Sociodemographic distributions and molecular characterization of colonized *Enterococcus faecium* isolates from locality hospitals in Khartoum, Sudan (#82432)

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Sociodemographic distributions and molecular characterization of colonized *Enterococcus faecium* isolates from locality hospitals in Khartoum, Sudan

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Background: In the last two decades, there has been a remarkable rise in the instances of nosocomial infections associated with antibiotic-resistant *Enterococcus faecium*. *E. faecium* is a significant opportunistic human pathogen with a broad host range, including humans and animals, have globally evolved towards a powerful and convergent adaption to the healthcare environment by acquiring a cocktail of key antimicrobial resistance enabling them to boom in the disturbed microbiota of hospitalized and non-hospitalized patients.**Objective:** This study aimed to determine the antimicrobial profile, demographic and clinical characteristics, along with the detection of virulence encoding genes, and to find out the clonal genetic relationship among colonized *E. faecium* strains. **Methodology:** A hospital-based cross-sectional study was carried out between October 2018 and March 2020 at four Khartoum locality hospitals in Sudan. The study comprised a total of 108 strains of *E. faecium* isolated from patients admitted to four locality hospitals in Khartoum. A self-structured questionnaire was used to gather information on sociodemographic traits. Data were analyzed using chi-square test. In all cases, P value ≤ 0.05 with a corresponding 95% confidence interval was considered statistically significant. Moreover, enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCR) was utilized to assess the prevalence of clonal relationships in the community and hospitals, and gel was analyzed using CLIQS software. **Results:** In this study, the isolation rate of colonized *E. faecium* strains was 108/170 (63.5%). 73(67.6%) were found multidrug-resistant (MDR) and 22 (20.4%) were extensively multidrug-resistant (XDR). 73 (67.6%) of patients were self-medication, 80 (74.1%) were low adherence to antibiotics, and 70 (64.8%) had previously taken antibiotics in the last 3 months. There are no significant associations between *E. faecium* colonization and sociodemographic and clinical characteristics except

with patients who had a previous history of antibiotics used ($P \leq 0.005$). Genotyping of virulence genes revealed that *asa*1 gene was predominant and yielded 22.2% among *E. faecium*. ERIC-PCR fingerprinting was used to genotype *E. faecium* isolates, resulting in DNA polymorphism bands ranging in size from 100 to 5000 base pairs. The genetic relatedness of *E. faecium* isolated revealed 7 identical clusters (A-G) with 100% genetic similarity indicating the possibility of clonal circulation in hospital environments and communities. **Conclusion:** This study found that the incidence of *E. faecium* isolated from locality hospitals in Khartoum was likely due to the spread of *E. faecium* clones, thereby highlighting the need for intensifying infection control measures to prevent spreading of nosocomial infection. These results also demonstrated that the use of ERIC-PCR is a reliable and rapid method for *E.faecium* genetic study.

Sociodemographic Distributions and Molecular characterization of Colonized Enterococcus faecium Isolates from Locality Hospitals in Khartoum, Sudan

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Abstract

Background: In the last two decades, there has been a remarkable rise in the instances of nosocomial infections associated with antibiotic-resistant *Enterococcus faecium. E. faecium* is a significant opportunistic human pathogen with a broad host range, including humans and animals, have globally evolved towards a powerful and convergent adaption to the healthcare environment by acquiring a cocktail of key antimicrobial resistance enabling them to boom in the disturbed microbiota of hospitalized and non-hospitalized patients. **Objective:** This study aimed to determine the antimicrobial profile, demographic and clinical characteristics, along with the detection of virulence encoding genes, and to find out the clonal genetic relationship among colonized *E. faecium* strains. **Methodology:** A hospital-based cross-sectional study was carried out between October 2018 and March 2020 at four Khartoum locality hospitals in Sudan. The study comprised a total of 108 strains of *E. faecium* isolated from patients admitted to four locality hospitals in Khartoum. A self-structured questionnaire was used to gather information on sociodemographic traits. Data were analyzed using chi-square test. In all cases, P value ≤ 0.05 with a corresponding 95% confidence interval was considered statistically significant. Moreover, enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCR) was utilized to assess the prevalence of clonal relationships in the community and hospitals, and gel was analyzed using CLIQS software. Results: In this study, the isolation rate of colonized *E. faecium* strains was 108/170 (63.5%). 73(67.6%) were found multidrug-resistant (MDR) and 22 (20.4%) were extensively multidrug-resistant (XDR). 73 (67.6%) of patients were self-

medication, 80 (74.1%) were low adherence to antibiotics, and 70 (64.8%) had previously taken antibiotics in the last 3 months. There are no significant associations between *E. faecium* colonization and sociodemographic and clinical characteristics except with patients who had a previous history of antibiotics used ($P \leq 0.005$). Genotyping of virulence genes revealed that asa1 gene was predominant and yielded 22.2% among *E. faecium.* ERIC-PCR fingerprinting was used to genotype *E. faecium* isolates, resulting in DNA polymorphism bands ranging in size from 100 to 5000 base pairs. The genetic relatedness of *E. faecium* isolated revealed 7 identical clusters (A-G) with 100% genetic similarity indicating the possibility of clonal circulation in hospital environments and communities. **Conclusion:** This study found that the incidence of *E. faecium* isolated from locality hospitals in Khartoum was likely due to the spread of *E. faecium* clones, thereby highlighting the need for intensifying infection control measures to prevent spreading of nosocomial infection. These results also demonstrated that the use of ERIC-PCR is a reliable and rapid method for E.faecium genetic study.

