nosocomial drug-resistant *Enterococci* acquisition [6].

Macrolides are bacteriostatic antibiotics that bind to the 50S ribosomal subunit and impede protein synthesis [7]. The *ermB* gene, which is found in multiple transposons and plasmids in *Enterococcus*, *Streptococcus*, *Staphylococcus*, and *Clostridium* spp., is the most widely distributed gene in *Enterococci* that confers resistance to macrolides [8].

Tetracyclines suppress protein synthesis by binding to the ribosome and preventing tRNA linking, which has a bacteriostatic effect on cells [9]. The *Enterococci* that have been linked to the *tet(K)* and *tet(L)* efflux pump genes or the *tet(M)*, *tet(O)*, and *tet(s)* ribosome protection genes have shown significant resistance to this family of antibiotics [8]. Out of the five human *Enterococci* strains, *tetM* seems to be the gene that is isolated from them the most frequently [8, 10]. This gene can be located on the chromosome or be transmitted via a plasmid [11].

Aminoglycosides attach to the aminoacyl-tRNA recognition site (A-site) of the 30S ribosomal subunit's 16S rRNA, inhibiting polypeptide synthesis and causing cell death. Resistance to aminoglycosides can emerge through numerous methods, including a. enzymatic modification and inactivation of the aminoglycosides, which is often reported in Gram-positive and Gram-negative

bacteria and is mediated by aminoglycoside acetyltransferases, nucleotidyl transferases, or phosphotransferases. b. increased efflux; c. lower permeability; and d. changes to the 30S ribosomal subunit that interferes with aminoglycoside binding [12, 13].

Aminoglycosides bind to the aminoacyl-tRNA recognition site (A-site) of the 16S rRNA of the 30S ribosomal subunit, blocking polypeptide synthesis and triggering cell death. Resistance to aminoglycosides can occur via a variety of mechanisms, including a. enzymatic modification and inactivation of the aminoglycosides, which is frequently observed in Gram-positive and Gram-negative bacteria and is mediated by aminoglycoside acetyltransferases, nucleotidyl transferases, or phosphotransferases. Changes to the 30S ribosomal subunit that interferes with aminoglycoside binding [12, 13].

So, the aim of our study was to detect the frequency and the antibiotic resistance profile, both phenotypically and genotypically, of MDR *Enterococci* isolated from the stools of children suffering from gastroenteritis who needed hospitalization.

## Materials and methods

### Design of the study

A cross-sectional study was conducted from October 2022 to February 2023 on 110 infants showing signs of typical gastroenteritis.

### Study setting

Microbiology & Immunology Department, Faculty of Medicine, Assiut University. Stool samples were collected from all studied patients (inpatients children) with sterile swabs and cultured on the different culture media. The samples were collected from Children Hospital, Gastroenterology and Hepatology unit, Faculty of Medicine, Assiut University.

### Sample size calculation

Sample size was calculated using statcalc program of EPI-info version 7.2 using population survey or descriptive study calculation, according to the prevalence of *Enterococci* from stool, prevalence of enterococcus species in stool was 11 % [12], acceptable margin of error was 5%, confidence interval 90 %, and the minimum required sample size will be 106 sample of stool to detect presence of *Enterococci* in it.

### Ethical statement

The study was approved by the Ethical Committee of the Faculty of Medicine at Assiut University in Assiut, Egypt, following Declaration of Helsinki number 17101901 of the World Medical Association.

### Isolation of *Enterococci*

Samples were cultured for 24 hours at 37° C on blood agar plates (Himedia), which were poured using 5% defibrinated sheep blood. Non-hemolytic white colonies with clean edges that were Gram positive cocci arranged in pairs or short chains [14].

### Identification of *Enterococci* isolates

Separate purified colonies were identified by catalase test, Gram stain, and culturing on bile esculin agar.

### Vitek 2 Compact System

Enterococcal colonies were confirmed by using Gram positive identification card which held 43 different biochemical tests applied on each sample [15].

### Testing for antimicrobial susceptibility

Minimum inhibitory concentrations (MICs) were determined by Vitek 2 Compact (BioMerieux Inc., France) for the following antibiotics: ampicillin, benzylpenicillin, high level streptomycin, high level gentamycin, ciprofloxacin, levofloxacin, erythromycin, quinupristin/dalfopristin, linezolid, nitrofurantoin, vancomycin, tetracycline and tigecycline [16]. A category interpretation will be reported along with a MIC, according to the interpretations defined by CLSI (2021) [17]. The result was interpreted as either sensitive, intermediate, or resistant as per guidelines. MDR *Enterococci* identified as Multidrug resistance (defined as resistant to at least one agent in three or more antimicrobial categories) [18].

### PCR for genotypic characterization of *Enterococci* isolates

DNA of bacterial isolates was extracted by boiling method [19]. For four genes: ermB, aph (3')IIIa, tetM and esp genes, the Multiplex PCR was

conducted for all isolated *Enterococci*. The primers used for detection of both resistant genes and virulence gene were showed in the following table (Table 1).

