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

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Background: *Enterococcus faecium* is an opportunistic pathogen of humans with diverse hosts, encompassing animals as well as human beings. In the past twenty years, there has been a rise in the instances of nosocomial infections that are linked to antibiotic-resistant *Enterococcus faecium*. The acquisition of diverse antimicrobial resistance factors has driven the global development of robust and convergent adaptive mechanisms within the healthcare environment. The presence of microorganisms in hospitalized and non-hospitalized patient populations has been significantly aided by the facilitation of various perturbations within their respective microbiomes. **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, and the gel was analyzed using CLIQS software. **Results:** In this study, the isolation rate of colonized *E. faecium* strains was 108/170 (63.5%). The colonization of *E. faecium* and its association with various sociodemographic and clinical features was examined. 73 (67.6%) of patients had Multidrug-resistant (MDR), and 22 (20.4%) had Extensively drug-resistant (XDR), 73 (67.6%) of patients engaged in self-

medication practices. Eighty patients (74.1%) were non-adherence to prescribed antibiotics, while 70 (64.8%) patients reported recent antibiotic usage within the 3 months. The present study suggests that demographic factors may not be significantly associated with the incidence of *E. faecium* infection except for patients who had a prior history of antibiotic use (P ≤ 0.005). The analysis of virulence genes showed a high prevalence of *asa1* gene (22.2%) among strains. In ERIC-PCR the genetic relatedness of *E. faecium* showed 7 identical clusters (A-G) with 100% genetic similarity. This implies clonal propagation in hospitals 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 the spreading of nosocomial infection.

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Abstract

21 **Background:**
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23 animals as well as human beings. In the past twenty years, there has been a rise in the instances of
24 nosocomial infections that are linked to antibiotic-resistant *Enterococcus faecium*. The acquisition
25 of diverse antimicrobial resistance factors has driven the global development of robust and
26 convergent adaptive mechanisms within the healthcare environment. The presence of
27 microorganisms in hospitalized and non-hospitalized patient populations has been significantly
28 aided by the facilitation of various perturbations within their respective microbiomes. **Objective:**
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30 along with the detection of virulence encoding genes, and to find out the clonal genetic relationship
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32 carried out between October 2018 and March 2020 at four Khartoum locality hospitals in Sudan.
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34 locality hospitals in Khartoum. A self-structured questionnaire was used to gather information on
35 sociodemographic traits. Data were analyzed using chi-square test. In all cases, *P* value ≤ 0.05
36 with a corresponding 95% confidence interval was considered statistically significant. Moreover,
37 enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCR) was
38 utilized to assess the prevalence of clonal relationships, and the gel was analyzed using CLIQS
39 software. **Results:** The colonization of *E. faecium* and its association with various

40   sociodemographic and clinical features was examined. 73 (67.6%) of patients had Multidrug-
41   resistant (MDR), and 22 (20.4%) had extensively drug-resistant (XDR), 73 (67.6%) of patients
42   engaged in self-medication practices. Eighty patients (74.1%) were non-adherence to prescribed
43   antibiotics, while 70 (64.8%) patients reported recent antibiotic usage within the 3 months. The
44   present study suggests that demographic factors may not be significantly associated with the
45   incidence of *E. faecium* infection except for patients who had a prior history of antibiotic use (P ≤
46   0.005). The analysis of virulence genes showed a high prevalence of *asa*1 gene (22.2%) among
47   strains. In ERIC-PCR the genetic relatedness of *E. faecium* showed 7 identical clusters (A-G) with
48   100% genetic similarity. This implies clonal propagation in hospitals and communities.
49   **Conclusion**: This study found that the incidence of *E. faecium* isolated from locality hospitals in
50   Khartoum was likely due to the spread of *E. faecium* clones, thereby highlighting the need for
51   intensifying infection control measures to prevent the spreading of nosocomial infection.
52   **Keywords:** *Enterococcus faecium,* Sociodemographic distribution, vancomycin-resistant
53   *Enterococcus faecium*, antibiotic resistance pattern; virulence encoding gene, ERIC-PCR, clonal
54   relationship, Khartoum locality hospital.
55

Introduction

57   In the past two decades, *Enterococcus faecium* has rapidly evolved as a worldwide nosocomial
58   pathogen by successfully adapting to conditions in a nosocomial setting and acquiring resistance
59   against glycopeptides (1),(2). The nosocomial pathogen *E. faecium* can survive for prolonged
60   periods on surfaces in the absence of nutrients, and also in hospital environments, these traits are
61   thought to contribute to the ability of *E. faecium* to transmit between patients in hospitals (3),(4).
62   It is commonly found in the intestines of humans and animals. While it is generally considered a
63   harmless commensal organism, sometimes causes infections, especially in individuals with
64   weakened immune systems or other underlying health conditions such as Urinary tract infections,
65   bacteremia, and endocarditis. The relative importance of *E. faecium* as a pathogen has increased
66   with the occurrence of high-level resistance to multiple antimicrobial drugs, such as amoxicillin
67   clavulanic acid, aminoglycosides, cephalosporin, and vancomycin (5). The prevalence of
68   multidrug-resistant (MDR) *E. faecium* infections is rising globally, although epidemiological
69   research remains generally scarce in specific regions such as African countries including Sudan
70   (3). Vancomycin-resistant *E. faecium* (VRE*fm*) is the most common multidrug-resistant
71   *Enterococcus* species and is one of the most serious concerns in healthcare settings (6),(7),
72   designated as a high-priority pathogen in need of therapeutic research and development according
73   to the World Health Organization (WHO) and the Centers for Disease Control and Prevention
74   (CDC) (8),(9). In the invasion process, enterococci use a variety of virulence factors including
75   (*asa*1, *cyl*A, *esp, gel*E, and *hyl* gene) for adhering to the infection site and colonizing (10),(11),
76   along with the presence of damage to the host tissue and antibiotic resistance, all help with the
77   invasion process. In the end, the clinical manifestation of infection in the targeted vital tissues is
78   the result of interactions between the host and enterococci.

