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Enterococcus faecalis a Major Cause of STD: A New Evidence

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ABSTRACT

The current study aimed to highlight the relationship of *Enterococcus faecalis* with sexually transmitted diseases (STD) as an uncommon cause of this infection. Thirty samples of urethral swabs were collected from male patients diagnosed by the specialist physician with sexually transmitted diseases according to clinical signs and history of the disease. The swabs were inoculated on the medium of blood and chocolate agar and diagnosed by classical methods, then confirmed using vitek-2 technique. The incidence of *E. faecalis* isolates was 16.6%.

This study also dealt with investigation of antibiotic resistance of bacteria to vancomycin, levofloxacin, erythromycin, and tetracycline depending on the minimum inhibitory concentration (MIC) using vitek-2 compact system, and the results showed that 20 %, 60 %, 80 % and 100 % of isolates were resistant to vancomycin, levofloxacin, erythromycin, and tetracycline respectively. This study concluded the existence of a relationship between this bacterium and STD by isolating it as the only cause in hypersexuality patients. 16S rRNA sequencing has been used for a more specific diagnosis, and the isolate designated as Enterococcus faecalis QaAm-IRAQ-1 was deposited in the GeneBank database under the accession number ON796012.1. The sequence similarity search using BLAST, phylogenetic analysis of the isolated bacteria was done by neighbor-joining (NB) method, the results showed that our isolate has a compatibility of 99% with many isolates including *E. faecalis* China, India, Japan, Germany and USA.

Keywords: *Enterococcus faecalis*, STD, Antibiotics test, Molecular diagnosis.

INTRODUCTION

Enterococcus faecalis is a gram-positive bacteria characterized by spherical oval shapes. It has individual, pairs, or chains arrangement. It was classified within genus Enterococcus in 1984 using DNA-DNA hybridize method. Researchers confirmed its role in many of human infection, including wounds infection, endocarditis, urinary tract infection, and teeth root canals infection. It possesses many virulence factors, including (gelatinase, extracellular surface proteins, cytolysin and hyaluronidase) which colonize the mucous membrane of the urethra and vagina for both men and women (Fisher and Phillips, 2009; Riedel *et al*., 2019).

The pathogenic mechanism of these bacteria relies on their ability to colonize the mucous membranes and then cause pathological changes in the host through toxic activity by inducing an inflammation process and avoiding the host's immune defense mechanisms (Weinstock *et al*., 2007).

There is a difference between the two terms, sexually transmitted infections (STIs) and sexually transmitted diseases (STDs) are often used interchangeably, STIs are infections that may develop into a STD. All STDs are preceded by STIs but not all STIs develop into a STD. People who have STIs may not show any symptoms or signs of the disease but can still be contagious and have the potential for the disease. (Ho, 2021)

E.faecalis also one of the most common pathogenic bacterial species in aerobic vaginitis, and it has been proven that its pathogenic effect causes spontaneous abortion, premature delivery, abscesses, and urinary tract infections (Jahić and Cerovac, 2022) as its presence in the digestive system and its transfer in various ways to other places can contaminate healthy skin. It may colonize the mucous membrane of the urethra (Fisher and Phillips, 2009)

Mainly recurrent urogenital infections are among the most common causes of infertility, especially those caused by sexually transmitted infections, urinary tract infections and imbalances and changes in the natural bacterial microbiota, the World Health Organization suggests improving sexual health and the need to provide treatment for sexually transmitted diseases (Rivera *et al*., 2022). Which include various pathogens such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, viruses such as Human immunodeficiency virus and Human papilloma virus, as well as parasites and fungi (Gimenes *et al*., 2014), in addition to other bacterial species such as *Staphylococcus aureus* and Streptococcus spp, which usually have negative effects on the density of spermatozoa chromatin (Zeyad *et al*., 2018). whereas, the bacteria that often colonize the anterior urethra may become potential causative agents for prostatitis and semen infections (Cardoso *et al*., 1998). The study aimed to highlight Enterococcus faecalis bacteria isolated from a urethral swab of a patient suspected of having a sexually transmitted disease as an uncommon causative agent of the disease

MATERIALS AND METHODS

Specimens

Thirty urethral swab specimens were collected from suspected sexually transmitted infected hypersexual males of age ranging from 20 to 45 years old, specimens were subjected to bacteriological and molecular tests.

