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Studies on Bacterial Pathogens Causing Respiratory Manifestations in Equine

A thesis Presented by

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Dedication

I wouid like to express my sincere gratitude and thanks to my family

My thanks and highest consideration for my parents, brothers, sister and my childern

Special thanks for my wife dr. samah adel

I appreciate their encouragement and support, I pray with supplication for all of them to acheieve pleasure and success in their life

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Abstract

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Abstract:

The current study aimed to characterize the phenotypic bacterial causes of respiratory diseases in clinical ,subclinical and carrier horses of different ages and to perform biochemical , serological,virulance and antibiogram tests of isolated bacteria from horses with respiratory diseases.

Total numbers of (250) nasal swabs were collected from into three groups :foregin breed (59), native breed (133) and arabien breed (58).

The main isolate is *s.equi subspp.equi* recovered from all breedes in an incidance of (13.2%),the others detected bacteria are *S.zooepidemicus* in an incidance of (8%),*R.equi* in an incidance of (6.8%),*S.aureus* in an incidance of (8.4%),*S.epidermidis* in an incidance of (10%),*E.coli* in an incidance of (15.2%),*K.pneumoniae* in an incidance of (5.6%) and *P. vulgaris* in an incidance of (1.6%).

This study include the virulance assay to all isolated bacteria as Hemolytic activity,Congo red binding activity,Vero cell cytotoxicity and Biofilm formation , in adittion to achieve antibiogram tests to isolated bacteria that shows the *Gram +ve* bacteria are sensitive to cefoxitin , gentamicin , ciprofloxacin ,azithromycin while they show high resistance for vancomycin, while *Gram – ve* bacteria are sensitive for imipenem , cefoperazone , aztreonam and ceftriaxone while they show high resistance for gentamicin , cefotaxime.

Keywords: Respiratory diseases- Bacterial isolation- bacterial Identification- Horses

Content

List of Tables

List of Figures

List of Abbreviations

IURD	Infectious upper respiratory disease
S. equi	*Streptococcus equi*
FNEB	fibronectin-binding protein
CR+	Congo red positive
PCR	polymerase chain reaction
NP	nasopharyngeal
GP	guttural pouch
SeM	*S.equi* M-like protein
SLO-like	streptolysin O-like
FNEB	fibronectin-binding protein
SclC	the cell surface collagen-like protein
A	Alpha
β	Beta
γ	Gamma
Fn	fibronectin protein
GBD	gelatin-binding domain
Scl	streptococcal collagen-like protein
LAD	lower airway disease
sAgs	superantigens
ChoE	cholesterol oxidase exoenzyme
LAMP	loop-mediated isothermal amplification assays
O	Somatic antigen
H	flagellar antigen
K	capsular antigen
CA-YE	Casamino acid-yeast extract- salts
TSBYE	Tryptic soy broth enriched with 0.6% yeast extract
PVC	polyvinyl chloride
OD	optical density
PBMCs	peripheral blood mononucleated cells

ETEC	Enterotoxigenic E. *coli*
EIEC	Enteroinvasive E. *coli*
VTEC	Verotoxin- producing E. *coli*
EPEC	Enteropathogenic *E. coli*
EAE	attaching-and-effacing factor
EAF	fimbrial adherence factor
XDR-TB	extensively drug-resistant tuberculosis
MDR-TB	multidrug-resistant tuberculosis
VRSA	vancomycin resistant *Staphylococcus aureus*
HIV	Human Immunodeficiency Virus

