Aerobic Bacteria Associated with Equine Wounds Infections in Khartoum State, Sudan.

By

Nugood Hussien Ahmed Mohammed
B.V.M. University of Khartoum 2003

Supervisor:
Prof.: Mohammed Taha Abd-allah Shigidi

A Thesis submitted in partial fulfillment of the Requirements for the Degree of M. Sc.

Department of Microbiology
Faculty of Veterinary Medicine
University of Khartoum

July 2010

List of content

Preface

This work was carried out in the Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, under the supervision of Prof. Mohammed Taha Abd-allah Shigidi.

Dedication

To my husband

To Soul of my father

To my mother

To my daughter and son

With infinite love

Acknowledgments

All praise is for God, Peaceful and merciful are for Prophet Mohammed.

I would like to express my grateful thanks to my supervisor Prof. Mohammed Taha Abd-allah Shigidi.

Thanks and gratitude is extended to all staff of the Department of Microbiology, Faculty of Veterinary Medicine and University of Khartoum.

I would like to express my thanks to my husband for his full support while, undertaking this study.

Abstract

This study was conducted to isolate and identify aerobic bacteria associated with wound infections in equines and to determine the sensitivity of these bacteria to some antibacterial agents commonly in use for treatment of infection in equines.

In this study 50 swabs were collected from infected wounds of equines, in Khartoum, Omdurman and Khartoum North, during the period from December 2009 to April 2010.

Wounds were in animals of various age groups, sexes, and in different sites of the body.

The swabs were transported to the laboratory in ice in a thermos flask, before being cultured on blood Agar and MacConKey's Agar. Isolates were identified on the basis of their microscopic appearance, staining reaction, cultural and biochemical characteristics.

A total of 68 bacterial species were identified which included *Staphylococcus aureus* (25%), *Streptococcus equisimilis* (20.6%), *Streptococcus zooepidemicus* (13.2%), *Streptococcus equi* (7.4%), *Micrococcus roseus* (7.4%), *Corynebacterium pseudotuberculosis* (5.9%), *E.coli* (2.9%), *Staphylococcus intermidius* (2.9%), *Staphylococcus epidermidis* (2.9%), *Rhodococcus equi* (2.9%), *Bacillus mycoides* (2.9%), *Neisseria lactamica* (2.9%), and *Proteus spp* (2.9%).

All isolates were found sensitive to Cephalexin, Ciprofloxacin, Cloxacillin, Co-trimoxazole and Gentamicin, except *E. coli*, which was found to be resistant to Co-trimoxazole and Gentamicin. All isolates were resistant to penicillin except *E.coli*. However, the isolates showed varying degrees of susceptibility to tetracycline.

The most frequently isolated bacteria from wound infections were *Streptococcus* spp (41.2%) followed by *Staphylococcus* spp (31.0%).

The choice of antibiotics for treatment should be made after antibiotics susceptibility tests.

ملخص الأطروحة

ارتكزت الدراسة على عزل وتعريف البكتيريا الهوائية المرتبطة باصابات الجروح في الفصيلة الخيلية؛ وتحديد المضادات الحيوية المناسبة والمستعملة في علاج هذه الاصابات.

في هذه الدراسة تم جمع 50 مسحة من الجروح الملتهبة من الفصيلة الخيلية في الخرطوم وأم درمان وبحري في الفترة من ديسمبر 2009 حتى أبريل 2010 وجمعت العينات من أعمار مختلفة ذكور وأناث من مناطق مختلفة من الجسم.

نقلت العينات في حافظات ثلج وتمت زراعتها في أجار الدم ووسط الماكونكي ثم العزل والتعرف على البكتيريا المسببة لتلوث الجروح اعتمادا على الشكل المجهري والتفاعل الصبغي وشكل الزراعة والخصائص الكيميائية.

بلغت البكتيريا المعزولة 68 عزلة وهي المكورات العنقودية الذهبية (25%)،المكورات العقدية شبيهة الخيلية (20.6%)، المكورات العقدية الحيوانية (13.2%)، المكورات العقدية الخيلية (7.4%)، جنس المكورات الدقيقة (7.4%)، الوتدية السلية الكاذبة (5.9%) و(2.9%) لكل من :المكورات العنقودية الجلدية، المكورات العنقودية الوسطية، الايشريكا القولونية، ذات الحبيبات الحمراء الخيلية، العصوية المخاطية، النيسيرية اللبنية وجنس المتقلبة.

أجريت ءاختبارات الحساسية لعدد من مضادات البكتيريا وقد وجدت حساسة للسيفالكسين والسيبروفلوكساسين والكلوكساسلين والترايموكسازول والجينتامايسين ما عدا الأشريكيا القولونية والتي كانت مقاومة للترايموكسازول والجينتامايسين. كل العينات مقاومة للبنسلين عدا الأشريكا القولونية. كما أظهرت العينات درجات متفاوتة من الحساسية للتتراسايكلين.

معظم البكتيريا التي تم عزلها كانت من نوع المكورات العقدية (41.2%) متبوعة بالمكورات العنقودية (31.0%).

اختبار المضاد الحيوي المناسب للعلاج يتم بعد اجراء اختبارات الحساسية لهذا المضاد الحيوي.

List of Tables

Chapter one

Introduction

Horses and donkeys play an important role in the developing countries. The importance of equines is steadily increasing in the Sudan as well as many other African countries, in which equines are being used for field operations. The population of equines in Khartoum State in 1997 was reported to be 29091.This number increased to more than 29916 in 2000.

Equines have been largely ignored in modern scientific literature on domestic animals, probably because they are not providers of meat and milk. Their contribution to the economy in the Sudan, however, is considerable and they are the principal means of transport both as draught and as riding animals. They are used mainly to carry wood and fuel and as for the distribution of domestic water in the larger villages and towns (RT Wilson 1978).

According to their use, equines are exposed to different types of wounds resulting from accidents and hitting or from biting by other animals. Bad saddles and girths, especially in animals used for pulling, may inflict wounds in equines. Wounds are one of the important causes of reducing working ability of the animal. These wounds may become infected with different types of pathogenic bacteria. Treatment of these infected wounds takes a long time resulting in considerable economic loss, or at least the output of the animal may be reduced. Infecting organisms may develop resistance to various drugs.

Bacteriological studies of wounds infections of equines are limited in the Sudan; therefore, this study was carried out to investigate bacteria associated with equines wound infections in Khartoum State**,** Sudan.

Objectives:

The objectives of this study were: -

1. To isolate and identify aerobic bacteria associated with wound infection in equines.
2. To determine the sensitivity of these bacteria to some antibacterial agents, commonly in use for treatment of wound infections in equines.

Chapter Two

1. Literature Review

1.1 Wounds

A wound is defined as an injury to the body by physical means that result in disruption of the continuity of the body structure (Douglas, 2002).

1.1.1 Types of wounds

1.1.1.1 Abrasions

Abrasions are wounds caused by blunt trauma or shearing forces that damage the skin leading to loss of the epidermis and portions of the dermis (Douglas, 2002).

1.1.1.2 Incised wounds

Are produced by a sharp-edged weapon or instrument (Vincent and Suzanna, 2007). Douglas (2002) defined an incised wound as a wound created by a sharp object. The edges are smooth and the trauma in the surrounding tissue is minimal.

1.1.1.3 Lacerated wounds

Are irregular wounds created by tearing of tissue resulting in a variable damage to both superficial and underlying tissue (Douglas, 2002).

1.1.1.4 Punctured wounds

Are penetrating wounds of the skin caused by a missile or sharp object. The superficial damage may be minimal, whereas the damage to the deeper structures may be substantial (Douglas, 2002).

1.1.1.5 Postoperative wounds

Surgical wounds are classified according to the likelihood of contamination and wound infection into three types: clean wounds, clean-contaminated wounds and contaminated wounds (Mann *et al*., 1996).

1.1.1.6 Infected wounds

Occurs when there are established infections (Jarrell *et al*., 2000).

1.2 Common bacteria isolated from infected wounds

1.2.1 Streptococcus spp

Streptococcus is described in Bergey's Manual (Hardie, 1986) as spherical or sometimes ovoid shaped cells, Gram-positive cocci, catalase negative bacteria that are usually facultative anaerobes. They grow in pairs or chains and their optimum temperature is usually 37 °C, even though minimum and maximum temperatures vary within the genus. Not all species form capsules, but it is not uncommon.