### PCR assays

Two separate multiplex PCR assays for each two gene sets (ermB and esp) and (aph (3') IIIa and tetM). PCR was performed in a 25 µl reaction mixture containing 0.5 µl each of forward and reverse specific primer pairs (0.5 µl x 4), 12.5 µl of PCR master mix (Thermo Fisher Scientific, United States), 5.5 µl of nuclease free water, and 5.0 µl of DNA template.

### ErmB and esp genes

Initial denaturation at 95°C for 5 minutes, 40 cycles, each cycle consisting of: denaturation at 95°C for 40 seconds, annealing at 55°C for 50 seconds, extension at 72°C for 50 seconds and final extension at 72°C for 5 minutes [10].

### TetM and aph (3')-IIIa genes

Initial denaturation at 95°C for 5 minutes, 40 cycle, each cycle consisting of: denaturation at 95°C for 40 seconds, annealing at 50°C for 50 seconds, extension at 72°C for 50 seconds and final extension at 72°C for 5 minutes [20, 21].

### Detection of the amplified products

After being stained with ethidium bromide and examined for 60 to 70 minutes. Under UV light at 80 volts, the resulting PCR amplicons were screened on a 1.5% agarose gel. As a negative control, distilled water was used in each PCR cycle [22].

### Statistical analysis

Data analysis was performed using statistical package for the social science (IBM-SPSS) version 26.0 software. Categorical data was presented in the form of frequencies and percentages. Numerical variables as age presented by mean  $\pm$  SD. Chi square ( $\chi^2$ ) test was used to compare proportion between groups as the difference in antibiotic resistance according to age groups. independent Sample T test compare mean age between positive and negative cases to *Enterococci*. The level of significance was considered at  $p$  value  $< 0.05$ .

**Table 1.** Primers used in detection of drug resistance and virulence genes.

Target gene	Sequence of primers	Size of amplified product(bp)	References
<b>Virulence gene</b>			
esp	F AGATTTTCATCTTTGATTCTTG R AATTGATTCTTTAGCATCTGG	510bp	10
<b>Resistance genes</b>			
ermB	F CCGAACACTAGGGTTGCTC R ATCTGGAACATCTGTGGTATG	139bp	10
tet(M)	F ACAGAAAGCTTATTATATAAC R TGGCGTGTCTATGATGTTTAC	170bp	20
Aph (3')-IIIa	F GGCTAAAATGAGAATATCACCGG R CTTTAAAAAATCATACAGCTCGCG	523bp	21

## Results

Out of 110 studied samples collected from infants suffering from gastroenteritis, about 36 (32.7%) isolates were found to be *Enterococci*. The distribution of isolated *Enterococci* among different age and sex groups is represented (**Table 2**).

Identification of bacterial isolates was done by Vitek 2 compact system. Most of the *Enterococci* isolated from stool were *E. faecium* (86.1%), followed by *E. avium* and *E. gallinarum* with percentages of 11.1% and 2.8%, respectively.

### Antimicrobial susceptibility pattern for *Enterococci*.

$\beta$ -lactams (ampicillin and benzylpenicillin) had the similar highest resistance with a percentage of 94.4%. The resistance to high level of streptomycin, tetracycline, and erythromycin were 88.9%, 66.7 %, and 100 %, about 41.7% had resistance to both (Ciprofloxacin and Levofloxacin) and nitrofurantoin, similarly, followed by high level gentamycin 30.5%, quinupristin /dalfopristin was

11.1%. Only 8.3% of the isolated *Enterococci* had vancomycin-resistance. Linezolid resistance was 2.8%. No resistance was found to tigecycline. The relationship between resistance to different antibiotics and children's age is illustrated in **table (3)**.

### Molecular detection

PCR assay which was conducted on the studied *Enterococci* from stool showed that ermB was found in 100% of all samples, tetM with the percentage of 91.7% and aph (3')IIIa with the percentage of 63.9% (**Figure 1, 2**). The esp gene (virulence gene) was found with 25% frequency in stool samples. The coexistence of three drug resistance genes and virulence esp gene in stool isolates was shown in **figure (1, 2)** and **table (4)**.

Relation between phenotypic and genotypic detection of *Enterococci* isolated from stool isolates. Thirty-six isolates (100%) of erythromycin-resistant strains carried ermB. Tetracycline-susceptible strains harbored the tetM resistance gene at a rate of 25% (9/36), while 66.7% (24/36) of tetracycline-resistant strains carried tetM. high-level streptomycin-resistant strains carried aph(3')IIIa with a percentage of 63.9%.