Isolation and Diagnosis

Specimens were cultivated on both blood and chocolate agar and then incubated for 24 and 48 hours at 37 C^o with 10% $CO_{2,}$ the bacterial growth was diagnosed after incubation according to macroscopic and microscopic features.

The diagnosis was confirmed by performing about 64 biochemical tests by using vitek-2 compact system which is one of the fast and most accurate diagnosis systems.

Antibiotics Sensitivity Test

Bacterial antibiotics sensitivity was evaluated by using vitek-2 compact system with AST-P580 card which contains the following antibiotics: vancomycin, levofloxacin, erythromycin, tetracycline

Molecular Diagnosis

1- DNA was extracted according to Geneaid kit's instructions.

2- 16S rRNA gene was amplified by PCR technique using forward and reverse primers
F 5 – AGAGTTTGATCCTGGCTCAG -3
R 5 – GGTTACCTTGTTACGACTT -3
the following thermal run was done according to this program
initial denaturation at 95°C for 3 min, followed by 30 cycles: 95°C for 30 s and 55°C for 30 s and 72°C for 1:30 min. final extension 72°C for 3 min

Investigation of Gene Sequence

Investigation of gene sequence was performed in biotechnology laboratories/Korea,
The genetic homology was performed by using BLAST software.

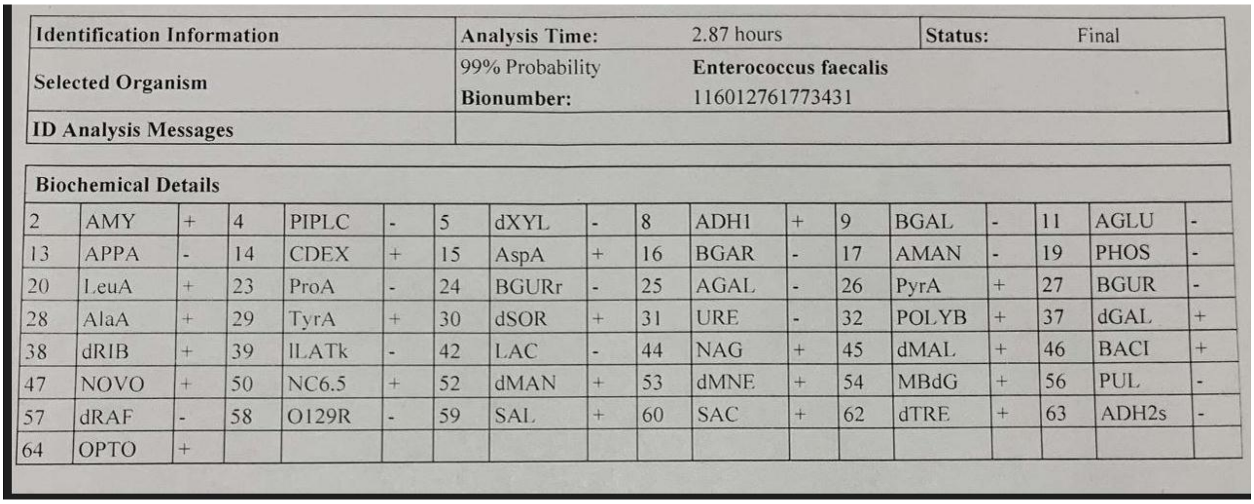
Phylogenetic Analysis

Neighbor Joining (NJ) algorithm within MEGA 6 software was used for production of the phylogenetic relationship and production of evolutionary tree.

RESULTS AND DISCUSSION

The results of cultivation depending on the macroscopic traits of colonies and pattern of hemolysis on blood agar exhibited small, white, transparent and non-hemolytic colonies with a total of 5 bacterial isolates with a ratio of 16.6%, Fig. (1) which appeared as gram positive cocci and diagnosed later as *E. faecalis* by using Vitek 2 compact system, Fig. (2).