Streptococci are responsible for a large number of important diseases in man and in animals. They are found on mucus membranes of the mouth, respiratory and urogenital tracts, as well as on the skin. The pyogenic groups of Streptococci are regarded as pathogenic or opportunistic pathogens of mammals (Hardie & Whiley, 1995). The most studied member of the pyogenic group is *Streptococcus pyogenes*, belonging to the Lancefield serological group A. It is the causative agents of various diseases in humans, ranging from mild infections of the skin do more severe diseases such as scarlet fever.

Streptococci can be divided into groups in different ways, e.g. the Lancefield Grouping, which based on differences in serological types (Lancefield, 1933).

1.2.1.1 Classification of *Streptococci*

The genus *Streptococcus* includes nearly 40 species. They consist of six groups:

a) The pyogenic group includes most species that are overt pathogens of man and animals.
b) The mitis group includes commensals of human oral cavity and pharynx, although one of the species, *Streptococcus pneumoniae*, is an important human pathogen.
c) The anginosus group
d) The salivarisus group. Both the anginosus and salivarisus groups are part of the commensal micro flora of the oral cavity and pharynx.
e) The bovis group found in the colon.
f) The mutans group of streptococci colonizes exclusively tooth surfaces of man and some animals; some species belonging to this group are involved in the development of dental caries.

Virtually, all the commensal species, including the enterococci, are opportunistic pathogens primarily if they gain access to bloodstream from the oral cavity or from the gut (Greenwood 2002).

The classification of the aerobic Streptococci has, however, given rise to considerable difficulty.

The criterion that has so far been of the most value in classification is the presence of polysaccharide haptens that can be extracted from the organisms by a variety of methods and which are demonstrable by precipitation tests. On the basis of these haptens, 18 groups of Streptococci (A to T) may be distinguished. All the strains in a particular group possess an antigenically identical polysaccharide (Stewart and Beswick, 1977).

1.2.1.2 Pathogenicity of *Streptococcus* spp

Streptococcal infections can result in a variety of diseases and their development depends on several factors such as animal species, bacterial species, body system affected, and portal of bacterial entry. Pyogenic

Streptococcus bacteria produce pus as part of their pathogenesis. When pyogenic *Streptococcus* bacteria enter tissue they cause an inflammatory response including vasodilation and invasion of neutrophils. As a result of chemotaxis (chemical signaling by bacteria) neutrophils move toward bacteria and phagocytose them. Following phagocytosis, bacteria may be digested, but some are resistant to enzymes in the neutrophil and will multiply in the neutrophil. Some *Streptococcus* bacteria will produce toxins that kill the neutrophils and the enzymes released upon death of the neutrophil will cause liquefaction of dead tissue. This liquefied mass becomes thick pus as a result of the large amount of protein from the nuclei of dead cells. Virulence factors vary among species of *Streptococcus* and include the following:

a- **Protein M**. Some strains have a membrane protein called protein M that inactivates complement and is antiphagocytic.

b- **Hyaluronic acid capsule**. Hyaluronic acid is a natural substance in the body and its presence in a capsule makes phagocytic leukocytes (WBCs) ignore the presence of bacteria with this virulence factor.

c- **Streptokinases**. There are two streptokinase enzymes that break down blood clots allowing bacteria to spread rapidly throughout infected tissue.

d- **Streptolysins**. There are two different, membrane-bound proteins called streptolysins, which lyse erythrocytes (RBCs), leukocytes, and platelets. These proteins interfere with the oxygen-carrying capacity of blood, immunity, and blood clotting. After phagocytosis, some *Streptococcus* bacteria will release streptolysins into the cytoplasm of

the phagocyte, causing the lysosomes to release their contents causing death to the phagocytes and release of bacteria.

e- **Enzymes**. Some species of *Streptococcus* produce proteases that break down proteins; others produce deoxyribonucleases (DNases) that reduce the viscosity of fluid containing DNA, whereas others produce hyaluronidases that promote the rapid spread of infection.

f- **Protein adhesin**. Some *Streptococcus* bacteria produce protein adhesin, a protein that causes binding of the cells to epithelial cells allowing bacteria a place to enter the cells. (Romich *et al*, 2008).

1.2.1.3 *Streptococcus zooepidemicus*

Streptococcus zooepidemicus is a Gram–positive sphere, which is arranged in pairs or chains, non-motile, non-sporing, aerobic and facultative anaerobic. It is catalase negative, oxidase negative and attacks carbohydrates fermentatively.

The most common site from which significant bacterial isolates were recovered was the respiratory tract, followed by wounds. *Streptococcus zooepidemicus* was the most common isolate from most infections in equines (Chris Clark *et al*, 2008). In foals, ulcerative lymphangitis is caused by *Streptococcus zooepidemicus* (Blood *et al*., 2000). It commonly causes wound infections in horses and it is a secondary invader in viral respiratory infections and infections of the mare reproductive organs (Bryans and Knight, 1972).

It is, by far, the most common cause of wound infections and respiratory tract infections of foals and young horses, including purulent

nasal discharge and abscesses of submandibular lymph nodes in some cases (Downar *et al*., 2001).

Streptococcus equi subspecies zooepidemicus causes pneumonia, wound infections, endometritis, arthritis and mastitis in animals. Animals can be carriers of this bacterium and horses are the animals most commonly affected by this organism (Romich *et al*, 2008).

1.2.1.*4Streptococcus equi*

Streptococcus equi subspecies *equi* occurs almost exclusively in equine causing strangles primarily in young animals without prior infection or immunization. It is found in the nasopharynx, upper and lower respiratory tract, and the genital mucous membranes of healthy equine and cattle (Romich *et al*, 2008).

1.2.1.5 Streptococcus equisimilis

It is occasionally recovered with *Streptococcus equi* from strangles, wound infections, genital infections and mastitis in the mare (Gordon 2004).

1.2.2 *Staphylococcus* **spp**

Gram-positive cocci in clusters, non-motile, non-spore forming, aerobic and facultative anaerobic, usually oxidase negative, catalase positive bacteria. They hydrolyse arginine and attack sugars by fermentation (Barrow and Feltham, 1993).

1.2.2.1 *Staphylococcus aureus*

It is a Gram-positive, sphere found in pairs or clusters, it is non-motile, non-sporing, aerobic, catalase positive, oxidase negative, attacks sugars fermentatively and is coagulase positive.

It is rarely encountered as a primary pathogen in horses. It has been isolated in pure culture from joint fluid, mastitis fluid, and surgical wounds, but in most cases it is a member of a mixed flora in the contaminated

wounds, resulting from castration, decubitus, or compound fractures (Bryan's and Knight, 1972).

The organism causes wound infections and septicaemia in horses (Blood and Henderson, 1968).

Staphylococcus aureus is a representative pyogenic bacterium that is often isolated from horses with abscess, wound infection, phlegmon and uteritis (Konia Shimozawa *et al*, 1997).

1.2.2.1.1 Pathogenicity of *Staphylococcus aureus*

Staphylococcus aureus elicits its pathological changes by secreting enzymes and toxins, which include:

a. Coagulase, an enzyme that clots plasma and coats staphylococcal cell, which probably prevent the bacteria from being phagocytosed and destroyed by macrophages.
b. Leucocidin, which kills the white blood cells.
c. Lytic toxins (exotoxine) that destroys red blood cells and platelets.
d. Deoxyribonuclease, that destroys deoxyribonucleic acid (DNA).
e. Hyaluronidase, that helps *Staphylococcus* to spread in the tissues.
f. Lipase, that breaks down fat.
g. Staphylokinase, that causes fibrinolysis.
h. Exfoliative toxin that causes peeling of the skin (Scalded skin syndrome).
i. Beta lactamase (antibiotic inactivating enzyme), that leads to penicillin resistance. (Cheesbrough, 1987).

1.2.2.2 *Staphylococcus intermedius*

Staphylococcus intermedius and *Staphylococcus aureus* have been isolated with about equal frequency from staphylococcal wound infections in

equines (Denny and William, 2003), particularly those of areas covered by the saddles and girths of horses (Collier *et al*., 1998).

1.2.2.3 *Staphylococcus epidermidis*

Recently, it has been shown that biofilms may exist in equine wounds, with *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* being the most common microorganisms found (Samantha, 2009).

Staphylococcus epidermidis is the organism most frequently associated with bacteraemia, wound infections and urinary tract infections in equines (Boyd and Hoerl, 1991).

1.2.3. *Corynebacterium pseudotuberculosis*

Corynebacterium pseudotuberculosis is a Gram-positive rod, which, under most conditions of growth, does not branch. It is non-motile, non-spore forming, non-acid fast, aerobic and facultative anaerobic. It is negative for catalase and oxidase, attacks sugars fermentatively and negative for aesculin hydrolysis.

