RESEARCH ARTICLE

Investigation of some changes and clonal relationship in enterococci isolates due to relocation of a hospital

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Abstract

Objective: To investigate the isolation rates, antimicrobial resistance rates, minimum inhibitory concentration values of antimicrobial agents, and clonal relationships of *Enterococcus faecalis* and *Enterococcus faecium* due to the relocation of a hospital to a newly constructed building.

Method: The comparative, prospective study was conducted at adult general intensive care units of the Mus State Hospital, Mus, Turkey, in two phases; before the relocation from January 25 to December 1, 2014, and after the relocation from February 10 to May 24, 2015. Rectal swab samples were collected 72 hours post-hospitalisation. Identification of *Enterococcus faecalis* and *Enterococcus faecium* isolates was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and antimicrobial resistance with minimum inhibitory concentration values was detected with Vitek 2 system. The clonal relatedness among the strains was investigated by pulsed-field gel electrophoresis. Data was analysed using SPSS 23.

Results: Of the 69 patients, 37(53.62%) were related to pre-relocation phase; 20(54.1%) females and 17(45.9%) males with mean age 62.81±21.71 years. There were 32(46.37%) patients in the post-relocation phase; 13(40.6%) females and 19(59.4%) males with mean age 62.69±21.35 years ($p>0.05$). Of the 84 enterococci strains isolated, 51(60.7%) were *Enterococcus faecium*; 28(55%) before relocation and 23(45%) after relocation ($p=0.77$). The remaining 33(39.3%) isolates were *Enterococcus faecalis*; 16(48.5%) before relocation and 17(51.5%) after relocation ($p=0.73$). Multiple strains were located in 7(18.9%) patients before relocation and in 7(21.9%) after relocation. In 1(3.1%) patient after relocation, 2(8.7%) *Enterococcus faecium* isolates with different resistance and pulsed-field gel electrophoresis patterns were detected. There were no significant differences between the isolation and antibiotic resistance rates before and after relocation ($p>0.05$), and a clonal relation between the isolates was not detected ($p>0.05$). Decreased minimum inhibitory concentration values were noted for some antibiotics.

Conclusion: Clonal relationship between the isolates and change in the rates of isolation and antimicrobial resistance of *Enterococcus faecalis* and *Enterococcus faecium* was not detected due to relocation. Minimum inhibitory concentration values could be used to reveal relocation-related changes in isolates obtained from patients hospitalised in intensive care units.

Keywords: *Enterococcus*, Antimicrobial drug resistance, Transmission, Hospital moving. (JPMA 74: 469; 2024)

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Introduction

Bacteria belonging to genus Enterococcus are found in the normal flora of the human digestive tract and are considered one of the most important pathogens in hospitals.[1] *Enterococcus (E.) faecium* and *E. faecalis* are major species of the genus Enterococcus responsible for approximately 5-10% and 85-90% of enterococcal infections, respectively.[2] These bacteria play a role in nosocomial infections, including bacteraemia, urinary tract infections (UTIs) and endocarditis. Treatment of infections caused by these bacteria is complex because of antimicrobial resistance.[3] Increasing antimicrobial resistance and development of very limited number of new antimicrobial agents is a serious problem.[4]

Transfer of hospitals is a rare event, and changes in infection rates, antimicrobial resistance rates, and minimum inhibitory concentrations (MIC) of antimicrobials may occur depending on the relocation.[5-10] It has been reported that a decrease in healthcare-associated infections (HAIs) has been detected due to the relocation of the hospital or intensive care units (ICUs).[5,6] Besides, the number of resistant microorganisms may decrease as a result of relocation. As a matter of fact, some studies have shown that the number of resistant microorganisms decreases significantly due to relocation.[7,8] Furthermore, the rates of antimicrobial resistance for isolates of the same species may also decrease.[9] In addition, although there is no