Keywords: Enterococcus faecium, Sociodemographic distribution, vancomycin resistant *enterococcus faecium*, antibiotic resistance pattern; virulence encoding gene, ERIC-PCR, clonal relationship, Khartoum locality hospital

Introduction

In the past two decades, *Enterococci faecium* has rapidly evolved as a worldwide nosocomial pathogen by successfully adapting to conditions in a nosocomial setting and acquiring resistance against glycopeptides (1),(2). The nosocomial pathogen *E. faecium* can survive for prolonged periods on surfaces in the absence of nutrients, and also in hospital environments, these traits are thought to contribute to the ability of *E. faecium* to transmit between patients in hospitals (3),(4). The relative importance of *E. faecium* as a pathogen has increased with the occurrence of high-level resistance to multiple antimicrobial drugs, such as amoxicillin clavulanic acid, aminoglycosides, cephalosporin, and vancomycin (5). The prevalence of multidrug-resistant (MDR) *Enterococcus faecium* infections is rising globally, although epidemiological research remains generally scarce in specific regions such as African countries including Sudan (3). Vancomycin-resistant *E. faecium* (VRE*fm*) is the most common multidrug-resistant Enterococcus species and is one of the most serious concerns in healthcare settings (6),(7) designated as a high-priority pathogen in need of therapeutic research and development according to the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) (8),(9).

In the invasion process, enterococci use a variety of virulence factors including (*asa1, cylA, esp, gelE*, and *hyl* gene) for adhering to the infection site and colonizing (10),(11), along with the presence of damage to the host tissue and antibiotic resistance, all help with the invasion process. In the end, the clinical manifestation of infection in the targeted vital tissues is the result of interactions between the host and enterococci.

Molecular typing is becoming a paradigm for understanding the fundamental mechanisms of enterococcus infections in hospital settings to investigate the clonal relationship among bacterial

strains, and to track the source of infections. PCR-based genotyping tools are used for determining different DNA fingerprints, among several PCR-based tools, the ERIC-PCR discriminating is a rapid, and cost-effective genotyping method for different types of strains. In hospital settings infection control, molecular typing methods are becoming an important tool to measure and trace the source and origin of infection during hospital outbreaks (12). In Sudan, in particular, no reports are available in epidemiological studies, assessing antibiotic resistance, molecular analysis, or other features of clinical *E. faecium* isolates.

Materials & Methods

Study Design, Setting, and Period

A hospital-based cross-sectional study was conducted in four tertiary hospitals in the Khartoum locality (The Academy Charity Hospital (ACH), Dar- Alelaj Specialized Hospital (DASH), Ibrahim Malik Teaching Hospital (IMTH)), and Yastabshiroon Hospital Riyadh (YASH)), from October 2018 to March 2020. Those hospitals provide different levels of care services in all disciplines, four wards were included: Medicine, ICU, Surgical, and Pediatric wards.

Patient and Statistical Analysis

Information on patient sociodemographic characteristics, risk factor data, and other independent variables were collected from each participant using a self-structured questionnaire. Data collection was done under the supervision of the project advisors. The information was gathered by conducting in-person interviews with patients in outpatient clinics or hospital wards by attending a nurse from the hospital. Qualitative data were described using numbers and percentages. Comparison between different groups regarding categorical variables was tested using Pearson's chi-square test ($P \leq 0.05$) to investigate the significance of *E. faecium* colonization association with sociodemographic distributions.

Isolation and Identification of *Enterococcus faecium*

A total of 108 *E. faecium* were isolated from fecal cultures, from patients in four Khartoum locality hospitals. All specimens were cultivated on the surface of the bile-esculin medium (HiMedia, India), and incubated at 37˚C for 24 hours. Colonies growing on bile-esculin medium with a dark brown halo and colonial morphology resembling enterococci were collected as presumptive isolates. All presumptive isolates of enterococci were further confirmed using phenotypes tests such as Gram staining, catalase, oxidase, and growth in 6.5% NaCl broth, as described elsewhere (13).

Antimicrobial Susceptibility Testing

All 108 strains of *E. faecium* were examined by using the disk diffusion technique according to Kirby–Bauer method (14) on Muller-Hinton agar (Hi-Media, India). Fifteen antimicrobial agents, include, Amoxicillin-clavulanic acid (30 µg), Ampicillin (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Ciprofloxacin (5 µg), Daptomycin (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Levofloxacin (15 µg), Linezolid (30 µg), Penicillin-G (10 IU), Rifampin (15 µg), Teicoplanin (30 µg), Tetracycline (10 µg), and Vancomycin (30 µg). The diameter of zone inhibition was measured and reported based on Hi-Media Antimicrobial Susceptibility

Systems guidelines, as susceptible (S), intermediate (I), or resistant (R). The reference control strain *E. faecalis* ATCC29212 was used while performing antibiotic susceptibility testing.

