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Antibiotic resistant Enterococcus species isolation and characterization in poultry complemented by an investigation of their virulence genes and disease occurrenc

Ahmed, Shabaan; Mai, M. Morsy; Shaimaa, H. Shaltot and Amera, F. Ebrahem

Reference laboratory for veterinary quality control on poultry production (RLQP),
Animal Health Research Institute (AHRI),
Agriculture Research Center (ARC), Egypt.

Review Article

Corresponding author:
Ahmed Shabaan
ahmedshabaansaad@gmail.com

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Abstract

Enterococci are Gram-positive cocci that can tolerate anaerobic conditions and do not produce spores. They can grow at 45 °C and hydrolyze aesculin in the presence of 40% bile and 6.5% sodium chloride. Some strains may be motile. The ideal growth temperature is 35-37°C, but some can grow at 45°C and 10°C. Enterococci are resistant to drying, lack oxidase activity, and ferment carbohydrates. They are leucine aminopeptidase (LAPase) and PYR positive. They can thrive in harsh environments and can be found throughout the body. Enterococcus, a genus of intestinal commensals, is related to the other streptococci but classified separately. The presence of antibiotic-resistant strains is still a global health concern. The primary Enterococcus species linked to poultry infections causing economic loss include *E. cecorum, E. faecalis, and E. faecium.* The most common diseases associated with these cases were locomotor abnormalities (mostly involving *E. cecorum),* septicaemia (including *E. cecorum* and *E. faecalis*), and omphalitis (primarily involving E. faecalis). Enterococcus strains with virulence factors can cause more severe infections than strains without them. Factors that influence enterococcal virulence include enterococcal surface protein (esp), gelatinase (*gel*E), hyaluronidase (*hyl),* ace, aggregation substance (*agg*), cytolysin (*cyl*A, *cyl*L), and biofilm formation. Enterococcus-associated diseases (EADs) on poultry farms in Egypt are limited, but a wide variety of enterococcal species, rising in prevalence, antibiotic resistance, virulence factors, and associated diseases affecting poultry production have been described worldwide.

Keywords: *Enterococci, antibiotic, resistant, virulence factors, E. cecorum, E. faecalis, and E. faecium.*

Introduction

Enterococcus spp. are facultative anaerobic lactic acid bacteria that are Gram-positive, non-spore-forming, catalase-negative. **Comerlato *et al.*, (2020)**. Enterococcus spp. are opportunistic pathogens that are commonly found in chickens digestive tract and can evolve into strains that can cause a variety of illnesses **Ribeiro *et al.*, (2023).** they can be present in the proventriculus, gizzard, and crop of the chicken (the proximal portions of the digestive tract). **Videnska *et al.*, (2013).** Enterococci are commensal gut bacteria that cause opportunistic infections in both humans and animals. Enterococci are prevalent in broiler accounting for 0.4% of all reported disorders, and are anticipated to climb to 12.9% by 2020. **Souillard *et al.,* (2022).** They are frequently employed as markers of fecal contamination and integrated hygiene standards for water and food products because they favor intestinal environments and are widely distributed, resilient, and simple to grow **Ramos *et al.*, (2020).** Although multiple researches have been unable to prove vertical transmission from breeder flock, the method of transfer is most likely a combination of vertical and horizontal transmission **Robbins *et al.*, (2012).**

On the other hand , antimicrobial-resistant bacteria can spread through the consumption of tainted animal source food, close animal contact, or environmental exposure **Ribeiro *et al.*, (2023).** Enterococci are widely known for their probiotic potential in birds including poultry. However, the presence of antibiotic resistant strains of enterococci remains a global health concern **Isichei-Ukah *et al.*, (2024).** Many studies indicated that young birds were already a source of multidrug-resistant bacteria in the post-hatching period **Jimenez-Belenguer *et al.*, (2016); Stępień-Pyśniak *et al.*, (2016); Moreno *et al.*, (2019). Stepięn-Pysniak *et al* (2021)** showed that about 80% of *E. faecalis* isolates considered as multidrug-resistant.