Fig. 1: *E. faecalis* on chocolate agar



Identification Information	**Analysis Time:** 2.87 hours		**Status:** Final
Selected Organism	99% Probability **Bionumber:**	**Enterococcus faecalis** 116012761773431	
ID Analysis Messages			

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	+	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	+	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	ILATk	-	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Fig. 2: The results of vitek-2 system revealing the diagnosis of *E. faecalis*

The bacteria appeared as small, white or milky yellow on blood agar while under the microscope it appeared as cocci or ovoid cell shape arranged in pairs or chains of different lengths with the inability to produce spores and capsules (Jaafar, 2022).

The results of antibiotic sensitivity test for five strains of bacteria by using the automated vitek-2 system exhibited the resistance of bacterial isolates to a number of antibiotics where 100% of isolates were resistant to tetracycline, in the same time the resistance ratio to both erythromycin and levofloxacin varied and it was 80% and 60% respectively while the isolates showed a minimum resistance ratio to vancomycin that was 20%, Fig. (3).

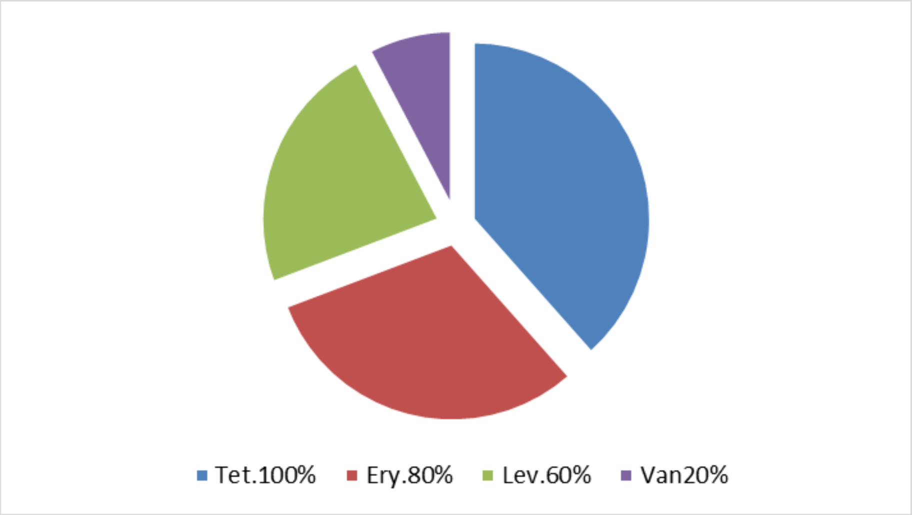


Fig. 3: Pattern of *E. faecalis* antibiotic resistance.

The ability of genitourinary diseases causing bacteria to colonize inside the epithelial cells of genitourinary tracts enables them to evade the antibiotics and immune system, therefore about 80% of recurrent genitourinary system infections are caused by the strain of the initial infection itself. (Ognenovska *et al*., 2022). The reason of bacterial resistance to levofloxacin and Erythromycin maybe due to the recurrent usage of antibiotics for treatment of genitourinary tract infections and other infections in the region of study. (Yilmaz *et al*., 2016)

In another study performed on *E. faecalis* and *E. faecium* in Kenia to determine the antibiotic sensitivity and the virulence factors and their relationship with clinical and demographic factors, the results exhibited a resistance to levofloxacin, vancomycin, tetracycline and erythromycin. in a ratio of 43.2%, 0%, 72.5% and 100% respectively. (Georges *et al*., 2022)

In two studies performed in Turkey, (Sibel *et al*., 2012) that include the evaluation of antimicrobial susceptibility of urine enterococci with the vitek-2 automated system and (Gök *et al*., 2020) that include the investigation of antibiotic resistance and virulence factors of Enterococcus strains isolated from clinical samples, our results were converged with the results of both studies regarding to Tetracycline. where 84.1% and 83.7% of isolates were resistant to it respectively while our results disagreed with both studies regarding Vancomycin. where 4.5% and 0% of isolates were resistant to it.