DNA Extraction and Detection of VRE*fm* Virulence genes by PCR

Genomic DNA was extracted using the G-spin Genomic DNA Extraction Kit (iNtRON, South Korea) following the manufacturer's instructions. Amplification was performed according to a published protocol (15), using a multiplex PCR to investigate the presence of five virulence genes in VRE*fm* isolates. All primer sequences are shown in (Table 1).

ERIC-PCR Typing

All isolates were genotyped using ERIC1A and ERIC2 primers using according to published protocol (16). ERIC-PCR products were resolved by gel electrophoresis (100 V for 90 min) and analyzed on 2% w/v agarose gel with ethidium bromide gel stain in TBE 1× electrophoresis buffer. A GelPilot 1 kb Plus Ladder (100) (Qiagen) was included as a molecular weight marker. PCR products were visualized using a UV-transilluminator. Nuclease-free distilled water was used as a negative control.

ERIC-PCR Data analysis

The clonal relatedness between the strains of *E. faecium* was analyzed using ERIC-PCR fingerprinting with the CLIQS 1D PRO software (TotalLab Ltd, Newcastle, United Kingdom) (17), Similarity distances between ERIC-PCR profiles were calculated using the Dice coefficient and dendrograms were constructed based on the unweighted pair group method with arithmetic mean (UPGMA). Normalization steps were included in the analysis of DNA polymorphism patterns produced by ERIC-PCR fingerprinting to ensure an adequate gel-to-gel banding pattern comparison. Isolates with an 80% level of similarity were grouped in the same cluster and were considered clonally related.

Ethical Considerations

Ethical approval for this study was obtained from the Graduate College-UMST and the Federal Ministry of Health, Sudan-Research Ethics Committee, Besides; the patients were informed about the study and the informed consent form was signed. Confidentiality was assured. No names were in the format used. The data were to be used for research only.

Results

Sociodemographic distributions

Demographic distribution and clinical characteristics among 108 patients were presented in (Table 2). The age of patients ranged from 1 to 82 with a median of 41.5 years. A higher frequency of *E. faecium* colonization (26.9%; 29/108) was observed in the age group of 35-49 years compared to the other age groups, 60 (55.6%) were males and 48 (44.4%) were females, about 61 (56.5%) of patients were University educated. 73 (67.6%) were from Urban residences, 31 (28.7%) were students by occupation. of which high frequency of *E. faecium* was observed in Academy Charity Hospital 33 (30.6%), out of which 66 (66.1%) were non-hospitalized patients. Moreover, in the clinical characteristic, regarding chronic comorbidities, most of the participants had one or more co-morbidities of which gastrointestinal tract infection was the most common 28 (25.9%). As presented in (Table 2) and based on wards distribution, enterococci were

identified most frequently in 17 (15.7%) treated patients in internal medicine wards. Thirty-six (33.3%) of patients stayed less than a week, 73 (67.6%) were antibiotics self-medicated, 80 (74.1%) were low adherence to antibiotics, and 70 (64.8%) had previously taken antibiotics in the last 3 months. Twenty-four (22.2%) of patients had a history of gentamicin use.

Seventy-three (67.6%) of colonized patients were categorized as MDR and 22 (20.4%) were categorized as XDR as shown in (Table 2). Forty (37%) of patients took antibiotics based on a physician's prescription. Sixty-one (56.5%) of patients used antibiotics according to consultation given according to pharmacist prescription. On the other hand, 71 (65.7%) of patients according to the study questionnaire mentioned that they asked relatives or friends for advice about antibiotic use. Only 26 (24.1%) of study participants visited the clinic for follow-up and received antibiotics prescriptions. However, 76 (70.4%) of patients requested antibiotics when they suffered from flu-like symptoms, and more than half of the *E. faecium* colonized patients lacked knowledge about antibiotic resistance as presented in (Table 2). The chi-square test indicates that there is no significant association between the sociodemographic characteristics and *E. faecium* colonization except with patients who had a previous history of antibiotics used ($P \leq 0.005$).

Prevalence of antimicrobial resistance among *E. faecium* strains

A total of 108 *E. faecium* were tested of which 33 (30.6%) were isolated from Academy Charity Hospital (ACH), 29 (26.9%) from Dar- Alelaj Specialized Hospital (DASH), 27 (25%) were from Ibrahim Malik Teaching Hospital (IMTH), and 19 (17.6%) were from Yastabshiroon Hospital Riyadh (YASH). Among them, 42 strains of colonized *E. faecium* were isolated from the hospitalized patient, while 66 strains were isolated from the non-hospitalized patient. Of these 18 (16.7%) were VRE*fm* strains. All of the strains were resistant to at least two antibiotics except one strain was susceptible to all antibiotics. However, a higher resistance was exhibited towards 86 (79.6%) ceftazidime, followed by 81(75%) amoxicillin-clavulanic acid, and 77 (71.3%) gentamicin, and only 6 (5.6%) strains were found resistant to daptomycin as shown in (Fig 1).

