ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

BACTERIOLOGICAL STUDIES ON THE RESPIRATORY TRACTS OF APPARENTLY HEALTHY AND PNEUMONIC CAMELS (*CAMELUS DROMEDARIES)* IN SELECTED DISTRICTS OF AFAR REGION, ETHIOPIA

BY

MU'UZ GEBRU SAHLE

JUNE, 2012

DEBRE-ZEIT, ETHIOPIA

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science (MSc) in Tropical Veterinary Microbiology

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BY

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LIST OF ABBREVIATIONS

a.s.l	Above Sea Level
ARS	Afar National Regional State
BUG	Biolog Universal Growth
CLSI	Clinical Laboratory and Standards Institute
CNS	*Coagulse negative staphylococcus species*
CSA	Central Statistics Agency
DCG	Drylands Coordination Group
ESAP	Ethiopian Society of Animal Production
EVA	Ethiopian Veterinary Association
GDP	Gross Domestic Product
H_2S	Hydrogen Sulfide
Hr	Hour
ILRI	International Livestock Research Institutes
KOH	Potassium Hydro Oxide
MR	Methyl Red
NCCLS	National Committee for Clinical Laboratory Standards
O-F	Oxidative-Fermentative test
OIE	Office International des Epizooties
PA	Pastoral Associations
PCDP	Pastoral Community Development Project
PIV3	Para Influenza Virus 3
PPR	Peste des Petits Ruminants
RDC	Respiratory Disease Complex
RSV	Respiratory Syncytial Virus
SIM	Sulfide Indole and Motility
Spp	Species
SPSS	Statistical Package for the Social Sciences
Subsp.	Subspecies

ABSTRACT

A cross-sectional study was conducted to isolate and characterize the normal bacterial flora and bacterial agents implicated in pneumonia from the respiratory tract of the apparently healthy and pneumonic camels in the selected districts of Asayita and Dubti woredas of Afar Region. A total of 532 samples including 384 nasal swabs, 74 lung tissues and the same number of corresponding tracheal swabs were collected for bacteriological examination from September 2011 to May 2012. Generally, 903 bacterial isolates that constituted 14 different genera were identified from both the field and abattoir based study. The 384 nasal specimens collected yielded a total of 641 bacterial isolates. *CNS*, 28.6%, *Bacillus*, 13.5%, S. *aureus*, 13.3% and *E. coli*, 7.8% were the leading bacterial species isolated from the nasal tracts of apparently healthy camels. Nasal swabs collected from the pneumonic camels revealed *S. aureus*, *CNS*, *Bacillus* species and *K. pneumoniae* at a rate of 28.0%, 11.7%, 11.3% and 7.0%, respectively. *E. coli*, 12.7%, *Proteus* species, 10.9%, and *K. pneumoniae*, 9.1% were recognized at higher proportions among the 55 bacteria identified from the normal lungs. On the other side, *S. aureus*, *S. equi subsp. equi* and *P. multocida* were the frequently encountered species in the pneumonic lungs with the incidence of 16.3%, 13.0% and 10.9%, respectively. The majority of the isolates colonized all the anatomical sites investigated. However, a general decrease in the isolation rate was observed as one goes down the respiratory passageways. There was a statistically significant difference between the health status of the camels as well as along the anatomical sites studied with the isolation rates of the major camel respiratory pathogens ($p<0.05$). *Streptococcus equi subsp. equi*, S. *equi subsp. zooepidemicus* and *S. pneumoniae* were identified using the Omnilog®-Biolog identification system at the Institute of Biodiversity Conservation, Addis Ababa. The majority of the strains of the camel respiratory pathogens were found susceptible to norfloxacin, streptomycin and gentamicin but resistant to the action of ampicillin and tetracycline on the *invitro* test. Further studies on the demonstration of the role of other microorganisms, the pathogenicity of *S. equi subsp. equi*, identification of the serotypes involved and development of polyvalent vaccine are recommended to allow the progress of preventive methods.

Key words: Abattoir, Aerobic bacteria, Antibiotic susceptibility, Asayita, Camel, Dubti, Nasal swab, Respiratory tract, *Streptococcus equi subsp. equi.*

1. INTRODUCTION

The livestock sector in Ethiopia contributes 12 and 33 % of the total and agricultural gross domestic product (GDP), respectively and provides a livelihood for 65% of the population. The sector also accounts for 12-15% of total exports (Ayele *et al*., 2005).

About 11.5 million of camels are living in the eastern part of Africa (Djibouti, Eritrea, Ethiopia, Somalia and Sudan) representing over 80% of the African and two thirds of the world camel population (Bekele, 1999). Camels (*Camelus dromedaries*) are a subset of the huge livestock resources in Ethiopia with the population estimated to be 1.7 million. This number ranks the country third in Africa after Somalia and the Sudan and fourth in the world (India included). The arid and semi-arid areas of the country that constitute more than 60% of the total area and home of 7.8 million pastoral and agro-pastoral communities (Abebe, 2000) are suitable for camel production. In these areas, the livelihood of the pastoral communities is certainly ensured by *dromedaries* (Teka, 1991; Wossene, 1991).

Camel (C*amelus dromedarius*) is an important domestic animal species in Ethiopia, uniquely adapted to the adverse climatic conditions and shortage of forage and water. It is also an indicator of social prestige and wealth (Bekele, 1999). The love, friendliness and admiration that the pastoral peoples have for the camel are very deep (Fekadu, 1989). Camels are a vital source of milk, meat, draft power and transportation service for the pastoralists in Eastern and Southern Ethiopia (Schwartz and Dioli, 1992a; Bekele, 1999).

Part of the Afar areas like the Asayita and Dubti districts community have considerable interest in camel production. Ecological changes, social conditions (religion, marriage linkage, conflict) and extensive seasonal migration have been the main driving forces behind the increased camel production in these communities (FARM-Africa-Pastoralists- Livelihood-Initiative-Project-Two-Years-Report, 2005-2007).

Despite all its ecological and economic importance and versatile uses to the livelihood of pastoral neglected domestic livestock in Ethiopia. Research agendas, promotion programs, regular vaccination and animal health service deliveries are almost always excluding camels. Little is

known about cons and pros of camel production and health problems of camels compared to other livestock (Zeleke and Bekele, 2000).

Due to the fact that camel production is in remote, migratory and poor infrastructure condition, available studies were based on small animal number, one time survey, interviewing, questionnaires, estimation and simulations. The depth of information on camels and camel production has not been adequate to solve its multifaceted problems (Bekele, 2010).

Although camels are well adapted to their environment and seem to be spared from devastating epidemic infections which threaten other livestock species in the same region, there are however a number of economically important diseases that affect camels (Kane *et al*., 2003; Dia, 2006). The *dromedary* of Ethiopia has suffered from different diseases like trypanosomiasis, camel pox, mange, respiratory disease complex, hemorrhagicsepticemia, pustulardermatitis, dermatomycosis, gastrointestinal parasites and acute plant poisoning for the past so many years (Tefera, 2004).

Respiratory tract diseases are among the emerging health hazards to camels worldwide that are incurring considerable loss of life and production (Abubakar *et al.*, 2008; Bekele, 2008; Intisar *et al*., 2009; Kebede and Gelaye, 2010). A variety of viral, fungal, bacterial and parasitic microorganisms have been associated with outbreaks of respiratory disease among camels. Viruses encountered in the respiratory tract infections of camels are *parainfluenza virus 3* (*PIV3*), *influenza viruses* A and B, *adenovirus*, *respiratory syncytial virus* (RSV) and *infectious bovine rhinotracheitis* (Intisar *et al*., 2010).

Camel respiratory problem has received little consideration even though it is an emerging disease in Ethiopia causing considerable loss of production and deaths (Rufael, 1996; Bekele, 1999). In Ethiopia few studies were conducted on the extent of respiratory problems of camels compared to other livestock species (Bekele, 2008). Some studies have been conducted on the bacterial population of the respiratory tract of domestic animals (Ajuwape *et al.*, 2006; Merga *et al*., 2006; Yimer and Asseged, 2007) but for the camel they mainly focus on lungs (Zubair *et al*., 2004) and studies on the bacteria of respiratory passageways of healthy camels are scarce (Bekele, 2008).

