Cairo University
Faculty of Veterinary Medicine
Department of Microbiology

Isolation and identification of *Enterococcus* spp from chicken

A Thesis Presented by

Shimaa Hamdy Okasha Ahmed

(B.V. Sc., Faculty of Veterinary Medicine, 2006, Cairo University)

For

Master Degree in Veterinary Medical Science,
Microbiology
(Bacteriology, Immunology and Mycology)

Under the Supervision of

Prof. Dr.
Nashwa Abd El-Salam
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Supervisors sheet

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Abstract:

This study was undertaken to estimate the occurrence of *Enterococcus* isolates recovered from broilers in Egypt showed different nervous manifestations such as torticollis (bent necks) and head tremors with signs of septicemia in post mortem investigation as enlarged, flaccid heart with hemorrhagic patches in the myocardium and focal granulomas could be found in many tissues as a result of septic emboli. Method: A total of 60 broilers internal organs (liver and spleen *n = 30* for each) was collected from broiler carcasses. Results revealed that the occurrence rate of *Enterococcus* positive samples was 18.3% (11/60). 6 out of the 11 isolates (54.6%) were *E. faecalis*, while 5 (45.4 %) were *E. faecium*.

Conclusion: The present study suggests that *Enterococcus* species could infect broilers and should be taken in consideration as an important bacterial pathogen affecting poultry.

Key words: *Enterococcus,* Prevalence, Broilers

ACKNOWLEDGMENT

From the beginning, it was clear to me that it was not what I could do, rather this is about what Allah wants me to do and how He will enable me to do it. So, gratitude thanks to our merciful Allah, who gives us the wishes and its achievement. This work would have never been accomplished without our Allah almighty and His blessings.

Thanks Allah, the merciful and the passionate, for providing me the opportunity to be able to step strong and smooth in this way.

I have also been supported and supervised by very kind professors to whom I would like to express my deepest gratitude; especially thanks to ***Prof. Dr. Nashwa Abd El-Salam Ezz El Din****, professor of Microbiology, Faculty of Veterinary Medicine, Cairo University, who shared me with a lot of her expertise and research insight. I cannot express my sincere thanks and heartfelt gratitude to her for his involvement, support, patience and enthusiasm.*

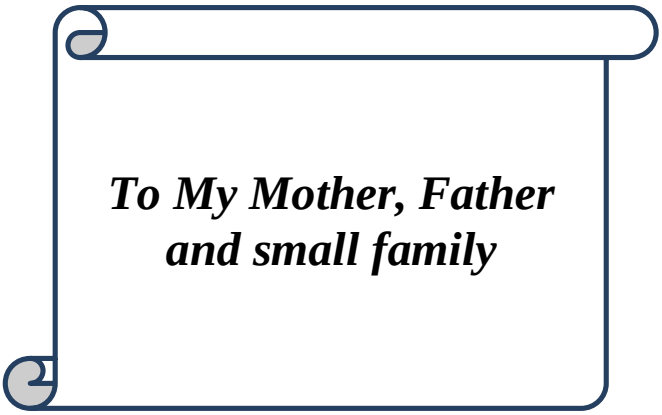
My sincere appreciation goes to ***Prof. Dr. Khaled Farouk EL Amry,*** *Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University for his help and support.*

Furthermore; thanks to the staff members of the Department of Microbiology, Faculty of Veterinary Medicine, Cairo University and all workers in this Department for their help and support to finish this work.

Special acknowledgement

For ***Dr. Ahmed Samir,*** *Assistant professor of Microbiology, Faculty of Veterinary Medicine, Cairo University , really there is no words will give him his value and effort in this study although he is not one of the study supervisors, he helps me in each step literally, my deepest greetings and appreciation for him. Allah blesses him. Really I thanked him a lot.*

DEDICATION



Who have provided never-ending support and taught me the value of a life dedicated to continued learning

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List of Abbreviation

AMP	Ampicillin
AST	Antibiotic Sensitivity Test
C	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical & Laboratory Standards Institute
E	Erythromycin
EDTA	Ethylene Diamine Tetra Acetic acid
CN	Gentamicin
ITS-PCR	Internal Transcribed Spacer-PCR
MDR	Multi Drug Resistant
MIC	Minimal Inhibitory Concentration
MRE	Multi-drug Resistance Enterococci
PBPs	Penicillin-Binding Proteins
PCR	Polymerase Chain Reaction
PBS	Phosphate Buffer Saline
PYR	Pyrrolidonyl Arylamidase
RD	Rifampin
TAE	Tris Acetate EDTA
TE	Tetracyclin
TS-YE	Tryptic Soya agar with Yeast Extract
Va	Vancomycin
VDE	Vavcomycin Dependant Enterococci
VRE	Vancomycin Resistant Enterococci
UTIs	Urinary Tract Infections

1.Introduction

Enterococcus comes from Greece word (έντερο), latin word (éntero), "intestine" and κοκκος, coccos, "granule"). they are group of bacteria not only a part of normal intestinal flora of human and animals specially Diplococci but also important pathogens which can cause serious infections.The genus *Enterococcus* include more than 17 species but only few cause clinical infections, with increasing antibiotic resistance,enterococci are recognized as feared nosocomial pathogens that can be challenging to treat **(Gilmore , *et al.*, 2002).**