79   Molecular typing is becoming a paradigm for understanding the fundamental mechanisms of
80   *Enterococcus* infections in hospital settings to investigate the clonal relationship among bacterial
81   strains, and to trace the source of infections during hospital outbreaks (12). PCR-based genotyping
82   tools are used for determining different DNA fingerprints, among several PCR-based tools, the
83   ERIC-PCR discriminating is a rapid, and cost-effective genotyping method for different types of
84   strains. Moreover, there are different numbers of ERIC sequence copies among bacterial species.
85   Interestingly, there is a significant diversity of copy numbers among different strains of *E. faecium*
86   (13). The use of homologous primers to ERIC sequences allows for assessing the clonal variability
87   found in various *E. faecium* strains, in which the resultant patterns can be utilized to evaluate the
88   phylogenetic closeness with the help of CLIQS 1D PRO software (14). In Sudan, in particular, no
89   reports are available in epidemiological studies, assessing antibiotic resistance, molecular analysis,
90   or other features of clinical *E. faecium* isolates.
91
Materials & Methods
93   **Study Design, Setting, and Period**
94   A hospital-based cross-sectional study was conducted in four tertiary hospitals in the Khartoum
95   locality (The Academy Charity Teaching Hospital (ACTH), Dar-Alelaj Specialized Hospital
96   (DASH), Ibrahim Malik Teaching Hospital (IMTH), and Yastabshiroon Hospital Riyadh
97   (YASH)), from October 2018 to March 2020. Those hospitals provide different levels of care
98   services in all disciplines, four wards were included: Medicine, ICU, Surgical, and Pediatric wards.
99   Ethical approval for this study was obtained from the Graduate College-UMST and the Federal
100 Ministry of Health Reference no. (ws-wk-aete-44-a), Sudan.
101 **Patient and Statistical Analysis**
102 Information on patient sociodemographic characteristics, risk factor data, and other independent
103 variables was collected from each participant using a self-structured questionnaire. Data collection
104 was done under the supervision of the project advisors. The information was gathered by
105 conducting in-person interviews with patients in outpatient clinics or hospital wards by attending
106 a nurse from the hospital. Qualitative data were described using numbers and percentages.
107 Comparison between different groups regarding categorical variables was tested using Pearson's
108 chi-square test ($P \leq 0.05$) to investigate the significance of *E. faecium* colonization association
109 with sociodemographic distributions.
110 **Collection and Identification of *Enterococcus faecium***
111 A total of 108 *E. faecium* were isolated from fecal cultures, from patients in four Khartoum locality
112 hospitals. All specimens were cultivated on the surface of the bile-esculin medium (HiMedia,
113 India), and incubated at 37°C for 24 hours. Colonies growing on bile-esculin medium with a dark
114 brown halo and colonial morphology resembling enterococci were collected as presumptive
115 isolates. All presumptive isolates of enterococci were further confirmed using phenotype tests such
116 as Gram staining, catalase, oxidase, and growth in 6.5% NaCl broth, as described elsewhere (15).
117 Catalase test aimed to detect the presence of catalase enzyme in microorganisms. This test was
118 performed by the addition of two or three drops of 3% H_2O_2 onto the cultures. The oxidase test is

119 a valuable technique for the differentiation of bacteria based on their enzymatic capabilities. The
120 test was performed by adding two droplets of oxidase reagent on sterile Petri dishes smeared with
121 a loopful of bacterial growth. Once obtaining pure isolates that met the criteria of being (Gram-
122 positive, and lacking catalase and oxidase activity), additional assays were performed. The isolates
123 also were subjected to incubation in BHI broth that contained 6. 5% NaCl at a temperature of 37°C
124 for a duration of 24 to 48 hours. Isolates were presumptively considered to be enterococci based
125 on the characteristic features: Gram-positivity and catalase-and oxidase-negativity, growth in a
126 medium containing 6.5% NaCl.

127 Antibiotic Susceptibility Testing
128 Utilizing the Kirby-Bauer method's disk diffusion procedure on Muller-Hinton agar (Hi-Media,
129 India) (16), Hundred-eight *E. faecium* strains were investigated. A total of fifteen antibacterial
130 drugs are included were previously described in Siddig *et al*., (2022) (5). According to the
131 recommendations of the Hi-Media Antimicrobial Susceptibility Systems, the width of the zone of
132 inhibition was determined and recorded as Sensitive (S), Intermediate (I), or Resistant (R).
133 Antibiotic susceptibility testing was carried out using the reference control organism *E. faecalis*
134 ATCC29212. All of the analyzed strains of *E. faecium* in this study were classified as Multidrug-
135 resistant (MDR), Extensively drug-resistant (XDR), and Pandrug-resistant (PDR). MDR is
136 defined as resistant to three or more antimicrobial classes (17). Non-susceptibility to at least one
137 agent in all but two or fewer antimicrobial categories is defined as XDR, while PDR is defined as
138 resistance to all agents in all antimicrobial categories.

139 DNA Extraction and Detection of VRE*fm* Virulence Genes by PCR
140 Genomic DNA was extracted using the G-spin Genomic DNA Extraction Kit (iNtRON, South
141 Korea) following the manufacturer's instructions. Amplification was performed according to a
142 published protocol (18), using a multiplex PCR to investigate the presence of five virulence genes
143 in VRE*fm* isolates. All primer sequences are shown in (Table 1).