The presence of virulence factors does not necessarily imply that the strains isolated from food cause diseases in humans, but they may have pathogenic potential because these factors have been shown to contribute to the severity of infection. Enterococcus strains with virulence factors induced a more serious infection than those without virulence factors. **Chajęcka-Wierzchowska *et al.* (2017)**. Poultry were hosts to various Enterococcus species with virulence factors that can be transferred to other organisms and are also significant for causing nosocomial infection. **Jung *et al.*, (2017).**

There is little information available regarding the epidemiology of *Enterococcus*-associated disease (EADs) on poultry farms in Egypt, despite the fact that a wide variety of enterococcal species, rising in prevalence, antibiotic resistance, virulence factors, and associated diseases impacting poultry production have been described worldwide. This review aims to provide insight on enterococcus in poultry farms.

1– **Isolation and identification**

Enterococcus spp are growing in 6.5% NaCl, 40% bile salts, and 0.1% methylene blue milk at pH 9.6. It grows at 10 and 45°C and resists 30 min at 60°C according to **Schleifer and kilpper (1984).**

Because of the prevalence of enterococci in various meals, feeds, clinical and environmental samples, a variety of media have been reported and proposed. Atypical media are often utilized, although they produce pale or slightly red colonies due to the presence of bile salt and crystal violet **The Food and Drug Administration (2002)**. Typical media are Two complex cultural media are more commonly used: first, Kanamycin Aesculin Azide (KAA) agar, enterococcus appears as a black colony due to Esculin hydrolysis **Mossel *et al.* (1978),** and second, Slantez and Bartleys (SB) agar, enterococcus colony appears red or marron due to reduction of triphenyltetrazolim chloride, **Slanetz and Bartley's (1957).** biochemical identification of *enterococcus* according to (**manero and blanch 1999**). is, positive aesculine , positive pyrrolidinyl arylamides and catalase negative,

Prevalence of enterococcal species

The principal *Enterococcus* species linked to poultry infections include *E. cecorum, E. faecalis, and E. faecium* **Dolka *et al.*, (2017).** The principal diseases connected with these cases were locomotor abnormalities (mostly involving *E. cecorum*), septicaemia (including *E. cecorum and E. faecalis*), and omphalitis (primarily involving *E. faecalis*) **Souillard *et al.,* (2022).**

Less prevalent Enterococcus species have also been shown to cause bacterial diseases in chickens. *Enterococcus hirae. Enterococcus durans, Enterococcus gallinarum, Enterococcus casseliflavus, and Enterococcus avium* have previously been identified in chicken diagnostic cases **Dolka *et al.*, (2017).** *E. cecorum* was originally identified as a poultry pathogen in 2002, including disease reports from Scotland **Wood *et al.,* (2002)** and the Netherlands **Devriese *et al.*, (2002).***E. faecalis* and *E. faecium* emerged for the first time in 2006. **(Souillard *et al* 2022)**

Hayes *et al.,* (2004) surveyed 82 poultry farms for Enterococci detection. 53% of isolates were

Enterococcus faecalis, followed by 31% by E. faecium. Multi-resistant antimicrobial phenotypes were observed among all species, with E. faecalis being more resistant to lincosamide, macrolide, and tetracycline antimicrobials, fluoroquinolones, and penicillins.

Isichei-Ukah *et al.,* (2024) collected 60 samples from five poultry farms and identified 32 Enterococcus isolates with a percentage of 53.3%. The most predominant Enterococcus spp were *E. faecium*, followed by *E. faecalis*, and the lowest prevalence were *Enterococcus casseliflavus* and *Enterococcus hirae*.

2- Enterococcus-associated disease (EADs)

- In chicken, *Enterococcus faecalis* and *Enterococcus faecium* are the dominant enterococcal intestinal microbiota in day-old chicks, while *Enterococcus cecorum* is more prevalent in adult layers and broilers. These bacteria cause omphalitis in young chicks due to faecal contamination or aerosol or oral transmission. Broilers are mainly affected by *E. cecorum*, leading to paralysis, lameness, and mortality. *E. cecorum* is involved in various loco-motor diseases, while *Enterococcus hirae* is associated with septicaemia, endocarditis, osteomyelitis, or brain necrosis in broilers and psittacine birds. *Entererococcus durans* has been isolated from various poultry organs, particularly from young chickens showing neurological signs. **Braga *et al.*, (2018), Jung *et al.*, (2018), Kolbjørnsen *et al.*, (2011), Chadfield *et al.*, (2005).**