In 1995 camel respiratory disease outbreak has occurred in Ethiopia which was characterized by a highly contagious nature with high rate of morbidity (over 90%) and a variable rate of mortality

(Roger *et al*., 2001). A definitive etiology of most respiratory diseases of camels has not yet been conclusively determined as a variety of viruses, fungi, bacteria and parasites are to be the possible causes of respiratory outbreaks among camels (Schwartz and Dioli, 1992a). Some previous reports indicate the involvement of peste des petits ruminants (PPR)-like virus, *Streptococcus equi subsp. equi*, *Pasteurella, Mannheimia* and *Mycoplasma* species (Yigezu *et al,* 1997; Bekele, 1999; Roger *et al*., 2001). Schwartz and Dioli (1992a) indicated that the most important predisposing factors are sudden climatic changes, poor management practices, exposure to various diseases, over traveling and low-grade nutrition.

Streptococcus species have been isolated from clinically healthy camels although they were not definitely identified and characterized (Shigidi, 1973; Mahmoud *et al*., 1988; Rana *et al*., 1993). On the other hand, *Streptococcus* species are described in active respiratory disease of camels. But there is no available information on the specific isolation of *S. equi subsp. equi* in camels (Rana *et al*., 1993).

Respiratory disease outbreak in camels characterized by sudden death has occurred in the Afar and Oromia regions during 2005/2006 (Wernery *et al*., 2006). In 2007 a similar disease was reported from the Somale and Oromia regions of Ethiopia. A further outbreak has also arisen recently in Asayita districts in 2011. Despite all efforts have been done so far for the identification of the real possible causes of the out breaks by a number of veterinary institutions and laboratories in Ethiopia, there was no substantial result obtained from the investigation of the disease. More investigative efforts are still in need for the identification of the true cause of the problem so as to design sustainable control strategies.

The present study was, therefore, designed to accomplish the following major objectives in the study areas.

General objectives:

- To isolate and characterize the possible bacterial pathogens associated with respiratory problems of camels.
- To isolate and characterize the bacterial flora in the healthy respiratory passageways of camels.

Specific objectives:

- ✓ To isolate and characterize *Streptococcus* species from different anatomical sites of the respiratory passageways of apparently healthy camels and camels with respiratory problems.
- ✓ To isolate and characterize the bacterial agents from the respiratory tract of camels if any camel respiratory problem is reported in the study areas during the study period.
- ✓ To determine the antibiotic sensitivity pattern of the leading bacterial agents in the respiratory problem to different types of antibiotics.

2. LITERATURE REVIEW

2.1. Distribution of camels (*Camelus dromedarius*)

Camelid was probably among the last of major domestic species to be put to regular use by man. The most likely time of domestication is about 4000 years before present or slightly earlier. The presumed area of domestication is the southern Arabian Peninsula, probably the area of Yemen and Oman. From presumed center of domestication, *dromedary* has subsequently been distributed to almost the rest of the world (Schwartz and Dioli, 1992b; Wilson, 1998).

Environmental, social and cultural factors have great influence on the distribution and production of camels. Arid and semi-arid zones of tropical and sub tropical countries of Africa and Asia are found to be convenient ecology. The greatest cultural influences in recent distribution of camels was the advent of Islam, when Arabs spread their gospel, consolidating its ranges north and east wards in Asia, and along the Mediterranean littoral. Generally, there has been steady increase in camel population since about 1980s. However, decrease in numbers has been observed in some countries for instance, where oil is the principal commodity and the nomadic way of life is no longer the major one (Wilson, 1998).

Eastern Africa is known to be the heartland for camel production as 80% and 63% of the Africa and world population, respectively produced in the region. Ethiopia ranks 4th in the world with total population estimated at 1.7 million. Subsistence camel production is practiced in dry areas of Ethiopia that covers 61% to 65% of the total land area (Abebe, 2000). The eastern part of the country is considered as the heartland for camel production, which is the home of two third of the nations camel population. Afar region is the major camel production area in the country. The pastoral districts in the administrative zone1 of the Afar region are the most important camel production areas (Getahun and Bruckner, 2000).

Thereafter, environmental, social and cultural factors, extensive seasonal migration and change in rangeland ecosystem(decreasing pasture for grazers and increasing browse vegetation species) are major factors for the expansion of dromedaries into the different camel-rearing areas (Biffa and Chaka, 2002).

2.2. Potential importance of camels

Camels are primarily the domestic animals of pastoral communities that ensure food security. They produce milk, meat, hair and hides, and also serve as a draught animal for agriculture and transport people as well as goods (Schwartz and Dioli, 1992b). Milk and meat are the important products that camels produce elsewhere. Long lactation and ability to maintain milk production over long dry spells are important facets of camel productivity. Apart from home consumption, majority of the households sell at least one-third of the produced milk to generate cash income (Getahun and Bruckner, 2000). Daily milk yield can be as high as 20 liters with improved management conditions (Schwartz and Dioli, 1992b).

Until the arrival of motorized transport in the arid and semi-arid zones, camels have been the sole means of transport in the areas where they are adapted. They are also used for wheel transport, water lifting and source of power for oil mill. Camel racing and other leisure activities such as camel safaris and trekking have recently become a tourist attraction and luxurious in some parts of the world (Schwartz and Dioli, 1992b; Wilson, 1998).

From global perspective, the economic production of camels seems minimal. But the breaking news is that the recent commencement of camel meat export from Ethiopia to the Middle East countries. The ELFORA modern slaughterhouse, located in Metehara town which is 170 kms far from Addis Ababa and the Hashim Ethiopian Livestock and Meat Exporter (HELIMEX) at Debre- Zeit have started slaughtering of camels collected from different camel-rearing districts of Afar, Somale and Oromia region. This is a good start to the country to increase the economic revenue from livestock. In Ethiopia, they are also the subset of huge livestock resource when considered from national economic point of view (Getahun and Bruckner, 2000). However, what makes the difference is its adaptation to harsh environments to produce milk from scanty and highly variable feed resources.

The most significant merits to perform in areas where other livestock species do not thrive and perhaps do not survive are attributed to the economic use of water in almost all metabolic functions and wide range of feed resource utilization (Yagil, 1985). In mixed species, the camel feeds on plants or part of plants that are not eaten by other conventional livestock due to its size

to browse the highest strata, thus reducing competitions and enhancing complementarities (Ayan, 1984; Teka, 1991).

2.3. Constraints to camel production

Camels are produced by pastoral societies of the third world who dwell in dry marginal areas. Due to the fact that the production is usually a migratory system, in remote areas with harsh living conditions and poor infrastructure, the animals are presumed to be inaccessible for research. This affects the depth of our knowledge on the general aspects of camels (Schwartz and Dioli, 1992b).

Generally, there is negligence towards the promotion of camel health and production. It is only recently that the camel became the subject of more intensive and systematic interest (Baumann and Zessin, 1992). However, this is yet not the case in Ethiopia, as no national or local research agenda featuring any aspect of this animal. So far, almost all research works were conducted by students as thesis research. This illustrates the existence of negligence by development planners and researchers (Bekele, 2010).

There are regular vaccination programs and other treatment and control services for cattle as well as other stocks, while almost nil for camels. It is important to notice that training program in Ethiopia has been insufficient to backup the veterinary personals to deal with multifaceted constraints and health problems of camels. Most camel diseases are known by herders' local vernacular names, but not in medical terms or English names with which most health workers are familiar. As a result, proper treatment is not given to them even with drugs and facilities at hand. The herders perceived this situation as "camel is regarded as property of pastoralist only", so that only owners are responsible for it. They further stated their complaint in that most government officials have been highlanders who never utilize camel products and as a result less attention was given to this animal. Accordingly, efforts to promote production and health care were not attempted when compared to other ruminants (Bekele, 2010).

Supporting the view of the herders, Megersa *et al*. (2008) have suggested that the camel represents something of an orphan commodity that neither animal scientists and veterinarians nor

wildlife conservationists feel responsible for. It is a poorly understood and highly underestimated animal in many cultures except amongst the pastoralists who have had experience with them. Thus, it is high time to change the situation and remove the stigma that has come to be associated with camel breeding as a backward activity. Currently with increasing attention to pastoral community and establishment of pastoral development institutions, there is a green light in future favoring promotion of camel husbandry and health care.