Corynebacterium pseudotuberculosis is reported to cause ulcerative lymphangitis in horses, a contagious disease characterized by inflammation of the subcutaneous lymphatic vessels, usually in the lower limbs (Roger, 2003**)**. Lesions of ulcerative lymphangitis in horses are mainly seen on hind limbs distal to the hocks as a result of the organism gaining entrance to the skin through wounds and spreading via the lymphatics (Hickman *et al*., 1985).

1.2.4 *Escherichia coli*

Gram-negative rods, often motile, aerobic and facultative anaerobic, catalase positive, oxidase negative, attack sugars fermentatively, normally with gas production and are usually citrate negative in Simmon’s medium (Barrow and Faltham, 1993). Most *E. coli* strains produce indol from

peptone water and reduce nitrate to nitrite. Colonies on blood agar after overnight incubation at 35-37 ℃ is 1-4 mm in diameter, may appear mucoid and some strains are haemolytic.

On MacConKey's agar most *E. coli* produce lactose-fermenting colonies (Cheesbourgh, 1987). They grow over a wide range of temperature 15-45℃ (Greenwood *et al*., 1997).

E. coli causes diarrhoea in human, cattle, swine, horses and other species. It is often associated with septic infections such as wound infections (Carter *et al*., 1995).

1.2.5 *Rhodococcus equi*

Rhodococcus equi is a strictly aerobic gram-positive bacterium displaying rod-to-coccus pleomorphism, with fragmenting and occasionally palisading forms. It is non fastidious. Colonies on blood agar from clinical specimens can be mucoid and coalescing. Typical salmon pink pigmentation develops on blood agar, but often only after 2 to 3 days incubation. Growth on Lowenstein-Jensen medium allows earlier detection of the pigment. Positive routine biochemical tests include catalase and urease.

Acid-fast staining of direct smears and fresh isolates is helpful in identification, but is rarely observed on subculture (Regina Linder, 1997).
The umbilical stump or open wounds in equines may serve as portal of entry. Many foals that succumb to this infection have a history of being undernourished or weak from birth, or suffering from debilitating injuries (Bryan's and Knight, 1972).

1.2.6 *Micrococcus spp*

It is commonly encountered in routine laboratories either as an environmental contaminant or as a commensal from normal skin (Barrow and Feltham, 1993). *Micrococcus spp* are Gram-positive spheres in pairs,

non-motile, non-spore forming, aerobic, catalase positive and attack sugars oxidatively or not at all (Carter, 1999).

1.2.7 *Proteus spp*

The species comprises Gram-negative rods which are motile, aerobic and facultative anaerobic. They are catalase positive, oxidase negative, attack sugar fermentatively, usually with gas production, and hydrolyze urea and gelatins (Barrow and Feltham, 1993).

When cultured aerobically, most *Proteus* strains produce characteristic swarming growth over the surface of blood agar and several other culture media. Swarming, however, is inhibited on media containing bile salts such as MacConKey's agar and Deoxycholate citrate agar (DCA). On these media, individual non-lactose fermenting colonies are produced after overnight incubation at 35-37°C. *Proteus* culture has distinctive smell (Cheesbrough, 1987).

Proteus mirabilis is a frequent cause of nosocomial infections and is most commonly isolated from the urinary tract and less frequently from wounds, burns, eyes, and other sites (R.C.Harmon *et al*, 1989).

1.2.8 *Pseudomonas aeruginosa*

Gram-negative rod, strict aerobe, catalase positive, oxidase positive, produces acid from many sugars in ammonium salt medium, and motile (Barrow and Feltham, 1993).

Pseudomonas aeruginosa is an obligate aerobe usually recognized by its yellow-green pyocyanin pigment. Culture has distinctive smell due to 2-aminoacetophenone productions. It usually produces large, flat hemolytic colonies on blood agar. The organism also grows well on nutrient, MacConKey's agar and when grown in Kligler iron agar, it produces a characteristic pink-red slope and butt (Cheesbrough, 1987).

Pseudomonas aeruginosa can be found in water, soil, sewage and vegetation. It can also be found in the intestinal tract of man and animals. It is frequently found in hospital environment especially in moist places (Cheesbrough, 1987).

Some conditions with which *Pseudomonas aeruginosa* has been associated are; all animals wound infections, abscess formation, diarrhoea, and ear, urinary and genital tracts infections (Carter *et al*, 1986).

1.2.9 *Bacillus spp*

Bacillus spp are rods, typically Gram-positive in young cultures; motile (non-motile forms occur), non-acid fast, produce spores, aerobic, catalase positive and oxidase variable. Some of them attack sugars. Most of them grow in nutrient agar but not on MacConKey's agar (Carter, 1999).
Most species of *Bacillus* are saprophytes widely distributed in air, soil and water. The majority of *Bacillus* species has no pathogenic potential but can occur as contaminants on laboratory media.

1.2.9.1 *Bacillus mycoides*

It is a Gram positive, facultative anaerobe, breakdown casein and gelatin, catalase positive, glucose fermentation positive, lactose fermentation negative, breakdown nitrate to nitrogen gas, does not produce hydrogen sulfide and methyl red positive. It forms non-motile chains of cells, and can form acid from glucose. Its cell body does not swell when sporulating, and is usually larger than 3 micrometres. Using the Vosges-Prostkauer test, *Bacillus mycoides* produces a positive result. It can also hydrolyse starch.

1.2.10 *Neisseria spp*

Neisseria is Gram negative and aerobic, but strains of some species grow weakly under anaerobic conditions. Cocci are single, but more often in pairs with adjacent sides flattened, or distinct short rods arranged as

diplobacilli and in short chains. They show tendency to resist Gram decolourisation, asporogenous, non capsulated, non-motile, some species are fastidious, some species produce a yellow-green carotenoid pigment, oxidase positive and catalase producing (except *Neisseria elongata*). Most species reduce nitrite and some species oxidise carbohydrates. Neisseriae are normal flora of mouth, nasopharynx, nose, external genitalia, anterior urethra, vagina, eye and skin.

1.2.10.1 *Neisseria lactamica*

Is a coccus, single, but more often in pairs with adjacent sides flattened, requires CO_2, usually grows on nutrient agar at 35 °C, and colonies are smooth, translucent, butyrous and usually yellow pigmented. It is glucose, maltose and lactose positive, sucrose and fructose negative and reduces nitrite (with gas), but not nitrate.

1.2.11 *Brucella spp*

Brucella is Gram-negative coccoid rods, non-motile, non-spore forming, and aerobic bacteria. *Brucella bronchiseptica* is the only motile species (Merchant and Packer, 1967). The *Brucellae* are obligate parasites of animals and human and are characteristically located intracellularly, they are relatively inactive metabolically.

1.2.11.1 *Brucella abortus*

Brucella abortus is a short, Gram-negative rod, non-motile, aerobic, partially acid fast, catalase positive, oxidase positive, urease positive and attacks sugars by oxidation.

Duff (1936) reported that *Brucella abortus* was isolated from 80% of fistulous withers samples in equines.

Fistulous withers (F.W): (Supra spinus bursitis) is an inflammatory disease that affects equines commonly over the second thoracic spine (Sisson and

Grossman, 1938). This bursa appears to the starting point of fistulous withers, which may be acute or chronic (Adams, 1974).

Cosgrove (1961) showed that 10.5% of sera collected from animals suffering from F.W were positive for antibodies against *Brucella abortus*. Thornton *et al*. (1981) reported the positive relationship between complement fixation test and *Brucella abortus* commonly present with *Actinomyces bovis* in F.W.

Brucella abortus is often found in chronic bursa enlargement as a secondary invader (Blood *et al*., 2000).

1.2.11.2 *Brucella suis*

It has also been recovered from fistulous withers in horses (Merchant and Packer, 1967).

1.2.11.3 *Brucella bovis*

It has been isolated from persistent necrotizing and purulent lesions involving the ligamentum nuchae in horses (Carter *et al*., 1995).

1.2.12 *Actinomyces spp*

Are Gram-positive rods, non-motile, non-spore forming, no acid fast, microaerophilic or anaerobic, catalase negative and attack sugars fermentatively.

They were isolated from horses with fistulous withers (Kimball and Frank, 1945). Roderick *et al*. (1948) reported that *Brucella abortus* and *Actinomyces bovis* were isolated routinely from affected horses.

1.2.13 *Actinobacillus spp*

Actinobacillus species are non-motile, Gram-negative rods, facultative anaerobes, ferment carbohydrates. producing acids with out gas. Most species are urease and oxidase positive.