Introduction

1. Introduction

Infectious upper respiratory disease (IURD) of horses has been a frequent problem. Risk factors for IURD include the season with a high transfer rate (summer and fall), the stabling period (≤ 3 months), and age (2 to 3 years old), suggesting that the movement and new environment may have depressed the immune system of the horses and decreased their ability to respond properly to pathogens. The bacterial strains isolated from IURD horses included *Pseudomonas spp.*, *Escherichia coli*, *Staphylococcus* spp., *Streptococcus equi subsp*. *equi* and *zooepidemicus (*Ryu et al., 2011*).Streptococcus equi* is the etiologic agent of a highly infectious upper respiratory disease of horses known as strangles. Asymptomatic carriers in the population may result in the spread of disease via introduction of *S. equi* to native populations. Bacterial culture and polymerase chain reaction (PCR) of nasopharyngeal (NP) washes and guttural pouch (GP) lavages have been used for both diagnostic testing and for the detection of *S. equi* in clinical and carrier animals but no definitive or gold standard test method has been shown to be optimal (Timoney and Artiushin, 1997; Sweeney et al., 2005; Holland et al., 2006; Boyle et al., 2012).

Introduction

Strangles is the main upper respiratory tract and a highly contagious disease of horses caused by *Streptococcus equi ssp. equi* (Libardoni et al., 2016). Clinical signs include fever, swollen lymph nodes of the head and neck, coughing, purulent nasal discharge and depression (Sweeney et al., 2005). Typical clinical signs are purulent nasal discharge, pyrexia, anorexia. The outcome is only rarely fatal due to complications (Sweeney et al., 2005). Complications include purpura hemorrhagica and metastatic abscessation. Control of outbreaks requires strict isolation protocols and hygiene measures. Detection of carriers is essential for preventing disease recurrence on a farm (Boyle, 2011).*S. equi* are *β-hemolytic streptococci*, belonging to Lancefield group C, and can be identified by conventional biochemical testing (Quinn et al., 1994) or by PCR. *Streptococcus equi subsp. zooepidemicus* (S. *zooepidemicus*) is generally considered a commensal and an opportunistic pathogen of the upper airways in horses. Establishing whether certain strains of *S. zooepidemicus* can cause upper respiratory disease as a host-specific pathogen of horses, and if there are certain genogroups of *S. zooepidemicus* that are more virulent than others is of major clinical importance (Lindahl et al., 2013). Transport for long periods frequently causes bacterial pneumonia in horses, the dominant pathogenic bacterium under such circumstances being *Streptococcus equi subsp.*

zooepidemicus (Oikawa et al., 1995). Other pathogens such as *Escherichia coli* became responsible for secondary bacterial pneumonia following the administration of antimicrobials (Racklyeft and Love, 2000). The mortality rate from pleuropneumonia and secondary bacterial pneumonia caused by these agents is high (Sweeney et al., 1985; Racklyeft and Love, 2000).Pneumonia is a major cause of disease and death in foals. *Rhodococcus equi* is considered the most important cause of pneumonia among foals between approximately 1 and 6 months of age (Prescott, 1991; Takai et al., 1999; Muscatello et al., 2007). The mortality rate of foals with clinical signs of pneumonia caused by *R. equi* is approximately 30% (Giguére, et al., 2004; Giguére, 2017). *Rhodococcus equi, is* a facultative intracellular pathogen and an important cause of pneumonia in foals, which is highly susceptible to killing by gentamicin in vitro (Burton et al., 2015). Susceptibility to *Rhodococcus equi* pneumonia in horses appears to be age-related. The disease occurs almost exclusively among foals, whereas adult horses or other species of animals generally do not develop signs of infection unless they are immune-compromised (Prescott, 1991; Takai, 1999; Muscatello et al., 2007). *R. equi* is an important pathogen of foals that causes severe pneumonia. There is no licensed vaccine effective against *R. equi pneumonia* of foals (Bordin et al., 2014). In equids,

susceptibility to disease caused by *Rhodococcus equi* occurs almost exclusively in foals. This distribution might be attributable to the age-dependent maturation of immunity following birth undergone by mammalian neonates that renders them especially susceptible to infectious diseases.

So the present study was performed to accomplish the following targets:

1) Isolation of bacterial causes from respiratory diseases in clinical, subclinical and carrier horses of different ages.
2) Identification of recovered microorganism by specific biochemical tests.
3) Serotyping of recovered microorganism
4) Virulence study for recovered microorganism
5) Application of antibiotic sensitivity testing against identified isolates to detect the multidrug resistant strain.

Review of Literature