All of the analyzed strains of *E.faecium* in this study were classified as MDR and XDR, and no ~~PDR~~ isolates were detected (Table 3). 73 (67.6%) were multidrug-resistance (MDR), while 22 (20.4%) were extensively drug-resistant (XDR). Overall, the highest rate of MDR of *E.faecium* isolates was detected among 48/100 (48%) of non-hospitalized patients compared to 25/70 (35.7%) among hospitalized patients, on the other hand, the highest rate of XDR was detected among 13 (13%) of non-hospitalized patients compared to 9/70 (12.9%) of hospitalized patients. According to Chi-square analysis, there was a significant association between antimicrobial categorization and patients in the community ($P = 0.021$) as shown in (Table 3).

Virulence genes among VRE*fm* strains

Out of 108 *E.faecium* isolates, 18 VRE*fm* strains were screened in this study to determine virulence genes. 11/18 (61%) were found positive for virulence genes as shown in (Table 4). The most common virulence encoding genes among VRE*fm* were *asa*1 4 (22.2%), followed by *esp* 3 (16.7%), *hyl* 1 (5.6%), *gelE* 1 (5.6%), and *gelE-hyl* 2 (11.1%), No *cyl*A gene was detected.

ERIC-PCR analysis of *E. faecium*

ERIC-PCR DNA fingerprinting among 108 *E.faecium* isolates produced ERIC profiles in ranges of 4-13 different banding patterns with molecular weights ranging from 100 to 5000 bp (Fig 2). The analysis of ERIC fingerprinting patterns results using the Dice coefficient and UPGMA revealed that ERIC-PCR profiles show high genetic variability among the isolates. The genetic relationship among *E. faecium* isolates rate was (13%; 14/108) when clustered with 100% similarity coefficient, (52.8%; 57/108) were clustered with 80% similarity. Whereas (24.1; 26/108%) isolates of *E. faecium* had less than 80% similarity, as shown in ERIC dendrogram analysis. In addition, 10 strains showing single lineage below 50% have been excluded, and fourteen patients showing identical fingerprinting were classified into clusters A-G as shown in (Fig 3).

Discussion

Until the 1980s, *Enterococcus* spp. were merely intestinal microbes of little clinical significance. Now, they are among the most common nosocomial pathogens, so physicians are becoming more worried (18). Resistance in enterococci was increased dramatically and the incidence of VRE colonization spread where vancomycin is one of the antibiotics of choice used to treat infections caused by Gram-positive multidrug-resistant organisms, such as Enterococci. In Sudan, no reports are available on the molecular epidemiology analysis of *E. faecium* and there are limited data on the prevalence of antibiotic resistance profiles (5). In this study, antibiotic resistance of *E. faecium* carrier rates, sociodemographic and clinical characteristics associated with *E. faecium* colonization was studied. VRE*fm* virulence genes and the genetic relationship among *E. faecium* strains isolated from Khartoum locality hospitals were investigated.

In the present study, the prevalence of *E. faecium* strains was (63.5%; 108/170). Our results were consistent with other studies from the United States revealing that the prevalence of colonization with *E.faecium* is higher than with other *Enterococcus* species (19). Antimicrobial resistance percentages among bacteria from human illnesses in the European Union and European Economic Area countries (EU/EEA) did not vary significantly between 2014 and 2020, except for *Enterococcus faecium*, where the proportion of vancomycin resistance increased from 9% in 2014 to 17% in 2020 (20), and this consists with our result where the prevalence of VRE*fm* was (16.6%). In our result, ceftazidime showed a high resistant rate against *E.faecium* (79.6%), while the current investigation found that daptomycin had the best residual inhibitory impact on *E. faecium*, with a resistance rate of 5.6%, which is consistent with prior research that found that most enterococcal isolates (>99.8%) are sensitive to daptomycin on a global scale (21).

Broad-spectrum antibiotics have the potential to harm the normal anaerobic flora of the gastrointestinal tract, resulting in infectious diseases due to their bactericidal impact against enterococci. Many studies have reported that previous use of broad-spectrum antibiotics is a risk factor for acquiring multidrug-resistant pathogens (22),(23). However, few investigations have been undertaken to evaluate the link between previous antibiotic exposure and the acquisition of *E. faecium* strains (23). Our findings indicated a significant association between prior antibiotic exposure and the acquisition of *E. faecium* infection ($P \leq 0.005$). Interestingly, our study data

findings supported that overusing antibiotics increased the likelihood of resistance while decreasing their efficacy, this was demonstrated by the high prevalence of antibiotic usage among hospitalized patients and non-hospitalized patients, as well as by their use of self-medication and erratic antibiotic regimen.

In several reported cases, gastrointestinal tract colonization generally precedes infection with antibiotic-resistant *E. faecium,* in particular, intestinal overgrowth by antibiotic-resistant enterococci is a recognized risk factor for disease (24),(25). Our study showed the prevalence of *E. faecium* colonization was higher among patients who had gastrointestinal tract infections (25.9%) compared to other chronic infections. The high prevalence rate of self-medication and antibiotic usage seen in this study could partly be explained by the patient's desire for a fast recovery from the disease. The economic situation is another major cause for self-medicates, and consultations of friends or relatives to avoid paying the physicians' fees.