Though currently not a problem, it is indicated that labor shortage for herding and attending camels is a future challenge. Increased human mobility, other job opportunity and increased school enrolment rates of pastoral children are the major reasons influencing labor demand in pastoral camel production. It has been demonstrated that labor needs for watering and herding camels are perhaps the major non biological factor affecting camel herd size and population expansion (Bekele, 2010).

Widespread diseases, poor veterinary service and lack of attention by the government are the primary constraints that hamper camel production. In most developing nations, much attention was not given to camels regarding improvement of health care and production aspects. The pastoralists have produced camels for centuries by their own efforts without much assistance. Pneumonia is one of the most serious infectious diseases of camels and widespread in camel rearing areas (Wossene, 1991; Köhler-Rollefson *et al*., 2001). Other diseases such as trypanosomiasis, pox infections, contagious skin necrosis, viral and parasitic infestations are of high clinical importance to affect the health of the camels in the camel-rearing areas (Demeke, 1998).

Besides the prevailing diseases, camel keeping is relatively a recent event in some districts of the Afar region like the Borena pastoral system. Those late comers have traditionally been based on cattle husbandry for milk production and wealth storage. They have recently developed considerable interest to shift to camel production as asset diversification for uncertainties and drought mitigations. Ecological changes, socio cultural conditions and extensive seasonal migration have been the main driving force behind their coming into camel production business.

Consequently, lack of appropriate traditional knowledge of husbandry practices, labor input requirement for its management and knowledge of its peculiar biology like inducible ovulation are perhaps the possible constraints of camel production in the area (Wernery and Kaaden, 2002). Respiratory problem is, therefore, one of the infectious diseases that affect the well being and production of camels.

2.4. Respiratory tract infection in camels

Respiratory tract infections are quite common in camels and occur in two forms: acute and chronic forms. The chronic form is associated with coughing showing specific signs of loud, severe and open mouth coughing, weight loss, restlessness, dullness, lacrimation and prolonged recumbency. While the acute form is associated with nasal discharge manifested by sneezing, mucus discharge from the nostrils, fever, depression and reduced feed intake. Mucus discharge eventually becomes pus and blocks the nostrils, leading to difficulties inbreathing and death. The most important and severe form is the acute respiratory infection, which can occur in the form of outbreak during the major wet season. Higher incidence of respiratory infection during the wet season is associated with change of climatic condition from warm dry period to wet, predisposing factors being draft, cold, rain, poor nutrition as well as migration that may distress the animals (Bekele, 2010).

Dust storms emerging during the first weeks of the rainy season in the African Sahel can also contribute to respiratory disease in camels. Hansen *et al*. (1989) described an outbreak of pneumoconiosis in Somali camels and reported the presence of large numbers of dust laden macrophages in the lungs of 94 out of 134 camels that had pneumonia. The crowding of pastoralist camels around limited watering points during the summer also contributes to the spread of respiratory pathogens as camels from different geographical regions often use the scanty open water sources. Likewise, more fatal and highly contagious camel disease with respiratory syndrome has also occurred as a large scale outbreak during wet season (April to May) of 2007 in Borana areas. The disease resulted in disastrous death losses causing 18% morbidity and over 50% mortality in affected herds (Melaku and Feseha, 1986).

Pneumonia is among the most important and commonly encountered disease of the camel. Despite low mortality and morbidity rates, the recovery period is quite long having negative impact on overall productivity. Nonetheless, it is becoming increasingly difficult to make an etiological diagnosis because, although a single agent may be a primary invader (Carter, 1984), when the local resistance of respiratory mucosa is lowered; bacteria growing in the nose and throat extend downwards; usually producing multiple bacterial infections (McSween and Whaley, 1992). Besides, most of the infectious agents that cause respiratory disease are ubiquitous in nature and are normal inhabitants of the nasopharynx (Radostits *et al*., 1994).

A variety of viral, bacterial, fungal and parasitic microorganisms have been associated with respiratory disease problems (Abbas and Omer, 2005). Although the exact causative agent still remain obscure, *Pasteurella* organisms (Bekele, 1999; Abubakar *et al*., 2008), *Streptococcus equi subsp. equi* (Yigezu *et al*., 1997), *Mannheimia haemolytica* (Al-Tarazi, 2001; Abubakar *et al*., 2008) and antibodies to *morbili* viruses (Roger *et al*., 2001) were detected from affected camels, and claimed as aetiology or having association with the disease. Numerous other bacteria including *Staphylococcus aureus, Bordetella species, Escherichia coli, Klebsiella pneumoniae, Rhodococcus equi* and *Neisseria species* have been incriminated in the aetiology of pneumonia in camels (Chauhan *et al.,* 1987; Al-Doughaym *et al*., 1999; Abubakar *et al*, 2008).

Lung abscesses due mainly to *Arcanobacterium pyogenes* and *Corynebacterium pseudotuberculosis* were reported by Abubakr *et al*. (1999) to affect both young and adult camels. There is very little in the literature with regards to the role played by *mycoplasmas* and other mollicutes in the aetiology of pneumonia in camels (Wernery and Kaaden, 2002).

Several authors have reported high antibody titers against numerous respiratory tract viruses such as *adenovirus*, *para influenza* 3, *respiratory syncytial* virus, and *morbili* viruses (Roger *et al.*, 2001) in camel sera. Viruses are believed to predispose to bacterial infections in two distinct ways. The first, viral agent can cause direct damage to the respiratory clearance mechanism and translocation of bacteria from the upper respiratory tract. The second way, viral infection can interfere with the immune system's ability to respond to bacterial infections. The viruses can affect the leukocytes causing impairment of their function and declining their number which result in increased susceptibility of the animal to bacterial infections (Jared *et al*., 2010).

2.5. Major respiratory tract pathogens

2.5.1. *Pasteurella multocida*

P. multocida cells are Gram negative, non motile, very small, ovoid bacilli, with straight axis, slightly convex sides and round ends; arranged singly, in pairs or in small bundles (Smith and Philips, 1990; Biberstein and Hirish, 1999b). *P. multocida* is a facultative anaerobe, which grows between 12 and 43^{o}c, but its optimum temperature for growth is 37^{o}c. Better growth of the species is obtained when blood or blood serum of ruminants, especially of sheep, is added to the media. Some strains fail to grow in media not containing blood serum (Smith and Philips, 1990). The colonies are usually evident after 24 hours of incubation at 37^{o}c.

They are moderate in size, round and grayish with a musty or mushroom sort of smell. They are non-haemolytic, 0.5-1.0mm in diameter, low convex, amorphous, grayish-yellow, translucscent colonies with a smooth, glistering surface along their entire edge. Most of the *P.multocida* strains do not grow on primary selective and differential MacConkey agar (Smith and Philips, 1990; Biberstein and Hirish, 1999b). Distinctly, some of the strains of *P.multocida* may produce relatively large, mucoid colonies due to their large capsule of hyaluronic acid (Quinn *et al*., 1994b). *P.multocida* is catalase and oxidase positive. It produces acid, no gas, in glucose, manose, galactose, sucrose, manitol, and usually sorbitol and xylose. Some strains ferment maltose, arabinose, trihalose or glycerol. Indole and H_2S are positive. Urea, Voges-Proskauer, methyl red and citrate are negative. Nitrate and methyl blue is reduced (Bisgaard *et al*., 1991).

The capsule, lipopolysaccharide, iron regulated outer membrane proteins, toxic outer membrane proteins and addhesins are among the most important cellular factors associated with pathogenicity of *P.multocida* (Boyce and Alder, 2000). The capsule plays many roles, the most important of which are the interference with phagocytosis and protection of the outer membrane from the deposition of membrane attack complexes generated by activation of the complement system. The hyaluronic acid capsule serves as an adhesion for respiratory tract epithelial cells in the case of *P.multocida* (Geschwend *et al*., 1997).