**Table 2.** Distribution of *Enterococci* isolates in stool of children according to some demographic variables.

Variables	Total (n=110)	Positive (No=36)	Negative (No=74)	P-value
<b>Age (months)</b>				
Mean ± SD	11.43±8.43 (2-31)	11.27±8.50 (4-30)	10.24±7.24 (2-31)	0.891
<6 months	37 (33.6%)	9 (24.3%)	28 (75.7%)	0.445
6-<12 months	43 (39.1%)	16 (37.2%)	27 (62.8%)	
12-24 months	22 (20.0%)	7 (31.8%)	15 (68.2%)	
>24 months	8 (7.3%)	4 (50.0%)	4 (50.0%)	
<b>Gender:</b>				
Female	59 (53.6%)	19 (32.2%)	40 (67.8%)	0.899
Male	51 (46.4%)	17 (33.3%)	34 (66.7%)	

No statistically significant difference between positive and negative cases to *Enterococci* regarding age, mean age of positive cases was 11.27±8.50 and ranged from 4-30 months and for negative cases was 10.24±7.24 and ranged from 2 to 31 months. Moreover, there was no statistically significant difference between different age groups regarding *Enterococci* presence (24.3%, 37.2%, 31.8% and 50.0% in < 6 months, 6-<12 months, 12-24 months and >24 months respectively).

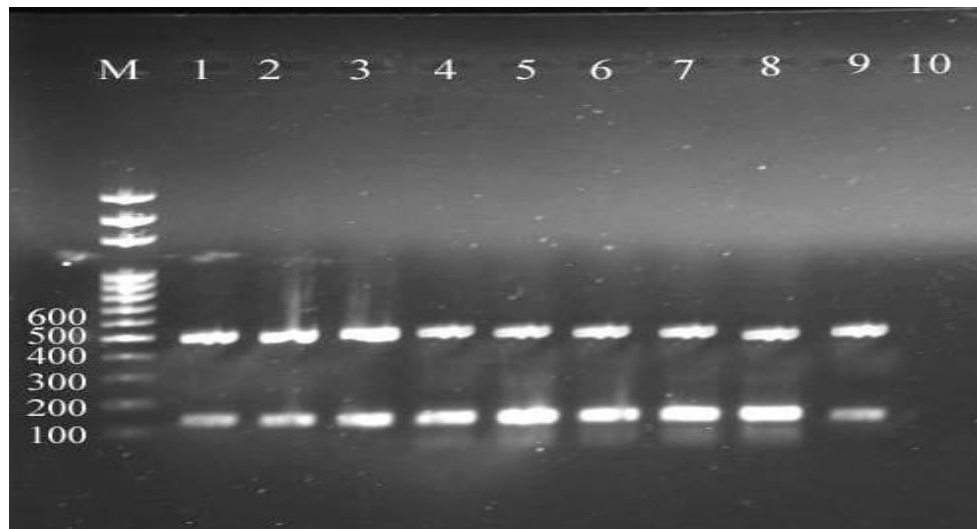
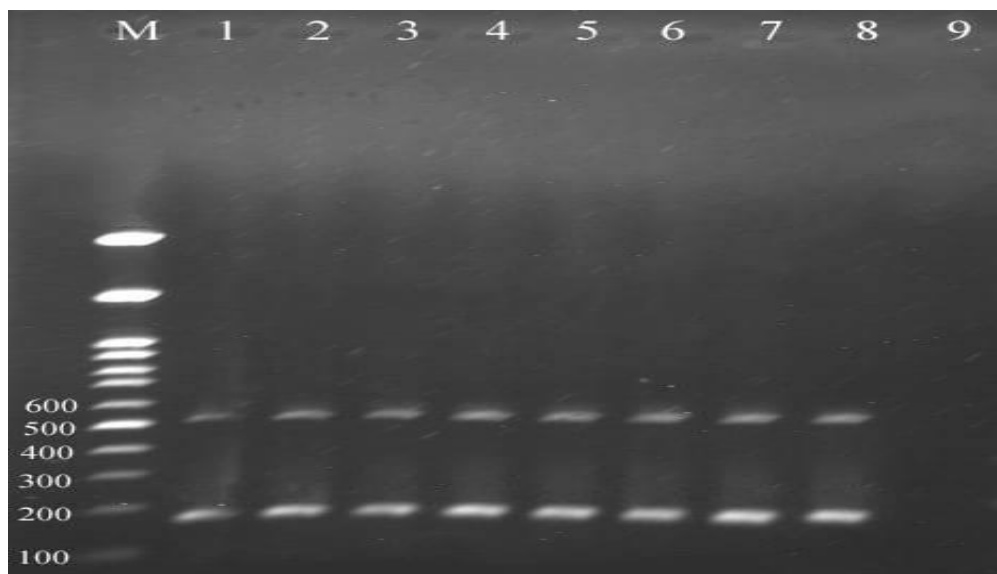
**Table 3.** Relationship between antibiotics resistance and age of children.