A recent study conducted in primary healthcare centers in Qatar reported that showed many factors contribute to the increased incidence of bacterial resistance to antibiotics, particularly; the misuse of antibiotics by physicians and the easy acquisition of antibiotics via non-physicians (26), and this study is in line with our results that showed (56.5%) of patients taken antibiotics according to pharmacist consultation, while (56.7%) taken antibiotic according to friends or relatives consultation. Most pharmacies in the developing world dispense antibiotics on patient demand. Research carried out in Addis Ababa's among community pharmacies reported that the tendency of selling antibiotics without a prescription is becoming more common. These results are attributable to commercial interests, consumer pressure, and lax rules (27). In this study, even in cases when they ~~weren't~~ necessary, such as flu-like, 76 (70.4%) of patients demanded antibiotics. This understanding was explained by their knowledge which led them to believe that antibiotics were helpful in such circumstances (28).

A multiplex PCR developed for the simultaneous detection of *E. faecium* virulence genes that encode for aggregation substance (*asa*1), gelatinase (*gel*E), cytolysin (*cyl*A), enterococcal surface protein (*esp*), and hyaluronidase (*hyl*). Our result showed that virulence genes *asa1* (22.2%), followed by *esp* gene (16.7%) are predominant in the virulent patterns of VRE*fm* isolated from hospitals and communities. Findings from our current study are consistent with a recent study from Southern California and Puerto Rico was reported the *asa1* gene is predominant in enterococci isolated from hospitals, the natural environment, animals, and wastewater (29).

According to the dendrogram, strains with 100% similar ERIC profiles were found in clusters, A (31/32), B (34/35), D (41/42), and cluster E (65/66) were isolated from patients among different hospital wards and communities. On the other hand, isolates presented in clusters, C (37 and 38), F (78 and 79), and G (97/98) with similar ERIC profiles isolated from patients within the same hospitals. *E. faecium* strains show high genetic diversity among isolates raising the possibility of circulation of various *E. faecium* strains between the hospitals and the community.

Conclusions

Our current study investigates the prevalence rate of *E. faecium* antibiotic resistance, sociodemographic characteristics, virulence genes, and the genetic relationship of *E. faecium* isolated from hospitalized and non-hospitalized patients from localized hospitals in Khartoum. Acquisition of *E. faecium* infection with the most supporting data showed that the previous history of antibiotic usage played a role as a risk factor. Our data also show that levofloxacin, linezolid, and daptomycin are still active against nosocomial *Enterococcus faecium* isolates. Appropriate antibiotic-resistance testing programs, as well as competent antibiotic stewardship, are critical in successfully lowering resistance to the aforementioned drugs, particularly in VRE isolates. This study also showed that there is an urgent need for education programs targeting all levels of the community and directed toward changing the public attitude and behavior to rationalize antibiotic use and limit self-medication and overuse. Furthermore, strict policies must be enforced to regulate the procurement of antibiotics and prohibit their purchase without a prescription. Our study also concluded that ERIC-PCR is a reliable typing method for discriminating different isolates of *E. faecium* isolated from hospitalized and non-hospitalized patients.

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Table 1(on next page)

Oligonucleotide Primers for targeted amplification of virulence gene and ERIC sequence

Table 1

1 **Table 1: Oligonucleotide Primers for targeted amplification of virulence gene and ERIC sequence**

Primers for Amplification of Virulence Genes of *Enterococcus faecium*			
Primer Name	**Sequence (5' -3')**	**PCR Product Size (bp)**	**Reference**
Aggregation substance (*asa1*)	F: GCACGCTATTACGAACTATGA R: TAAGAAAGAACATCACCACGA	375	(16)
Gelatinase (*gelE*)	F: TATGACAATGCTTTTTGGGAT R: AGATGCACCCGAAATAATATA	213	
Cytolysin *(cylA)*	F: ACTCGGGGATTGATAGGC R: GCTGCTAAAGCTGCGCTT	688	
Enterococcal surface protein (*Esp*)	F: AGATTTCATCTTTGATTCTTGG R: AATTGATTCTTTAGCATCTGG	510	
Hyaluronidase (*Hyl*)	F: ACAGAAGAGCTGCAGGAAATG R: GACTGACGTCCAAGTTTCCAA	278	
Primer for Amplification of ERIC sequences			
ERIC1	F: TGTAAGCTCCTGGGGATTCAC	100-10000	(17)
ERIC2	F: AAGTAAGTGACTGGGGTGAGCG		

2

Table 2(on next page)

Sociodemographic and Clinical Characteristics of Enterococcus faecium isolates among the patients at Khartoum locality hospital, Sudan

Table 2

Table 2: Sociodemographic and Clinical Characteristics of *Enterococcus faecium* isolates among the patients at Khartoum locality hospital, Sudan