The expression of adhesins depends on the environmental cues. That is, adhesins are expressed while the microorganism inhabits the epithelial surface, but repressed when the microorganism is

inside the host where adherence to phagocytic cell would be disadvantageous (Dabo *et al*., 1997; Geschwend *et al*., 1997). Strains of *P.multocida* may produce extracellular products such as O-sialoglycoprotien endopeptidase, neuraminidase and hyaluronidase. The role played by these enzymes in the pathogenesis of disease is somewhat unclear. Neuraminidase has been postulated to play a role in the colonization of the epithelial surface by removing terminal sialic acid residues from mucin, there by modifying normal host immunity (Straus and Purdy, 1995).

More than one hundred domestic and wild mammalian and avian species are known to harbor *P.multocida* strains on the mucous surfaces of the nasal cavity (cattle, sheep and other ruminants), on the pharyngo-laryngeal to upper tracheal area (all animal species) and in the mouth (cats and dog) (Rusvai and Fodor, 1998). Their presence in the lower part of the respiratory tract (i.e below the tracheal bifurcation to the lung) is always associated with a state of disease. Some of the strains may be also be recovered from the mucous membranes of the genitals and from the alimentary canal (Corbeil *et al*., 1985). *P.multocida* has been isolated from the lung of pneumonic camels in Nigeria (Abubakar *et al*, 2008).

2.5.2. *Mannheimia haemolytica*

Advances in understanding the phylogenies have come from genomic studies and defined species belonging to *sensustricto* definitions of the genus *Pasteurella.* In 1981, Mannheim showed that the phylogenetic structure of the family *Pasterellaceae* was complex and that more than three genera would be required to accommodate the array of species, which has previously been misclassified (Mannheim, 1981). Yet, the studies suggested that some rearrangement of the organism in the various genera was indicated, especially as *P. haemolytica* biotype A was more closely linked with *A. lignieresi* than with *pasteurella*; this supported an earlier suggestion by Mraz. Based on this, the trehalose positive strains, which had no close association with the genus *Pasteurella,* were named *P. trehalosi* (Sneath and Stevens, 1990).

The phenotypic and genotypic diversity of the trehalose- negative *P. haemolytica* complex has been investigated and reclassified to a new genus *Mannheimia* with five species in which *Mannheimia haemolytica* is considered as a type species (Tefera and Smola, 2001).

The name *Mannheimia* was given in tribute to the Germen scientist Walter Mannheim for his significant contribution in the recent taxonomy of the family *Pasteurellaceae*.

The cells of *Mannheimia haemolytica* are Gram negative, non motile, short, evenly stained rods and coccobacilli (Smith and Philips, 1990). The growth characteristics of *Mannheimia haemolytica* are similar to *P. multocida* though the need for enrichment of the media is greater. The colonies of *Mannheimia haemolytica* on sheep blood agar are circular, glistering and convex up to 2mm in diameter, surrounded by a narrow zone of haemolysis. Haemolysis can sometimes be discerned only under the colony when the latter is spread off the blood agar. Agar plates made with the blood of very young lambs gives rise to a double zone haemolysis; a narrow inner zone and a wide incomplete outer zone that increases in size at room temperature (Biberstein and Hirish, 1999a).

The strains will lose their haemolytic capacity on repeated culture. The hemolytic activity returns, however, upon passage of the culture through mice or chicken egg embryo. In contrast to *P. multocida*, *Mannheimia haemolytica* grow on MacConkey agar as pink to red colonies (Wessman, 1995). *Mannheimia haemolytica* is catalase and oxidase positive, D-sorbitol, D-xylose, maltose and dextrin are fermented. Trehalose is not fermented. No strains ferment L-arabinose or glucosides. Indole, H_2S, urea, VP, MR and citrate are negative. Nitrate and MB are reduced (Tefera and Smola, 2001).

The main virulence factors that have been associated with *M. haemolytica* are capsule, lipopolysaccharide and Leukotoxin (Confer *et al*., 1990). The capsule inhibits complement mediated serum killing as well as phagocytosis and intracellular killing of *Mannheimia haemolytica*. It enhances neutrophil-directed migration and adhesion of *Mannheimia haemolytica* to the alveolar epithelium. *Mannheimia haemolytica* is confirmed mainly to ruminants. All the strains are found on the mucous membrane of the upper respiratory tract of healthy carriers. Some of the strains excrete substances with bactericidal activity against other respiratory tract bacteria to protect their nich (Quinn *et al*., 1994b).

One of the most fascinating aspects of the strain is the specificity they show for the host species, both with regard to peaceful commensalism and with regard to the initiation of disease (Biberstein and Hirish, 1999a).

Bekele (1999) has isolated *Mannheimia haemolytica* from respiratory tract of camels while investigating a camel respiratory infection out breaks in Shinile zone of the Ethiopian Somali region and he recorded the importance of *Mannheimia haemolytica* as a causal agent of respiratory disease of camels. In addition to this Abubakar *et al*. (2008) as well as Al-Tarazi (2001) have reported *M. haemolytica* from pneumonic lungs of camels in Nigeria and Jordan, respectively.

2.5.3. *Streptococcus equi subsp. equi*

Streptococcus species are considered a diverse group, ranging from commensal organisms which occupy various niches of animals and human body to pathogens that have the capacity to infect a wide range of hosts (Fishcetti, 2000). Further, *Streptococcus* species are considered among the most important pathogens affecting humans and mammals and have been reported to affect cold blooded animals including fish. They live as commensals in the mucus membrane of the upper respiratory, alimentary and lower genital tract. Many infections of *Streptococci* are probably endogenous and stress related (Eldar *et al*., 1994).

Streptococci species have been isolated from clinically healthy camels although they were not definitely identified and characterized (Shigidi, 1973; Mahmoud *et al*., 1988; Rana *et al*., 1993). On the other hand, *Streptococcus* species such as β-haemolytic *streptococci*, *Streptococcus viridans, S. pneumoniae* and *S. pyogenes* (Thabet, 1994) are described in active respiratory disease of camels. An outbreak of contagious cough in camels has been described in the Gobi desert and the bacteriological examination revealed α- haemolytic *pneumococcus*.

Streptococcus equi subsp. equi is believed to have evolved from an ancestral strain of *Streptococcus equi subsp. zooepidemicus*, and the two organisms share 97% sequence identity (Chalker *et al*., 2003). The bacteria produce a novel M-like protein, SeM, which has been linked to its increased virulence over its evolutionary parent, *Streptococcus equi subsp. zooepidemicus* (Timoney *et al*., 1997). SeM actively binds fibrinogen and immunoglobulin G (IgG) and inhibits the deposition of C3b on the bacterial surface, resulting in an anti phagocytic action similar to that of the M proteins of group A streptococci (Meehan, 2000).

It is assumed that this bacterium played a role in the epizootic occurring in camels in Ethiopia. However, more bacteriological surveys and serological investigations need to be carried out to prove this. As far as synergism with other pathogenic organisms is concerned, the morbilli virus prevalence observed may have favored the development of infection with *S. equi subsp. equi* as a secondary bacterial agent (Yigezu *et al*., 1997).

2.5.4. *Bordetella bronchiseptica cluster*

The genus *Bordetella* in the family *Alcaligenaceae* comprises five species, four of which cause infections of the upper respiratory tract in different host organisms. *Bordetella pertussis* is an obligate human pathogen and is the etiological agent of whooping cough (pertussis), an acute respiratory infection more serious among infants. *Bordetella parapertussis* causes a mild pertussis like disease in humans and is very closely related to *B. pertussis*. An animal pathogen of the *Bordetella* species is *Bordetella bronchiseptica*, a flagellated bacterium that rarely infects humans but can result in pneumonia occasionally accompanied by bacteremia (Beier *et al*, 1996). *Bordetella avium* is a pathogen of poultry. *Bordetella hinzii*, which was only recently recognized as a member of the genus *Bordetella*, is frequently associated with poultry, but does not necessarily cause disease (Falbo *et al*., 1993).

The three main species of *Bordetella*; *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* are respiratory pathogens of mammals and have an important economic impact on both human and animal health. Historically, the classification in to species was based on the host range and severity of clinical disease. However, it is now apparent that by true genetic criteria, they actually comprise a single group of highly related sub species. *Bordetella pertussis* and *B. parapertussis* appear to be more host-adapted and differentiated and are less representative of the group as a whole than are *B. bronchiseptica* strains. As a result, the designation *Bordetella bronchiseptica* cluster has been adopted to more accurately refer to this group of highly related organisms (Mattoo and Cherry, 2005).

