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Antimicrobial Resistant *Enterococcus* sp. Isolated from Clinical Samples

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Authors' contributions

This work was carried out in collaboration among all authors. Author FA did the implementation, conducted the study procedure, performed the study and drafted the manuscript. Author MJA conducted the data analysis. Author AS conceived, designed the study and supervised all laboratory procedures. All authors read and approved the final manuscript.

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ABSTRACT

The present study has been targeted towards; investigation of molecular epidemiology and analysis of antibiotic resistance in different bacterial sp. Total 120 new bacterial isolates has been obtained having majority of bacteria *Enterococcus sp*. from 4 regional hospitals of 92 patients. The antimicrobial susceptibility test has been performed using 18 different antibiotics and resistant strains have been analyzed. Additionally, the isolated strains were tested for antibiotic resistance and polymerase chain reaction (PCR) has been performed for van A and van B genes. In the series of antimicrobial bacterial species *Enterococcus sp*. has emerged as one of the potential cause to raise the healthcare problems. This study has significant impact on such kind of molecular epidemiology investigations and may be useful in producing the basic knowledge on the local microorganism to refine and resolve the antimicrobial resistance issues faced by hospitals in the world.

Keywords: Enterococcus sp.; antimicrobial resistance gene; multidrug-resistance.

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1. INTRODUCTION

Enterococci are common type of bacteria, generally found in the organ of both animals and humans. These bacteria are capable to grow in any kind of adverse climatic conditions. Additionally, these bacterial genera are responsible for different kinds of fermented foods having some special organoleptic properties [1,2]. Enterococcus are capable to tolerate different types of antibiotics and exhibittheir resistive nature towards drugs because of chromosomes, transfer of transposon acquisition or plasmids which contains sequence of genetics that create obstacle in growth of other microbes [3]. During last few years Enterococci have grown through different mutation process, resulting as the most prevalent nosocomial pathogens. It is responsible for many infections in community as well as hospital infections such as urinary tract infection, life threating bloodstream infections, meningitis and endocarditis [4,5].

Enterococci belong to the group of nosocomial pathogen which can be transferred from person to person. Moreover, it can transmit through contaminated environment and foods. Till today more than 50 different species of enterococcus have been identified [6]. Some common types of species found in human wounds are *Enterococcuus faecalis* and *Enterococcus faecium* whereas in plants and animals some common species are *Enterococcuus faecium, Enterococcuus cecorum, Enterococcuus faecalis* along with some *Enterococcuu shiraet* can affect wound, soft tissues and cause bacteremia, endocarditis or urinary tract [7,8,9].

In recent years *Enterococcuus faecium* has been found to be more resistance towards different drugs such as ampicillin (ARE), vancomycin (VRE) and aminoglycosides (HLAR) as compared to *Enterococcuus faecalis.* The nature of high drug tolerance capability is mainly achieved due to the horizontal gene transfer. Some of the common resistance found in *Enterococcus faecalis* and *Enterococcus faecium* are amino-glycosides, cephalosporins, sulphonamides and macrolides; also dalfopristin and clindamycin in *Enterococcuus faecalis* [10,11,12,13]. Some of the most common antimicrobials used against the multi drugs resistance enterococci infections are vancomycin, gentamicin and ampicillin, but tremendous application of these drugs developed a raise in quantity of vancomycin resistance (VRE), which induces serious risk issue. Enterococci are resistance towards antimicrobials drugs along with vancomycin (VRE) that perform the most important role in both, inter as well as intra species transfer of antimicrobial resistive genes [14-18].

Therefore, the motive of the present study is to perform genomic examination and susceptible nature of the antibiotic newly bacterial isolates namely *Enterococcus species.* This study also includes pathogenic evaluation efficiency between the effects of enterococci pathogen on hospital-to-hospital, person-to-person transfer as well as between different genders. The isolates were examined in order to check the availability of antimicrobial resistance gene and presence of virulence by using 18 different antibiotics.

2. MATERIALS AND METHODS

2.1 Sample Processing/Collection

During October 2018 to September 2019 total of one hundred and twenty positive *Enterococci spp*. samples were collected from the outpatients and hospitalized patients in different wards of King Khalid Hospital, Hail General Hospital. All patient identifiers were removed and isolates were serially numbered, only details of age, and sex were retained.