Demographic Characteristics	Enterococcus faecium (n=108) No (%)	Other Enterococcus (n=62) No (%)	Frequency (n= 170) No (%)	Chi-square Test
Gender				X2=0.086 ,P= 0.768*
Male	60 (55.6)	33 (53.2)	93 (22.9)	
Female	48 (44.4)	29 (46.8)	77 (45.3)	
Age (Years) Mean±sd				
Age groups				X2=3.930,P= 0.415 *
Less than 20 years	25 (23.1)	7 (11.3)	32 (18.8)	
20-34 years	28 (25.9)	20 (32.3)	48 (28.2)	
35-49 years	29 (26.9)	20 (32.3)	49 (28.8)	
50-64 years	18 (16.7)	10 (16.1)	28 (16.5)	
65 years and above	8 (7.4)	5 (8.1)	13 (7.6)	
Educational status				X2=2.401 ,P= 0.662 *
Illiterate	6 (5.6)	5 (8.1)	11 (6.5)	
Under school age	5 (4.6)	5 (8.1)	10 (5.9)	
Primary	13 (12)	6 (9.7)	19 (11.2)	
Secondary	23 (21.3)	9 (14.5)	32 (18.8)	
University	61 (56.5)	37 (59.7)	98 (57.6)	
Residence				X2=1.080 ,P= 0.298 *
Urban	73 (67.6)	37 (59.7)	110 (64.7)	
Rural	35 (32.4)	25 (40.3)	60 (35.3)	
Occupation				X2= 4.91 ,P= 0.672 *
Employed	29 (26.9)	15 (24.2)	44 (25.9)	
Under age	11 (10.2)	2 (3.2)	13 (7.6)	
Freelancer	11 (10.2)	7 (11.3)	18 (10.6)	
Farmer	6 (5.6)	3 (4.8)	9 (5.3)	
Student	31 (28.7)	18 (29)	49 (28.8)	
Housewife	11 (10.2)	7 (11.3)	18 (10.6)	
Merchant	6 (5.6)	7 (11.3)	13 (7.6)	
Retired	3 (2.8)	3 (4.8)	6 (3.5)	
Hospital Code				X2=0.377 ,P= 0.944 *
ACH	33 (30.6)	20 (32.3)	53 (31.1)	
DASH	29 (26.9)	18 (29)	47 (27.6)	
IMTH	27 (25)	13 (21)	40 (23.5)	
YASH	19 (17.6)	11 (17.7)	30 (17.6)	
Comorbidities				X2=0.923 ,P= 0.336 *
Yes	67 (62)	43 (69.4)	110 (64.7)	
No	41 (38)	19 (30.6)	60 (35.3)	
Comorbidities if Yes				
Gastrointestinal tract infection	28 (25.9)	14 (22.6)	42 (24.7)	

Renal and kidney-associated disease	10 (9.3)	7 (11.3)	17 (10)	
Urinary tract infection	7 (6.5)	7 (11.3)	14 (8.2)	
Cardiovascular Disease	2 (1.9)	7 (11.3)	9 (5.3)	
Respiratory tract infection	8 (7.4)	4 (6.5)	12 (7.1)	
Diabetes	7 (6.5)	2 (3.2)	9 (5.3)	
Prostatitis	4 (3.7)	1 (1.6)	5 (2.9)	
Wound infection	1 (0.9)	1 (1.6)	2 (1.2)	
Wards (n=70)				X2=1.676 ,P= 0.642 *
Surgery	13 (12)	10 (16.1)	23 (13.5)	
Medical	17 (15.7)	13 (21)	30 (17.6)	
Pediatric	5 (4.6)	1 (1.6)	6 (3.5)	
ICU	7 (6.5)	4 (6.5)	11 (6.5)	
Patient setting				X2=0.639 ,P= 0.423 *
Hospitalized patient	42 (38.9)	28 (45.2)	70 (41.2)	
Community patients	66 (61.1)	34 (54.8)	100 (58.8)	
Duration of stay (Days) Mean±sd	(5±2)			
Duration of stay (n=70)				X2=0.603 ,P= 0.437 *
Less than week	36 (33.3)	22 (35.5)	58 (82.9)	
Week and more	6 (5.6)	6 (9.7)	12 (17.1)	
Self-Medication				X2=1.854 ,P= 0.173 *
Yes	73 (67.6)	48 (77.4)	121 (71.2)	
No	35 (32.4)	14 (22.9)	49 (28.8)	
Antibiotic adherence				X2=1.492 ,P= 0.221 *
Yes	28 (25.9)	11 (17.7)	39 (22.9)	
No	80 (74.1)	51 (82.3)	131 (77.1)	
Used Antibiotic in last 3 months				X2=0.212 ,P= 0.645 *
Yes	70 (64.8)	38 (61.3)	108 (63.5)	
No	38 (35.2)	24 (38.7)	62 (36.5)	
Antibiotic exposure				X2=29.55 ,P= 0.005**
Ceftazidime	17 (15.7)	3 (4.8)	20 (11.8)	
Ceftriaxone	13 (12)	4 (6.5)	17 (10)	
Clindamycin	1 (0.9)	4 (6.5)	5 (2.9)	
Amoxicillin	5 (4.6)	8 (12.9)	13 (7.6)	
Gentamicin	24 (22.2)	4 (6.5)	28 (16.5)	
Ciprofloxacin	4 (3.7)	6 (9.7)	10 (5.9)	
Azithromycin	2 (1.9)	3 (4.8)	5 (2.9)	
Chloramphenicol	2 (1.9)	1 (1.6)	3 (1.8)	
Erythromycin	5 (4.6)	2 (3.2)	7 (4.1)	
Metronidazole	3 (2.8)	2 (3.2)	5 (2.9)	
Penicillin	2 (1.9)	4 (6.5)	6 (3.5)	
Tetracycline	9 (8.3)	3 (4.8)	12 (7.1)	
Vancomycin	2 (1.9)	5 (8.1)	7 (4.1)	
Not remembered	19 (17.6)	13 (21)	32 (18.8)	

