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ANTIBIOGRAM FOR *STREPTOCOCCUS* SPP. RECOVERED FROM STRANGLES SUSPECTED SAMPLES

By

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ABSTRACT

In the present study 150 swabs "100 nasal swabs and 50 pus swabs" were collected from horses suffered clinically from strangles symptoms for examination of *Streptococcus* spp. The microbiological and biochemical results revealed that there were 2 *S.equi* isolates" all from pus swabs and 2 *S.zooepidemicus* isolates" one from nasal swabs and one from pus swabs " , these result confirmed serologically by using specific antisera and also molecularly by using PCR with specific primers for each species. Antibiogram for the recovered *S.equi* and *S.zooepidemicus* isolates showed that they were sensitive for penicillin except one isolates of *S.zooepidemicus* "and cephalosporins "except one isolates of *S.equi* ", while they show high resistance for bacitracin, erythromycin, tetracycline and amikacin.

Keywords:

(Streptococcus, Occurrence, Antibiogram*)*.

INTRODUCTION

Streptococcus equi subspecies *equi* (*S. equi*) is the causative agent of equine strangles, characterized by abscessation of the lymph nodes of the head and neck. Rupture of abscesses formed in retropharyngeal lymph nodes into the guttural pouches leads to a proportion of horses becoming persistently infection carriers **(Jorm *et al*., 1997**). These carriers transmit the organism to naive horses and play an important role in disease spread. *S. equi* is believed to have evolved from an ancestral strain of *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) which is associated with a wide variety of diseases in horses and other animals including humans **(Webb *et al* ., 2004)**. Horses that maintain the organism within the guttural pouch and/or cranial sinuses are responsible for persistence of infection as well as introduction of the organism to other herds **(Newton *et al*, 2000)**. Strategies to minimize antimicrobial resistance in the interest of public health and animal well-being have been

promoted by governments, medical and veterinary organizations, researchers and clinicians since the problem was first recognized. Early efforts included Great Britain's Penicillin Act of 1947 and subsequent Aureomycin and Chloramphenicol Regulation of 1951 that eliminated free access to antibiotics by the general public, theoretically reducing the risk of selective pressure from unnecessary use or inappropriate dosing. As the microbiological complexities of antibiotic resistance became clearer, myriad other proposed strategies focused on reduced use of antimicrobials, implementation of more appropriate dosing regimens, development of new antimicrobial drugs and vaccines, susceptibility testing of anaerobic isolates, increased attention to the effect of antibiotic residues in the environment and the role of commensals as reservoirs of resistance, isolation of patients harboring resistant bacteria, and uniform preparation of annual antibiogram to track resistance and improve therapy **(Pakyz., 2007)**. While antibiotic resistance in clinical veterinary medicine has traditionally paralleled discoveries in human clinical medicine, the two have been inextricably linked by the issue of antibiotic use in food animals. A similar point of debate has been the significance of working in close proximity to animals on human acquisition of multi drug resistant zoonosis **(Fluharty *et al*., 1991)**, so the aims of the present study are detection the occurrence of *S.equi and S.zooepidemicus.* In equines respiratory problems with special attention with antibiogram for the recovered isolates.

MATERIAL AND METHODS

Sample:

Physical examination was employed for horses which suffered clinically from strangles signs as: respiratory distress, nasal discharge, swelling of mandibular lymph node, followed by collection of swabs from nasal discharge and/or opened lymph node. A total of 150 swabs as shown in (Table 1)**.**

Table (1): Numbers of examined samples

Animal species	Nasal swabs	Pus swabs	Total
Horses clinically suffered from strangles signs	**100**	**50**	**150**

***Isolation and identification of Streptococcus spp.* (Patterson, 1996).**

The swabs were inserted into a tube of nutrient broth, incubated overnight and then contents of the tube were mixed. The swab was pressed against the wall of the tube then rolled over on

plate containing Blood Agar soya tryptase base with sulfamethoxazole and trimethoprim. Theinoculated plates were incubated at37ºC for 24 - 48 hours and examined for bacteriological growth.The suspected pathogenic bacterial colonies were subcultured on Edward media and preserved on soft agar for further identification. Serological identification by using specific antisera and confirmed molecularry by using PCR with specific primers showed in (Table 2).

Table (2): Oligonucleotide primer sequence.