The genus *Bordetella* encompasses a group of Gram-negative, small coccobacilli bacteria. They are catalase positive, oxidase positive, unreactive on O-F and are motile peritrichous bacteria. They are obligate aerobes and fail to ferment carbohydrates such as glucose. Because they cannot utilize carbohydrates, they drive their energy mainly from the oxidation of amino acids and have

no special growth requirement. They grow on MacConkey agar without fermenting lactose (Quinn *et al*., 2002).

Most of the members have adapted to live in close association with higher organisms, either as overt primary pathogens or in commensal association that occasionally result in opportunistic diseases. All members of the *B. bronchiseptica* clustr have been implicated in respiratory disease in mammals (Mattoo and Cherry, 2005). *B. bronchiseptica* has been reported from the pulmonary lesions of camels (Nesibu *et al*., 2010) and are known to play a determinant role in the development of canine kennel cough (Thompson *et al*, 1976).

2.5.5. *Klebsiella pneumoniae*

Bacteria of the genus *Klebsiella* belong to the family *Enterobacteriaceae* and are named after the German pathologist Edwin Klebs. They are opportunistic pathogens found in the intestines of humans and animals and in the environment, where they can multiply in the water or on the surface of leaves. The forming of a capsule is characteristic (Bockemuehl, 1992). In culture they appear as mucoid colonies. *Klebsiella* are Gram-negative rods, grow on MacConkey and non-motile which form a capsule (Henton, 1994).

The classification of capsular types (done with the quelling reaction according to Neufeld) gives a much clearer indication of pathogenicity than biochemical typing (Henton, 1994). Currently 82 defined polysaccharide-capsule-antigens can be differentiated, which can be expressed by any of the capsule producing *Klebsiella* species (Bockemuehl, 1992). In respiratory tract infections capsule type 1 to 6 were found while in pneumonia cases type 1 is more prominent than type 2 and type 3. Infections of the urinary tract are associated with type 2 and above type 6. Capsular type 1 and 2 followed by the less frequent type 3 and 4 are the most pathogenic capsular types for humans and animals (Euzéby, 2004). Apart from the capsule, also endo and entero toxins and adhesion antigens are virulence factors. However, the virulence is rather low, as *Klebsiella* is a typical pathogen of multifactorial diseases.

Very little is known about the involvement of *Klebsiella* species in infections of camels in general and respiratory tract infections in particular. *Klebsiella pneumonia* (capsular type 11) has been isolated together with *Diplococci* from two dead adult camels, which suffered respiratory distress, pyrexia and prolonged cough in Haryana, India. Histological examination showed

microbial broncho-pneumonia, characterized by acute capillary hyperaemia and the presence of serofibrinous exudates in the alveoli. The authors suggest that *Klebsiella pneumoniae* and/or the isolated *Diplococci* were involved in the development of the disease in camels (Arora and Kalra, 1973). *Klebsiella pneumoniae* has been isolated from healthy and pneumonic camel lungs with higher prevalence in the lungs having lesions (Abubakar *et al*, 2008).

2.6. Respiratory disease complex (RDC) in camels

A combination of various diseased conditions of lower respiratory tract of camel is called respiratory disease complex. A variety of viral, fungal, bacterial and parasitic microorganisms have been associated with outbreaks of respiratory disease complex among camels. The most common predisposing factors for RDC are sudden changes of climate, generally poor management and lowered nutritional status. Animals under other forms of stress such as overcrowding, unsanitary conditions, draft, cold, rain and those suffering from other health problems and young stock are the classes most at risk. Mode of infection and spread depends solely on the infectious agent (Khan *et al*., 2003).

Typical clinical signs of acute onset of lower respiratory diseases are a change in respiratory rate and depth, wheezing, coughing, uni or bi lateral nasal discharge (serous, purulent or haemorrhagic), increased temperature, anorexia, reluctance to move or work, hyper lacrimation, abnormal posture such as abduction of the elbows, extended neck, head to neck angle is wider than usual, swelling above the sinus frontalis. General immuno-depression makes the affected animals more prone to other infections (Khan *et al*., 2003).

Typical signs of respiratory diseases of viral origin are often masked by secondary bacterial invasion. Bacteriological and histological examination should be performed if clear-cut etiological diagnosis is needed. Principal treatment of affected animals includes antimicrobial therapy, improved management practices such as better housing, hygiene and good nursing care. High doses of long-acting broad spectrum antibiotics should be used in case of bacterial infections. Fistula formation between the sinus and the nasal cavity is a common sequel in most cases of sinus infection. In case of nasal airway obstruction due to purulent discharge, relief can

be achieved by regular cleaning and flushing of the nasal cavity with saline solution. If it is treated early, prognosis is usually good (Schwartz and Dioli, 1992a).

Table 1. Pathogenic agents associated with respiratory disease outbreaks in camel

Agent	Prevalence	Disease
Parainfluenza type 1, 2, 3	Widespread	Pneumonia, Influenza
Influenza type A/B	Regional	Influenza
Adenovirus	Regional	Influenza
Respiratory syncytial virus	Regional	Influenza
Infectious bovine rhino-tracheitis	Regional	Influenza, Pneumonia
Pasteurella multocida type A	Widespread	Bronchopneumonia
Mycobacterium bovis	Uncommon	Miliary/nodular tuberculosis
Streptococcus, Corynebacterium, Actinomyces ,Staphylococcus species and Klebsiella pneumoniae	Very common	Plumonary abscessation
Mycoplasma mycoides	Regional	Pleuropneumonia
Rickettisia species	Widespread	Pneumonia
Trypanosoma species	Very common	Pleuropneumonia
Dictyocaulus viviparus	Widespread	Verminous pneumonia
Echinococcus granulosus, Cysticercus dromedarii	Very common	Hydatid disease
Dipetalonema evansi	Regional	Pneumonia, pleuritis
Fly larvae (Nasal myiasis)	Widespread	Rhinitis
Leeches	Regional but common	Rhinitis

Source: Modified from Schwartz and Dioli (1992b).

3. MATERIALS AND METHODS

3.1. Description of the study area

The present study was conducted from September 2011 to May 2012 in the selected pastoral and agro pastoral residences of Asayita and Dubti districts of the Afar region (Figure 1). The Afar National Regional State (ARS) is located in the northeast part of the country. The region borders four national regional states i.e. in the North and Northwest, Tigray region; in West and South West, Amhara region; in South, Oromia region and in South West, Somalia region. The ARS also shares international borders with Djibouti and Eritrea to the West and North West, respectively.

Administratively, the region is divided in to five zones, which are further subdivided into 32 woredas and 358 kebeles. The total population of the region is estimated at 1.2 million of which 90% are pastoralists and 10% agro-pastoral. In general, there are 10,179,277 live stocks in the region of which 4,267,969 (41.93%), 2,463,632 (24.20%), 2,336,483 (22.95%), 852,016 (8.37%) are Goat, sheep, cattle and camel, respectively (Afar Finance and Economy Bureau, 2006).

Pastoralism and agro-pastoralism are the two major livelihood ways practiced in the region. Animal husbandry in Afar region is characterized by extensive pastoral production system and seasonal mobility. Due to the shortage of rainfall, in the dry season, the pastoralists are forced to move their animals to far distance for water and grazing land. Camel herd movement may move the whole herd to water points and to relatively better areas where green fodder is available, or by herd splitting where lactating and young animals are kept around homesteads and moving the rest to distant located forage areas (Afar Pastoral Agriculture and Rural Development Office).

The administrative zone 1 of the Afar regional state contains 6 woredas namely Mille, Elidar, Chifra, Dubti, Asayita and Afambo. 1,171,790 Goats, 658,756 Cattle, 755,902 sheep and 257,312 camels constitute the largest percentage of the total live stoke population of zone 1 (CSA, 2004).