2.2 Culture Analysis and Species Identification

Stool specimens were plated on the surface of the bile Esculin Agar (BEA) medium (HiMedia, India), and incubated at 37°C for 24 hours; colonies with a brown halo and different morpho-types were randomly chosen and sub-cultured into Muller Hinton Agar. A presumptive identification of *Enterococcus* was made on the basis of colonial morphology, gram staining result, catalase production, growth in 6.5% salt broth, and litmus milk production.

Definitive identification was done with automated system MicroScan® (Siemens) to confirm the species identification and Antimicrobial susceptibility testing of *Enterococcus* isolated. The isolated samples were stored in BHI broth containing 20% glycerol and charcoal at −80°C in cryogenic vials for further analysis [19].

2.3 Antimicrobial Susceptibility Testing (AST) of Bacterial Isolates

The susceptibility to antimicrobial agent's AMP, Ampicillin; VAN, Vancomycin; CAZ, Ceftrazidime;

AML, Amoxicillin/Amoxycavulinic acid; ERY, Erythromycin; GEN,Gentamycin; CIP, Ciprofloxacin; CTR, Ceftrixone; PEN, penicillin; DAP, Daptomycin; LZ, Linezolid; RA, Rifampin; LVX, Levofloxacin; Strepsynergy; Synercid; Gentsyrine; TET, Tetracycline; and TPL, Teicoplanin; were performed using the disk diffusion method (Kirby-Bauer), according to the NCCLS guidelines. The plates were incubated at 37°C for 18–24 hours. The quality control strain used in our study will be ATCC, *E. faecalis* 29212.

2.4 DNA Extraction

DNA was extracted from VRE isolates using QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instruction.

2.5 Polymerase Chain Reaction (PCR) Amplification of Van A and Van B Genes

The PCR assay was performed in a total volume of 25 µl containing 10 mMTris-HCl (pH 8.3), 50 mMKCl, 1.5 mM MgCl2, 0.2 mM each deoxynucleotide triphosphate (dAT P, dCT P, dGT P, and dTT P), and 0.5 U of Taq DNA polymerase with the following primer F:5′-CATGAATAGAATAAAAGTTGCAATA-3′ and 5′-CCCCTTTAACGCTAATACGATCAA-3′ for amplification of van A and F: 5′-GTGACAAACCGGAGGCGAGGA-3′ and R: 5′-CCGCCATCCTCCTGCAAAAAA-3′ for amplification vanB gene. DNA amplification was carried out with the following thermal cycling profile: initial denaturation at 94°C for 5 min, 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min), and a final extension at 72°C for 10 min. *E. faecium* BM4147 (vanA-positive) and *E. faecalis* V583 (vanB-positive) were used as positive controls. PCR products were analyzed on a 1% agarose gel with 0.5 × Tris-borate-EDTA buffer. A 100-bp DNA ladder (New England Biolabs, Beverly, Mass.) was used as the molecular size marker. The gels were stained with gel red and photographed under UV light.

2.6 Statically Analysis

Resistance results were interpreted according to CLSI guidelines all the experiment has been performed in triplicate.

3. RESULTS

3.1 Demographic and Clinical Characteristics

All positive bacteria have been identified as hospital outpatient. Total 120 isolates have been isolated by classical culture and stain as mentioned in the material and methods section and confirmed using automated system MicroScan® (Siemens). In 59.50% of the cases, infection was community acquired, and 40.50% cases was hospital acquired. Isolates were identified from out patients suffered earlier with number of diseases mainly categorized in infection caused disease like, urinary tract infection (UTI), respiratory tract infection, pregnancy as well as gastrointestinal tract infection. It was also observed that the infection caused by isolates was also gender specific as the Male recorded more sensitive towards the isolate then female. In *Enterococcus* sp. isolated *E. faecium* was the most dominating specie having 89 colonies while *E. faecalis* 16-second dominating species following *E. duran/hirae* 8, *E. gallinarum* 5 and *E. casseliflavus* 2 [Fig. 1].