- In Turkey, *E. faecalis* and *E. faecium* may cause omphalitis in young poultry **Sander *et al.*, (1998)** owing to Skin wounds contamination, such as after vaccination of pullets for Marek's disease or fecal contamination **Landman *et al.* (2000).** *E. faecalis* has been linked to hepatic granuloma lesions in turkeys, geese, and racing pigeons **Jung *et al.*, (2014); Dolka *et al.*, (2017).**

- In ducks, *E. faecalis* has been linked to arthritis **Bisgaard, (1981); Souillard *et al.* (2022)**. *E. faecium* has been linked to mortality and septicaemia in Pekin ducklings **Sandhu, (1988); Souillard *et al.* (2022).**

3- Pathogenesis of infection

After an oral infection, pathogenic Enterococcaceae (EC) strains colonize the intestines of broiler chickens and spread to the bloodstream, causing septicemia and organ damage. EC can also colonize osteochondrosis dissecans (OCD) lesions, causing lameness and paresis. This process is influenced by virulence factors, caecal microbiota composition, and the intestinal barrier **Borst *et al.,* (2017), Schreier *et al.,* (2022).**

4-Virulence factors

The presence of virulence factors does not necessarily indicate that the strains isolated from food cause diseases in humans; however, these factors have been shown to contribute to the severity of infection. Enterococcus strains with virulence factors caused a more severe infection than strains without them. **Chaje˛cka-Wierzchowska *et al.* (2017**). These bacteria can survive for several months in challenging conditions, including severe pH (4.8 to 9.6), temperature (10°C to 45°C), and high NaCl concentrations (up to 6.5%), **Torres *et al.*, (2018).** When 40% bile salts are present, the majority of them can hydrolyze esculin; this ability is used as method for phenotypic identification. **Lebreton *et al.*, (2014).** Enterococcal virulence is determined by the presence of specific factors such as enterococcal surface protein (*esp*), gelatinase (*gel*E), hyaluronidase (*hyl*), enterococcal surface adhesion (ace), aggregation substance (*agg*), cytolysin (*cyl*A, *cyl*L), and biofilm formation **Fiore *et al.*, (2019).** Gelatinase production is one of the most common virulence factors in enterococci, and foodborne enterococci can synthesize gelatinase at similar levels as clinical isolates **Graham *et al.*, (2020).** The *Gel*E gene can hydrolyze host extracellular matrix proteins (gelatine, elastin, collagen, and hemoglobin). However, our findings indicated that some isolates containing *gel*E did not exhibit phenotypic gelatinase activity. **Zarzecka *et al.* (2022).** The asa1 gene encodes an enterococcal surface protein known as Aggregation substance (AS), which facilitates bacterial conjugation by forming bacterial aggregates. **Dubin and Pamer, (2017).** The esp gene has been shown to be strongly related with biofilm development in enterococci. Furthermore, it has been demonstrated that biofilm development plays a crucial role in the sharing of antibiotic resistance genes between cells and in building resistance to antibiotics. **Chajęcka-Wierzchowska *et al.*, (2017).** Cytolysin is a bacteriocin-type exotoxin that lyses erythrocytes, leukocytes, and macrophages. The majority of the isolates (69.6%) possessed the gel E gene, indicating the infections potential pathogenicity. **Sava *et al.*, (2010).** The isolates also expressed virulence characteristics (haemolysin, gelatinase, biofilm formation) and virulence genes (asa1, cylA, gelE**). Ngbede *et al.*, (2017).** There were 283 isolates from broiler that tested positive for virulence genes. Only the virulence genes gelE, asa1, esp, and cylA were found. Gel E was the most common virulence gene (234, 69.6%), followed by asa1 (160, 47.6%), esp (37, 11%), and cylA (2.0.6%). **Aslantaş *et al.*, (2018).**

5- Metal tolerance among Enterococcus spp.