144 ERIC-PCR Typing
145 All isolates were fingerprinted using primers ERIC1A ('5-ATGTAAGCTCCTGGGGATTCAC-
146 3') and ERIC2 ('5-AAGTAAGTGACTGGGGTGAGCG-3') according to a published protocol
147 (19). ERIC-PCR products were resolved by gel electrophoresis and analyzed on 2% w/v agarose
148 gel with ethidium bromide gel stain in TBE 1× electrophoresis buffer. A GelPilot 1 kb Plus Ladder
149 (100) (Qiagen) was included as a molecular weight marker. PCR products were visualized using
150 a UV-transilluminator.

151 ERIC-PCR Data Analysis
152 The clonal relatedness between the strains of *E. faecium* was analyzed using ERIC-PCR
153 fingerprinting with the CLIQS 1D PRO software (TotalLab Ltd, Newcastle, United Kingdom)
154 (14), Similarity distances between ERIC-PCR profiles were calculated using the Dice coefficient
155 and dendrograms were constructed based on the unweighted pair group method with arithmetic
156 mean (UPGMA). Normalization steps were included in the analysis of DNA polymorphism
157 patterns produced by ERIC-PCR fingerprinting to ensure an adequate gel-to-gel banding pattern

158   comparison. Isolates with an 80% level of similarity were grouped in the same cluster and were
159   considered clonally related.
160

Results

162   **Sociodemographic Distributions**
163   The presentation of the demographic distribution and clinical characteristics of 108 patients is
164   delineated in (Table 2). The age distribution of patients ranged from 1 to 82 years, with a median
165   age of 41.5 years. A higher incidence of *E. faecium* colonization (26.9%; 29/108) was observed in
166   individuals aged between 35 and 49 years. Moreover, the majority of the cohort was comprised of
167   males (55.6%) and approximately (44.4%) were females. Furthermore, it was noted that (56.5%)
168   of the patients held a University education, (67.6%) were from Urban residences, and (28.7%)
169   were students by occupation. A high frequency of *E. faecium* was observed in Academy Charity
170   Hospital (30.6%). In relation to clinical characteristics, the majority of the participants exhibited
171   at least one instance of chronic comorbidity. Gastrointestinal tract infection was the most
172   frequently encountered comorbidity, with 28 participants (25.9%) presenting with this condition.
173   Table 2 reveals that, in accordance with their distribution across wards, the prevalence of
174   enterococci was highest amongst patients who received treatment in internal medicine wards, with
175   17 individuals (15.7%) identified as carriers. A sizeable proportion of patients (33.3%) had a stay
176   duration of less than one week. The majority of patients (67.6%) reported self-medication with
177   antibiotics. Non-adherence to prescribed antibiotics was prevalent among an overwhelming
178   majority of patients (74.1%). Additionally, a high proportion (64.8%) of patients had a history of
179   previous antibiotic intake within the preceding three months. In the study, a total of 80 individuals
180   exhibited antibiotic non-adherence behaviors. Among these participants, 45 (56.3%), 65 (81.3%),
181   52 (65%), and 50 (62.5%) were identified as male, engaging in self-medication, residing in urban
182   areas, and having attained university education, respectively. Additionally, 44 (55%) of the
183   participants demonstrated inadequate knowledge regarding appropriate antibiotic use.
184   According to the findings reported in (Table 2), a significant proportion of the colonized patients
185   (67.6%) were classified as multidrug-resistant (MDR), while a notable subset of 22 patients
186   (20.4%) exhibited characteristics of extensively drug-resistant (XDR) infections. Approximately
187   thirty-seven percent (37%) of patients were administered antibiotics through a prescription from a
188   physician. Fifty-six point five percent (56.5%) of individuals under medical care utilized
189   antibiotics as per the recommendations relayed by the pharmacist's prescription. Conversely, a
190   majority of 71 individuals, accounting for 65.7% of patients, ascertained through the study
191   questionnaire that they sought counsel from acquaintances or family members concerning the
192   application of antibiotics. Only 26 (24.1%) of study participants visited the clinic for follow-up
193   after receiving antibiotics prescriptions. According to the findings, a significant proportion of
194   patients, specifically 76 (70.4%), made requests for antibiotics when presenting with flu-like
195   symptoms. Moreover, over 50% of individuals with *E. faecium* infection exhibited lacked
196   knowledge about antibiotic resistance as presented in (Table 2). Based on the results of the chi-
197   square test, there is no statistically significant relationship observed between sociodemographic
198   factors and the occurrence of *E. faecium*. The colonization of *E. faecium* was observed to occur

199 exclusively in subjects without prior history of antibiotic administration, as indicated by a
200 statistically significant *P*-value of ≤ 0. 005.

Prevalence of Antimicrobial Resistance among *E. faecium* Strains

202 A total of 108 *E. faecium* isolates were subjected to analysis, with 33 (30.6%) retrieved from
203 Academy Charity Teaching Hospital (ACTH), 29 (26.9%) obtained from Dar-Alelaj Specialized
204 Hospital (DASH), 27 (25%) sourced from Ibrahim Malik Teaching Hospital (IMTH), and 19
205 (17.6%) originating from Yastabshiroon Hospital Riyadh (YASH). Among them, Forty-two
206 strains of colonized *E. faecium* were isolated from the hospitalized patient, while Sixty-six strains
207 were isolated from the non-hospitalized patient (Fig 1). Out of the total samples collected, 18
208 (16.7%) were found to be VRE*fm* strains. In our previous work, the majority of *E. faecium* strains
209 have been demonstrated to exhibit resistance to antibiotics with the exception of a single strain
210 that displays susceptibility to all known classes of antibiotics. However, higher and lower
211 resistance rates in *E. faecium* stains were shown against ceftazidime (79.6%), and daptomycin
212 (5.6%) respectively (5).