Antimicrobial Agent/ total no. (36)	Age groups/month				Total	P-value
	< 6 months (9)	6- <12m (16)	12-24m (7)	>24 months (4)		
<b>Macrolide:</b> Erythromycin	R	R	R	R		
	9 (100%)	16 (100%)	7 (100%)	4 (100%)	100%	NA
<b>Tetracycline:</b> Tetracycline	5 (55.6%)	10 (62.5%)	5(71.4%)	4(100%)	66.7%	0.441
<b>β – lactams:</b>						
Ampicillin	9 (100%)	16 (100%)	5 (71.4%)	4(100%)	94.4%	0.032
Benzylpenicillin	9 (100%)	16(100%)	5(71.4%)	4(100%)	94.4%	0.032
<b>Fluoroquinolones:</b>						
Levofloxacin	3 (33.3%)	8(50%)	2(28.6%)	2(50%)	41.7%	0.723
Ciprofloxacin	3 (33.3%)	8(50%)	2(28.6%)	2(50%)	41.7%	0.723
<b>Nitrofurantoin:</b> Nitrofurantoin	5 (55.6%)	5(31.3%)	3(42.9%)	2(50%)	41.7	0.715
<b>Aminoglycoside:</b>						
High Level Streptomycin	9 (100%)	12(75.0%)	7(100%)	4(100%)	88.9%	0.131
High level Gentamicin	2 (22.2%)	5(31.3%)	2(28.6%)	2(50%)	30.5%	0.795
<b>Streptogramin:</b> Quinupristin/Dalfopristin	0(0.0%)	1(6.3%)	1(14.3%)	2(50%)	11.1%	0.052
<b>Glycopeptide:</b> Vancomycin	1(11.1%)	0(0.0%)	1(14.3%)	1(25%)	8.3 %	0.344
<b>Oxadiazoles:</b> Linezolid	(0.0%)	(0.0%)	(0.0%)	1(25%)	2.8%	0.041
<b>Glycylcycline :</b> Tigecycline	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)	NA

R: resistant. NA (non-applicable to calculate the significance between groups). There were statistically significant differences between resistance to β – lactams drugs (Ampicillin and Benzylpenicillin) and age group distribution, as the lower resistance to the drugs was among 12-24 months groups (71.4%) and the other age groups the resistance was 100.0%. Moreover, there was statistically significant no resistance to linezolid among all age groups (0.0%) except > 24 months, the resistance was 25.0%. No statistically significant difference between resistance to other drugs and age distribution. In the current study, MDR *Enterococci* was found to be 94.4% (34/36) in total isolates, which were resistant to more than 3 classes of antibiotics.

**Table 4.** Coexistence of drug resistance genes and esp gene in stool isolates.

No. of genes	Genes	No. of isolates (36)	No. of MDR <i>Enterococci</i>
Two genes	ermB, tetM	17	15/ 17 (88.2%)
Three genes	eEsp, ermB, tetM	1	1 (100%)
Three genes	ermB, tetM, aph(3')IIIa	10	10 (100%)
Four genes	esp, ermB, tetM, aph(3')IIIa	8	8 (100%)

**Figure 1.** Agarose gel electrophoresis of the products of PCR to detect ermB (139 bp) and esp (510 bp) genes. Lane (M) shows the DNA (100-1500bp) standard marker, All lanes (1 to 9) show ermB and esp positive isolates. Lane (10) is negative.**Figure 2.** Agarose gel electrophoresis of the products of PCR to detect tetM (170 bp) and aph(3')IIIa (523 bp) genes. Lane (M) shows the DNA standard marker(100-1500bp), All lanes (1 to 8) show tetM and aph(3')IIIa positive isolates. Lane (9) is negative.

## Discussion

With significant morbidity and death, *Enterococci* have become a major factor in a number of illnesses. Treatment of enterococcal infections is difficult due to their ability to acquire resistance as well as innate resistance to several antimicrobials [20]. Previously thought to be benign intestinal commensals with little clinical importance, *Enterococci* has developed into a significant nosocomial pathogen [2].

In the current study, 32.7% (36/110) of children with gastroenteritis had *Enterococcus* species. In a similar study conducted in Iran, **Alebouyeh et al.** [23] found the percentage of *Enterococcus* species isolated from rectal swabs to be 34.6% which comes in accordance with our results. A study from Iraq (2019) found that 48.3% of children with diarrhoea were infected with *Enterococci* [3].

In the present study, different species were isolated from children. *Enterococcus faecium* was the most predominant isolate in the samples with a prevalence of 86.1% (31/36), followed by *E. avium* and *E. gallinarum* with prevalence of 11.1% (4/36) and 2.8% (1/36), respectively. No detection of other species of *Enterococcus* was found in the current study. **Ngah et al.** [24] found that the prevalence of *E. faecalis* was 10.5% and that *E. faecium* was 36.9% in the stool in another study done in Cameroon.