Metal tolerance (MeT) operons, including arsA, merA, and tcrB, are acquired on mobile genetic elements that also carry antibiotic resistance genes (ABR). The incidence of MeT genes among Enterococcus spp. is low, and their genetic diversity and phenotype have been investigated in a large collection of Enterococcus spp. and available genomes recovered during the last 120 years. The plasmid-acquired tcrYAZB operon, which is associated with Cu efflux and tolerance, has been the most researched MeT element among Enterococcus spp., although genetic evidence demonstrating tolerance to other important metal pollutants, such as Hg or As, is extremely scarce in this species. Copper, a key component in chicken diets, has been employed as a cofactor for metabolic enzymes and gut bacteria in broilers **Rebelo *et al.*, (2021).**

Copper is used in poultry diets for tissue and bone development and metabolic enzymes. Enterococcus with Cu tolerance genes, tcrB ± cueO, are primarily found in feces. The prevalence and load of these Enterococcus vary throughout chickens lifespan in flocks supplemented with trace mineral diets.**(Rebelo *et al.*, 2023).**

6- Antibiotic resistant

Antimicrobial resistance (AMR) is a global health crisis, with migratory birds potentially being a source for antibiotic-resistant bacteria. A study in Bangladesh found that Enterococcus spp. were found to be resistant to ampicillin, streptomycin, meropenem, erythromycin, and gentamicin, with all isolates showing multidrug resistance. This suggests that migratory birds traveling to Bangladesh could carry and spread antibacterial resistant (ABR) bacteria, potentially posing public health risks. **Islam *et al.,* (2021).** while ,in Poland and the Netherlands, E. faecalis was found to be highly multidrug resistant, with resistance to antibiotics from tetracycline (category D) , lincomycin, erythromycin, aminoglycosides, and chloramphenicol (category C), and ciprofloxacin(category B). No resistance was observed to penicillin, ampicillin, gentamicin, tigecycline, or linezolid **Stępień-Pyśniak *et al.,* (2021). In Egypt, Ahmed *et al.,* (2020)** Recorded that the *E.feacalis* showed the highest resistance, followed by *E.faecium*, *gallinarum*, and *avaim* to teicoplanin and vancomycin and Vancomycin resistance genes were found in 14 isolates. On the other hand in Abu Dhabi Emirate. All isolates isolated from chilled chicken carcasses did not show resistance to ampicillin, teicoplanin, vancomycin, or tigecycline **Habib *et al.,* (2022).** In Lusaka and Copperbelt provinces of Zambia, 308 isolates showing resistance to tetracycline, erythromycin, quinupristin/dalfopristin, ampicillin, vancomycin, linezolid, ciprofloxacin, nitrofurantoin, and chloramphenicol. The prevalence of enterococci resistant to at least one antibiotic was 99.4%, with 86% being MDR. **Mudenda *et al.,* (2022).**

All species exhibited multi-resistant antimicrobial phenotypes, with *E. faecalis* being more resistant to lincosamide, macrolide, and tetracycline antibiotics, fluoroquinolones, and penicillins**. Hayes *et al.* (2004)**. Also, **Noh *et al.* (2020)** discovered that *Enterococcus faecalis* has developed resistance to multiple antimicrobials, with tetracycline being the most resistant, followed by doxycycline and erythromycin **Alzahrani *et al.* (2022)** divided *Enterococcus* spp. into four groups: *Enterococcus faecalis* (50%), *Enterococcus faecium* (33.33%), *Enterococcus hirae* (13.33%), and *Enterococcus avium* (3.33%). The isolates were highly resistant to tetracycline (55.6%), erythromycin (31.1%), and ampicillin (30%). However, all of the isolates were sensitive to linezolid. **Yu *et al.* 2022** discovered that 61 *E. faecalis* strains had drug resistance rates ranging from 96.72 to 8.20% against 11 antibiotics in 5 classes

Conclusion

Enterococci grow in hard environments and are similar to other streptococci, although they are categorized differently. Streptococcus and enterococcus differ in biochemistry, serology, and genetics. Specifically, enterococcus can grow in 6.5% NACL, bile esculin, arginine, and pyrrolidinyl arylamides, but streptococcus cannot. Streptococcus are categorized according to Lancefield classification, which is based on carbohydrate on the surface of bacteria , into groups A (*streptococcus pyogens*), B (*streptococcus agalactiae*), C (*streptococcus equi),* and D (enterococci). A genetically distinct 16 s rRNA sequence. Enterococci strains containing virulence factors can cause more severe infections, including locomotor abnormalities, septicaemia, and omphalitis. More study is needed on pathogenicity in vitro and in vivo, as well as molecular features and prevalence rates of Enterococcal-associated infections on poultry farms on Egypt.

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