Antimicrobial categorize				X2=3.560 ,P= 0.059 *
MDR	73 (67.6)	44 (71)	117 (68.8)	
XDR	22 (20.4)	5 (8.1)	27 (15.9)	
Taking antibiotics according to physician consultation				X2=0.710 ,P= 0.399 *
Yes	40 (37)	19 (30.6)	59 (34.7)	
No	68 (63)	43 (69.4)	111 (65.3)	
Taking antibiotics according to pharmacist consultation?				X2=0.164 ,P= 0.684 *
Yes	61 (56.5)	37 (59.7)	98 (57.6)	
No	47 (43.5)	25 (40.3)	72 (42.4)	
Taking antibiotics according to friends or relative consultation?				X2=0.491 ,P= 0.483 *
Yes	71 (65.7)	44 (71)	115 (67.6)	
No	37 (34.3)	18 (29)	55 (32.4)	
Would you visit a physician for a follow-up after taking antibiotics?				X2=0.504 ,P= 0.477 *
Yes	26 (24.1)	18 (29)	44 (25.9)	
No	82 (75.9)	44 (71)	126 (74.1)	
If ill with flu-like symptoms and the doctor doesn't prescribe antibiotics, do you take an antibiotic?				X2=0.284 ,P= 0.594 *
Yes	76 (70.4)	46 (74.2)	122 (71.8)	
No	32 (29.6)	16 (25.8)	48 (28.2)	
Do you aware of miss use of antibiotics leads to resistance of bacteria?				X2=2.538 ,P= 0.111 *
Yes	59 (54.6)	26 (41.9)	85 (50)	
No	49 (45.4)	36 (58.1)	85 (50)	

3

Table 3(on next page)

Antimicrobial categorization of *E.faecium* isolates among the hospitalized and non-hospitalized patients

Table 3

Table 3: Antimicrobial categorization of *E.faecium* isolates among the hospitalized and non-hospitalized patients

Antimicrobial categorize	Hospitalized (N=70)		*P*	Non-hospitalized (N=100)		*P*
	E. faecium	Other Enterococcus		*E. faecium*	Other Enterococcus	
Susciptible	8 (11.4)	6 (8.6)	0.751	5 (5)	7 (7)	0.021**
MDR	25 (35.7)	18 (25.7)		48 (48)	26 (26)	
XDR	9 (12.9)	4 (5.7)		13 (13)	1 (1)	

Table 4(on next page)

Virulence gene patterns among the VRE*fm* isolates

Table 4

1

Species	Virulence factors	Number of positive isolates
VREfm (n=18)	*asa1*	4 (22.2%)
	hyl	1 (5.6%)
	gelE	1 (5.6%)
	esp	3 (16.7%)
	gelE-hyl	2 (11.1%)

2 **Table 4: Virulence gene patterns among VRE*fm* isolates**

3

Figure 1

Percentage of antibiotic resistance in *E.faecium* strain isolated from hospitalized and non-hospitalized patients.

Percentage of antibiotic resistance in *E.faecium* strain isolated from hospitalized and non-hospitalized patients. ERY: Erythromycin; AMC: amoxicillin-clavulanic acid; AMP: ampicillin; PEN: penicillin-G (10 IU); CRO: ceftriaxone; CAZ: ceftazidime; GEN gentamicin; CIP: ciprofloxacin; LEV: levofloxacin; DAP: daptomycin; TET: tetracycline; LNZ: linezolid; TEC: teicoplanin; RIF: rifampin; VAN: vancomycin.