Prevalence of antibiotic resistance was highest in the  $\beta$ -lactam group (ampicillin and benzylpenicillin), at 94.4% (34/36), similarly. The high resistance of  $\beta$ -lactams group may be due to the frequent use of unictam (combination of sulbactam and ampicillin) in treatment of gastroenteritis in children hospital. In a similar study conducted in Korea, **So et al.** [25] discovered an 82.9% prevalence of  $\beta$ -lactam resistance. This was nearly in agreement with our results. Our finding was higher than the prevalence detected by **Jannati et al.** [26], who detected the percentage of  $\beta$ -lactams resistance was 41.3%. **Al-Dahmoshi et al.** [3], found the prevalence of  $\beta$ -lactam resistance was 53.6%. According to **Subramanya et al.** [27], this resistance is due to the organism's inherent resistance to various antibiotics, including  $\beta$ -lactams.

Our research revealed that a significant portion of isolated samples were high resistant to high-level aminoglycoside antibiotics, with a

percentage of 88.9% (32/36) being resistant to high-level streptomycin, about 30.5% (11/36) being resistant to high-level gentamycin. The highest resistance to aminoglycoside may be due to the use of amikacin in the treatment of gastroenteritis. The results of our research were higher than those discovered by **So et al.** [25], who reported that 46.8% of high-level aminoglycosides were resistant (streptomycin was 13.2% and gentamycin was 33.7%). This was less than that of our results. In contrast to our findings, a study conducted in Iran found that the high-level gentamycin resistance was 2.7% and the high-level streptomycin resistance was 17.4% [26].

The high rate of tetracycline resistance observed in the present study was 66.7% (24/36). This is in accordance with **Jannati et al.** [26], who detected the resistance of tetracycline at 66.7%. Tetracycline resistance was shown to be 87.5% in an Iranian study, according to **Yadegarynia et al.** [28]. In a study done in Iraq, **Al-Dahmoshi et al.** [3] found that the prevalence of tetracycline resistance was 57.14%.

In our study, the rate of erythromycin resistance was 100% (36/36). **Al-Dahmoshi et al.** [3] and **Yadegarynia et al.** [28] revealed the high resistance rate of erythromycin, as their results were 85.71% and 95.8%, respectively. In a study established in Iran, the prevalence of erythromycin resistance was 41.3%, which was lower than our results [26].

Resistance to fluoroquinolones (ciprofloxacin and levofloxacin) was obviously marked in our results with a 41.7% (15/36), equally. Another study done in Iraq, it was found that the frequency of fluoroquinolones was 60.7% [3]. **Jannati et al.** [26] detected the low resistance rate of fluoroquinolones, that is was 15.9%. In the absence of antibiotic exposure, the presence of antibiotic-resistant genes in bacteria present in the infant's gut may indicate that these strains were transferred from a carrier mother to the child, as suggested by **Patangia et al.** [32].

Our findings showed a prevalence of nitrofurantoin resistance of 41.7%, which was more than that reported by **Jannati et al.** [26], who found a prevalence of nitrofurantoin resistance of 19.3%. **Al-Dahmoshi et al.** [3] found the prevalence to be 64.29%, which is greater than the data we found, and so found that this rate of nitrofurantoin resistance was the highest.

The lowest resistance rates in the current study were found for quinupristin/ dalfopristin, Vancomycin, and Linezolid, with respective rates of 11.1%, 8.3%, and 2.8%. **So et al.** [25] and **Subramanya et al.** [27] found no resistance to linezolid in their studies. The three VRE strains isolated were multi drug resistant, two of them were *E. faecium* and the other one was *E. avium*. Vancomycin resistance was found at 44.4% and 46.4%, respectively, by **Alebouyeh et al.** [23] and **Dahmoshi et al.** [3], which was more than our values. Vancomycin resistance was reported to be 17.1% according to a study by **So et al.** [25], although no Vancomycin resistant *Enterococci* were observed, according to **Jannati et al.** [26].

Vancomycin Resistant *Enterococci* (VRE) sepsis is emerging as a significant problem in the intensive care setting. The infection can be acquired from the carrier mother or as cross infection from the hospital [29]. Vancomycin resistant *Enterococcus faecium* (VRE-fm) was first reported in 1988, a time point when it had rarely been isolated in neonates. Its prevalence has significantly increased globally during the following decades [30]. Almost any surface can become contaminated with VRE, which can also persist in the environment for lengthy periods of time (more than one week). Healthcare workers can also spread these organisms from one patient to another. This highlights the urgent need for effective measures to control the spread of these pathogens [23].

The high prevalence of MDR *Enterococci* in infant stool may be due to vertical antibiotic transmission across the mother-baby axis via lactation or during birth.

Natural resistance to a variety of antibiotics exists in *Enterococci*, and they are also capable of transferring resistance genes to other microbes via plasmid conjugation mechanisms [33]. **Ben Braiek et al.** [34] mentioned that intrinsic antibiotic resistance of *Enterococci* includes resistance to cephalosporins, sulphonamides, lincosamides,  $\beta$ -lactams, and aminoglycosides, located in the chromosomes.