Actinobacillus spp were isolated from a higher than expected percentage of horses that developed postoperative wound infections after clean, elective surgery (Meredith, 2002).

1.2.13.1 *Actinobacillus mallei*

Is highly pathogenic for horses, mules and asses. Farcy can be a manifestation of local wound infection. Infection is contracted in most instances through inhalation and through wound infections (Hagan's *et al*., 1977).

1.2.13.2 *Actinobacillus equuli*

Actinobacillus equuli has been isolated from bacterial granuloma. Bacterial granuloma is frequently associated with wound contamination of limbs or scrotum. Lesions are non-puritic, firm, poorly circumscribed, nodular growths often with draining tracts and ulceration. White or yellowish granules may be present in the purulent discharge in equines (Andrew and Jack, 2006).

1.2.14 *Nocardia spp*

Nocardia are Gram-positive rods and filaments, non-motile, aerobic, catalase positive and attacks sugars by oxidation. They cause a chronic progressing disease characterized by suppurating granulomatous lesions in equines.

Rare granulomas caused by *Nocardia* or *Actinomyces spp*, which may act as wound contaminants, are sometimes seen in immunocompromised horses, or horses with hyperadenocortcism (Andrew and Jack, 2006).

Chapter three

2.1 MATERIALS AND METHODS

2.1.1 Materials

2.1.1.1 Sampling

2.1.1.1.1 Study area

In this study, the fieldwork was carried out in selected areas in Khartoum State. The areas, namely Mayou, Halaieb and Kuku, represent the main donkey-gathering sites in the three Provinces of the State, Khartoum, Omdurman and Khartoum North, respectively. (Table1).

The study was conducted during the period from december.2009 to April 2010.

2.1.1.1.2 Sampling

Fifty samples were collected using commercial sterile swabs from equines (2 horses and 48 donkeys) that were suffering from infected wounds. A single sample was collected from each animal randomly. Samples were collected from different anatomical sites of animals suffering from different types of the wounds. Fourty-three, (86%) samples were collected from the saddle area, four (8%) samples from the forelimbs and three (6%) samples from other different sites of the body. (**Table2**).

Table (1): Location and number of samples collected from infected wounds of equines

Location	*Number of samples*	*Percentage*
Khartoum	11(donkeys)	22%
Omdurman	37(donkeys)	74%
Khartoum North	2(horses)	4%
Total	50	100%

Table (2): Anatomical site and number of samples collected from infected wounds of equines

Anatomical site	*Number of samples*	*Percentage*
Saddle area	43	86%
Forelimbs	4	8%
Different other sites	3	6%

2.1.1.2 Media

Different types of media (solid, semi solid and liquid media) were used for the isolation and identification of the isolated bacteria. All media were prepared according to the manufacturer′s description.

2.1.1.2.1 Solid Media

2.1.1.2.1.1 Nutrient Agar (OXOID, CM 3)

Twenty – eight grams of the powder were added to one liter distilled water, dissolved and the pH was then adjusted to 7.4. The medium was sterilized by autoclaving at 121°C for 15 minutes before being poured into sterile Petri dishes in 20 ml volumes.

2.1.1.2.1.2 Blood Agar (OXOID, CM55)

To every 90 ml of sterile nutrient agar 10 ml of defibrinated sheep blood were added aseptically at 45 – 50 °C, mixed and poured into sterile Petri dishes in 15 – 20 ml volumes.

2.1.1.2.1.3 MacConkey's Agar (OXOID, CM7)

Fifty-two grams of medium were suspended in one liter of distilled water, dissolved and the pH was then adjusted to 7.4. The medium was sterilized by autoclaving at 121 °C for 15 minutes and then poured aseptically in sterile Petri dishes in 15 – 20 ml volumes.

2.1.1.2.1.4 Diagnostic Sensitivity Test Agar (DST) (OXOID, CM261)

Forty grams of medium were dissolved completely by boiling in a one liter of distilled water and the pH was adjusted to 7.3. The medium was then sterilized by autoclaving, cooled and distributed into sterile Petri dishes, 20 ml each.

2.1.1.2.1.5 Urea Agar (OXOID, CM53)

About 2.4 grams of medium were suspended in 95ml of distilled water, which was brought to boiling to dissolve the powder completely. The

medium was then sterilized by autoclaving at 121℃ for 15 min, cooled to 50℃ and aseptically, 5 ml of sterile 40% urea solution were added, mixed and then distributed in 10 ml volumes into sterile bijou bottles and allowed to solidify in a slant position.

2.1.1.2.1.6 Simmon's citrate Agar (OXOID, CM155)

Twenty – three grams were suspended in one liter of distilled water, boiled to dissolve completely and the pH was adjusted to 7.0. The medium was sterilized by autoclaving at 121℃ for 15 minutes and distributed into bijou bottles in portions of 5ml each and left to solidify in a sloped position.

2.1.1.2.2 Semi-Solid Media

2.1.1.2.2.1 Hugh and leifson's (O.F) Medium (OXOID)

The medium was prepared by dissolving 10.3 grams of solid in one liter of distilled water by heating, and the pH was adjusted to 7.1. Filtered bromothymol blue 0.2% aqueous solution was added and then the medium was sterilized at 115℃ for 20 minutes. Sterile solution of glucose was aseptically added to give a final concentration of 1%. The medium was mixed and distributed aseptically as 7ml volumes in sterile test tubes.

2.1.1.2.2.2 Motility Medium (OXOID)

Thirteen grams of nutrient broth was add to 4 grams of agar and dissolved in one liter of distilled water and the pH was then adjusted to 7.2. The medium was distributed as 5ml volumes in test tubes containing carigie-tubes and sterilized by autoclaving at 115 ℃ for 15 minutes.

2.1.1.2.3 Liquid Media

2.1.1.2.3.1 Nutrient Broth

Thirteen grams of the medium were suspended in one liter of distilled water, dissolved and the pH was adjusted to 7.4. The medium was

distributed as 5ml volumes in test tubes and sterilized by autoclaving at 121℃ for 15 minutes.

2.1.1.2.3.2 Peptone Water

Fifteen grams of the medium were dissolved in one liter of distilled water and the pH was adjusted to 7.2 .The medium was distributed into 5 ml volumes and sterilized by autoclaving at 121 ℃ for 15 minutes.

2.1.1.2.3.3 Glucose Phosphate Broth (M.R-VP medium)

Five grams of peptone water and 5 grams of potassium phosphate were dissolved in one liter of distilled water and the pH was adjusted to 7.5. Then Five grams of glucose were added, mixed and the medium was distributed into 5ml volumes in test tubes and sterilized by autoclaving at 121℃ for 15 minutes.

2.1.1.2.3.4 Peptone Water Sugars

Nine hundred ml of peptone water was prepared and the pH was adjusted to 7.1-7.3 before 1 ml of Andrade's indicator was added. Ten grams of appropriate sugar were added to the mixture, which was distributed into 5 ml tubes. Which were sterilized by autoclaving at 115℃ for 10 minutes.

2.1.1.2.3.5 Nitrate broth

One gram of nitrate was dissolved in one liter of nutrient broth, then distributed into tubes and sterilized by autoclaving at 121℃ for 15 minutes.

2.1.1.3 Biological Materials

2.1.1.3.1 Sheep Blood

Defibrinated sheep blood was used for the preparation of blood agar.

2.1.1.3.2 Human plasma

This was used for the detection of coagulase production by staphylococci.

2.1.1.4 Reagents

2.1.1.4.1 Hydrogen Peroxide

Hydrogen Peroxide was prepared as 3% aqueous solution, protected from light and stored in the refrigerator at 4°C. It was used for the catalase test.

2.1.1.4.2 Oxidase test reagent

Tetramethyl p-phenylene diamine dihydrochloride was prepared as 1% aqueous solution and used for the oxidase test.

2.1.1.4.3 Kovac's reagent

This regent is composed of 5 grams of Para- dimethyl- amino Benz aldehyde, 75 ml amyl alcohol and 25 ml concentrated hydrochloric acid. Cooled and the acid were added carefully. The regent was stored at 4 ºC for later use in the indole test.

2.1.1.4.4 Lead acetate

Filter paper strips, 4-5 mm wide and 50-60 mm long were impregnated in lead acetate saturated solution and then dried. It was used for the hydrogen sulphide test.

2.1.1.4.5 Methyl red solution

This solution was prepared by dissolving 0.04 grams of methyl red powder in 40 ml ethanol and the volume was made up to 100 ml with distilled water.

2.1.1.4.6 Alpha- naphthol solution

It was prepared as 1% aqueous solution and used for the (VP) test.

