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Review of multidrug sensitivity and resistance in *Enterococcus*

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Abstract
This study aimed to isolate and identificate *Enterococcus spp*. from human, dogs and cats, a total of 100 human samples group (G)1 including (100 nasal and 100 oral swabs) were collected from pet animal (dogs and cats) owners when visiting the Baghdad Veterinary Teaching Hospital for their animals, and stool samples were collected from another group as a group(G)2 from private laboratories. The samples were collected from both gender and of different ages. A total of 150 samples including oral, nasal, and fecal were collected from dogs (25) and cats (25) from both gender and different ages and breeds, the study was done from the 2ed Nov. 2021 to the 28th April. 2022. Antimicrobial resistance profile indicated that human *enterococci* isolates were 100% resistant to Ceftriaxone, Clindamycin, and Gentamycin, while 93.54% resistant to Erythromycin, Azithromycin and Doxycycline, MDR were 100% with high-risk of the isolates, Dogs isolates were resistant 100% against Cefitrixone, Clindamycin, and Gentamycin, 75% to Azithromycin and Doxycycline, MDR were 100% with high risk, cats isolates were resistant to Cefitrixone, Erythromycin, Azithromycin, Doxycycline, and Gentamycin. Most of the isolates 74% could form biofilm using congo-red method and crystal violate assay methods, 77.77% of them were weak, and 22.22% were moderate. *E. gallinarum* were recorded 27.7% of them were moderate and 72.3% were weakly formed, *E. faecium* were 75% weak, and 25% were moderate. Whereas, *E. faecalis* 85.71% were weak and 14.28% were moderate.

Keywords: *Enterococcus* spp., Antimicrobial resistance, Biofilm formation, Human and animal isolates

1. Introduction
Enterococci species spp. are Gram-positive cocci, non-spore-forming, facultative anaerobic lactic acid microorganisms, naturally found in the gastrointestinal tract of both human and animals (Escobedo-Hinojosa and Pardo-LópezL., 2017; Comerlato *et al.*, 2020) [19, 14]. In addition, *enterococci* spp. are one of the sources of contamination of food, especially antimicrobial strains (Dubin and Pamer., 2017; Zaheer *et al.*, 2020; Arias and Murray., 2012; Kamal & Al-haddad, 2022) [16, 55, 6, 28].
Recurrent and continues antimicrobial resistance (AMR) of opportunistic microorganisms is one of the serious public health risks.
Enterococci spp. Are often intrinsic resistant to antibiotics classes routinely used in treatment such as Cephalosporins, Macrolides, Sulfonamides, β lactams and Aminoglycosides. Furthermore, this genus can acquire resistance to several antibiotics horizontally by transfer genes, in the same time are able to transfer of antimicrobial-resistant (AMR) genes to other microorganisms (Hollenbeck and Rice., 2012; Werner *et al.*, 2013; Asgin and Otlu., 2020) [24, 52, 8]. In addition, prevalence of MDR among *enterococci* spp. has been reported worldwide, and is account as a major problems on public health (Ali *et al*., 2017; Castano-Arriba *et al.*, 2020; Kamal & Al-hadad, 2023) [3, 12, 27], and *enterococci* has the ability to form a biofilm that increased of infections with this bacteria, due to accorelation between biofilm production and both MDR and MAR index (El-Zamkan *and mohamed*., 2021; Oliva *et al.*,2021; Alzahrani *et al.*, 2022) [18, 38, 5]

Review
Taxonomy and Classification of *enterococci spp*
At the end of the ninth century in 1899, a saprophytic cocci bacteria recognize predominantly in the gastrointestinal tract of the diarrheal patient, after that this bacterium transmitted from

the intestine to the blood caused septicemia of intestinal origin and was described as a diplococci Gram-positive bacterium and named "Enterocoque" indicated to its morphology and intestinal origin. In the same year, a similar bacterium was detected in acute endocarditis in human and termed Micrococcus Zymogenes which now named *Enterococcus faecalis* (*E. faecalis*) **(**MacCallum and Hastings., 1899; Thiercelin., 1899; Mohammed, H. F., & Al-Gburi, 2023) [35, 46, 37].

Characteristics of the *Enterococci* genus

The genus of *enterococci* are positive for gram stain, appear as coccobacilli, oval or spherical, arranged in double or short chains of different lengths, non-capsulated, most of the spp are nonmotile, and the motile spp. are *E. casseliflavus* and *E. gallinarum* (Arias and Murray, 2012; Ch'ng *et al*., 2019; Mahmood *et al*., 2014) [6, 13, 36].