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significant change in the rates of resistance for isolates of the same species, a decrease in the MIC values of antimicrobial agents may be detected due to relocation. A study reported that the MIC values of some antibiotics decreased due to hospital relocation.[10]

In addition to these changes in antimicrobial resistance due to hospital relocation, it has been reported that resistant isolates detected before were also detected in the new hospital after the relocation. In some studies, resistant isolates belonging to the same species were detected before and after relocation, and were considered to be the same clone.[11,12]

Environmental contamination is an important source of HAIs. Therefore, environmental improvements provided by relocation or renovation will reduce HAIs.[8] The current study was planned to investigate the isolation rates, antimicrobial resistance rates, MIC values of antimicrobial agents, and clonal relationships of *E. faecalis* and *E. faecium* due to the relocation of a hospital to a newly constructed building.

Materials and Methods

The comparative, prospective study was conducted at adult general ICUs of the Mus State Hospital (MSH), Mus, Turkey, in two phases; before the relocation from January 25 to December 1, 2014, and after the relocation from February 10 to May 24, 2015.

MSH is a second-level hospital located in the centre of Mus. The hospital was first built in 1954. The old hospital had a capacity of 300 beds before it was moved. The hospital, which was moved to a new building on December 4, 2014, subsequently had a bed capacity of 485. Before the hospital was moved, adult general ICUs had 15 beds and 16 nurses. After the move, there were 13 beds and 18 nurses.

Permission was obtained from the MSH chief physician for the use of enterococcal isolates that were isolated for routine Vancomycin-resistant enterococcus (VRE) screening before and after the relocation, and they were stored at -800C for the current study. After approval from the institutional ethics review committee, data of the patients from whom the isolates had been taken were obtained retrospectively from the hospital records. Experimental analyses, including identification of the isolates, determination of antimicrobial resistance rates and MIC values of antimicrobial agents, and clonal relationship between the isolates were performed prospectively.

The sample size was calculated with 95% confidence level, 5% margin of error and effect size of 0,85 using GPower 3.1.9.7 software.[13] Enterococci isolated from rectal swabs of male and female patients older than 18 years of age admitted to adult general ICUs due to various reasons for at least 72 hours were included. Enterococcal isolates from patients who developed enterococcal infection within 72 hours and patients transferred from other hospitals were excluded. All patients who met the inclusion criteria were included.

Rectal swab samples were collected 72 hours post-hospitalisation. The strains were previously identified by classical methods and their antibiograms were applied. The isolates had been kept in the modified broth medium containing 30% glycerol (1% peptone, 0.5% sodium chloride [NaCl] [pH 7]) at -80°C till the analyses.[14] Pure cultures of isolates had been obtained by making passages from storage media to sheep blood agar. For the current study, re-identification of isolates was performed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and antimicrobial resistance with MIC values was detected with Vitek 2 system. The clonal relatedness among the strains was investigated by pulsed-field gel electrophoresis (PFGE).

MALDI-TOF MS (Bruker Daltonik, Germany) was used for the identification of isolates. For this purpose, ethanol-formic acid extraction procedure was performed. For each isolate, a loopful of bacterial material was diluted in 300µL of distilled water, and 900µL of ethanol was added. The bacterial suspension was centrifuged at 17,000xg for 2 minutes, and the supernatant was removed. Centrifugation was performed for the second time and the ethanol residue was removed. The resulting pellet was air-dried and reconstituted with up to 50µL of formic acid-water (70:30, vol/vol) depending on the amount, and then added to an equal volume of acetonitrile. The resulting suspension was centrifuged at 17,000xg for 2 minutes, and 1µL of the formed supernatant was transferred to MALDI target plates and allowed to dry at room temperature before coating with 1µL matrix solution. Acquisition and analysis of mass spectra was performed on a Microflex LT mass spectrometer (Bruker Daltonik, Germany) using the reference database version 3.1.2.0 and the MALDI Biotyper software package version 3.0. Values ≥2.0 were used for species-level identification, while values in the range of ≥1.7 and <2.0 were used for genus-level identification. Values <1.7 were considered unreliable.