213 Table 3 reveals that a majority of strains, totaling 73 (67.6%), exhibited MDR, while 22 (20. 4%)
214 XDR. None of the identified isolates demonstrated Pandrug-resistance (PDR). In general, the
215 prevalence of multidrug-resistant (MDR) *E. faecium* isolates was observed to be highest among
216 non-hospitalized patients, with a frequency of 48/100 (48%), in contrast to a rate of 25/70 (35.7%)
217 among hospitalized patients. Conversely, the occurrence of extensively drug-resistant (XDR)
218 isolates was equal among the hospitalized and non-hospitalized patients, with a rate of 13%. The
219 results of a chi-square analysis revealed a statistically significant correlation between the
220 categorization of antimicrobial agents and community-based patients (*P*= 0.021), as evidenced by
221 the data presented in (Table 3).

Virulence Genes among VRE*fm* Strains

223 Among 108 *E. faecium* isolates, a total of 11 VRE*fm* strains tested positive for virulence genes as
224 shown in (Table 4). The present study reveals that the most common virulence-encoding genes
225 among VRE*fm* were *asa*1 4 (22.2%), followed by *esp* 3 (16.7%), *hyl* 1 (5.6%), *gel*E 1 (5.6%), and
226 *gel*E-*hyl* 2 (11.1%). Notably, no instance of the *cyl*A gene was discerned within the studied
227 population.

ERIC-PCR Analysis of *E. faecium*

229 The present study employed the ERIC-PCR DNA fingerprinting technique on a total of 108 *E.*
230 *faecium*. Subsequent analysis revealed ERIC profiles spanning a range of 4-13 distinct banding
231 patterns, with molecular weights ranging from 100 to 5000 bp (Fig 2). The assessment of ERIC
232 fingerprinting patterns utilizing the Dice coefficient and UPGMA presents that ERIC-PCR profiles
233 demonstrate considerable genetic diversity among the isolated samples. The rate of *E. faecium*
234 isolates was found to be 13% (14/108) when clustering was based on a 100% similarity coefficient.
235 Additionally, 52.8% (57/108) of the isolates were found to cluster with an 80% similarity
236 coefficient. Whereas 24.1% (26/108) isolates of *E. faecium* had less than 80% similarity.
237 Furthermore, 10 strains demonstrating a singular lineage below 50% were omitted, and a grouping

238 analysis revealed that fourteen patients with identical fingerprint patterns were allocated to clusters
239 A through G, as depicted in (Fig 3).

Discussion

241 Until the 1980s, *Enterococcus* spp. were merely intestinal microbes of little clinical significance.
242 Now, they are among the most common nosocomial pathogens, so physicians are becoming more
243 worried (20). Resistance in enterococci was increased dramatically and the incidence of VRE
244 colonization spread where vancomycin is one of the antibiotics of choice used to treat infections
245 caused by Gram-positive multidrug-resistant organisms, such as Enterococci. In Sudan, no reports
246 are available on the molecular epidemiology analysis of *E. faecium* and there are limited data on
247 the prevalence of antibiotic resistance profiles. We previously conducted a study on "Prevalence
248 and Antimicrobial Profile of Colonized Enterococcus Species Isolated from hospitalized and non-
249 hospitalized Patients, Khartoum, Sudan" (5), but the study did not address risk factors of
250 acquisition of *E. faecium* infection associated with Sociodemographic characteristics. In this study,
251 antibiotic resistance of *E. faecium* carrier rates, sociodemographic and clinical characteristics
252 associated with *E. faecium* colonization were studied. VRE*fm* virulence genes and the genetic
253 relationship among *E. faecium* strains isolated from Khartoum locality hospitals were investigated.
254 In the present study, the prevalence of *E. faecium* strains was (63.5%; 108/170). Our findings
255 showed that a high prevalence of *E. faecium* occurred in non-hospitalized patients of
256 Yastabshiroon Hospital (70%), whereas, (67.7%) showed in hospitalized patients of Ibrahim Malik
257 Teaching Hospital. These results were consistent with a previous study done by Tedim A. *et al*.,
258 (2015) revealing that the prevalence of colonization with *E. faecium* is higher in non-hospitalized
259 patients compared to hospitalized patients (21). Antimicrobial resistance percentages among
260 bacteria from human illnesses in the European Union and European Economic Area countries
261 (EU/EEA) did not vary significantly between 2014 and 2020, except for *E. faecium*, where the
262 proportion of vancomycin resistance increased from 9% in 2014 to 17% in 2020 (22), and this
263 consists with our result where the prevalence of VRE*fm* was (16.6%).
264 Broad-spectrum antibiotics have the potential to harm the normal anaerobic flora of the
265 gastrointestinal tract, resulting in infectious diseases due to their bactericidal impact against
266 Enterococcus. Many studies have reported that previous use of broad-spectrum antibiotics is a risk
267 factor for acquiring multidrug-resistant pathogens (23),(24). However, few investigations have
268 been undertaken to evaluate the link between previous antibiotic exposure and the acquisition of
269 *E. faecium* strains (24). Our findings indicated a significant association between prior antibiotic
270 exposure and the acquisition of *E. faecium* infection ($P \leq 0.005$). Interestingly, our study data
271 findings supported that overusing antibiotics increased the likelihood of resistance while
272 decreasing their efficacy, this was demonstrated by the high prevalence of antibiotic usage among
273 hospitalized patients and non-hospitalized patients, as well as by their use of self-medication and
274 erratic antibiotic regimen.
275 In several reported cases, gastrointestinal tract colonization generally precedes infection with
276 antibiotic-resistant *E. faecium*, in particular, intestinal overgrowth by antibiotic-resistant
277 enterococci is a recognized risk factor for disease (25),(26). Our study showed the prevalence of