Enterococci are chemo-organotrophic, facultative anaerobic bacteria, and they can live in aerobic conditions, the optimum temperature for growth ranges between35-37°C. *Enterococci* can grow at PH 9.6, and 6.5% NaCl, and some spp. can growth at 45°C-62°C and even at low temperatures of 10°C, resistance to ethanol 22%, high concentration of bile 40%, and sodium dodecyl sulfate, and survive in drying environments thus can be found in hospital environments (Sedláček., 2007; Švec *et al*., 2009; Parija., 2012; Ch'ng *et al*., 2019; Al-Shammary, 2019) [42, 45, 39 13, 4].

Enterococci spp required media rich with nutrients for cultivation, in ordinary media the colonies are milky white, and some spp produce carotenoid pigments on this media such as *E. casseliflavus*, *E. sulfureus*, and *E. mundtii*. On blood agar, 1-2mm diameter colonies and alpha hemolytic (actually nonhemolytic; alpha hemolytic is due to peroxidase rather than hemolysin), some spp. produce beta hemolysis on horse, rabbit or human blood agar, but not on blood agar contain blood sheep, *E. durans* is beta hemolysis on even sheep blood agar; tiny and magenta colored colonies on MacConkey agar; black colonies on potassium tellurite agar. Selective media include Columbia colistin-nalidixic acid agar and Bile-esculin-azide medium. Biochemical reaction characteristics are negative for catalase, ferment sorbitol, sucrose, and mannitol, positive for the Pyrrolidonyl Arylamidase (PYRase) test, negative for the Christie–Atkins–Munch-Peterson (CAMP) test, and hydrolysis of esculin and arginine, while negative hydrolysis hippurate (Švec *et al*., 2009; Parija., 2012; Lebreton *et al*., 2014; Khalaf & Rejah, 2024)[45, 39, 34, 30].

Epidemiology of Enterococci

In Human

A number of *enterococci* infections and antimicrobial resistance *enterococci* spp. from diseased or healthy humans were reported. In the intestinal tract of ICU patients, *enterococci* were investigated, *E. faecalis* was at 31%, and *E. faecium* at 56% (BelloGonzalez *et al*., 2017) [11]. Different clinical samples from outpatient and inpatient, *enterococci* infection was 6.2%, and most of the isolates were from urine at 41.6%, wounds 25%, and blood at 20.8% (Yilema *et al*., 2017) [54]. Of UTIs patients *E. faecalis* was detected at 3.2% (Khalid., 2016) [32], another study found a high percentage of *E. faecalis* at 83%, and 17% of *E. faecium* from patients with UTIs.

In animals

Enterococci is one of the causative agents for mastitis in animals, it was identified in cows with clinical mastitis at 28% as *E. faecium*, *E. hirae*, *E. faecalis*, *E. gallinarum*, *E. durans*, and *E. raffinosus* (Kateete *et al*., 2013) [29], *E. faecalis* were detected in 22.3% in an outbreak of infective mastitis in sheep farm (Sanciu *et al*., 2013) [41]. *Enterococci* were at 30% in milk collected from cows with mastitis (Hamzah and Kadim., 2018) [23], from bovine mastitis and subclinical mastitis at 17.32% were investigated as *E. avium*, *E. faecalis* and *E. faecium* (Gao *et al*., 2018) [21], and from subclinical mastitic milk from goats and sheep, *enterococci* were identified as 73.33% from sheep and 39.47% from goat milk, *E. faecalis* was the predominant spp. followed by *E. faecium* (El-Zamkan and Mohamed., 2021) [18].

Antimicrobial resistance

Enterococci spp. have a range of intrinsic and acquired resistance genes leading to development of an intrinsic and acquired resistance to drugs and these genes may transfer to other microorganisms, most antibiotics that *enterococci* are resistant to are β-lactam antibiotics, lincosamides, Cphalosporins, Streptogramins and Aminoglycosides. Acquired resistance exists to Macrolides, Tetracyclines, Glycopeptides (Vancomycin), Linezolid and Chloramphenicol (Hollenbeck *et al*., 2012; van Harten *et al*., 2017; Torres *et al*., 2018; Fiore *et al*., 2019) [23, 49, 47, 20]. Resistance to Erythromycin was detected in human clinical in 1951; and resistance in *enterococci* from animals decreased spectacularly after the ban (Aarestrup *et al*., 2001; Atiyah & Hamood, 2021; Elaywe, 2007) [1, 9, 17].