The erythromycin-resistant gene (*ermB*) was found to be present in 100% of isolates. The prevalence of the *ermB* resistant gene was determined to be 33.3% in research by **Mišić et al.** [35], which is much lower than our findings. According to **Tian et al.** [36] and **Ben Sallem et al.**

[37], there were 67.7% and 84.6%, respectively, of *ermB*-positive clinical isolates.

*Aph(3')IIIa* gene was detected in 63.9% (23/36) of isolated strains. Approximately 63.9% of all high-level streptomycin resistant strains carried *aph(3')IIIa*, while 25% (9/36) of the high-level streptomycin resistant strains detected did not carry the *aph(3')IIIa* gene. This could be due to the existence of unknown resistance mechanisms, as suggested by **Ben Sallem et al.** [37]. According to **Tian et al.** [36], 64.9% of isolated samples carried the *aph(3')IIIa* gene, which is consistent with our findings. With rates of 32%, and 54.5%, respectively, **Moussa et al.** [38], and **Jannati et al.** [26] indicated the low prevalence of the *aph(3')IIIa*-resistant gene. In contrast, the percentage of *aph(3')IIIa* was very high in a study conducted in Egypt by **Diab et al.** [39], as they detected 86.5% of resistant strains carrying the *aph(3')IIIa*-resistant gene.

The *tetM* gene was found in isolated strains with 91.7% (33/36) percentage. Surprisingly, a tetracycline resistance gene was present in 25% (9/36) of tetracycline-susceptible strains while 66.7% (24/36) was the percentage of tetracycline-resistant strains harboring *tetM* resistant gene. **Said et al.** [20] and **Ben Sallem et al.** [37] stated that the percentage of *tetM* was found to be 50% and 70% in the isolated strains, respectively. In another study conducted in China, **Tian et al.** [36] documented the prevalence of *tetM* was 100% which comes nearly to our finding and reported that there was no tetracycline susceptible strain carrying *tetM* found in their study.

The *esp* virulence gene was found in 25% (9/36) of stool samples. Both **Ben Sallem et al.** [37] and **Sattari-Maraji et al.** [31] found the prevalence of the *esp* virulence gene to be 17.5% in their studies.

Thirty-four of the thirty-six multidrug resistant bacteria included the four genes that were the subject of the study in *Enterococci* isolates, and they were distributed as follows: fifteen MDR strains carried two resistance genes (*ermB* and *tetM*). Eleven MDR strains carried three different genes; 10 of them carried the *ermB*, *tetM* and *aph(3')IIIa* genes, and the other one carried the *esp*, *ermB*, and *tetM* genes. Eight isolates of multi drug-resistant bacteria carried the four genes (*esp*, *ermB*, *tetM* and *aph(3')IIIa*).



The emergence of clinical infection is mostly correlated with high colonization pressure and risk factors, the most significant of which are: young age, use of invasive devices, administration of antimicrobial drugs, immunosuppression, low birth weight, and underlying malignancy [30].

### Conclusion

MDR *Enterococci* were isolated in community acquired diarrhea in young aged children. More than one resistance genes: ermB, aph(3')IIIa, tetM and virulence gene (esp) were detected in isolates. The presence of MDR strains is risky at a young age as it limits treatment options.

### Study limitation

- 1-No financial fund for more molecular work
- 2-We don't collect from healthy children beside diseased ones and compare between them.

### Disclosure of potential conflicts of interest

The authors report no conflicts of interest.

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Not declared.

### References

- 1-Abdeltawab A.A., Mohamed R.S., Kotb M.A. Molecular detection of virulence and resistance genes of *Enterococci* spp isolated from milk and milk products in Egypt. Nature and Science 2019;17(9).
- 2-Krupa-Kotara K., Grajek M., Grot M., Czarnota M., Wypych-Ślusarska A., Oleksiuk K., Głogowska-Ligus J., Słowiński J. Pre- and Postnatal Determinants Shaping the Microbiome of the Newborn in the Opinion of Pregnant Women from Silesia (Poland). Life (Basel). 2023 Jun 13;13(6):1383.
- 3-Al-Dahmishi H.O., Rabeea H.W., Aridhee A.S., Al-Khafaji N.S., Al-Allak M.H., Lazm A.M., Jebur M.S. Phenotypic investigation of vancomycin, teicoplanin and linezolid resistance among *Enterococcus* spp. isolated from children diarrhea. J Pure Appl Microbiol. 2019 Mar 1;13(1):531-6
- 4-Dubin, K., Pamer E.G. *Enterococci* and their interactions with the intestinal microbiome. Microbiol. Spectr. 2016, 5 .
- 5- Ramos S., Silva V., Dapkevicius Md L.E., Igrejas G., Poeta P. *Enterococci*, from Harmless Bacteria to a Pathogen. Microorganisms. 2020; 8(8):1118.
- 6-El-Kersh, T.A., Marie, M.A., Al-Sheikh, Y.A., Al-Agamy, M.H., Al Bloushy, A.A. 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. Saudi Med. J. 2016, 37, 280–287.
- 7-Schroeder M.R., Stephens D.S. Macrolide resistance in *Streptococcus pneumoniae*. Frontiers in cellular and infection microbiology. 2016 Sep 21; 6:98.
- 8-Torres C., Alonso C.A., Ruiz-Ripa L., León-Sampedro R, Del Campo R, Coque T.M. Antimicrobial resistance in *Enterococcus* spp. of animal origin. Antimicrobial resistance in bacteria from livestock and companion animals. 2018 Oct 1:185-227.
- 9-Miller W.R., Munita J.M., Arias C.A. Mechanisms of antibiotic resistance in *Enterococci*. Expert review of anti-infective therapy. 2014 Oct 1;12(10):1221-36.
- 10-Kim Y.B., Seo KW, Jeon HY, Lim SK, Sung HW, Lee YJ. Molecular characterization of erythromycin and tetracycline-resistant *Enterococcus faecalis* isolated from retail chicken meats. Poultry science. 2019 Feb 1;98(2):977-83.
- 11-Fioriti S., Coccitto S.N., Cedraro N., Simoni S, Morroni G, Brenciani A, Mangiaterra G, Vignaroli C, Vezzulli L, Biavasco F, Giovanetti E. Linezolid resistance genes in *Enterococci* isolated from sediment and