278 *E. faecium* colonization was higher among patients who had gastrointestinal tract infections
279 (25.9%) compared to other chronic infections. The high prevalence rate of self-medication and
280 antibiotic usage seen in this study could partly be explained by the patient's desire for a fast
281 recovery from the disease. The economic situation is another major cause for self-medicates, and
282 consultations of friends or relatives to avoid paying the physicians' fees.
283 A recent study conducted in primary healthcare centers in Qatar reported that many factors
284 contribute to the increased incidence of bacterial resistance to antibiotics, particularly; the misuse
285 of antibiotics by physicians and the easy acquisition of antibiotics via non-physicians (27). In the
286 developing world, it is common practice for pharmacies to distribute antibiotics based on patient
287 requests, and this study is in line with our results that showed (56.5%) of patients have taken
288 antibiotics according to pharmacist consultation. The findings of the study also reveal more than
289 half of patients received antibiotics based on recommendations provided by friends or relatives. In
290 addition, even in cases when they weren't necessary, such as flu-like symptoms, 76 (70.4%) of
291 patients demanded antibiotics. This understanding was explained by their knowledge which led
292 them to believe that antibiotics were helpful in such circumstances (28). According to a study
293 conducted among community pharmacies in Addis Ababa, an increase in the frequency of over-
294 the-counter sales of antibiotics has been observed. These results are attributable to commercial
295 interests, consumer pressure, and lax rules (29).
296 Proper adherence to antibiotic therapy is an essential element among various measures necessary
297 to combat the rising issue of antimicrobial resistance (30). In this paper, we describe this burden
298 and its consequential effects on medication adherence in patients at Khartoum locality hospitals.
299 We assigned everyone who was not perfectly adherent to the use of antibiotics as "Non-
300 adherence". The overall prevalence of drug non-adherence was found to be 74%. The study
301 findings are consistent with studies done in the USA (70%) (31), Ethiopia (60.1%) (30), and
302 Nigeria (63.4%) (32). Our findings are also higher than previous studies conducted in Malaysia
303 (34.8%) (33).
304 A multiplex PCR developed for the simultaneous detection of *E. faecium* virulence genes that
305 encode for aggregation substance (*asa*1), gelatinase (*gel*E), cytolysin (*cyl*A), enterococcal surface
306 protein (*esp*), and hyaluronidase (*hyl*). Our result showed that virulence genes *asa*1 (22.2%),
307 followed by *esp* gene (16.7%) are predominant in the virulent patterns of VRE*fm* isolated from
308 hospitals and communities. Findings from our current study are consistent with a recent study from
309 Southern California and Puerto Rico was reported the *asa*1 gene is predominant in enterococci
310 isolated from hospitals, the natural environment, animals, and wastewater (34).
311 According to the dendrogram, strains with 100% similar ERIC profiles were found in clusters, A
312 (31/32), B (34/35), D (41/42), and cluster E (65/66) were isolated from patients among different
313 hospital wards and communities. On the other hand, isolates presented in clusters, C (37/38), F (78
314 /79), and G (97/98) with similar ERIC profiles isolated from patients within the same hospitals. *E.*
315 *faecium* strains show high genetic diversity among isolates raising the possibility of circulation of
316 various *E. faecium* strains between the hospitals and the community.
317

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. 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. In this study, antibiotic non-adherence was considerably high among the participants. As a result, community service providers must provide relevant prescription information as well as appropriate counseling to antibiotic non-adherent patients. 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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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: ATGTAAGCTCCTGGGGATTCAC	100-10000	(17)
ERIC2	R: AAGTAAGTGACTGGGGTGAGCG		

Sociodemographic and Clinical Characteristics of *Enterococcus faecium* isolates among the Khartoum locality hospital patients, Sudan (2018-2020).

Academy Charity Teaching Hospital (ACTH); Dar-Alelaj Specialized Hospital (DASH); Ibrahim Malik Teaching Hospital (IMTH); Yastabshiroon Hospital Riyadh (YASH); *Multidrug-resistant* (MDR); and extensively drug-resistant (XDR).

1   **Table 2: Sociodemographic and Clinical Characteristics of *Enterococcus faecium* isolates among the Khartoum**
2   **locality hospital patients, Sudan (2018-2020).**

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 *
High Adherence	28 (25.9)	11 (17.7)	39 (22.9)	
Non-adherence	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)	

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

1   **Table 3: Antimicrobial categorization of *E.faecium* isolates among the hospitalized and non-hospitalized**
2   **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)	

Virulence gene patterns among the VRE*fm* isolates

Enterococcal aggregation substance (*asa1*); hyaluronidase (*hyl*); gelatinase (*gelE*); Enterococcal surface protein (*esp*).

1   **Table 4: Virulence gene patterns among VRE*fm* isolates.**

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

Figure 1

Distribution of *E.faecium* among the patients at Khartoum locality hospital, Sudan, 2018-2020.