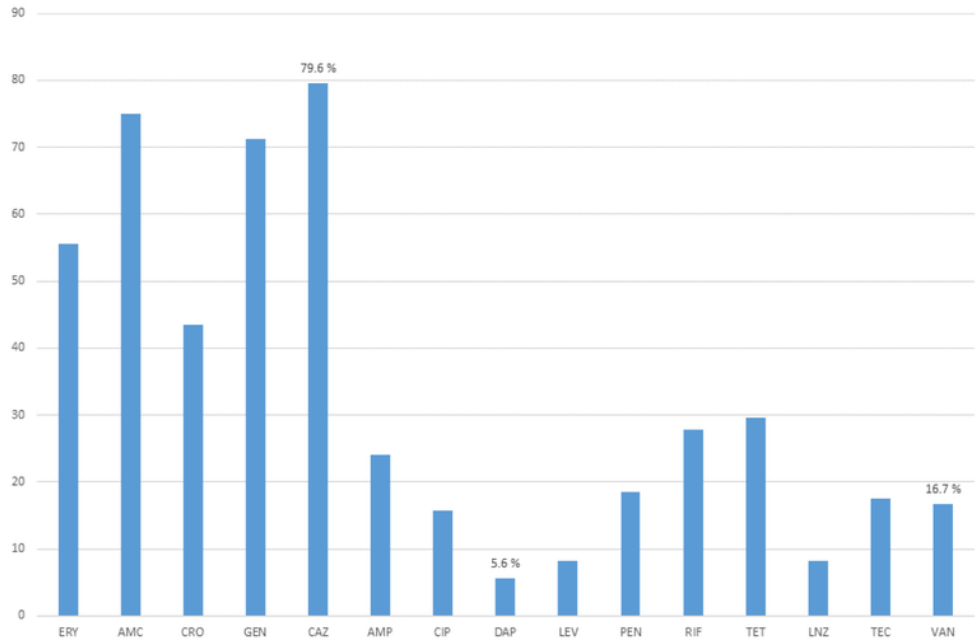

Figure 2

Representative genetic profiles yielded by the ERIC-PCR analysis of *E. faecium* strains isolated from different Khartoum locality hospitals. Lanes 1 and 21= Gel Pilot 1 Kb Plus ladder; Lanes 2–20, 22-40 = A representation of the ERIC profiles for[i]

Fig 2

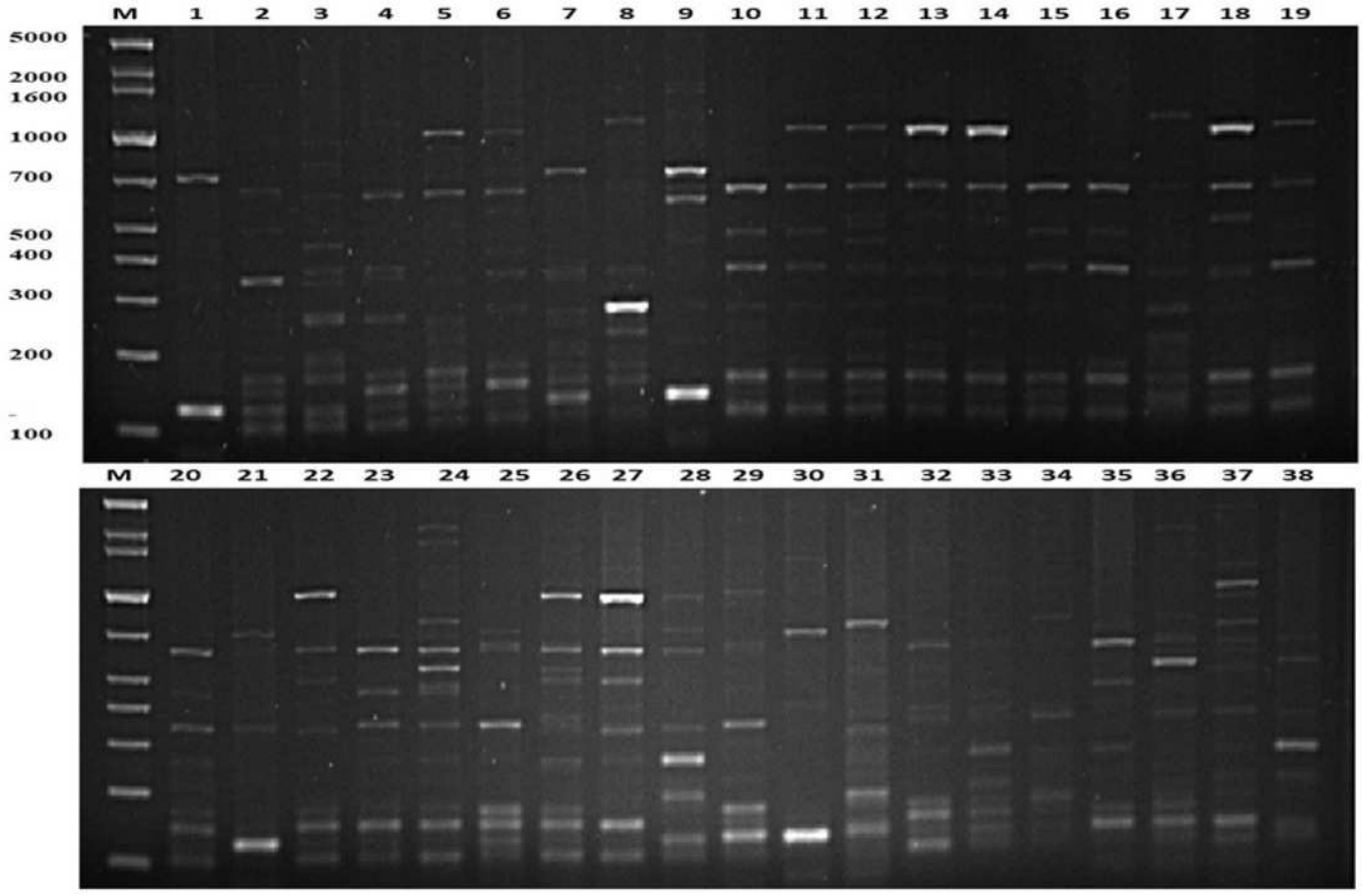

Figure 3

A represented dendrogram of ERIC-PCR using CLIQS fingerprint data software and UPGMA with arithmetic averages at 80% similarity on 108 strains of *Enterococcus faecium* isolated from Khartoum locality hospitals.