- zooplankton in two Italian coastal areas. *Applied and Environmental Microbiology*. 2021 Apr 13;87(9): e02958-20.
- 12-Doi Y., Wachino J.I., Arakawa Y.** Aminoglycoside Resistance: The Emergence of Acquired 16S Ribosomal RNA Methyltransferases. *Infect Dis Clin North Am*. 2016 Jun;30(2):523-537.
- 13-Chow J.W.** Aminoglycoside resistance in *Enterococci*. *Clinical Infectious Diseases*. 2000 Aug 1;31(2):586-9.
- 14-Ekin İ.H., Sevil A.T., Tollu G., Gulaydin O., Gurturk K., Ilhan Z., Ozturk C.** The presence and prevalence of *Enterococcus faecalis* and *Enterococcus faecium* strains in urine and stool samples. *Turkish Journal of Veterinary Research*. 2018;2(1):14-8.
- 15-Crowley E., Bird P., Fisher K., Goetz K., Boyle M., Benzinger, Jr M.J., et al.** Evaluation of the VITEK 2 gram positive (GP) microbial identification test card: collaborative study. *Journal of AOAC International*. 2012 Sep 1;95(5):1425-32.
- 16-Elanany M.G., Mansour T.M., Hassan R.M., Maher H.** Early Identification of Bacterial Isolates from Positive Blood Culture Bottles using MALDI-TOF MS and Early Antimicrobial Susceptibility Testing on Pediatric Oncology Patients. *Int J Biomed Sci*. 2018;14(1):26-31.
- 17-Clinical and Laboratory Standards Institute. 2021.** Performance standards for antimicrobial susceptibility testing. CLSI supplement M100, 31st ed. Clinical and Laboratory Standards Institute, Wayne, PA.
- 18-Admasu A., Mihret T., Saba Gebremichael T., Melaku A. B.** Prevalence, Antimicrobial Susceptibility Patterns, and Risk Factors Associated with *Enterococci* among Pediatric Patients at Dessie Referral Hospital, Northeastern Ethiopia", *BioMed Research International*, vol. 2021, Article ID 5549847, 9 pages, 2021.
- 19-Fayyad R.J. and Dwaish A.S.** New modified protocol of DNA extraction comparison with other extraction methods for polymerase chain reaction analysis of genomic DNA from Cyanophyceae isolates. *Advances in Environmental Biology*. 2016 Sep 1;10(9):77-83.
- 20-Said H.S. and Abdelmegeed E.S.** Emergence of multidrug resistance and extensive drug resistance among enterococcal clinical isolates in Egypt. *Infection and Drug Resistance*. 2019 May 7:1113-25.
- 21-Khani M., Fatollahzade M., Pajavand H., Bakhtiari S., Abiri R.** Increasing prevalence of aminoglycoside-resistant *Enterococcus faecalis* isolates due to the aac (6')-aph (2'') gene: A therapeutic problem in Kermanshah, Iran. *Jundishapur journal of microbiology*. 2016 Mar;9(3).
- 22-Isbir T., Kirac D., Demircan B., Dalan B.** Gel Electrophoresis. *Brenner's Encyclopedia of Genetics (Second Edition)*, 165-167, 2013.
- 23-Alebouyeh M., Shirvani F., Hassanzadeh R., Azimi T., Ghandchi G., Abdollahi N., Gholinejad Z., et al.** Intestinal colonization of vancomycin-resistant *Enterococcus* in children admitted to Mofid children's hospital intensive care unit at admission and at discharge. *Molecular Biology Reports*. 2023 Apr; 50(4):3271-81.
- 24-Ngah E., Assiene S.D., Ayangma C.R., Assiene A.J., Gueguim C., Adiogo D.** Antibiotic resistance profile of *Enterococcus* spp. strains isolated from the intestinal tract at the Yaounde Military Hospital Laboratory. *GSC Biological and Pharmaceutical Sciences*. 2022;19(1):009-19.