Antimicrobial susceptibility of all strains was determined with Vitek 2 System (bioMerieux, France) using AST-GP67 cards.[15] An isolate resistant to at least one antimicrobial agent in three or more antimicrobial categories was defined as multidrug-resistant (MDR).[16]

For PFGE, isolation and deproteinisation of the genomic deoxyribonucleic acid (DNA) were obtained, according to

the protocol of Asgin and Otlu.[17] Electrophoresis was applied for 20 hours with initial pulse time 3.5 seconds, end pulse time 23.5 seconds, pulse angle 120°, current 6 V/cm², and temperature 14°C in the CHEF-DR II PFGE system (Bio-Rad Laboratories, Nazareth, Belgium) using the Sma I enzyme (New England Biolabs). Gels were stained with ethidium bromide (2mg/mL, Sigma) for 25 minutes and washed 3 times with distilled water for 15 minutes, and visualised using an ultraviolet (UV) trans-illuminator. PFGE patterns were examined using the GelCompar II software system version 6.5 (Applied Maths, Sint-Martens-Latem, Belgium). Dice correlation coefficient was used to determine similarity for band analysis, and Unweighted Pairwise Grouping Mathematical Avenging (UPGMA) method was utilised for cluster analysis. Strains similar for ≥95% according to the dice correlation coefficient were considered to be in the same clone, and strains with <95% similarity were considered different from each other.

Data was analysed using SPSS 23. Chi-square test was utilised for categorical variables. For continuous variables, Mann-Whitney U test was utilized. P<0.05 was taken as statistically significant.

Results

Of the 69 patients, 37(53.62%) related to pre-relocation phase; 20(54.1%) females and 17(45.9%) males with mean age 62.81±21.71 years. There were 32(46.37%) patients in the post-relocation phase; 13(40.6%) females and 19(59.4%) males with mean age 62.69±21.35 years (p>0.05). Of the 84 enterococci strains isolated, there were 51(60.7%) *E. faecium* and 33(39.3%) *E. faecalis* isolates (p=0.06). Among *E. faecium* isolates, 28(55%) related to pre-relocation phase and 23(45%) to post-relocation phase(p=0.77). Among *E. faecalis* isolates, 16(48.5%) related to pre-relocation phase and 17(51.5%) to post-relocation phase (p=0.73). Multiple strains were located in 7(18.9%) patients before relocation and in 7(21.9%) after relocation. In 1(3.1%) patient after relocation, 2(8.7%) *E. faecium* isolates with different resistance and PFGE patterns were detected (Figure [Nos 46 and 78]).

Table-1: MIC values of *Enterococcus (E.) faecium* isolates against various antibiotics.

Antimicrobials/MDR	Before/after relocating	MIC values			Resistance (%)	*p*-value*
		MIC Range	MIC50	MIC90		
Vancomycin	Before relocating	≤0.5->4	1	**2**	10.7	**
	After relocating	≤0.5-1	1	**1**	Nil	
Teicoplanin	Before relocating	≤0.5->4	≤0.5	**>4**	21.4	0.14
	After relocating	≤0.5->4	≤0.5	**≤0.5**	4.4	
Ampicillin	Before relocating	≤2->8	>8	>8	57.1	1
	After relocating	≤2->8	>8	>8	73.9	
Linezolid	Before relocating	1->4	2	4	3.6	1
	After relocating	1->4	2	4	4.4	
Levofloxacin	Before relocating	≤1-4	>4	>4	67.9	0.72
	After relocating	≤1-4	>4	>4	56.5	
Streptomycin-Syn	Before relocating	≤1000->1000	≤1000	>1000	46.4	0.50
	After relocating	≤1000->1000	≤1000	>1000	30.4	
Gentamicin-Syn	Before relocating	≤500->500	≤500	>500	28.6	0.57
	After relocating	≤500->500	≤500	>500	17.4	
Multidrug Resistance (MDR) (%)	Before relocating	42.86				0.81
	After relocating	34.78				

MIC: Minimum inhibitory concentration. *Statistical analysis was performed to compare resistance rates.
**No antimicrobial resistance was detected in one group.