Hospitalized patients (IP); Non-hospitalized patients (OP); Academy Charity Teaching Hospital (ACTH); Dar-Alelaj Specialized Hospital (DASH); Ibrahim Malik Teaching Hospital (IMTH); and Yastabshiroon Hospital Riyadh (YASH). (Siddig LA. et al., 2022).
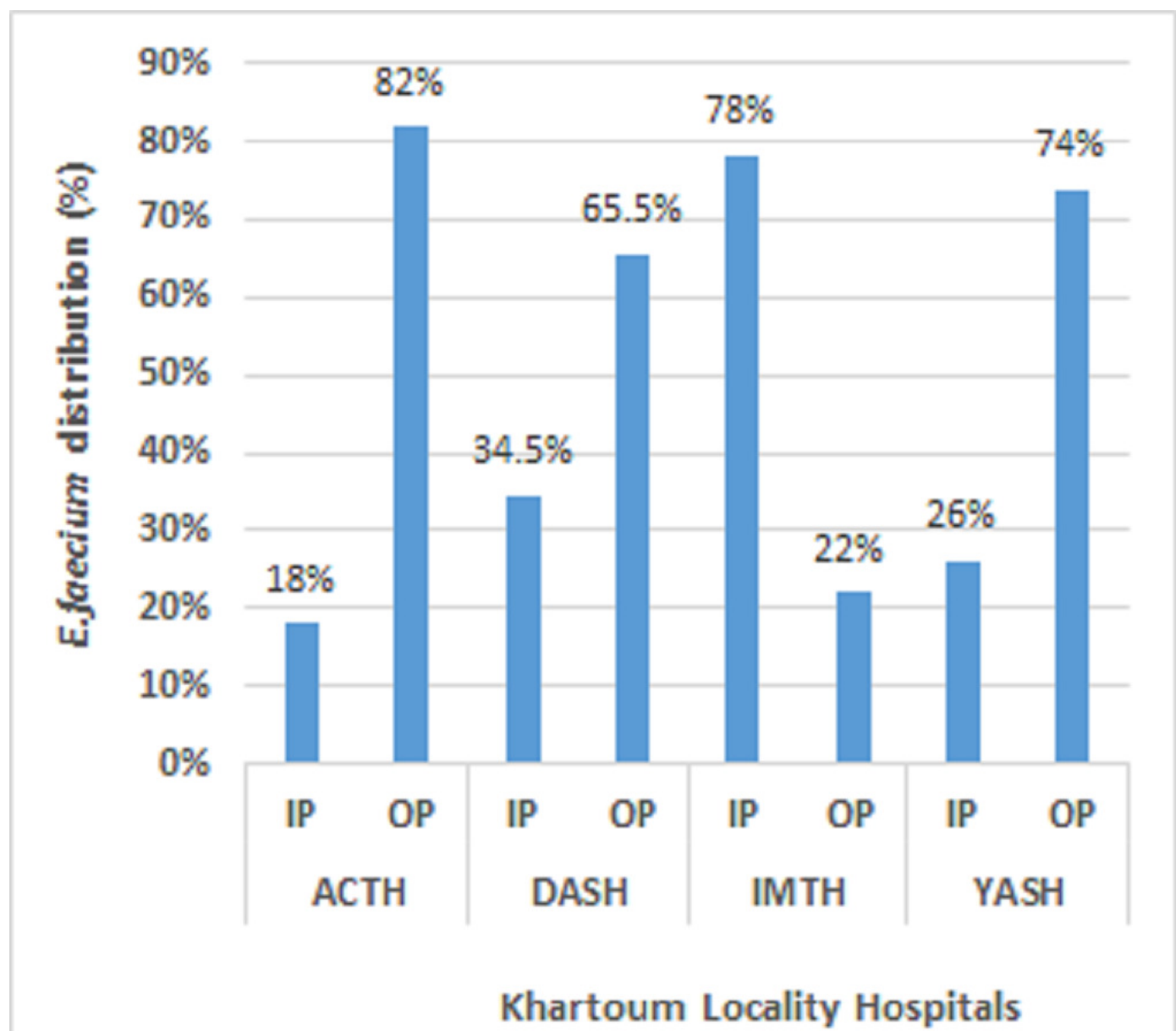
Figure 2

Representative genetic profiles yielded by the ERIC-PCR analysis of *E.faecium* strains isolated from different Khartoum locality hospitals.

Lanes M = Gel Pilot 1 Kb Plus ladder; Lanes 1–19, 20-38 = A representation of the ERIC profiles for *E.faecium* isolates.
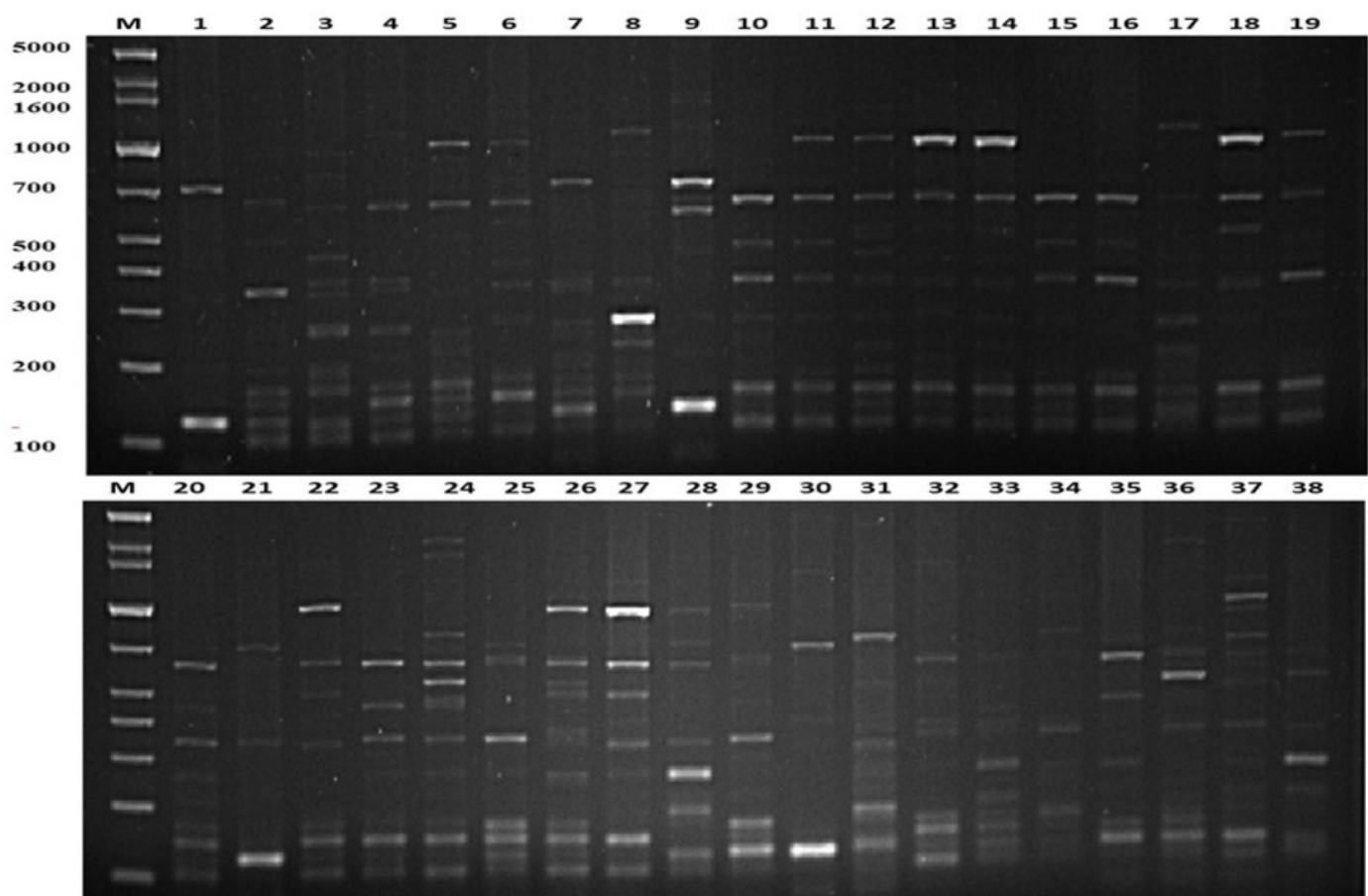
Figure 3