- 25-So H and Lee J.** Prevalence and Antimicrobial Resistance of Enterococcus species in a Tertiary Center Children's Hospital in Korea. In Open Forum Infectious Diseases 2022 Dec (Vol. 9).
- 26-Jannati E., Amirmozaffari N., Saadatmand S., Arzanlou M.** Faecal carriage of high-level aminoglycoside-resistant and ampicillin-resistant Enterococcus species in healthy Iranian children. Journal of global antimicrobial resistance. 2020 Mar 1;20:135-44.
- 27-Subramanya, S. H., Amberpet, R., Chaudhary D., Nayak N., Padukone S, Bairy I. Gokhale, S.** Neonatal sepsis due to glycopeptide resistant Enterococcus faecium from colonized maternal gut-rare case evidence. Antimicrobial Resistance & Infection Control, 8(1), 1-4. (2019).
- 28-Yadegarynia D., Roodsari S.R., Arab-Mazar Z.** Evaluation of antimicrobial susceptibility among enterococcus species by e-test method at khatam-ol-anbia hospital during 2013–2014. Archives of Clinical Infectious Diseases. 2016 Jan 1;11(1).
- 29-Shantala G.B., Nagarathnamma T., Pooja D.R., Harsha T.R., Karthik R.** Neonatal septicemia caused by vancomycin resistant Enterococcus faecium-a case report. Journal of clinical and diagnostic research: JCDR. 2014 Nov;8(11):DD03.
- 30-Marom R., Mandel D., Haham A., Berger I., Ovental A., Raskind C., Grisar-Soen et al.** A silent outbreak of vancomycin-resistant Enterococcus faecium in a neonatal intensive care unit. Antimicrobial Resistance & Infection Control. 2020 Dec;9(1):1-6.
- 31-Sattari-Maraji A., Jabalameli F., Node Farahani N., Beigverdi R., Emancini M.** Antimicrobial resistance pattern, virulence determinants and molecular analysis of Enterococcus faecium isolated from children infections in Iran. BMC microbiology. 2019 Dec;19(1):1-8.
- 32-Patangia D.V., Ryan C.A., Dempsey E., Stanton C., Ross R.P.** Vertical transfer of antibiotics and antibiotic resistant strains across the mother/baby axis. Trends in Microbiology. 2022 Jan 1;30(1):47-56.
- 33-Golaś-Prądyńska M., Łuszczynska M., Rola J.G.** Dairy Products: a potential source of Multidrug-Resistant Enterococcus faecalis and Enterococcus faecium Strains. Foods. 2022 Jan;11(24):4116.
- 34-Ben Braiek O. and Smaoui S.** Enterococci: between emerging pathogens and potential probiotics. BioMed research international. 2019 Oct;2019.
- 35-Mišić M., Kocić B., Arsović A., Čukić J., Vidanović D., Šekler M., Baskić D.** Human enterococcal isolates as reservoirs for macrolide-lincosamide-streptogramin and other resistance genes. The Journal of Antibiotics. 2022 Jul;75(7):396-402.
- 36-Tian Y., Yu H., Wang Z.** Distribution of acquired antibiotic resistance genes among Enterococcus spp. isolated from a hospital in Baotou, China. BMC research notes. 2019 Dec; 12:1-5.
- 37-Ben Sallem R., Klibi N., Klibi A., Ben Said L., Dziri R., Boudabous A., Torres C., Ben Slama K.** Antibiotic resistance and virulence of *Enterococci* isolates from healthy humans in Tunisia. Annals of Microbiology. 2016 Jun; 66:717-25.
- 38-Moussa A.A., Md Nordin A.F., Hamat R.A., Jasni A.S.** High level aminoglycoside resistance and distribution of the resistance genes in Enterococcus faecalis and Enterococcus faecium from teaching hospital in

Malaysia. Infection and Drug Resistance. 2019 Oct 21:3269-74.

**39-Diab M., Salem D., El-Shenawy A., El-Far A., Abdelghany A., Awad A.R., El Defrawy I. et al.** Detection of high-level aminoglycoside resistance genes among clinical isolates of *Enterococcus* species. Egyptian Journal of Medical Human Genetics. 2019 Dec;20(1):1-6.

Abdel Hamid RF, Nafadi HKM, Ahmed AS, Galal SM, Abd El-Kareem DM, Rashwan RS. Multidrug resistant *Enterococci* as a community acquired infection in diarrheal cases of children. Microbes Infect Dis 2023; 4(4): 1275-1286.