2.1.1.4.7 Andrade's Indicator

Five grams of acid fuchsin were dissolved in one liter distilled water, and then 150 ml of alkali solution (NaOH) were added. It was used in the peptone sugar medium.

2.1.1.4.8 Bromothymol blue solution

This indicator was prepared by dissolving 0.2 gram of bromthymol blue powder in 100 ml distilled water. It was used for the oxidation fermentation test.

2.1.1.4.9 Normal saline

Physiological or isotonic saline was prepared by dissolving 8.5 grams of sodium chloride in one liter of distilled water to obtain 0.85 % concentration.

2.1.1.4.10 Nitrate test reagent

This reagent was composed of two types of solution:

Solution A: Sulphanilic acid 0.33% in 5 N-acetic acid dissolved by gentle heating.

Solution B: Dimethyl- a-naphthyl amine 0.6% in 5N- acetic acid.

The complete reagent was used to detect nitrate reduction.

2.1.2 Methods

2.1.2.1 Sterilization

2.1.2.1.1 Flaming

Flaming was used to sterilize slides, cover slips and glass rods.

2.1.1.1.2 Hot air oven

Hot air oven was used, at 160 ºC for one hour, to sterilize glassware such as pipettes, Petri dishes, tubes, flasks and glass rods.

2.1.2.1.3 Moist heating (Autoclaving)

Media, solutions, screw-capped bottles, rubber, Stoppard, flasks and plastic ware were sterilized by autoclaving at 121ºC for 15 min and 110ºC for 10 min for sugar media.

2.1.2.1.4 Disinfection

Alcohol (70%) and phenolic solution were used for disinfecting working places in the media preparation room and the floors and benches in the laboratory.

2.1.2.2 Cultural methods

2.1.2.2.1 Primary isolation

For the primary isolation, the swabs were cultured on blood agar and MacConkey's agar. The plates were incubated aerobically at 37ºC for 1-2 days.

2.1.2.2.2 Examination of cultures

Cultures were examined visually for the detection of growth, pigmentation and colonial morphology.

2.1.2.2.3 Purification and storage of the isolates

Isolated bacteria were purified by repeated sub culturing on blood agar and nutrient agar plates. Subculturing was repeated several times until pure colonies were obtained. Then incubated at 37ºC for 24 hours until pure colonies were obtained. The purified bacteria were stored at 4 ºC.

2.1.2.2.3.1 Identification of the isolates

Identification of the isolates was carried out according to Barrow and Feltham (1993).

2.1.2.2.3.1.1 Primary identification

2.1.2.2.3.1.1.1 Preparation of smears

Smears were prepared by emulsifying small inocula of the bacterial culture in a drop of sterile normal saline and spreading them on a clean slide. The smears were allowed to dry and fixed by gentle heating.

2.1.2.2.3.1.1.2 Gram's stain

This was done as described by Barrow and Feltham (1993). It was used to study morphology, shape and gram staining reaction of each isolate.

Gram's staining method:

1. Crystal violet was added to fixed smear for 30 seconds.
2. Washed with distilled water.
3. Lugol′s iodine was added for 30 seconds.
4. Decolorized with acetone-alcohol for 2-3 seconds.
5. Washed with distilled water.
6. Counter stained with dilute carbol fuchsin for 30 seconds.
7. Washed with distilled water.
8. Dried with filter paper and examined under microscope by oil immersion objective lens.

Gram-positive bacteria appeared purple, while Gram-negative bacteria appeared red.

2.1.2.2.3.1.1.3 Biochemical tests

All the following biochemical tests were conducted and preformed according to Barrow and Feltham (1993).

2.1.2.2.3.1.1.3.1 Primary tests

2.1.2.2.3.1.1.3.1.1 Catalase test

To differentiate those bacteria that produce the catalase enzyme, a drop of 3% hydrogen peroxide was placed on a clean slide and a colony of the test organism cultured on nutrient agar was picked with glass rod and added to the drop of the solution. A positive result was indicated by immediate production of air bubbles.

2.1.2.2.3.1.1.3.1.2 Oxidase test

Commercial oxidase paper was used. The test organism was picked using a sterile bent glass rod and rubbed on a filter paper, saturated with oxidase reagent. The development of dark purple color within 10 seconds indicated a positive result.

2.1.2.2.3.1.1.3.1.3 Oxidation fermentation test (O.F)

This test was used to determine the way by which the bacterium attacks a carbohydrate (by fermentation or by oxidation). The test was performed by growing the bacterium in two tubes of Hugh and Leifson's medium. One of them was covered with a layer of sterile paraffin oil.

All tubes were incubated at 37 ºC and examined daily for 14 days. Fermentative organisms produced a yellow color on both tubes while oxidative organisms produced a yellow color only on open tube. When the two tubes were green, the result was negative.

2.1.2.2.3.1.1.3.1.4 Motility test

By a sterile wire, a small piece of colony was picked and stabbed in the center of the semi-solid agar in the Craigie tube. This preparation was incubated overnight at 37 ºC. The growth out side the Craigie tube and turbidity in the medium indicated that the organism was motile.

2.1.2.2.3.1.1.3.1.5 Gas from glucose

Tubes of glucose sugar medium were inoculated with the test culture and incubated for up to 7 days at 37 ºC. Production of acid was indicated by the development of a pink color in the medium and gas production was indicated by the presence of an empty space in the inverted Durham's tube.

2.1.2.2.3.1.1.3.2 Secondary tests

2.1.2.2.3.1.1.3.2.1 Voges- proskauer (VP) test

Test tubes or screw–capped bottle containing glucose phosphate broth were inoculated with the test organism and incubated overnight at 37 ºC. Then 0.2 ml of 40% KOH and 0.6 ml of 5% alpha-naphthol solution were added to one ml of the culture and shacked. The tubes were then placed in sloped position and examined; a positive test was indicated by a strong red color within half an hour.

2.1.2.2.3.1.1.3.2.2 Indole test

Peptone water was inoculated with the isolate under test and incubated at 37ºC for 48 hours. Kovac's reagent was added to the culture and shacked well. The development of a red ring on the surface of the culture indicated a positive result.

2.1.2.2.3.1.1.3.2.3 Methyl red test (MR)

Culture in peptone water was incubated over night and then 3-5 drops of methyl red were added to the culture. Production of a red color in the medium indicated a positive test.

2.1.2.2.3.1.1.3.2.4 Urease test

The test bacteria were inoculated on a slope of urea agar medium incubated at 37 ºC and examined for up to 5 days. The change of the color of the medium to pink indicated a positive result.

2.1.2.2.3.1.1.3.2.5 Citrate utilization test

Simmon's citrate medium was inoculated with the test organism, incubated at 37ºC and examined daily for 7 days. Change of color to blue indicated a positive test and a yellow indicated a negative test.

2.1.2.2.3.1.1.3.2.6 Hydrogen sulphide (H_2S) production

A filter paper soaked in a 10% lead acetate solution was let to dry. The dry filter paper was put at upper side of the tube containing the culture, pushed with cotton cover and incubated overnight. In positive tubes the culture changed the color of the paper used to black.

2.1.2.2.3.1.1.3.2.7 Acid production from carbohydrate

Peptone water sugar (glucose, lactose, sucrose, maltose, xylose, mannitol and sorbitol) medium was used. It was inoculated with the organism under test, incubated at 37 ºC and examined daily up to 7 days for acid production. Development of a pink or a red color indicated a positive result.

2.1.2.2.3.1.1.3.2.8 Nitrate reduction test

The test organism was grown into 5 ml nutrient broth and incubated for 48 hours after that 1 ml of nitrate reagent A was added followed by 1 ml of reagent B. Development of a red color indicated the reduction of nitrate to nitrite. To tubes that did not show red color, zinc powder was added and allowed to stand. Formation of red color indicated the nitrate was present and the test organism did not reduce it.

2.1.2.2.3.1.1.3.2.9 Coagulase test

For the coagulase test, plasma of rabbit or human was used. Plasma contains fibrinogen that is converted to fibrin by the staphylococcal coagulase enzyme.

2.1.2.2.3.1.1.3.2.9.1 Slide coagulase test

Two separate drops of normal saline were placed on a clean slide and a small amount of the test organism was emulsified in each of the drops to make two thick suspensions.

A drop of undiluted human plasma was added to one of the suspensions and mixed gently. The development of clumping within 10 seconds was reported as a positive result.