The low ratio of bacterial resistance to vancomycin that belongs to Glycopeptides antibiotics may be due to the exclusion of fecal isolates as the infectious isolates were only included as well as the study was confined on a limited number of isolates. (Gök *et al*., 2020; Ramos *et al*., 2020)

In a study regarding to asymptomatic urinary tract infection performed in Iraq, *E. faecalis* were isolated from 1.4% of males and diagnosed by using the standard biochemical tests as well as vitek-2 compact system where the ratio of bacterial resistance to both levofloxacin and erythromycin was 50% while didn't exhibit any resistance (0%) to both tetracycline and vancomycin (Al-Azawi and Abbas, 2020)

Semen is considered a transport medium for bacteria, where male reproductive system can be a reservoir for bacterial infection to the male himself or to the female reproductive system. (Viscarra *et al*., 2013). Opportunistic pathogens are associated with 68% of seminal fluid infections. (Emokpae *et al*., 2009)

Our results were agreed in the ratio of isolated bacteria with the results of (Bamberger *et al*., 2005) which dealt with the investigation of sexually transmitted infections in hematospermia patients where the ratio of *E. faecalis* isolation was 17%. While our study disagreed with the study (Rivera *et al*., 2022) about the relationship between sexually transmitted bacteria and microbiota and seminal quality of asymptomatic men where the targeted bacteria weren't isolated (0%).

After isolation and diagnosis by vitek-2system, molecular diagnosis was performed by PCR technique depending on 16S rRNA gene via the investigation of the results of DNA segment multiplication of the gene and performance of agarose gel electrophoresis. Electrophoresis results of the 16S rRNA gene showed a band indicating the amplified of the target gene Fig. (4).

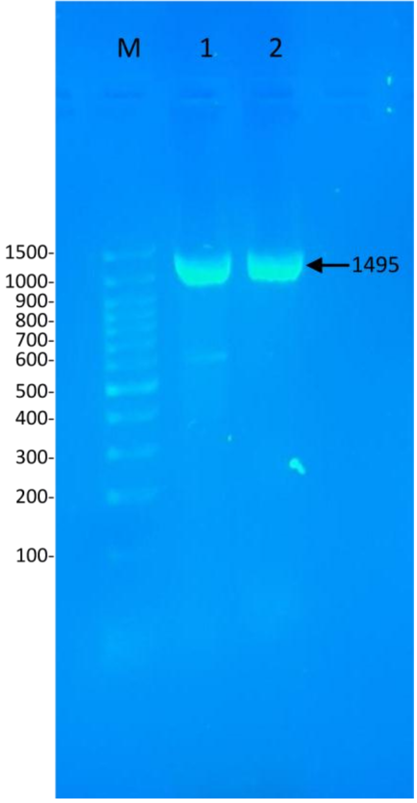


Fig. 4: Agarose gel electrophoresis showing amplification of 16s rRNA on1495 bp fragment using 1500bp DNA Ladder.

16S rRNA gene is playing an important role in the accurate diagnosis of bacterial isolates and discovery of new strains in the field of microbiology as its sequences have a great importance in slow growing, fastidious and rare bacteria as well as this test is more accurate than other diagnostic tests. (Woo *et al*., 2008)

Phylogenetic analysis

After the molecular diagnosis was confirmed by the investigation of 16S rRNA gene, its nucleotide sequence was determined and the results were compared within NCBI, and by using BLAST software the molecular results of isolated bacteria exhibited matching ratio of 99% *E. faecalis* USA and Germany Fig. (5) and (Table 1), the isolates were also registered in GeneBank with the name of *E. faecalis* strain QaAm-IRAQ-1 and the code ON796012.

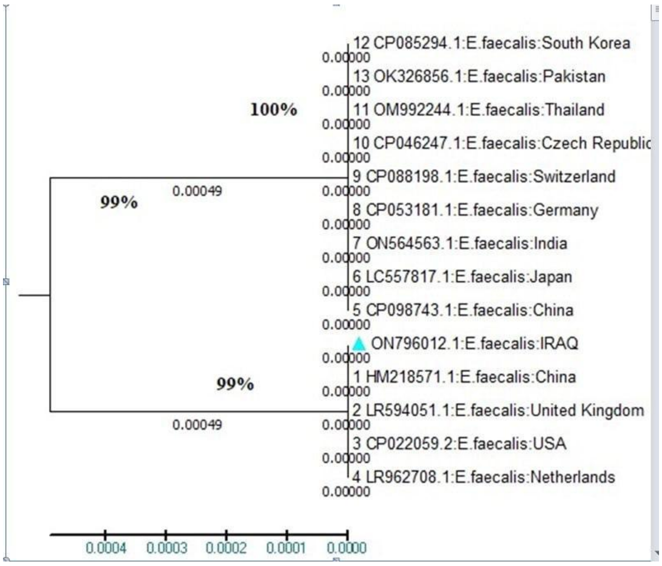


Fig. 5: Matching results of *E. faecalis* QaAm-IRAQ-1 by using BLAST software.