Table-2: MIC values of *Enterococcus (E.) faecalis* isolates against various antibiotics.

Antimicrobials/MDR	Before/after relocating	MIC values			Resistance (%)	*p*-value*
		MIC Range	MIC50	MIC90		
Vancomycin	Before relocating	1-2	1	2	Nil	**
	After relocating	1-2	1	2	Nil	
Teicoplanin	Before relocating	0.5->4	≤0.5	≤0.5	6.3	1
	After relocating	0.5->4	≤0.5	≤0.5	5.9	
Ampicillin	Before relocating	≤2->8	≤2	≤2	6.3	**
	After relocating	≤2-≤2	≤2	≤2	Nil	
Linezolid	Before relocating	≤0.5-2	1	2	Nil	**
	After relocating	≤0.5->4	1	2	5.9	
Levofloxacin	Before relocating	≤1-4	**>4**	>4	56.3	0.08
	After relocating	≤1-4	**2**	>4	17.7	
Streptomycin-Syn	Before relocating	≤1000->1000	≤1000	>1000	43.8	0.57
	After relocating	≤1000->1000	≤1000	>1000	29.4	
Gentamicin-Syn	Before relocating	≤500->500	≤500	>500	37.5	0.53
	After relocating	≤500->500	≤500	>500	23.5	
Multidrug Resistance (MDR) (%)	Before relocating	25				1
	After relocating	29.41				

MIC: Minimum inhibitory concentration. *Statistical analysis was performed to compare resistance rates. **No antimicrobial resistance was detected in both groups or in one group.

Vancomycin resistance was detected in 3(10.7%) pre-relocation *E. faecium* isolates. The resistance for ampicillin was detected 1(6.25%) pre-relocation *E. faecalis* isolate, while linezolid resistance was detected only 1(5.88%) post-relocation *E. faecalis* isolate.

No significant difference was detected for teicoplanin (p=0.14), linezolid (p=1), levofloxacin (p=0.72), streptomycin-syn (p=0.50), gentamycin-syn (p=0.57)) and MDR (p=0.81) before and after relocation in *E. faecium* isolates (Table 1).