3.2 Antimicrobial Susceptibility and AMR Pattern Analysis

Among 120 isolated *Enterococcus sp.*, five strains are identified in which *E. faecium* is the most dominating sp. Total 18 antimicrobial drug were tested on these isolates and most of the isolates were mainly resistant for four antibiotics, namely, Ceftrazidime, Gentamycin, Amox/Clav. and Erythromycin. Whereas, the bacterial strains either showed sensitivity or intermediate effect [Fig. 2]. The majority of the *E. faecalis isolates* showed 88% MDR pattern on 18 used antibiotics whereas 100% resistant towards the antibiotic Ceftrazidime showed by all isolates of *Enterococcus* species. Further, antibiotic, namely, Vancomycin, Ciprofloxacin, Ampicillin, Daptomycin, Gentsyrine, levofloxacin, Linezolid, Pencillin, Rifampin, Strepsynergy, Synercid, Teicoplanin, Tetracycline showed majority of sensitivity on these drugs [Table 1]. In total 18 antibiotics with different patterns of resistance, sensitivity and intermediate effect, 89 species of *E. faecium* were recorded for 4 antibiotics and the most dominating pattern of resistance involved four antibiotics (Ceftrazidime, Gentamycin, Amox/Clav. and Erythromycin). Second strong pattern was observed in *E. faecalis* followed by *E. gallinarum* bacterial community [Fig. 2].

3.3 PCR Analysis of Vancomycin Resistance Genes

Identification of van A and van B were performed by PCR reaction for all resistance isolates for vancomycin. From the total number of all samples screened were found only 11 isolates (6.6%) resistance to vancomycin, 7 samples resistant to *E. faecium* (63.6%) and 4 resistance (36.4) to *E. faecalis*. From the clinical Samples that found resistant to vancomycin van A was the highest with percentage of detection of vancomycin resistant genes with total number of 8 (72.7%) isolates, and van B is the lowest by 3 (27.3%) isolates.

Table 1. Represents, antimicrobial susceptibility pattern of 18 antibiotics on *Enterococci species*

Bacterial Strains:	**Sensitive**					**Resistance**					**MDR**
E. faecium- A	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**strains**
E. faecalis-B											
E. gallinarum-C											
E. duran/hirae-D											
E. casseliflavus-E											
Erythromycin	19	4	0	6	2	45	8	3	0	0	A,B,C
Amox/Clav.	4	0	1	2	2	76	10	3	6	0	A,B,C,D
Ceftriaxone	28	5	2	3	2	26	3	2	3	0	A,B,C,D
Gentamycin	7	2	1	2	2	70	11	3	6	0	A,B,C,D
Ceftrazidime	9	1	1	2	2	71	13	3	6	0	A,B,C,D
Vancomycin	82	11	1	8	2	5	5	3	0	0	A.B,C
Ciprofloxacin	76	14	5	7	2	6	0	1	1	0	A,C,D
Ampicillin	77	13	5	8	2	10	2	0	0	0	A,B
Daptomycin	85	13	5	8	2	2	0	0	0	0	A
Gentsyrine	84	16	5	8	2	3	0	0	0	0	A
levofloxacin	76	15	5	6	2	4	0	0	0	0	A
Linezolid	79	11	5	8	2	0	5	0	0	0	B
Pencillin	77	11	5	8	2	10	5	0	0	0	A,B
Rifampin	53	7	2	4	2	19	4	0	3	1	A,B,D,E
Strepsynergy	76	14	1	8	2	11	2	4	0	0	A,B.C
Synercid	24	6	0	8	0	7	8	1	0	0	A.B,C
Teicoplanin	84	10	1	8	2	3	6	0	0	0	A,B
Tetracycline	71	10	5	8	2	16	5	4	0	0	A,B,C

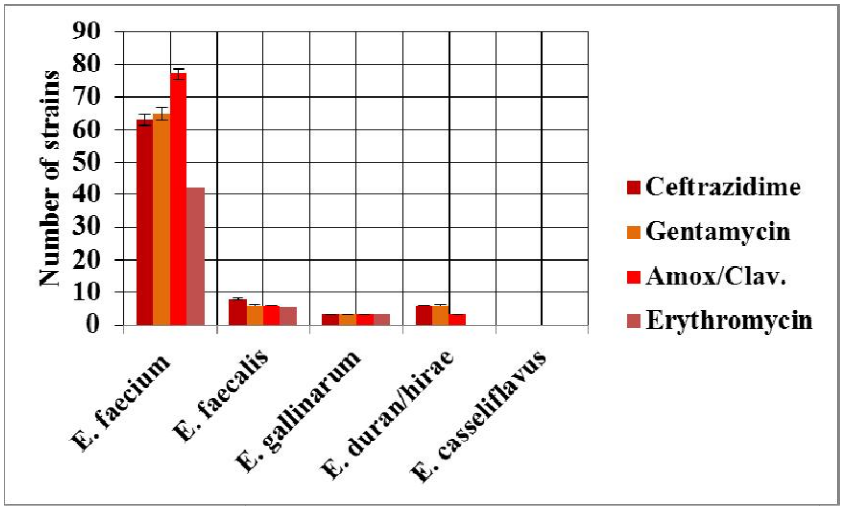

Fig. 1. Represents resistance impact of 4 antimicrobial drugs on different strains of *Enterococci sp.*

