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Original Article

Detection of Antibiotic Resistance Genes (*CTX-M*, *Van* A and *Van* B) of *Enterococcus faecalis* Isolated from Children with Bacteremia by RT-PCR

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Abstract

Fever is one of the most common diseases affecting humans, as it results from any disease or development and worsening of the disease for most people with widespread infections in the body. Therefore, this study aimed to evaluate antibiotic resistance genes (*CTX-M*, *Van* A and *Van* B) of *Enterococcus faecalis* isolated from children with bacteremia by RT-PCR. A total of 200 children was enrolled in the study, 100 children with fever and 100 healthy children (not suffering from any problem); that is, they are a control group for the detection of antibiotic resistance genes (CTX-M, *Van A* and *Van B*) of *Enterococcus faecalis* by RT-PCR. The age of the two groups ranged from one to five years. Four ml of venous blood sample was collected from each child; the venipuncture area was sterilized first with alcohol at a rate of 70%, followed by medical iodine and then sterilized with alcohol again to avoid contamination with skin flora. The blood samples were cultured on media for isolating bacteria. Then, the resistant isolates of *E. faecalis* to Vancomycin and cefotaxime antibiotics were taken and kept in special nutrient agar media where the DNA of the bacteria was extracted using (Zymogene Extraction kit, Japan). The detection of the exact genes (CTX-M, *Van A* and *Van B*) was done using Real-Time PCR technology according to the protocol mentioned by the company (Sacace biotechnology, Italy). The study presented that 40% of children with fever have positive blood cultures compared with 5% in the control group, with a significant difference between the two groups (P<0.001). The study found that 32.5% of bacteremic children were due *to S. aureus*, 30%, 5%, and 4% were due to *E. faecalis*, *E. coli*, *P. aeruginosa* and *Klebsiella* spp, respectively, with significant difference (P<0.01). The study showed that 91.67% of *E. faecalis* isolates were sensitive to Levofloxacin, 83.33% to Amoxiclav, 66.67% to Erythromycin, 58.33% to Amikacin, 50% to Ampicillin, 33.33% to cefotaxime and Ceftriaxone and 25% toward Vancomycin. From 9 isolates resistant to Vancomycin, the study presented that 88.89% of them were observed with *Van A* gene production as detected by real-time PCR (P<0.001). The study also showed that 77.78% were observed with *Van B* gene production as detected by real-time PCR (P<0.001). The study revealed that all *E. faecalis* isolates resistant to cefotaxime and Ceftriaxone were characterized by CTX gene production as detected by real-time PCR (P<0.001).

Keywords: Antibiotic resistance, CTX-M, Vancomycin, *Enterococcus faecalis* RT-PCR

1. Introduction

Fever is one of the most common diseases affecting humans, as it results from any disease or development and worsening of the disease for most people with general infections in the body (1). Bacteremia in humans, especially children, is one of the most important diseases characterized by high temperatures as a result of the spread of many types of bacteria that are distinguished by their virulence and their ability to cause pathological events, given that they have many toxins and outputs that

ultimately lead to high temperatures in these people (2). Among those bacteria, the *Enterococcus faecalis* is one of the most important causative bacteremia pathogens. *Enterococcus faecalis* is a gram-positive bacterium that causes several human infections (3). The problem of antibiotic resistance in bacteria isolated from sick people is one of the biggest problems facing medical personnel, children's burden and general surgeons because the increased rate of bacterial resistance to antibiotics makes them more dangerous as they lead and threaten the lives of patients at a very high rate, especially in societies where do not pay great attention to antibiotic resistance in bacteria (4). Several recent studies were conducted to identify and reveal genes that cause antibiotic resistance in many bacteria that cause bacteremia in children and adults worldwide (5-7). Therefore, this study aimed to evaluate antibiotic resistance genes (*CTX-M, Van A* & *Van B*) of *Enterococcus faecalis* isolated from children with bacteremia by RT-PCR.