2.1.2.2.3.1.1.3.2.9.2 Tube Coagulase Test

This test was used to detect free Coagulase. Fresh plasma was diluted 1:10 in physiological saline and 0.5 ml of diluted plasma was placed in a sterile test tube. Then 0.5 ml of an overnight broth culture of the test organism was added. The tube was then incubated at 37 ºC and examined after 1, 3, and 24 hours. A positive test was indicated by coagulation of the tube content.

2.1.2.2.3.1.1.3.2.10 Antibiotic susceptibility test

The sensitivity of each isolate to different commonly used antibiotics was evaluated using the disc diffusion method.

Peptone water medium was inoculated by the test organism and incubated at 37 ºC for 24 hours. Then about 1 ml was poured onto diagnostic susceptibility test medium agar plate. Excess fluid was removed by using Pasteur pipettes and the plates were allowed to dry.

Sterile forceps were used to place the discs on the plates and lightly press them down to ensure that the antibiotic discs were in contact with the agar. After 30 minutes, the plates were incubated aerobically at 37 ºC for 24 hours. Susceptibility to antibiotics was indicated by zones of inhibition around the antibiotic discs. The diameters of zones were measured in millimeter. The isolates were then reported as susceptible, intermediate or resistant according to the size of inhibition zone (National Committee for Clinical Laboratory Standards, 1997). (Table 3).

Table (3) the antibiotics used for susceptibility tests

Antibiotic	Code	Concentration	R	I	S
Penicillin G	P	10 units	20	21-28	=29
Co-trimoxazole	BA	25 mcg	= 11	12-16	=17
Cephalexin	PR	30mcg	= 14	15-17	=18
Tetracycline	TE	30mcg	= 14	15-18	=19
Cefotaxime	CF	30mcg	= 14	15-22	=23
Ciprofloxacin	CP	5mcg	= 15	16-20	=21
Pefloxacine	PF	5mcg	= 15	16-20	=21
Ofloxacine	OF	5mcg	= 15	16-20	=21
Cloxacillin	CX	5mcg	= 9	10-13	=14
Erythromycin	E	15mcg	= 13	14-22	=23
Clindamycin	CD	2mcg	= 14	15-20	=21
Gentamycin	GM	10mcg	= 12	13-14	=15

R= Resistant, I= Intermediate, S=Susceptible.

Chapter Four

Results

4.1 Isolation of bacteria

Fifty samples from wounds in different animals (2horses and 48 donkeys) were investigated bacteriologically. Aerobic bacteria were isolated from 48 samples and 68 isolates were recovered.

The isolates represented 9 different bacterial genera Table (4). The most frequently isolated bacteria were *Streptococcus spp* (41.2%), followed by *Staphylococcus spp* (31.0%), *Micrococcus spp* (7.4%), *Corynebacterium spp* (5.9%), *Escherichia spp* (2.9%), *Neisseria spp* (2.9%), *Rhodococcus spp* (2.9%), *Bacillus spp* (2.9%) and *Proteus spp* (2.9%) Table (5).

Identification of the isolates was carried out according to Barrow and Feltham (1993).

The biochemical tests used to identify the different bacterial isolates are shown in tables 6 to 14.

Table (4): Types and frequency of bacteria isolated from infected wounds of equines

Type of bacteria	**Number of isolates**	**Frequency of isolates**
Streptococcus spp.	28	41.2%
Staphylococcus spp.	21	31.0%
Micrococcus spp.	5	7.4%
Corynebacterium spp.	4	5.9%
Escherichia spp.	2	2.9%
Bacillus spp.	2	2.9%
Rhodococcus spp.	2	2.9%
Neisseria spp.	2	2.9%
Proteus spp.	2	2.9%
Total	**68**	**100%**

Table (5): Species of bacteria isolated from infected wounds of equines

Bacterial species	**Number of isolates**	**Frequency of isolates**
Streptococcus equisimilis	14	20.6%
Streptococcus zooepidermicus	9	13.2%
Streptococcus equi	5	7.4%
Staphylococcus aureus	17	25.0%
Staphylococcus intermidius	2	2.9%
Staphylococcus epidermidis	2	2.9%
Corynebacterium pseudotuberculosis	4	5.9%
Escherichia coli	2	2.9%
Bacillus mycoides	2	2.9%
Neisseria lactamica	2	2.9%
Rhodococcus equi	2	2.9%
Micrococcus roseus	5	7.4%
Proteus spp.	2	2.9%
Total	**68**	**99.8%**

Table (6): Results of biochemical tests used to identify *Streptococcus spp*

Biochemical test	*S.equi*	*S.zooepdemicus*	*S.equisimilis*
Gram stain	G +ve cocci	G +ve cocci	G +ve cocci
Haemolysis	ß	ß	ß
Catalase	-	-	-
Oxidase	-	-	-
Motility	-	-	-
Glucose	+	+	+
O.F	F	F	F
Urease	-	-	-
VP	-	-	-
Sucrose	+	+	+
Lactose	+		+
Trehalose	-	+	-
Sorbitol	-	-	+
Mannitol	-	-	-

O.F = Oxidation Fermentation test.

VP = Voges- Proskauer test.

G = Gram, + = Positive, - = Negative and F = Fermentative.

Table (7): Results of biochemical tests used to identify *Staphylococcus spp*

Biochemical test	*S.aureus*	*S.epidermidis*	*S.intermidius*
Gram stain	G +ve cocci	G +ve cocci	G +ve cocci
Catalase	+	+	+
Oxidase	-	-	-
Motility	-	-	-
Glucose	+	+	+
O.F	F	F	F
Urease	+	+	+
VP	+	+	-
Nitrate	+	+	+
H_2S	-	-	-
Sucrose	+	+	+
Lactose	+	-	+
Maltose	+	+	-
Xylose	-	-	-
Mannitol	+	-	+
Coagulase	+	-	+

O.F =Oxidation Fermentation test.

VP =Voges- Proskauer test.

G = Gram, + = Positive, - = Negative and F = Fermentative

Table (8): Results of biochemical tests used to identify *Corynebacterium pseudotuberculosis*

Biochemical test	*Corynebacterium pseudotuberculosis*
Gram stain	G +ve rods
Catalase	+
Oxidase	-
Motility	-
Glucose	+
O. F	F
Urease	+
VP	-
Nitrate	+
H_2S	-
Sucrose	-
Lactose	-
Maltose	+
Xylose	-
Manitol	-

O.F =Oxidation Fermentation test.

VP = Voges- Proskauer test.

G = Gram, + = Positive, - = Negative and F = Fermentative.

Table (9): Results of biochemical tests used to identify *E. coli*

Biochemical test	*E. coli*
Gram stain	G -ve rods
Catalase	+
Oxidase	-
Motility	-
Glucose	+
O.F	F
MacConKey growth	+
VP	-
Simmon's citrate	-
Urease test	-
H_2S	-
Indole	+
Methyl Red	+
Maltose	+
Xylose	+
Mannitol	+

O.F = Oxidation Fermentation test.

VP = Voges- Proskauer test.

G = gram, + = Positive, - = Negative and F= Fermentative.

Table (10): Results of biochemical tests used to identify *Bacillus mycoides*

Biochemical test	*Bacillus mycoides*
Gram stain	G +ve rods
Motility	-
Catalase	+
Oxidase	-
Glucose	+
O.F	O
VP	+
Haemolysis	+
Growth on nutrient agar	+
Galactose	-
Xylose	-
Nitrate reduction	+
Spore position	Central

O.F = Oxidation Fermentation test.

VP = Voges- Proskauer test.

G= Gram, + = Positive, - = Negative and O = Oxidative.

Table (11): Results of biochemical tests used to identify *Neisseria lactamica*

Biochemical test	*Neisseria lactamica*
Gram stain	G -ve cocci
Catalase	+
Oxidase	+
Glucose	+
O.F	O
Sucrose	-
Lactose	+
Maltose	+
Haemolysis	-
Nitrate reduction	-

O.F =Oxidation Fermentation.

G= Gram, += Positive, - = Negative and O = Oxidative.

Table (12): Results of biochemical tests used to identify *Micrococcus spp*

Biochemical test	*Micrococcus spp.*
Gram stain	G +ve cocci
Motility	-
Catalase	+
Oxidase	+
Glucose	+
O.F	O
VP	-
urease	+
Sucrose	-
Nitrate reduction	-

O.F = Oxidation Fermentation test.

VP = Voges- Proskauer test.

G = Gram, + = Positive, - = Negative and O = Oxidative.

Table (13): Results of biochemical tests used to identify *Proteus spp*

Biochemical test	*Proteus spp.*
Gram stain	G -ve rods
Catalase	+
Oxidase	-
Glucose	+
O.F	F
VP	-
urease	+
H_2S	+
Sucrose	+
Lactose	-
Maltose	-
Xylose	+
Mannitol	+
MacConKey growth	+
MR	+

O.F = Oxidation Fermentation test. MR =Methyl Red.