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.

The different clusters at 80% similarity are arbitrarily designated as sub-group 1–53. In the code, the first number refers to the patient, the second letter refers to the strain, and the third refers to admitted hospital's name. ERIC-PCR strains of *E. faecium* were investigated in this study into 7 groups, every strain with 100% genetic similarity represented in clusters (A to G).

No	Code	Strain Location	Antibiotic resistance pattern	
1	23FMD	Dar-Alelaj Specialized Hospital	EY,AC,CO,GN,CZ,RF,LZ	
2	4FMBI	Ibrahim Malik Teaching Hospital	AC,CO, AP,PN,RF,TT	
3	147FMAC	Academy Charity Hospital	EY,AC,GN,CZ	
4	104FMY	Yastabshiroon Hospital	AC,CO,GN,CZ	
5	150FMD	Dar-Alelaj Specialized Hospital	EY,AC,CO,TC,VN AC,CO,GN,CZ,TC,VN	
6	118FMD	Dar-Alelaj Specialized Hospital	AC,CO,GN,CZ,TC,VN	
7	83FMAC	Academy Charity Hospital	EY,CO,AP,CP	
8	123FMAC	Academy Charity Hospital	CO	
9	122FMD	Dar-Alelaj Specialized Hospital	AC,CO, AP,PN,RF,TT	
10	71FMD	Dar-Alelaj Specialized Hospital	EY,AC,GN,CZ	
11	137FMD	Dar-Alelaj Specialized Hospital	EY,AC,CO,GN,CZ,AP,CP,LV,PN,RF,TT,TC,VN	
12	158FMAC	Academy Charity Hospital	AC,GN,RF,TC,VN	
13	142FMAC	Academy Charity Hospital	EY,AC,GN,CZ,PN,RF,LZ,TC,VN	
14	167FMB	Ibrahim Malik Teaching Hospital	AC,GN	
15	2FMAC	Academy Charity Hospital	EY,AC,CO,GN,CZ,AP,DP,TC,VN	
16	165FMAC	Academy Charity Hospital	EY,AC,GN,CZ,VN	
17	155FMAC	Academy Charity Hospital	EY,AC,CO,GN,CZ,AP,PN,RF,TC,VN	
18	136FMBI	Ibrahim Malik Teaching Hospital	AC,GN,CAZ,RF	
19	102FMY	Yastabshiroon Hospital	EY,AC,CO,GN,CZ,AP,PN,TT,TC,VN	
20	157FMBI	Ibrahim Malik Teaching Hospital	EY,AC,GN	
21	154FMYI	Yastabshiroon Hospital	EY,CO,CP	
22	41FMBI	Ibrahim Malik Teaching Hospital	EY,AC	
23	138FMAC	Academy Charity Hospital	AC,GN,CZ	
24	58FMBI	Ibrahim Malik Teaching Hospital	EY,AC,CZ,TT	
25	161FMAC	Academy Charity Hospital	EY,AC,CO,GN,CZ,AP,CP,LV,PN,RF,TT	
26	60FMDI	Dar-Alelaj Specialized Hospital	EY,AC,CO,GN,CZ,AP,PN,TT	
27	96FMY	Yastabshiroon Hospital	AC,CZ	
28	174FMY	Yastabshiroon Hospital	GN,CZ,DP	
29	89FMAC	Academy Charity Hospital	EY,AC,CO,GE,RF	
30	95FMAC	Academy Charity Hospital	AC,GN,AP,PN,TT	
31	42FMBI	Ibrahim Malik Teaching Hospital	EY,AC,GN,CZ,TT	A
32	153FMAC	Academy Charity Hospital	AC,CO,GN,CZ,AP	
33	110FMY	Yastabshiroon Hospital	AC,CO,GN,CZ,RF	
34	97FMAC	Academy Charity Hospital	EY,AC,GN,CZ	B
35	80FMY	Yastabshiroon Hospital	EY,CO,GN ,CZ,LV,TT,LZ	
36	169FMBI	Ibrahim Malik Teaching Hospital	EY,AC,CO,GN,CZ,CP	
37	81FMACI	Academy Charity Hospital	EY,AC,CO,GN,CZ,AP,PN,RF,TC,VN	C
38	140FMAC	Academy Charity Hospital	EY,AC,GN,CZ,TC,VN	
39	90FMYI	Yastabshiroon Hospital	AC,CO,GN,CZ,RF	
40	39FMBI	Ibrahim Malik Teaching Hospital	GN,CZ,RF	
41	94FMBI	Ibrahim Malik Teaching Hospital	CO,TT	D
42	92FMAC	Academy Charity Hospital	EY,CO,GN,TT,LZ	
43	77FMB	Ibrahim Malik Teaching Hospital	EY,AP,CP,DP,LV,PN,RF,TT	
44	13FMD	Dar-Alelaj Specialized Hospital	EY,AC,CZ	