Dubti Woreda

Dubti is located at about 610 km north-east of Addis Ababa, the Ethiopian capital. The woreda is bordered on the south by the Somali region, on the southwest by Mille, on the west by Chifra, on

the northwest by administrative zone 4, on the north by the administrative zone 2, on the northeast by Elidar, on the east by Asayita, and on the southeast by Afambo. Towns in Dubti include DateBahri, Dubti, Logiya, Sardo and Semera. The average elevation in this woreda is 503 meters above sea level (a.s.l) (Hailu, 2008) the highest point in Dubti is mount MandaHararo (600 meters a.s.l). Rivers include the Awash River, which splits the woreda into northern and southern parts, and its tributary the Logiya (Robert *et al*., 1992).

The area is characterized by high temperature which ranges from 25^0C to 42^0C. May-June is the driest season of the year, '*hagay'*. It is said to be unsuitable for browsing since bushes dry up. The main rainy season *'Karima'*, which accounts for above 60% of the annual total rainfall is from July to September. This is followed by the best grazing season of '*Kayra'* that occurs from September to November. Another minor rainy season is '*Sugum'*and appears during March and April. '*Gilal'* is less severe dry season with relatively cool temperatures (November to March). Occasional rainfalls called*'dada'*may interrupt*'Gilal'* (Dubti district pastoral agriculture and rural development office).

Asayita Woreda

The woreda is located at about 667 kms northeast of Addis Ababa and lies at 11°34′ North latitude and 41°26′ East longitude. Part of the administrative zone1, Asayita is bordered on the south by Afambo, on the west by Dubti, then on the north by the Awash River which separates it from Elidar, and on the east by Djibouti. The major town in Asayita woreda is Asayita. Asayita is a town in northeastern Ethiopia, and was the capital of the Afar region of Ethiopia before 2007. Part of the shoreline of Lake Gargori lie within the boundaries of this woreda (Asayita district public communication and information office).

Based on the 2004 Census conducted by the Central Statistical Agency of Ethiopia (CSA), this woreda has a total population of 50,803, of whom 27,284 are men and 23,519 women; with an area of 1,678.28 square kilometers. Asayita has a population density of 30.27. While 16,052 or 31.60% are urban inhabitants, a further 9,358 or 18.42% are pastoralists.

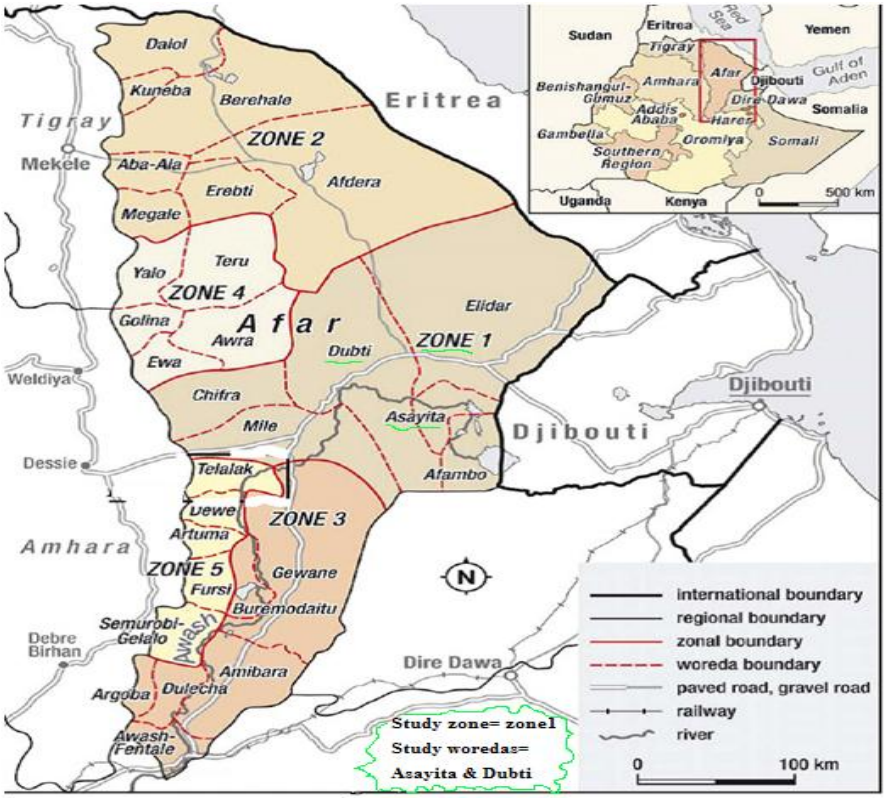

Figure1. Administrative location of Afar region showing the study zone and woredas.

3.2. Study animals

The society in both of the study districts keeps a higher proportion of breeding females. They keep few males for mating and transportation. These larger proportions of females in herds in the areas indicate a strong desire of herdsmen to maximize herd size and the importance of milk production in pastoral areas. The people manage their camels broadly in two ways. They freely release the adult camels (> 2 years) to the field during the day time and keep them in a wider area during the night. But the herdsmen keep the young camels (< 2 years) in a confined and fenced area around the homesteads.

The camel population of Asayita district is about 3277 and that of the Dubti woreda is about 5966, which were considered as study population (CSA, 2004) for this study. Live camels with clinical signs of respiratory diseases and apparently healthy camels found in the study areas were used as sources of nasal swabs. Moreover, any camel respiratory problem reported in the study areas during the study period was investigated. In addition lungs with and without pneumonic

lesions together with their correspondence trachea were taken from the slaughtered camels for bacterial isolation. Although records were lacking regarding the age and previous health status, nearly all of the slaughtered camels were adult males and were clinically normal during ante-mortem examination.

3.3. Sample size determination

There was no previous investigation about the prevalence of the bacterial species in the study districts. Hence, the average expected prevalence rate was assumed to be 50% for the area within 95 % Confidence Intervals (CI) at ± 5% desired accuracy. Subsequently, the number of study animals was determined following the formula published in Thrusfield (2007).

$$n = \frac{1.96^2 \times P_{exp} \times (1-P_{exp})}{d^2} \quad \text{..... (Thrusfield, 2007)}$$

Where n= required sample size, d= desired absolute precision, P_{exp}= expected prevalence (50%)

Therefore, based on this the desired sample size to be collected with 50% prevalence rate, absolute precision value of 5% and 95% confidence interval was 384. In addition, 74 tracheal swabs and 74 lung tissue samples were included from the locally slaughtered camels for the abattoir based survey.

3.4. Study design

A cross-sectional study was designed and conducted to isolate and characterize the bacterial species found in the respiratory tract of the apparently healthy and pneumonic camels (*Camelus dromedaries)* in the study districts.

3.5. Sampling strategy

Camel herds found in Asayita and Dubti woredas were considered as the study population. This area coverage was conveniently chosen to do daily laboratory analysis of nasal and tissue samples at Semera veterinary regional laboratory and due to previous report of camel respiratory infection out breaks. A total of 10 kebelles (pastoral associations (PAs)) were selected from the

two weredas. Pastoral associations (PAs), namely Galifagie, Kerebuda, Keredura, Gaheretu and Rumaytu (from Assayita) and Gurmudalie, Bebedeta and Korile, Debel, Airolab, and Beyahalie (from Dubti) were included in this study. The PAs were selected purposively based on accessibility to the villages by vehicle or proximity to road, previous and existing report of camel respiratory problems, awareness of the society and camel population.

Subsequently, 87 pneumonic camels (from Dubti) and 104 (from Asayita) were purposively sampled. On the other hand 79 and 114 apparently healthy camels were randomly selected from Asayita and Dubti, respectively for the study. Out of the total 191 pneumonic camels, 75 were < 2 years old and 116 were > 2 years old. On the other side, 90 and 103 of the entire 193 apparently healthy camels were < 2 and > 2 years old, respectively. In addition to the live camels, 46 and 28 lungs together with their analogous trachea were sampled from Dubti and Asayita, respectively out of the slaughtered camels for the abattoir based study.

Camel herds belonging to the selected PAs were visited and sampled early in the morning before released to the field, and the collected samples were processed in the afternoon at Semera regional veterinary laboratory. But the sample collection was prolonged at the watering points at about 4:30 am if not fully managed in the morning.