VP = Voges- Proskauer test.

G = Gram, += Positive, - = Negative and F= Fermentative.

Table (14): Results of biochemical tests used to identify *Rhodococcus equi*

Biochemical test	*Rhodococcus equi*
Gram stain	G +ve cocci
Catalase	+
Oxidase	+
Glucose	-
O.F	-
Motility	-
urease	+
Heamolysis	-
Sucrose	-
Maltose	-
Nitrate reduction	+

O.F = = Oxidation Fermentation test.

VP = Voges- Proskauer test.

G = Gram, += Positive, - = Negative.

4.2 Antibiotic susceptibility

Twenty- one isolates representing the recovered bacteria were subjected to antibiotic sensitivity test. All isolates were found sensitive to Cephalexin (PR), Ciprofloxacin (CP), Cloxacillin (CX), Co-trimoxazole (BA) and Gentamicin (GM), except *E. coli*, which was found to be resistant to Co-trimoxazole (BA), and Gentamicin (GM). All isolates were resistant to penicillin (P). However, the isolates showed varying degrees of susceptibility to tetracycline (TE). (Table 15).

Table (15): Susceptibility of the bacterial isolates to different antibacterial drugs

Bacterial spp/ Antibiotic	**P**	**BA**	**PR**	**TE**	**CP**	**CX**	**GM**
Staph. aureus	R	S	S	S	S	S	S
Staph. epidermidis	R	S	S	S	S	S	S
Staph. intermidius	R	S	S	S	S	S	S
Strept. equi	R	S	S	R	S	S	S
Strept. zooepidimicus	R	S	S	M	S	S	S
Strept.equisimilis	R	S	S	M	S	S	S
Corynebacterium pseudotuberculosis	R	S	S	M	S	S	S
E.coli	R	R	S	R	S	M	R
Neisseria lactamica	R	S	S		S	S	S
Rhodococcus equi	R	S	S	M	S	S	S
Micrococcus spp	R	S	S	R	S	S	S
Bacillus mycoides	R	S	S	-	S	S	S

P= Penicillin, BA= Co-trimoxazole, PR= Cephalexin, TE= tetracycline, CP= Ciprofloxacin, CX= Cloxacillin, GM= Gentamicin.

R = Resistant, S = Sensitive, M = Moderate and - = Not tested.

Chapter Five

Discussion

Equines in the Sudan in both rural and urban areas play a very important role in supporting poor families, thus maintaining these animals in good health will be valuable to those low-income people

The objectives of the present study were to isolate and identify aerobic bacteria associated with wound infections in equine in Khartoum State, Sudan and study their sensitivity to various antibacterial agents.

In the present study, it was observed that most wounds (86%) were in the saddle area (back and abdomen), 8% were in forelimbs area, whereas only 6% were distributed in other 5 different sites. Dania (2007), reported that most sites of wounds were in the back (71.6%), whereas 14.2% were in the abdominal region. However, the incidence of the back wounds was higher than those found by Sells *et al*, (2010), who reported an incidence of 54% of back wounds in equines in Morroco.

In this study most of the wounds examined were superficial and a few were deep. This finding may indicate that most causes of the wounds were mechanical injuries. It was suggested that carrying heavy weights causes wounds on legs, while wounds on the back and abdominal region of the animal are caused always by repeated friction and bruising from the saddle with subsequent devitalization and necrosis of the tissue (Pallister, 1964, Soulsby, 2000).

Most of the infections were found to be due to *Streptococcus spp*, as 41.2% of the swabs examined revealed *Streptococcus spp*, the most frequent of these were *Streptococcus equisimilis* (20.6%), followed by *Streptococcus zooepidemicus* (13.2%) and *Streptococcus equi* (7.4%).

Ibrahim (2007) isolated 26% *Streptococcus equisimilis* and 8% *Streptococcus equi* from 50 wound swabs in equines.

The frequency of *Streptococcus spp* was followed by *Staphylococcus spp*, which were isolated from 31.0% of the swabs examined.

Twenty five percent of the staphylococci were *Staphylococcus aureus* and 2.9% were *Staphylococcus epidermidi*s, which agrees with the findings of Asha *et al*, (2006) who recovered *Staphylococcus aureus* from 20.3% and *Staphylococcus epidermidis* from 2% of swabs. The 2.9% *Staphylococcus intermidius* isolated in this study is in agreement with the results of Collier *et al*, (1998), who isolated *Staphylococcus intermidius* from 3% of wounds in the saddle and girth areas of equine.

Corynebacterium pseudotuberculosis was isolated from 5.9% of the swabs studied. This confirms the results of Asha *et al*, (2006), who isolated 6% of *Corynebacterium pseudotuberculosis* from infected wounds. This organism was reported to cause lesions of ulcerative lymphangitis in horses, which are usually seen on hindlimbs distal to the hocks as a result of the organism gaining entrance to the skin through wounds and spreading via the lymphatics (Roger, 2003).

Escherichia coli was isolated from (2.9%) and *Micrococcus spp* from (7.4%) of the swabs examined. Ibrahim (2007) isolated *Escherichia coli* from (2%) of his samples from wounds in equines, while Shimozawa *et al*, (1997), isolated *Micrococcus spp* from infected wounds of racehorses.

Rhodococcus equi was isolated from 2.9% of the samples, which was much lower than that reported by Dania (2007), who isolated *Rhodococcus equi* from 31.7% of wounds infection in equines in Sudan.

Bacillus mycoides, *Neisseria lactamica* and *Proteus spp* were isolated from 2.9% of swabs each. These results differed from those isolated by

Dania (2007), who isolated *Bacillus mycoides* and *Neisseria lactamica* from 5.0% and 1.2%, respectively of wounds.

Sensitivity testing showed that all isolates were sensitive to Cephalexin (PR), Ciprofloxacin (CP), Cloxacillin (CX), Co-trimoxazole (BA) and Gentamicin (GM), except *Escherichia coli*, which was found to be resistant to Co-trimoxazole and Gentamicin. All isolates were resistant to penicillin. This result is significant as penicillin is the drug of choice for the treatment of wound infections in equines. Isolates showed varying degrees of susceptibility to tetracycline (TE). Similar results were obtained by Dania (2007). This calls for susceptibility testing of bacteria to antibiotics before commencing treatment.

Conclusions

- *Staphylococcus aureus, Staphylococcus intermidius, Staphylococcus epidermidis, Streptococcus equisimilis, Streptococcuszooepidemicus, Streptococcusequi, Corynebacterium pseudotuberculosis, Escherchia coli* and *Rhodococcus equi* were the commonest causative aerobic bacteria of infected wounds in equine in Khartoum State.
- *Micrococcus spp, Bacillus mycoides, Neisseria lactamica* and *Proteus spp* can also be isolated from infected wound as non-pathogenic agents.
- *Brucella* spp and *Actinomyces* spp were not isolated from cases of saddle infections.
- All isolates were found sensitive to Cephalexin, Ciprofloxacin, Cloxacillin, Co-trimoxazole and Gentamicin, except, *Escherchia coli*, which was found to be resistant to Co-trimoxazole, and Gentamicin. All isolates were resistant to Penicillin. However, the isolates showed varying degrees of susceptibility to tetracycline.

Recommendations

It is recommended that

- Early diagnosis and treatment of the wounds is important to reduce the risk of wound infections.
- The choice of antibiotics for treatment should be made after antibiotic susceptibility tests.
- Owners of equines may decrease the prevalence of wounds and, therefore, improve welfare and working capability of these equines by using proper saddling.
- Further researches are required to identify reasons for regional difference in prevalence of pack wounds in equines.

Chapter Six

References

Adams, O.R. (1974). *Lameness in Horses*. 3rd ed., Philadephia, Lea and Febiger. Pp: 910.

Andrew Higgins, and Jack R. Snyder (2006). *The Equine Manual*. W.B. Saunder Company, USA. 2nd ed, pp: 325.

Asha, M.E.; Salim, M.O.; Amel, O.B. and Ibrahim, A.A. (2006). The Aerobic Bacteria of Equine Wound Infection Associated with Type of Animal Use and Sampling. *Sud. J. Vet. Sci. and Anim.Husb*. **45** (1 and 2): 1-2.

Barrow, G.I. and Feltham, R.K.A. (1993). *Cowan and Steels Manual for the Identification of Bacteria*, 3rd ed., Cambridge University, Cambridge, pp: 1172.