45	37FMAC	Academy Charity Hospital	EEY,AC,CO,GN,CZ,TT	
46	35FMACI	Academy Charity Hospital	EY,CO,GN,CZ,PN,TT	
47	9FMAC	Academy Charity Hospital	Y,AC,GN,CZ	
48	70FMD	Dar-Alelaj Specialized Hospital	GN,CZ,RF	
49	46FMDI	Dar-Alelaj Specialized Hospital	AC,CZ,TT	
50	115FMD	Dar-Alelaj Specialized Hospital	AC,GN,CZ,RF	
51	85FMY	Yastabshiroon Hospital	EY,AC,GN,CZ,RF,TT	
52	82FMDI	Dar-Alelaj Specialized Hospital	AC,GN,CZ,AP,DP,PN,RF,TT	
53	86FMY	Yastabshiroon Hospital	GN,CZ,CP	
54	87FMBI	Ibrahim Malik Teaching Hospital	AC,CZ	
55	168FMDI	Dar-Alelaj Specialized Hospital	EY,AC,CZ,TT	
56	3FMBI	Ibrahim Malik Teaching Hospital	0	
57	120FMAC	Academy Charity Hospital	AC,CZ	
58	69FMD	Dar-Alelaj Specialized Hospital	EY,AC,CO,CZ,AP,CP,DP,LV,PN,RF,TT,TC,VN	
59	25FMY	Yastabshiroon Hospital	EY,CO,CZ,CP	
60	56FMYI	Yastabshiroon Hospital	EY,CO,GE,CZ,AP,CP,LV,PN,TT,LZ,TC	
61	173FMY	Yastabshiroon Hospital	AC,CZ	
62	146FMD	Dar-Alelaj Specialized Hospital	EY,AC,GN,CZ	
63	101FMBI	Ibrahim Malik Teaching Hospital	CO,CZ,AP	
64	68FMY	Yastabshiroon Hospital	EY,GN,CZ	
65	98FMB	Ibrahim Malik Teaching Hospital	EY,AC,CO,CP,PN,RF,TT,LZ	E
66	8FMY	Yastabshiroon Hospital	EY,AC,CO,GN,CZ,AP,PN,TT	
67	107FMB	Ibrahim Malik Teaching Hospital	EY,AC,GN,CZ,LZ	
68	106FMDI	Dar-Alelaj Specialized Hospital	AC,CO,GN,CZ,TC,VN	
69	12FMAC	Academy Charity Hospital	EY,GN,CZ	
70	78FMD	Dar-Alelaj Specialized Hospital	EY,CO,GN,CZ	
71	152FMAC	Academy Charity Hospital	EY,AC,GN,CZ,RF,TC,VN	
72	105FMDI	Dar-Alelaj Specialized Hospital	EY,AC,CO,CP,PN,RF,TT,LZ	
73	103FMY	Yastabshiroon Hospital	AC,GN ,CZ,RF	
74	27FMAC	Academy Charity Hospital	EY,AC,GN	
75	79FMY	Yastabshiroon Hospital	EY,AC,GN,CZ,RF	
76	124FMAC	Academy Charity Hospital	AC,CO,GN,CZ,AP,PN	
77	99FMD	Dar-Alelaj Specialized Hospital	AC,CO,CZ,AP	
78	7FMBI	Ibrahim Malik Teaching Hospital	GN,CZ,AP	F
79	5FMBI	Ibrahim Malik Teaching Hospital	AC,CO,CZ, AP	
80	75FMBI	Ibrahim Malik Teaching Hospital	AC,CZ	
81	141FMDI	Dar-Alelaj Specialized Hospital	AC,GN,CZ	
82	108FMAC	Academy Charity Hospital	EY,AC CO,CZ,TC,VN	
83	128FMACI	Academy Charity Hospital	CZ,AP,CP,DP,LZ,TC	
84	61FMBI	Ibrahim Malik Teaching Hospital	GEN,CZ	
85	53FMBI	Ibrahim Malik Teaching Hospital	EY,AC,CO,CZ,AP,CP,LV,PN,TT	
86	143FMAC	Academy Charity Hospital	EY,AC,GN,CZ	
87	135FMAC	Academy Charity Hospital	AC,GN,CZ	
88	30FMACI	Academy Charity Hospital	CO,GN,CZ	
89	72FMAC	Academy Charity Hospital	EY,AC,GN,CZ,AP,CP,LV,PN,RF,TT,TC,VN	
90	134FMAC	Academy Charity Hospital	AC,GN,CZ	
91	1FMD	Dar-Alelaj Specialized Hospital	EY,AC,CO,GN,CZ,TT	
92	133FMAC	Academy Charity Hospital	EY,AC,GN,CZ,RF	
93	131FMBI	Ibrahim Malik Teaching Hospital	EY,AC,GN,CZ,CP,LV,RF,TT	
94	148FMAC	Academy Charity Hospital	AC,CO,GN,CZ,TC,VN	
95	19FMBI	Ibrahim Malik Teaching Hospital	EY,CO,CZ,AP	
96	129FMBI	Ibrahim Malik Teaching Hospital	AC,GN,CZ,TT	
97	14FMD	Dar-Alelaj Specialized Hospital	GN,CZ,TT	G
98	113FMD	Dar-Alelaj Specialized Hospital	EY	

% Similarity