Enterococcus is a genus of lactic acid bacteria of the family Enterococcaceae, order Lactobacillales, class Bacilli ,phylum Firmicutes, kingdom Bacteria. They are Gram positive cocci that often occur in pairs (diplococci) or short chains, none motile, they are catalase negative, they are facultative anaerobic organisms, i.e., they are capable of cellular respiration in both oxygen-rich and oxygen-poor environments**(Fischetti *et al.*,2000).**Though they are not capable of forming spores, enterococci are tolerant of a wide range of environmental conditions as extreme temperature(10-45°C), pH (4.5-10.0) and high sodium chloride concentrations**(Fisher *et al.*,2009).** Enterococci are usually considered strict fermenters because they lack a Kreb's cycle and respiratory chain **(Willett *et al.*,1992).**

Enterococci are typically exhibit gamma-hemolysis on sheep's blood agar **(Ryan and Ray,2004).**

Members of the genus *Enterococcus* were classified as Group D *Streptococcus* until 1984, when genomic DNA analysis indicated a

separate genus classification would be appropriate **(Schleifer and Kilpper-Balz ,1984).**

The term *Enterococcus* was first used by **Thiercelin** in a paper from France published in 1899; the name was proposed to emphasize the intestinal origin of this new Gram-positive diplococcus **(Sherman, 1937).**

The name *Enterococcus faecalis* (*faecalis*, relating to feces) was first coined in **1906** by **Andrewes** and **Horder**, who isolated this organism from a patient with endocarditis and considered that this Enterococcus was "so characteristic of the human intestine that the term *'Enterococcus faecalis'* may justly be applied to it".

In **1919**, **Orla-Jensen** described a second organism of this group, *Enterococcus faecium*, which differed from the fermentation patterns of *Enterococcus faecalis.* A third species called *Enterococcus durans* proposed by Sherman and Wing, was similar to *Enterococcus faecium* but of less fermentation activity.

In the 1930, with the establishment of the Lancefield serological typing system, Enterococci were classified as group D streptococci and were differentiated from the non enterococcal group D streptococci such as *Streptococcus bovis* by distinctive biochemical characteristics **(Sherman, 1937).**

In an excellent review in **1937**, **Sherman** emphasized that the term Enterococcus had been used to mean different things ranging from the broad definition of any fecal streptococcus to a restricted definition of organisms that appeared to be identical to *Enterococcus faecalis*,

Sherman proposed a classification scheme which separated streptococci into four divisions: pyogenic, viridans, lactic, and *Enterococcus.*

Sherman further recommended that the term "*Enterococcus*" should be used specifically for streptococci that grow at both 10 and 45°C, at pH 9.6, and in 6.5% NaCl and survive at 60°C for 30 min. These organisms were also noted to hydrolyze esculin in the presence of bile **(Sherman, 1937).**

A number of studies in the 1940s and 1950s showed that organisms referred to as *Enterococcus faecium* had biochemical characteristics that distinguished them from *Enterococcus fecalis.* Such differences included inhibition by potassium tellurite, fermentation reactions, and failure to reduce tetrazolium to formazan **(Barnes,1956 , Deibel 1964 and Hartman *et al.*,1966).**

Although *Enterococcus faecium* was not officially recognized as a separate species in 1957 Bergey's Manual of Determinative Bacteriology, the species status of these organisms was nonetheless widely accepted and was incorporated into official nomenclature by the mid-1960s.During this period, *Enterococcus durans* was sometimes listed as a separate species and sometimes referred to as a variant of *Enterococcus faecium* **(Breed et al.,1957 , Buchanan et al., 1966 and Deibel 1964).**

In **1967**, **Nowlan** and **Deibel** added *Enterococcus avium* to the enterococcal group. In **1970** ,**Kalina** proposed that a genus for the enterococcal *streptococci* be established and suggested that, based on cellular arrangement and phenotypic characteristics, *Streptococcus*

faecalis and Streptococcus faecium and the subspecies of these two taxons be named *Enterococcus.*

In the 1980, based on genetic differences, *enterococci* were removed from the genus *Streptococcus* and placed in their own genus, *Enterococcus*. Genetic evidence that *Streptococcus faecalis* and *Streptococcus faecium* were significantly different from the other members of the genus to merit a separate genus was provided by **Schleifer** and **Kilpper Balz**. Since then it has been generally accepted that the genus *Enterococcus* is valid (**Schleifer and Kilpper Balz,1984).**

Although a dozen *Enterococcus* species have been identified, only two are responsible for the majority of human infections. Until recently, *Enterococcus faecalis* had been the predominant enterococcal species, accounting for 80 to 90% of all clinical isolate **(Maki and Agger, 1988, Kuhnen et al.,1988).**

Enterococcus faecium and *Enterococcus faecalis* are part of the normal animal and human gut flora. These bacteria are also ranked among the leading causes of nosocomial infections.

Enterococcus spp are normal microflora found in the intestinal tract of poultry and other bird species; infections are usually secondary to another disease. These bacteria usually are found in large numbers in food of animal origin, such as cattle, pig, and poultry carcasses **(Giraffa, 2002)**, and their presence is an indication of fecal contamination, which commonly occurs during slaughter of the animals **(Hammerum et al., 2010).**