2. Materials and Methods

2.1. Participants and Study Design

The study was conducted in the city of Kirkuk from April 2019 to January 2020 and included 100 children with fever and 100 healthy children not suffering from any problems as the control group. The ages of the two groups ranged from 1-5 years. The study included collecting blood samples from the children included in the study, where 4 ml of venous blood was collected from each child, where the withdrawal area was sterilized well with alcohol at a rate of 70%, followed by the use of medical iodine, and then sterilized with alcohol a second time to avoid contamination with skin flora.

2.2. Bacterial Isolation

Characterization of the isolated enterococci to the genus level was performed using Gram staining, blackening of Bile Aesculin Azide Agar (Oxoid), and culture on nutrient broth at 10°C, 45°C, with 6.5% NaCl. Then the motility test, sugar fermentation tests (L-Arabinose, Mannitol, Sorbitol Glycerol D-Lyxose Mannitol, Galactose, and Hippurate), and arginine dihydrolase and pyruvate utilization test were used for characterization of the isolated enterococci to the species level.

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of the isolates was determined by disk diffusion method for the following antibacterial agents; Erythromycin (15 µg), Ampicillin (10 µg), Amoxiclave (30 µg), Levofloxacin (30 µg), Vancomycin (30 µg), Amikacin (30 µg), Cefotaxime (30 µg), Ceftriaxone (5 µg) (Bioanalyse, Turkey). Muller-Hinton agar plates were inoculated with 0.5 McFarland standard suspensions of the strains; antimicrobial disks were placed into plates and then incubated at 37°C for 24 h. The agar dilution method determined the minimum inhibitory concentrations (MICs) of Vancomycin and Erythromycin. Zone diameters were assessed according to the Clinical Laboratory Standard Institute guidelines.

2.4. DNA Extraction

Then, *E. faecalis* isolates, resistant to Vancomycin and Cefotaxime, were taken, isolated and kept in special nutrient agar media where the DNA of the bacteria was extracted using (Zymogene Extraction kit, Japan). Then, the exact genes (*CTX-M, Van A* and *Van B*) were detected using Real-Time PCR technology and according to the protocol mentioned by the company (Sacace biotechnology, Italy).

2.5. Statistical Analysis

Categorical variables were analyzed using the chi-square test using SPSS software (version 20). *P*-values of < .05 were considered statistically significant.

3. Results

The study presented that 40% of children with fever have positive blood cultures compared with 5% in the control group, with a significant difference between the two groups ($P<0.001$) (Table 1).

Table 1. Prevalence of Bacteremia in children with and without fever

Result	Children with fever		Healthy group	
	No.	%	No.	%
+ve blood culture	40	40	5	5
Negative	60	60	95	95
Total	100	100	100	100

$P<0.001$

The study found that 32.5% of bacteremic children were due to S. aureus, 30% to *E. faecalis*, 5% of *E. coli*, 4% of *P. aeruginosa* and *Klebsiella spp*. (*P*<0.01) (Table 2).

Table 2. Distribution of bacterial isolates from bacteremic children

Bacterial isolates	Children with fever	
	No.	%
S. aureus	13	32.5
E. faecalis	12	30
E. coli	5	12.5
P. aeruginosa	4	10
Klebsiella spp	4	10
S. epidermidis	2	5
Total	40	100

P<0.01

Table 3 shows that 91.67% of *E. faecalis* isolates are sensitive to Levofloxacin, 83.33% to Amoxiclave, 66.67% to Erythromycin, 58.33% to Amikacin, 50% to Ampicillin, 33.33% to Cefotaxime and Ceftriaxone and 25% toward Vancomycin.

Table 3. Rate of antibiotics sensitivity toward isolated *E. Faecalis*

Antibiotics	Rate of antibiotic sensitivity (*E. faecalis*, n:12)	
	No.	%
Ampicillin	6	50
Levofloxacin	11	91.67
Erythromycin	8	66.67
Vancomycin	3	25
Ceftriaxone	4	33.33
Amikacin	7	58.33
Amoxiclav	10	83.33
Cefotaxime	4	

From 9 isolates which were resistant to Vancomycin, the study presented that 88.89% of them were observed with *Van A* gene production as detected by real-time PCR (*P*<0.001) (Table 4).