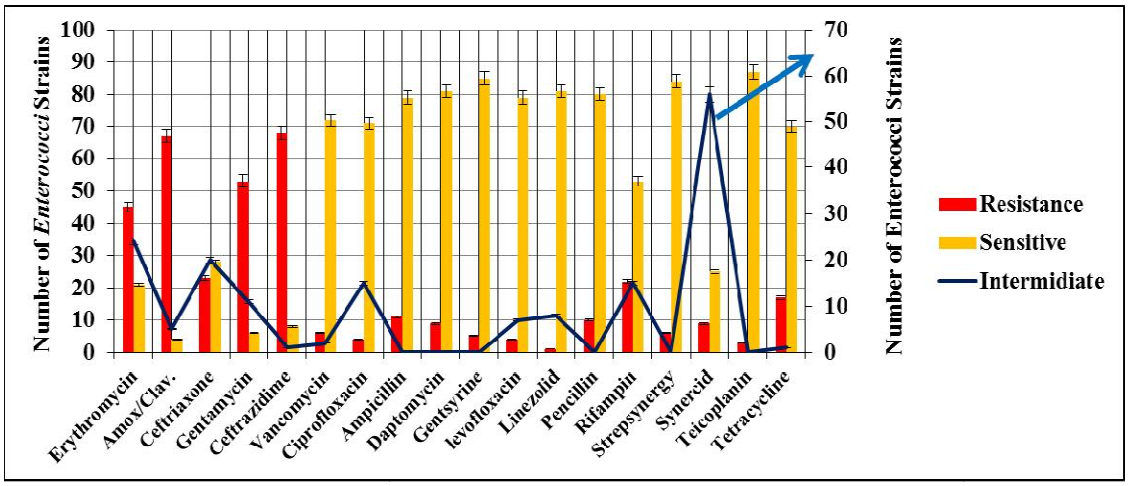

Fig. 2. Represents, impact of all 18 antibiotics on *Enterococci sp*., resistance, sensitivity and intermediate impact