3.6. Sample collection

Field sample (Nasal swabs): The external nares of the camel was properly cleaned and disinfected with 70% denatured alcohol. Nasal swabs were collected by inserting a sterile cotton-tipped applicator sticks into the nasal passageways from the healthy and clinically diseased camels for bacteriological examination. The sterile cotton-tipped applicator stick was introduced directly into nasal cavity and rubbed smoothly against the mucosa in a circular motion. The cotton-tipped swab was allowed to remain in contact with the secretions for about 1 minute. The swabs collected from each animal were placed in a separate sterile test tubes containing tryptone soya broth, was labeled, kept in a cool box and transported to the nearby Semera regional veterinary laboratory on the day of collection for immediate incubation at 37 °C for 24 hours (OIE, 2008).

Abattoir survey: Tracheal swabs and lung tissue samples were collected from the slaughtered camels so as to investigate the lower respiratory tract. Ante-mortem (by Visual inspection) and post-mortem examination was carried out for the evidence of respiratory problems using the routine visualization, palpation and incision methods (Taiwo, 2005). However, as the number of slaughtered camels were so small (one animal per week from each wereda), it was not possible to get adequate abattoir data, and only 74 camels were used as a source of tracheal swab and lung tissue samples for the abattoir based study.

Tracheal swab: Following the slaughter of the camel, the trachea was grasped with tissue forceps and partially opened by sterile scalpel blade. Then, a sterile, long cotton-tipped swab was inserted into the opened tracheal tube, ensuring effective contact with the mucosa. The swabs were put in separate sterile test tubes into which 3ml tryptone soya broth was added (Carter, 1984), labeled and kept in a cool box, and transported to the nearby Semera regional veterinary laboratory on the day of collection for immediate incubation at 37°C for 24 hours (Quinn *et al*., 1994a).

Tissue samples (Lung tissue): Before collecting lung samples, the external surfaces were disinfected with 70% alcohol (Carter, 1984) to minimize surface contamination. Using sterile blade and thumb forceps, pieces of the lung tissues were collected separately into sterile screw-capped universal bottles. After labeling, these were transported in an icebox to Semera regional veterinary laboratory. Then the samples were flamed with Bunsen burner and about 10g of each was transferred into another universal bottle containing 3ml tryptone soya broth and incubated aerobically at 37°C for 24-48 hours. The grown samples were conveyed to microbiology laboratory of the college of veterinary medicine and agriculture of Addis Ababa University for further processing within 6-7 days of collection.

3.7. Isolation and identification of bacterial species

3.7.1. Identification based on morphological, cellular and biochemical characteristics

The incubated samples (swabs and lung tissues) were thoroughly agitated to aid mixing after 24 hrs. Then, a loopful of the broth culture was taken and streaked over an identified petri-plate

containing blood agar base (Oxide, Hampshire, England) supplemented with 7% sheep blood for isolation of the aerobic as well as microaerophilic bacteria (Quinn *et al*., 1994a). The plates were labeled and incubated aerobically at 37°C for 24 hrs with further re-incubation for 36 – 72 hrs, if no growth observed after 24 hrs (Carter, 1984; Ajuwape and Aregbesola, 2002).

Single colony of different colony types of the positive cultures was subcultured on blood agar plates so as to obtain a pure culture for further analysis. The pure cultures were subjected to Gram's staining to study staining reaction and cellular morphology under light microscope, at 100X magnification. Potassium hydro oxide (KOH) was used to further confirm the Gram reaction when ever there was doubt on the Gram staining. Gram-negative bacteria were sub-cultured on MacConkey agar plates (Oxide, Hampshire, England) for further analysis (Quinn *et al*., 1994a).

Primary identification of the bacteria was performed using colony morphology, Gram reaction, cellular morphology, catalase and oxidase tests and growth on MacConkey agar (Oxide, Hampshire, England) of the pure colony (Duerden *et al*., 1998). Pure cultures of single colony type were subjected to a series of secondary biochemical tests (Annex 8) for final identification, following standard procedures (Carter, 1984; Quinn *et al*., 1994a).

3.7.2. Fatty acid analysis using Biolog-Microstation identification system

Pure isolates of *Streptococcus* species (based on biochemical tests) were prepared for Biolog-Microstation identification system (Biolog, California, USA) which is the latest molecular identification technique based on each microbial species surface fatty acid substrates, at the Institute of Biodiversity Conservation, Addis Ababa, Ethiopia. The colonies of *Streptococcus* species were inoculated on the Biolog Universal Growth (BUG) agar with 5% sheep blood and incubated at 37^{o}c for 24hrs. Sub culturing was done using the same culture media to have pure culture colonies before identification was done by Omnilog.

Bacterial suspension was prepared from the grown pure culture colonies with an appropriate level of bacterial density (20% turbidity in 16-18 inoculating fluid), as recommended in the protocol of the instrument. The suspension was inoculated in to Biolog micro plates aseptically. Then the micro plates were loaded in to the Omnilog and incubated at 37°C for 24 hrs after the necessary

information regarding the bacteria was registered on the software of the instrument (Omnilog user guide, 2008). Finally the micro plates were read by the Omnilog identification system and the bacterial species were identified using the inbuilt Biolog database.

3.8. Antibiotic susceptibility test

The clinical and laboratory standards institute (CLSI, formerly the National Committee for Clinical Laboratory Standards) defines an antibiogram as an overall profile of antimicrobial susceptibility results of a microbial species to a battery of antimicrobial agents (CLSI, 2005). Antibiotic susceptibility tests were done on the representative isolates of the leading bacterial isolates including the major respiratory pathogens found in the respiratory tract of camels.

The antibiogram was conducted by the disk diffusion method using Oxoid antibiotic disks including Ampicillin (AMP), Erythromycin (E), Doxycycline (DO), Kanamycin (K), Norfloxacin (NOR) Tetracycline (TE), Chloramphenicol (C), Streptomicine (S) Gentamicine (CN) and Vancomycine (VA). The bacterial species selected for antibiotic susceptibility test were cultivated on a blood agar medium (Oxide, Hampshire, England) under optimal incubation conditions to obtain a fresh overnight grown culture.

A number of distinct colonies (3-5 morphologically similar colonies) were harvested from the fresh grown plate culture and transferred to a tube containing 4-5ml sterile distilled water. The suspension was mixed gently until turbidity corresponding to 0.5McFarland standard is reached to obtain a homogeneous suspension and then poured onto Mueller-Hinton agar plate (Oxide, Hampshire, England). Blood agar (Oxide, Hampshire, England) enriched with 7% sheep blood was used for *streptococcus* species (Janosi and Kaszanyitzki, 2003). After the inoculum on the plate was dried, antibiotic disks were distributed over the surface of the inoculated plates at equal distance from each other using sterile forceps. The disks were gently pressed onto the agar to ensure firm contact and then incubated for 24 hrs at 37^{o}C.

After 24 hrs, the diameters of the zones of complete inhibition around the disks were measured to the nearest whole mm from the back of the plate using a mm ruler. Zone margins were read as the area showing no obvious growth detected by the unaided eye.

It was read again after 48 hrs of incubation if the isolates were not sufficiently grown. Every test was conducted twofold and then an average of the two results was taken as a final outcome. Growth inhibition zones were interpreted according to the manufacturer's recommendation and NCCLS standards.

3.9. Data processing and statistical analysis

The data obtained from the collected samples were entered in to Microsoft Excel 2007 spread sheet and transferred to SPSS® Version 17 software for statistical analysis. The results generated from the study were summarized using Descriptive statistics. Percentages were used to express the relative abundance of each genera/species to the total number of isolates. Furthermore, chi-square test was computed so as to observe the relationship between the variants. A p-value of <0.05 was considered indicative of a statistically significant difference.

4. RESULTS

4.1. Health status of the sampled camels

During this study a total of 384 individual live camels as well as respiratory tracts of 74 slaughtered camels were used. In total, 384 nasal swabs from the live animals, 74 tracheal swabs and 74 lung tissues from slaughtered camels were examined bacteriologically. Among the 384 live camel samples, 193 (50.26%) were apparently healthy and 191 (49.74%) showed respiratory problems such as nasal discharge, cough, fever of 40-42^{0}c, lacrimation, depression and loss of appetite. Twenty six (35.14%) of the lungs possessed one or more types of lesion. The remaining 48 (64.86%) of the lungs had no evidence of gross lesion. The collected nasal and tracheal swabs as well as lung tissue samples were processed for aerobic bacteriological isolation and characterization.