Gene	Primer		bp.	Reference
	Forward (5-3)	Reverse(5-3)		
Streptococcus spp.	TAAGCACCATGCCACCTATG	TTGCCCTCTGAGATTGGTGT	480	Båverud *et al* ., 2007
S.equi	TTACCTCCATTACTTGACAATCCAT	GATTTGCAACATGAAACATTTACAG	201	
S.zooepidemicus	CTTTTCTTCACCGCCCACT	TGAGCTTTGGAGAAATGGAA	158	

Antibiogram for Streptococcus isolates:

All purified isolates were tested by the standard disc diffusion method **(CLSI, 2014)** and were subjected to a susceptibility panel of antibiotics (Oxoid) belonging to different drug classes. Isolates were cultured in trypticase soy broth (TSB) supplemented with 0.6% yeast extract, and transferred to Mueller - Hinton agar (Oxoid). The plates were incubated at 37ºC for 48 hours.

RESULTS

Occurrence of *Streptococcus* spp. recovered from strangles samples

Microbiological, biochemical, serological and molecular characterization of *Streptococcus* spp. from suspected strangles samples revealed that from 150 samples recoverey of 4 isolates (2.7%) which showed positive amplification of the 380 bp fragment of primer specific for the genus *Streptococcus* in all of the examined *Streptococcus* isolates **(**n= 4), two were 158 bp for *S. zooepidemicus* while the other two isolates it was 201 bp for *S.equi*.

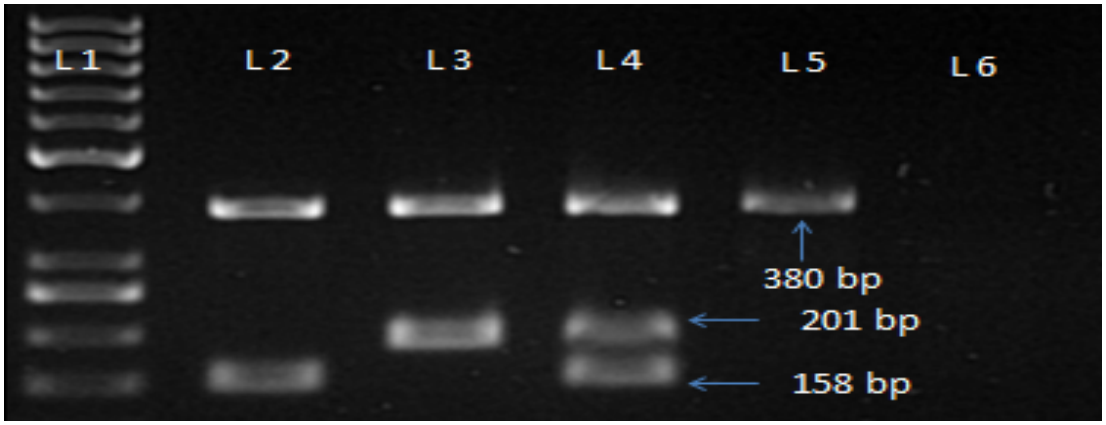

Fig. (1) : Agarose gel electrophoresis showing positive amplification of product 480 bp fragment of 16srRNA gene of *Streptococcus* spp. performed with specific primer .

A):

L1: 100-1000bp DNA ladder

L2: *S.zooepidemicus* nasal swab

L3: *S.equi* from pus swab

L4: *S.equi* and *S.zooepidemicus* from pus swab

L5: control positive (*S.pyogenes* local isolates)

L6: control negative (*S.aureus* local isolates)

In vitro antibiotic resistance pattern:

The recovered *S. equi* and *S. zooepidemicus* isolates were sensitive for Penicillin " except one isolates of *S. zooepidemicus* "and cephalosporin's " except one isolates of *S.equi* " ., while they show high resistance for bacitracin , erythromycin , tetracycline and amikacin (Table 3).

Table (3): Result of Antimicrobial sensitivity for recovered *Streptococcus* spp.

Isolates	**Isolates (1)** ***S.equi*** ***(from pus swabs)***	**Isolates (2)** ***S.equi*** ***(from pus swabs)***	**Isolates (3)** ***S.zooepidemicus*** ***(from pus swabs)***	**Isolates (4)** ***S.zooepidemicus*** ***(from nasal swabs)***
Antimicrobials	**n=of sensitive isolates**	**n=of sensitive isolates**	**n=of sensitive isolates**	**n=of sensitive isolates**
Bacitracin	**0/1**	**0/1**	**0/1**	**0/1**
Penicillin G	**1/1**	**1/1**	**0/1**	**1/1**
Tetracycline	**0/1**	**0/1**	**0/1**	**0/1**
Erythromycin	**0/1**	**0/1**	**0/1**	**0/1**
Amikacin	**0/1**	**0/1**	**0/1**	**0/1**
Cefuroxime	**1/1**	**0/1**	**1/1**	**1/1**
Cefoperazone+ sulbctam	**1/1**	**1/1**	**1/1**	**1/1**
Cefoperazone	**1/1**	**1/1**	**1/1**	**1/1**