Blood, D.C. and Henderson, J.A. (1968). *Veterinary Medicine*, 3rd ed. Bailliere Tindall and Cassell Ltd., London, pp: 927.

Blood, D.C.; Radositis, O.M. and Henderson. (2000). *Veterinary Medicine*. 7th ed. Balliere Tindall, London.

Boyd, R.F. and Hoerl, B.G. (1991). *Basic Medical Microbiology*. 4th ed., Little, Brown and Company, Boston, p: 402.

Bryans, J.T. and Knight, H.D. (1972). *Equine Medicine and Surgery*. 2nd ed., American Veterinary Publications Inc. Wheaton, pp: 960.

Carter, G.R. (1999). *Essentials of Veterinary Bacteriology and Mycology*. 3rd ed., Lea and Febiger. Philadephia, pp: 193.

Carter, G.R.; William, C.G. and Rikihisa, Y. (1986). *Essential of Veterinary Bacteriology and Mycology*. 3rd ed., Lea and Febiger, Philadelphia, pp; 261.

Carter, G.R; Chengeappa, M.M; Roberts, A.W; William, C. and Rikihisa, Y. (1995). *Essentials of Veterinary microbiology*. 5th ed., William and Wilkins, Philadelphia, pp: 393.

Cheesbrough, M. (1987). *Medical Laboratory manual for tropical countries*.2nd ed. ELBS. London: 1.

Chris Clark, Sarah Greenwood, Joe O. Boison, Manuel Chirio-Trejo, and Patricia M. Dowling. (2008). Bacterial Isolates from Equine Infection in Western Canada (1998-2003). *Can Vet J.* **49** (2): 153-160.

Collier, L.; Balows, A. and Sussman, M. (1998). *Topley and Wilson's microbiology and microbial infections*, 9th ed. **(3)**, Oxford University Press, New York, pp. 1163.

Cosgrove, J.S. (1961*). Clinical aspect of equine brucellosis*. Vet. Rec. **73**: 1377-1382.

Dania E.A. (2007). *Some Aerobic Bacteria Associated with External Wounds of Donkeys in Khartoum State*. M.Sc. thesis, University of Khartoum.

Denny W.Scott; William H.Miller. (2003). *Equine Dermatology*. Elsevier health sciences, USA. pp: 221.

Douglas H. Slatter. (2002). *Text Book of Small Animal Surgery*. Elsevier health sciences, USA 3rd ed., pp: 66.

Downar, J.; Willey, B.M.; Sutherland, J.W.; Mathew, K. and Low, D.E. (2001). Streptococcal meningitis resulting from the contact with an infected horse. *J. Clin. Microbiol.* **39**: 2358-2359.

Duff, H.M.M. (1936). *Fistulous withers and poll evil due to Brucella abortus*. Vet Rec. **48**: 75-177.

Pallister, E.F. (1964). Proper Care of Wounds in Horses. *Can. Vet. J.* **5** (9), pp: 231.

Francois Roger and Bojia Endebu (2003). Comparative Study on the Occurrence and Distribution of Epizootic Lymphangitis and Ulcerative Lymphangitis in Ethiopia. *Int. J. of App. Res. in Vet Med* **1**(3), pp: 1.

Gordon, R. Carter, Darla, J. Wise (2004). *Essentials of Veterinary Bacteriology and Mycology*. 6th ed., Wiley-Blackweel, Iowa. pp: 185-186.

Greenwood, O.D.; Richard, C.S. and John, F.P. (2002). *Medical Microbiology*. 16th ed. Churchill Livingstone, Edinburgh, pp: 174-175.

Greenwood, O.D.; Slack, R.C.B. and Peutheren, J.F., (1997). *Medical Microbiology*. 15th ed. Funded by ELST Publishers with Churchill Livingstone, Hong Kong, p: 267.

Hagan, W.A.; Bruner, D.W. and Gillespie, J.H. (1977*). Infectious Diseases of Domestic Animals.* 6th ed. Cornell University Press in United Kingdom, London, pp: 1385.

Hardie, J.M. and Whiley, R.A. (1995). *The Genus Streptococcus*. The Genera of Lactic Acid Bacteria. London, Blackie Acadimic and Professional. **2**: 55-124.

HardieJ.M. (1986). *Streptococcus genus in Bergey's Manual of Systemic Bacteriology*. 1st ed. Springer, New York, US. Pp: 1043-1056.

Hickman, J. (1985). *Equine surg. and Med*. Vol. **(1).** Harcourt Brace Jovanovich Publishers, London. P: 398.

Ibrahim A. Mohammed (2007). *Bacteria of Infected Wounds in Equines in Khartoum State.* M.Sc. Thesis, University of Khartoum.

Jarrell, B.E.; Carabasi, R.A. and Radomisks, J.S. (2000). *National Medical Series for Independent Study*. 4th ed. Lippincott Williams and Wilkins, Sydney, p: 19.

Kimball, A; Franck, E.R. (1945). The Isolation of Actinomyces bovis from Fistulous Withers and poll evill. *A J. Vet. Res*. **6**: 39-44.

Koneman, E.W.; Allen, S.D.; Janda, W.M.; Achrekenberger, P.C. and Winn, W.C. (1992). *Diagnostic Microbiology*. 4th ed., J.B. Lippincott company, Philadelphia. Pp. 433.

Kunio Shimozawa, Toru Anzai, Masonobu Kamada and Takatori Kosuke. (1997). Fungal and Bacterial Isolation from Racehorses with Infections Dermatosis. *J of equ. sci*. Vol. **8** (4) p: 89-93.

Lancefield R. (1933). A Serological Differentiation of Human and Other groups of hemolytic Streptococci. *J. Exp. Med*. **57**, 571-595.

Mann C.V.; Russell, R.C.G. and William, N.S. (1996). *Bailey and Lovers. Short Practice of Surgery*, 22nd ed. Chapman and Hall Medical, Weinhem, pp: 527.

Meredith A. Smith (2002). Postoperative infection with *Actinobacillus* spp in horses. *JA.VM.A.* **221**(9): 1306-1310.

Merchant, I.A. and Packer, R.A. (1967). *Veterinary Bacteriology and Virology*. 7th ed., Iowa State University Press, Iowa, pp: 752.

Sells. G. Pinchbeck; J. Ibourki and M. Crane. (2010). Back Wounds of Donkeys and Mules in Nothern High Atlas and Lowlands of Morroco. *Eq. Vet.J.* **42** (3): 219-226.

National Committee for Clinical Laboratory Standards. (1997). Specialty Collection: Susceptibility Testing. SC21L. M7-A4. NCCLS, Wayne, PA.

Wilson RT. (1978). Studies on the livestock of southern Darfur, Sudan Notes on equines. *Trop. Anim. Helh Prod*. **10** (3): 183-9.

Regina Linder. (1997). *Rhodococcus equi* and *Aracanobacterium haemolyticum*: two bacteria increasing recognised as agents of human infection. *Emerging Infectious Disease*.**3** (2): 145-146.

Richard C. Harmon, Richard L. Rutherford, Hisu-Mei Wu, and Micheal S. Collins (1989). Monoclonal Antibody-mediate protection and neutralization of motility in experimental *Proteus mirabilis* infection. *Inf. and Immun.* **57**: 1936-1941.

Roderick, L.M; Kimball, A; Franck, E.R. (1948). A study of equine fistulous withers and poll evil. A complex infection with Actinomyces bovis and Brucella. *AJVR.* **30**: 5-10.

Romich, Janet Amundson (2008). *Understanding Zoonotic Disease.* Thomson/ Delmar Learning, Michigan. Pp: 327.

Samantha K. Hart (2009). *Elution of Antibiotic from Novel Cross- Linked Dextran Gel: In Vivo Quantification.* Thesis submitted to the Faculty of Virginia Polytechnic Institute and State University. Leesburg, pp: 1.

Sisson, S; Grossman, J.D. (1938). *The Anatomy of Domestic Animals*. 3rd ed. W.B. Saunders C.O, Philadelphia. Pp: 214-215.

Soulsby, E.J.L. (2000). *The Professional Handbook of the Donkey*. 3rd ed. By Elisabeth, D.S.M.B.E. Whittet Book Limited, London. Pp: 5-6.

Stewart,E.S. and Beswick,S.L.(1977). *Bacteriology, Virology and Immunity*. 10th ed. Bailliere indall, London.pp: 486.

Vincent J.M.Di Maio, and Suzanna E. Dana. (2007). *Hand Book of Forensic Pathology*. Taylor and Francis group, NewYork. 2nd ed. Pp: 117.