High Gentamicin resistance in *enterococci* isolated from animal origin was first reported in Denmark in 1998, and in the United States in 2001 (Bager and Emborg., 1998; Donabedian *et al*., 2003 Khalaf & Rejah, 2024) [10, 15, 31]. Although the family of Tetracyclin included several active antibiotics and was mostly used in treatment, resistance to these family were reported in many studies (Roberts., 2005) [40]. Quinolones and Fluoroquinolones have less antimicrobial to *enterococci*, and Levofloxacin and Moxifloxacin the most active compounds, acquired resistance is occured due to mutations genes (Arsène and Leclercq., 2007) [7].

Resistance *enterococci* against Linezolid has emerged in human and animals, in some cases the resistance mechanisms developed during treatment with Oxazolidiones, as well as, nosocomial transmission of Linezolid resistant *enterococci* such as *E. faecalis* and *E. gallinarum* were reported (klare *et al*., 2015) [33].

E. faecium were detected highly resistance against Erythromycin 96%, Trimethoprim 67%, Tetracycline 57%, and Gentamicin 55% (Wajda *et al*., 2022) [51]. *E. faecalis* isolated from urine reported resistance to HLG 3.1%, HLS at 53% (Shridhar and Dhanashree., 2019) [3]. *E. faecium* was high resistance to Erythromycin 88.9%,Gentamicin 77.8%,Amoxicillin-Clavulanate 63.9%,Ofloxacin at 44.4%,Teicoplanin 19.4% and Vancomycinat 16.7%, while *E. faecium* was resistance to Teicoplanin at 27.7%, and Vancomycin at 13.8% (Adesida *et al*., 2017) [2].

High levels of resistance of *enterococci* isolates against Streptomycin 94.1%, Neomycin 90.2%, Gentamicin 68.6%, Enrofloxacin 74.5%, Ciprofloxacin 66.7%, Oxacillin 98%, Clindamycin 84.3%, Tetracycline 78.4%, and quinupristin–dalfopristin 78.4%, Vancomycin 13.7%, Teicoplanin 3.9% and Amoxicillin/clavulanic acid 11.8%, MDR was 86.27% (Stępień-Pyśniak *et al*., 2021) [44], and MDR was detected at 32.14% of human, and 43.14% of turkey *enterococci* isolates (Woźniak-Biel *et al*., 2019) [53].

Enterococci isolated from animals were MDR, resistance to Oxacillin at 89.2%, Vancomycin 75.7%, and Lineozolid at 70.3% (El-Zamkan and Mohamed., 2021) [18], and that isolated from human, dogs and cats were resistance against Ampicillin 18.7%, Amoxicillin/clavulanate 12.5%, Erythromycin 25%, Tetracycline 6.2%, Ciprofloxacin 6.2%, Teicoplanin 4.2%, Vancomycin 6.2% (Iseppi *et al*., 2020) [25]. *E. faecalis* detected from the dogs and cats were resistant against Erythromycin 96%, Ciprofloxacin 93%, Gentamicin 29%, Kanamycin at 33%, Streptomycin 24%, and MDR at 78% (Trościańczyk *et al*., 2021) [48]. Výrostková *et al*. (2021) [50] reported a high resistance of *E. faecalis* to Rifampicin 100%, vancomycin 85.7%, Teicoplanin and Erythromycin 71.4%, Minocycline and Nitrofurantion at 57.1%, Ciprofloxacin and Levofloxacin at 14.3%. Also *enterococci* were high resistant against Clindamycin 100%, Linezolid 91.6%, Teicoplanin 91.6%, Erythromycin 87.5%, and Tetracycline 29.1% (Hammad *et al*., 2022) [22].

Conclusion

1. Isolated enterococci from humans and their pet animals were high antibiotic resistance, MDR and high risk.
2. All isolates from humans, dogs and cats showed absolute resistance to same antimicrobial (Ceftrixone, Clindamycin and Gentamycin).
3. Enterococci spp. exhibit significant resistance to multiple antibiotics, including β-lactams, aminoglycosides, macrolides, tetracyclines, and glycopeptides. Their ability to acquire and transfer resistance genes poses a major public health concern. High resistance rates have been observed in both human and animal isolates, with increasing multidrug-resistant (MDR) strains. Effective antimicrobial stewardship and surveillance are essential to control the spread of resistant enterococci and preserve treatment options.

Conflict of Interest

Not available

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Not available

Reference

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