DISCUSSION

Opportunistic infection of *S. zooepidemicus* in horses typically results in pneumonia, rhinitis, placentitis, endometritis, cervicitis, abortion, keratitis, mastitis, cellulitis and non-strangles lymph node abscessation. *S. zooepidemicus* has long been recognized as an important pathogen in equine reproduction and presently is the most frequently isolated pathogen associated with abortion in mares **(Erol *et al*., 2012)**. The pathogenesis of *S. zooepidemicus* placentitis is not completely understood. The external genitalia of the mare and stallion are suggested as sources of the organism which opportunistically penetrate the cervix via natural or artificial insemination, iatrogenically or due to poor conformation of the internal and external genitalia of the mare causing infection. Little is known about the pathogenesis of S. *zooepidemicus* in the lower respiratory tract of horses. The organism has been associated with lower airway disease of young horses with compromised or overwhelmed immune system due to stress (intense training, transportation) or secondary infection **(Sweeney and Beech, 1991)**. In the present study from 150 swabs "100 nasal swabs and 50 pus swabs" were collected from horses suffered clinically from strangles symptoms , the microbiological and biochemical results revealed that there were 2 *S.equi* isolates" all from pus swabs and 2 *S.zooepidemicus* isolates" one from nasal swabs and one from pus swabs " , these result confirmed serologically by using specific antisera and also molecularly by using PCR with specific primers for each species Veterinary personnel historically have been the target of such research, with variable conclusions drawn over time. Attention is increasingly being focused on the transmission of resistant pathogens between companion animals, horses and their owners, particularly as concerns methicillin-resistant *Staphylococcus aureus* **(Stenberg., 1999)**. Furthermore, organisms such as *Streptococcus zooepidemicus* that are typically associated with equine disease occasionally are reported to cause serious disease in humans that live or work in proximity to horses **(Downar *et al* ., 2001)**. *Rhodococcus equi* emerged as a pathogen of human concern following reports of increased incidence in patients with Human Immunodeficiency Virus (HIV) infection. Both veterinary and human literature demonstrate a rise in concern for public health in venues such as petting zoos , a topic gaining importance in human medicine due to the prevalence of animal assisted therapies for the ill, disabled, elderly and immunocompromised **(Khan *et al* ., 2000)**. In light of this, an in-depth

understanding of the institutional, local and regional microbial population is a prerequisite for effective and responsible antimicrobial use by veterinary hospital clinicians and field practitioners. Consideration of the potential impact of biosecurity and antibiotic protocols on the health of patients, staff, clients, and the general public is of paramount importance **(Brosnahan.,2008)** . Current literature in equine medicine encompasses a broad range of topics in both clinical and research microbiology, including nosocomial infections, zoonotic transmission of disease and multi-drug resistance. Efforts to describe the microbiological environment encountered in equine practice range from broad retrospective surveys of multiple pathogens to detailed molecular characterization of individual isolates. Salient characteristics including disease presentations, reported trends in antimicrobial resistance and zoonotic concerns are summarized below for the principal bacterial organisms encountered in equine clinical practice **(Moodley *et al.,* 2010)**. Documentation of significant resistance trends was not apparent in current literature, though concern was transiently expressed about the possibility of resistance to trimethoprim sulfamethoxazole. A recent large-scale evaluation of equine isolates submitted to a university diagnostic lab showed susceptibility of *Streptococcus equi* zooepidemicus to ceftiofur (100%), cephalothin 99%), penicillin (95%), ampicillin (92%), enrofloxacin (91%), erythromycin (91%), amoxicillin/CA (87%), spectinomycin (87%), gentamicin (85%), tetracycline (59%), trimethoprim sulfamethoxazole (55%), neomycin (20%) and amikacin (5%) **(Clark *et al*., 2008)**. Antibiogram for the recovered *S.equi* and *S.zooepidemicus* isolates in the present study showed that they were sensitive for Penicillin " except one isolates of *S.zooepidemicus* "and cephalosporins " except one isolates of *S.equi* " ., while they show high resistance for bacitracin , erythromycin , tetracycline and amikacin

CONCLUSION

Our study concluded that ***S.equi*** **and** ***S.zooepidemicus*** **represent the main pathogens in upper respiratory tract** in equines with emergence of antibiotic resistance isolates which act as public health hazard due to its zoonotic importance

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