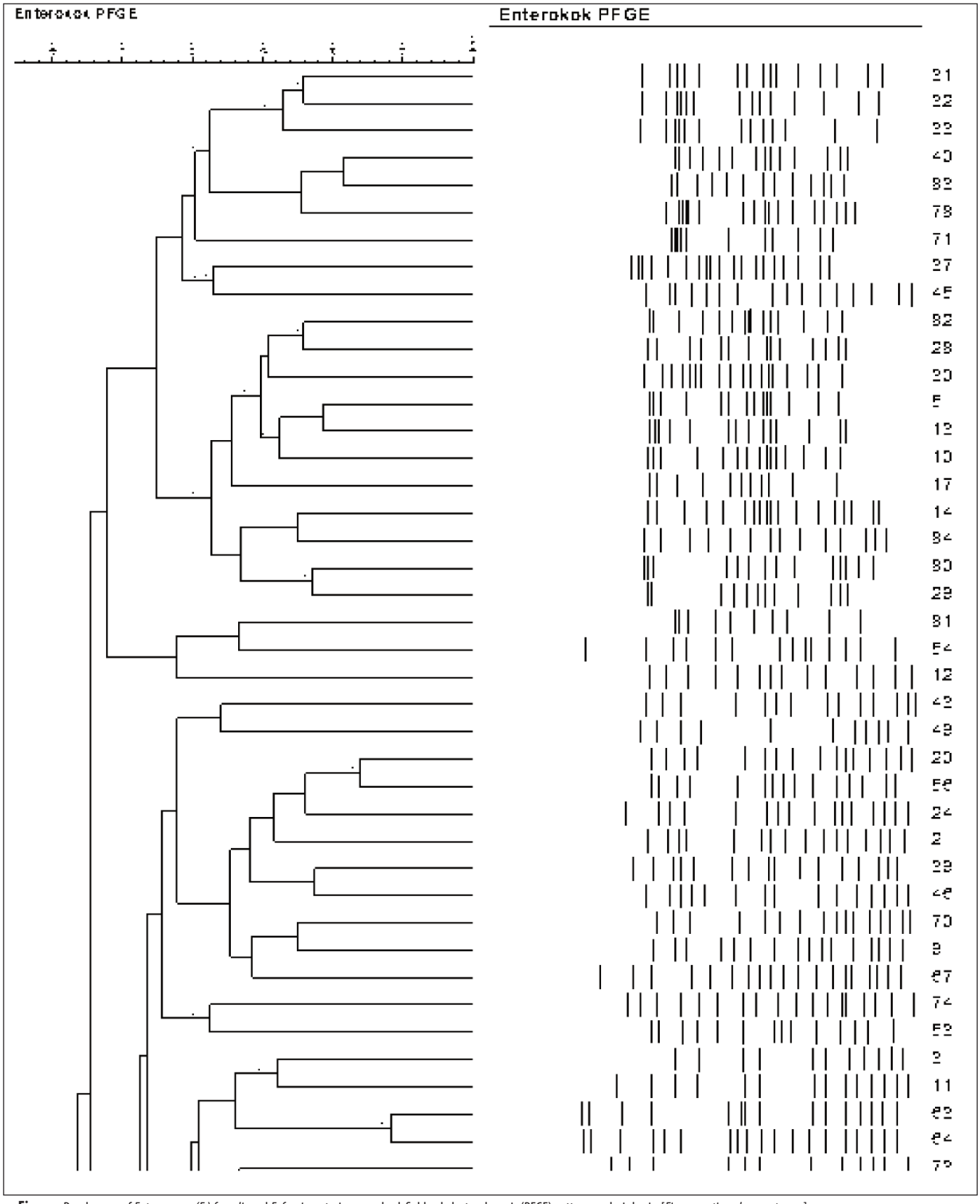

Figure: Dendogram of *Enterococcus (E.) faecalis* and *E. faecium* strains on pulsed-field gel electrophoresis (PFGE) pattern analysis basis. [*Figure continued on next page*]