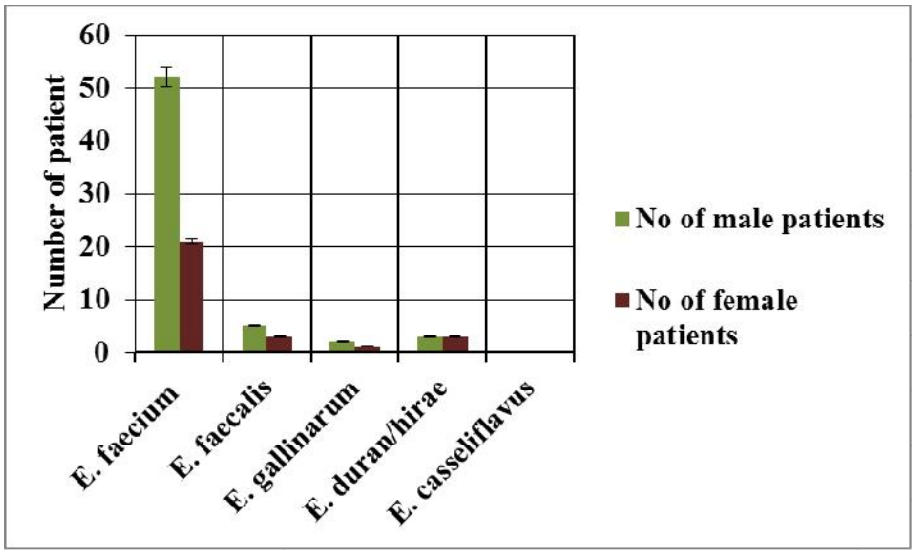

Fig. 3. Distribution of *Enterococci sp*. infection based on gender of patients

4. DISCUSSION

Following the trend worldwide enterococcus infections show increasing pattern in Saudi Arabia, and *E. faecalis* is one of the most common dominating species genus identified in isolated hospital-associated infections due to resistance to a broad range of antibiotics [20]. The tremendous increase in number consider as major performer challenge facing the health care system. Enterococcus has broad range to transfer and grow in human and animal through plasmids and transposons, which make them a thread to infection control authorities. Moreover, ribosomal inhibition, cell membrane permeability, interference with DNA and antimetabolite action is the huge category mechanismoption obtain by this pathogen to spread infection rapidly in surrounding of environment. In addition, this pathogen genus are flexible to grow in ubiquitous media, hence its adaptability is very easy to reach out the infections [21].

In one of the study from Saudi Arabia showed 21–25% resistance to high-dose gentamycin [22,23], high dose of which is highly concerning and given for treatment for enterococcus infections [20] and generally, gentamycin is used

as a synergistic antibiotic with other similar effect antibiotic for the microbial infection treatment [24,25], which we found in this study that *E. faecium* showing high resistance to gentamycin in hail region. The higher resistance of noted antibiotics has been confirmed. In contrast to a Saudi Arabia other studies also shows authenticity of the current study. Moreover, well-developed MDR Enterococcus sp. has been reported in many studies in Saudi Arabia [26]. According to one of the study the confirmed rate of resistance recorded by the National Healthcare Safety Network during 2009–2010 was around 6.2% and 9.8% for *E. faecalis* [27] in contrast to our study we found that *E. faecium* is more resistance than other species of Enterococcus. Apart from molecular mechanism, geographical area, environment surroundings and gender have shown significant impact on MDR pathogens [28].

E. faecalis isolate analyses in one of the study also possessed gyrA, and parC, genes showed resistance to quinolone groups of antibiotics namely ciprofloxacin, levofloxacin, and moxifloxacin which are frequently used for urinary and respiratory infections [29] which is not the case in our current study. In addition to thus, aminoglycoside-modifying enzyme genes (aac(6′)-Ie-aph(2″)-Ia as well as ant(6′)-Ia) were most commonly tracked in *E. faecalis* community which shows resistant to high-dose gentamycin and streptomycin [30].

In our study high Sensitivity was observed for gentsyrine, teicoplanin, strepsynergy, deptomycin, ampicillin, ciprofloxacin, vancomycin and tetracycline where other studies showed some of these antibiotics resistance to Enterococcus [26].

Formation of biofilm is one of the known strong properties *of E. faecalis*, which enhanced its pathogenicity and give it strength to hold against antimicrobial agents around 100- to 1000-fold more concentrations. Cho et al. (2019) isolated 637 strain and about 97% (445/458) of the samples were positive for enterococci. In these sample *enterococcus casseliflavus* (33.6 percent) is the predominant species followed by *Enterococcus faecalis* (26.5 percent) and *Enterococcus hirae* (13.2 percent). Regardless of the species, lincomycin (88.5 percent) and tetracycline (13 percent) were the highest levels of resistance; isolates also displayed resistance to newer antimicrobials, daptomycin (8.9%) and tigecycline (6.4%). Study showed that surface waters contain a large population of diverse *enterococci sp*ecies that are immune to antimicrobials including resistance to new antimicrobials [31].

The vancomycin resistant to *Enterococcus* sp. in our study is higher than other study conduct in Saudia Arabia [20]. Therefore measurements aiming at prevent the spread of VRE should applied as soon as possible including infection controls measurements and prescription of antibiotics which should focus on reducing use of glycopeptides and third generation cephalosporin. There are studies showed higher use of glycopeptides followed by an increase in VRE occurrence [31,32,33,34].

5. CONCLUSION

The study presents a layout of the impact of antimicrobial drug resistance properties in Enterococcus species. It was found that among total 120 newly isolated *Enterococcus species,* 87 *E. faecalis* isolates exhibited strong antimicrobial resistance toward the antibiotics namely, Ceftrazidime Gentamycin Amox/Clav. Erythromycin. Whereas 14 antimicrobial drugs either showed intermediate or sensitive impact on bacterial strains and can be consider for prescription. Regulations for prescription of antibiotic and frequent surveillance are needed to stop the threat of more antibiotic to be resistance.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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