Table1: Matching ratios of *E. faecalis* QaAm-IRAQ-1 with diagnosed isolates which are registered in NCBI gene bank.

	Accession	Country	Source	host	Compatibility
1.	ID: HM218571.1	China	Enterococcus faecalis	fermented dairy products	99%
2.	ID: LR594051.1	United Kingdom	Enterococcus faecalis	-------------	99%
3.	ID: CP022059.2	USA	Enterococcus faecalis	Homo sapiens\urine	99%
4.	ID: LR962708.1	Netherlands	Enterococcus faecalis	-------------	99%
5.	ID: CP098743.1	China	Enterococcus faecalis	Chicken manure	99%
6.	ID: LC557817.1	Japan	Enterococcus faecalis	Homo sapiens\ hands, dyer	99%
7.	ID: ON564563.1	India	Enterococcus faecalis	cheese whey	99%
8.	ID: CP053181.1	Germany	Enterococcus faecalis	Gallus gallus\ choane	99%
9.	ID: CP088198.1	Switzerland	Enterococcus faecalis	Sus scrofa domesticus	99%
10.	ID: CP046247.1	Czech Republic	Enterococcus faecalis	Rhodnius prolixus	99%
11.	ID: OM992244.1	Thailand	Enterococcus faecalis	-------------	99%
12.	ID: CP085294.1	South Korea	Enterococcus faecalis	pig	99%
13.	ID: OK326856.1	Pakistan	Enterococcus faecalis	-------------	99%

CONCLUSION

The study concluded a high prevalence rate of *Enterococcus faecalis* isolation from the samples of patients with suspected sexually transmitted disease, and suggesting the bacteria as a new causative agent for this disease.

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المكورات المعوية البرازية سبب رئيسي للأمراض المنقولة جنسياً: أدلة جديدة

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MARA / ماليزيا

الملخص

تسلط الدراسة الحالية الضوء على علاقة جرثومة *Enterococcus faecalis* مع الامراض المنقولة جنسيا كمسبب غير شائع للإصابة، جمعت ثلاثين مسحة من احليل مرضى تم تشخيص اصابتهم بالأمراض المنقولة جنسيا بواسطة اطباء اختصاصيين اعتمادا على الاعراض السريرية وتاريخ المرض. تم زرع العينات على وسط اكار الدم المطبوخ وشخصت بالطرق التقليدية وتم تأكيد التشخيص باستخدام تقنية vitek 2 compact system. تم عزل جرثومة*Enterococcus faecalis* بنسبة 16.6%. تناولت هذه الدراسة ايضا التحري عن مقاومة الجراثيم لعدد من المضادات الحيوية وهي Vancomycin و Levofloxacin و Erythromycin و Tetracycline اعتمادا على التركيز المثبط الادنى باستخدام تقنية vitek 2 compact system حيث اظهرت النتائج ان 20%، 60%، 80% و100% من العزلات كانت مقاومة لكل من Erythromycin and Tetracycline, Vancomycin, Levofloxacin على التوالي. استنتجت الدراسة وجود علاقة بين هذه الجرثومة والامراض المنقولة جنسيا من خلال عزلها كمسبب وحيد من معظم العينات. شخصت الجرثومة باستخدام تقنية 16S rRNA كطريقة تشخيص اكثر تخصصية حيث تم تسجيل سلالة جديدة في بنك الجينات NCBI بأسم *Enterococcus faecalis* QaAm-IRAQ-1 كما تم استخدام نظام BLAST و طريقة NB(neighbor-joining) لتحديد التشابه في التسلسل الجيني و رسم الشجرة التطورية للجرثومة المعزولة و اظهرت النتائج عزلتنا الجرثومية متطابقة بنسبة 99% مع العديد من العزلات من ضمنها China, India, Japan,Germany and USA *E.faecalis*.

الكلمات الدالة: المكورات المعوية البرازية، الأمراض المنقولة بالاتصال الجنسي، اختبار المضادات الحيوية، التشخيص الجزيئي.