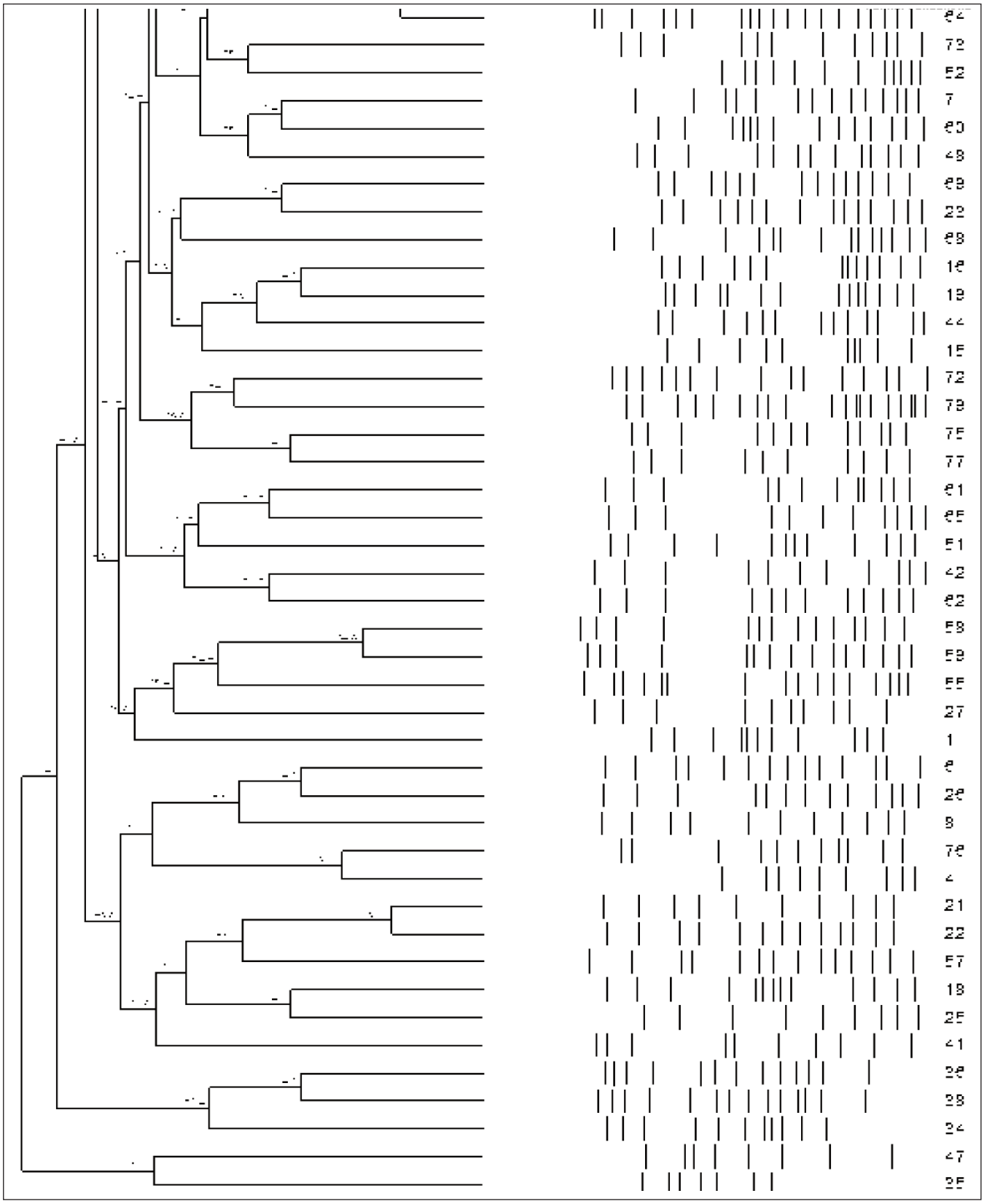

Figure: Dendogram of *Enterococcus (E.) faecalis* and *E. faecium* strains on pulsed-field gel electrophoresis (PFGE) pattern analysis basis. [*Figure continued from previous page*]

Similarly, no significant difference was detected for teicoplanin (p=1), levofloxacin (p=0.08), streptomycin-syn (p=0.57), gentamycin-syn (p=0.53) and MDR (p=1) before and after relocation in *E. faecalis* isolates (Table 2).

MIC required to inhibit the growth of 90% of organisms (MIC90) values of vancomycin and teicoplanin decreased in *E. faecium* isolates before and after relocation, while no significant change was detected in MIC required to inhibit the growth of 50% of organisms (MIC50) and MIC90 values of other antibiotics (Table 1). For *E. faecalis* isolates, MIC50 value of levofloxacin decreased, while there was no significant change in MIC50 and MIC90 values of other antibiotics (Table 2).

No strain had >95% similarity when PFGE profiles were analysed, indicating that there was no clonal transmission between the pre-relocation and post-relocation phases (Figure).

Discussion

Enterococci species can be detected at different rates from rectal swab or stool samples.[17,18] In the current study, significant difference was not found for the isolation ratio of enterococci isolates before and after the relocation of the hospital.