Table 4. Rate of *Van A* gene production (by RT-PCR)

Van A gene	No.	%
Present	8	88.89
Absent	1	11.11
Total	9	100

P<0.001

The study also showed that 77.78% were observed with *Van B* gene production as detected by real-time PCR (*P*<0.001), as in table 5.

The study revealed that all *E. faecalis* isolated who were resistant to cefotaxime and Ceftriaxone were characterized by *CTX* gene production as detected by real-time PCR (*P*<0.001) (Table 6).

Table 5. Rate of *Van B* gene production (by RT-PCR)

Van B gene	No.	%
Present	7	77.78
Absent	2	11.11
Total	9	100

P<0.001

Table 6. Rate of *CTX-M* gene production (by RT-PCR)

CTX-M gene	No.	%
Present	8	100
Absent	0	0
Total	8	100

P<0.001

4. Discussion

The study presented that 40% of children with fever have positive blood cultures compared with 5% in the control group, with a significant difference between the two groups (*P*<0.001). The results that arrived in our study were similar to the results of studies that were previously conducted in different countries of the world, as these studies confirmed that the most common causes of fever in children are bacteremia and sepsis, as the rates of bacteria isolated from blood samples with persons similar to the offending persons in our study reached a rate of up to to 45% in those studies (6, 7). The high temperature in people with bacteremia is due to the bacterial secretion of many toxins that lead to a high level of Interleukin 1 and gamma interferon, which leads to a rise in the temperature in the body (8). The study found that 32.5% of bacteremic children were due to *S. aureus*, 30% to *E. faecalis*, 5% of *E. coli*, 4% of *P. aeruginosa*

and *Klebsiella spp*. (P<0.01), (Table 2). Table 3 shows that 91.67% of *E. faecalis* isolates are sensitive to Levofloxacin, 83.33% to Amoxiclave, 66.67% to Erythromycin, 58.33% to Amikacin, 50% to Ampicillin, 33.33% to Cefotaxime and Ceftriaxone and 25% toward Vancomycin. Several studies also found similar findings, as *S. aureus* and *E. faecalis* were the predominant bacterial isolates of bacteremic children (9, 10). Other studies indicated that most *E. faecalis* were resistant to Vancomycin (6, 8). Another study found that most *E. faecalis* isolates were resistant to Vancomycin and Ceftriaxone, with a rate reaching 80% (11). The reasons for the high resistance of these bacteria to many antibiotics are the fact that they are present and in abundance in the medical body as well as the community hospital environment (12) and that the excessive and wrong use of antibiotics in the community has a negative impact on these bacteria and made them resistant to antibiotics, which are used frequently and in excess in all pathological conditions such as urinary tract infection, diarrhea and coughing. On the other, by any means, it is more harmful than other types resistant to antibiotics (13, 14). It is worth noting that most of the types of antibiotic resistance genes in *Enterococcus faecalis* are the *Van A* and *Van B* gene and *CTX B* genes (15). As very recent studies reported that the highest percentage of antibiotic-resistance genes genetically isolated from bacteria that are resistant to antibiotics and that the most significant cause of antibiotic resistance in most types of bacteria, in addition to the bacteria mentioned above, is genetics, meaning that the reason is the transfer of genes from one bacterium to another through means like transformation and transduction (16-18).

Bacteria children were with Vancomycin and cephalosporin-resistant *E. faecalis* bacteria due to CTX-M, *Van A* and *Van B* production.

Authors' Contribution

Study concept and design: A. M. S.
Acquisition of data: A. M. S.
Analysis and interpretation of data: V. I. H.
Drafting of the manuscript: S. A. H.
Critical revision of the manuscript for important intellectual content: S. A. H.
Statistical analysis: V. I. H.
Administrative, technical, and material support: A. M. S.

Ethics

The study protocol was approved by the Northern Technical University, Mosul, Iraq ethics committee.

Conflict of Interest

The authors declare that they have no conflict of interest.

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