In the study, antimicrobial resistance rates of enterococcal isolates were compared. Glycopeptide antibiotics are effective for the treatment of healthcare-associated β-lactam-resistant enterococcal infections.[19] However, enterococci that are normally considered weak pathogens can cause fatal infections if they have vancomycin resistance.[20] In the current study, no significant difference was found for vancomycin and teicoplanin resistance rates of the isolates of the two species related to relocation.

MDR bacterial infections can lead to inadequate or delayed antimicrobial therapy.[4] As a result, antimicrobial treatment options are decreasing.[1] In the current study, no significant difference was found in terms of MDR rates of the isolates before and after relocation. Linezolid is a valuable antimicrobial option for VRE infections.[21] In the current study, significant difference was not found in resistance ratios of linezolid of *E. faecium* strains due to relocation. Since monotherapy, including bactericidal β-lactams, often exerts only bacteriostatic action, infections caused by enterococci are difficult to treat. Ampicillin-aminoglycoside combination is generally used against susceptible agents to obtain a bactericidal effect. However, ampicillin and vancomycin resistance necessitates the use of other antibiotics.[22] In the current study, significant ampicillin resistance rate was found for *E. faecium* strains. However, no significant difference was detected in terms of the resistance against ampicillin.

The high level of resistance against gentamicin and streptomycin complicates the treatment for severe diseases caused by enterococci. This situation eliminates the synergy in the combined application of aminoglycosides and cell-wall-active antibiotics.[1] In the current study, no significant difference was found in terms of high-level gentamicin and high-level streptomycin resistance ratios before and after relocation. Another antibiotic evaluated in the study was levofloxacin, which is in the fluoroquinolone group. This antibiotic has broad-spectrum activity.[23] It is widely used in urology due to its efficacy and low price.[24] In the current study, significant difference was not found in resistance ratios of levofloxacin due to relocation. Hatsuda et al. detected a decrease in the rates of resistance to some antibiotics due to the relocation of a hospital to a new building.[9] However, in the current study, significant difference was not found for antimicrobial resistance of strains of both species before and after relocation.

In the current study, a decrease was detected in vancomycin and teicoplanin MIC90 values of *E. faecium* isolates after relocation. A decrease in the levofloxacin MIC50 value of *E. faecalis* isolates was also observed after relocation.

Schonfeld et al. reported that changes in MIC values, even if small, reveal changes due to relocation.[10] The reason for this change can be relocation to a new building in addition to the renovation of beds and other equipment in ICUs.

Nakamura et al., reported that the identical extended spectrum beta-lactamase (ESBL)-positive Klebsiella (K.) pneumoniae strain (TUM19831) isolated from the old hospital was also isolated post-relocation.[12] Regarding enterococci, it has been reported that VRE faecium (VREf) sequence type 142 can spread within and between hospitals.[11] However, the same clone which shows transmission with high clonal diversity among isolates was not detected in the current study. Consistent with the current finding, Asgin and Otlu also reported the absence of a predominant clone among enterococci strains.[17]

The limitation of the current study is its inability to evaluate enterococcal strains isolated at the time of hospitalisation since rectal swab samples were collected 72 hours after hospitalisation.

Conclusion

No clonal relationship between the isolates was detected. No change was detected in the rates of isolation and antimicrobial resistance of *E. faecalis* and *E. faecium* due to relocation. In contrast, there was a decrease in MIC90 values of vancomycin and teicoplanin in *E. faecium* isolates and in the MIC50 value of levofloxacin in *E. faecalis* isolates after relocation.

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Author Contribution:

HK: Substantial contributions to the conception and design of the work, acquisition, analysis, interpretation of data, drafting, revising it critically, final approval.

GH: Substantial contributions to the conception and design of the work, acquisition, analysis, interpretation of data, identification and antimicrobial susceptibility test, drafting, revising it critically, final approval.

CC: Substantial contributions to the conception and design of the work, acquisition, analysis, interpretation of data, drafting, revising it critically, final approval.

SS: Drafting, revising it critically, final approval.

OA: Final approval.

EST: Pulsed-field gel electrophoresis, final approval.