4.2. Isolation and identification of bacterial species from the nasal cavity of camels

Out of 384 nasal specimens a total of 641 isolates representative of different genera and species were identified from both study woredas, namely, Asayita and Dubti. The isolates constituted 14 different genera of bacteria including *Streptococcus*, *Pasteurella*, *Mannheimia*, *Staphylococcus*, *Micrococcus*, *Enterococcus*, *Pseudomonas*, *Bacillus*, *Klebsiella*, *Bordetella*, *Neisseria*, *Corynebacterium*, *Proteus* and *E. coli* (Table2). More than one bacterial species were isolated from all of the nasal swabs collected from the pneumonic camels and from 101 (52.33%) nasal swabs of apparently healthy camels. Only one species from 89 (46.11%) and no bacterial growth was obtained from 3 (1.55%) of the nasal swabs collected from the apparently healthy camels.

4.2.1. Isolation rates of bacterial species from the nasal cavity of camels

In the present study a total of 641 bacterial species were isolated from the nasal tracts of both apparently healthy and camels showing signs of respiratory problems. Coagulase negative *staphylococcus* (*CNS*), 47 (25.5%), *Bacillus*, 29 (15.8%), *Staphylococcus aureus*, 29 (15.8%) and *E. coli*, 21 (11.4%), were the leading bacterial species isolated from the nasal tracts of apparently healthy young camels.

Whereas, *CNS*, 63 (31.5%) *Bacillus*, 23 (11.5%) and *S. aureus*, 22 (11.0%) were the most frequently encountered bacterial isolates in the nasal tracts of apparently healthy adult camels (Table2).

Nasal swabs collected from the young camels with signs of respiratory infection revealed that there was a high prevalence of *S. aureus* which accounted for 42 (33.6%) of the total bacterial isolates. This was followed by *Bacillus* and *K. pneumoniae* species each contributed for 13 (10.4%) and 12 (9.6%) of the total bacterial count, respectively. *S. aureus*, 30 (22.7%), *CNS*, 18 (13.6%), *and Bacillus*, 16 (12.1%), were among the bacterial species isolated at a relatively higher rates in the clinically diseased adult camels. The further bacterial isolates and their absolute/relative abundance are presented on Table2.

Table 2. Bacterial species isolated from the nasal cavities of apparently healthy and pneumonic camels.

	Healthy			Pneumonic		
Bacterial species	Young camels	Adult camels	Total	Young camels	Adult camels	Total
S.aureus	29 (15.8%)	22 (11.0%)	51 (13.3%)	42 (33.6%)	30 (22.7%)	72 (28.0%)
CNS	47 (25.5%)	63 (31.5%)	110 (28.6%)	12 (9.6%)	18 (13.6%)	30 (11.7%)
Bacillus spp	29 (15.8%)	23 (11.5%)	52 (13.5%)	13 (10.4%)	16 (12.1%)	29 (11.3%)
K. pneumoniae	6 (3.3%)	2 (1.0%)	8 (2.1%)	12 (9.6%)	6 (4.5%)	18 (7.0%)
Neisseria spp	7 (3.8%)	12 (6.0%)	19 (4.9%)	1 (0.8%)	8 (6.1%)	9 (3.5%)
Micrococcus spp	9 (4.9%)	6 (3.0%)	15 (3.9%)	4 (3.2%)	1 (0.8%)	5 (1.9%)
Proteus spp	8 (4.3%)	14 (7.0%)	22 (5.7%)	5 (4.0%)	11 (8.3%)	16 (6.2%)
Corynebacterium spp	9 (4.9%)	7 (3.5%)	16 (4.2%)	3 (2.4%)	7 (5.3%)	10 (3.9%)
Enterococcus spp	3 (1.6%)	10 (5.0%)	13 (3.4%)	0 (0%)	5 (3.8%)	5 (1.9%)
M. haemolytica	1 (0.5%)	0 (0%)	1 (0.3%)	4 (3.2%)	1 (0.8%)	5 (1.9%)
B. bronchiseptica	1 (0.5%)	0 (0%)	1 (0.3%)	6 (4.8%)	2 (1.5%)	8 (3.1%)
E. coli	21 (11.4%)	9 (4.5%)	30 (7.8%)	1 (0.8%)	5 (3.8%)	6 (2.3%)
P. multocida	2 (1.1%)	0 (0%)	2 (0.5%)	7 (5.6%)	1 (0.8%)	8 (3.1%)
S. equi subsp. equi	0 (0%)	2 (1.0%)	2 (0.5%)	1 (0.8%)	1 (0.8%)	2 (0.8%)
S. equi subsp. zooepidemicus	1 (0.5%)	10 (5.0%)	11 (2.9%)	0 (0%)	3 (2.3%)	3 (1.2%)
P. aeruginosa	0 (0%)	1 (0.5%)	1 (0.3%)	12 (9.6%)	3 (2.3%)	15 (5.8%)
Unidentified spp	11 (6.0%)	19 (9.5%)	30 (7.8%)	2 (1.6%)	14 (10.6%)	16 (6.2%)
Total	184 (100%)	200 (100%)	384 (100%)	125 (100%)	132 (100%)	257(100%)

According to the Pearson-Chi-Square comparison, there was a statistically significant difference between the health status of the nasal tract and isolation rate of S. *aureus*, *B. bronchiseptica and K. pneumoniae* ($p<0.05$). But there was no statistically significant difference among *S. equi subsp. equi*, *M. haemolytica* and *P. multocida* with reference to the nasal health ($p>0.05$). On the other hand, a statistically significant difference was observed between the age groups with the isolation rate of *P. multocida* and *K. pneumoniae* ($p<0.05$). While no statistically significant difference was seen in the isolation rate of the rest camel respiratory pathogens between the age groups ($p>0.05$).

4.3. Isolation and identification of bacterial species from trachea and lung of camels (Abattoir survey)

Only 39 (52.7%) of the lung tissue samples and 33 (44.59%) of the tracheal swabs yielded bacterial colonies and identified either to the genus or species level. The remaining 4 (5.4%) of the lung lesions, 31 (41.89%) of the apparently healthy lungs and 41 (55.4%) of the tracheal samples examined did not reveal any bacterial growth. More than one bacterial species were isolated from 20 (27.02%) pneumonic lungs, 6 (8.11%) apparently healthy lungs and 18 (24.32%) tracheal samples. On the other hand, only one bacterial isolate was identified from 2 (2.7%) pneumonic lungs, 11 (14.86%) apparently healthy lungs and 15 (20.27%) tracheal samples investigated.

A total of 262 bacterial isolates were identified from 74 lung and the same number of corresponding tracheal samples; out of which 115 (43.89%) and 147 (56.11%) were recovered from trachea and lung, respectively.

Totally, 92 bacteria were isolated from the pneumonic camel lungs from which *S. aureus*, 15 (16.3%), *S. equi subsp. equi*, 12 (13.0%) and *P. multocida*, 10 (10.9%) were the dominant isolates. *S. equi subsp. zooepidemicus* and *S. pneumoniae* were recovered at the lowest rate; each comprised 1% (Table 3).

E. coli and *Proteus* species took 7 (12.7%) and 6 (10.9%), respectively, of the total 55 bacteria demonstrated from the healthy lungs. On the other side, *S. equi subsp. equi, B. bronchiseptica and P. aeruginosa*, each 1 (1.8%), were isolated at the lowest proportion. *M. haemolytica* was not recovered from the normal lungs.

Table 3. Bacterial species isolated from the trachea and lung of camels.

	Trachea			Lung		
Bacterial species	Healthy	Pneumonic	Total	Healthy	Pneumonic	Total
S. equi subsp. equi	0 (0%)	0 (0%)	0 (0%)	1 (1.8%)	12(13.0%)	13 (8.8%)
P. multocida	0 (0%)	1 (2.0%)	1 (9.0%)	4 (7.3%)	10 (10.9%)	14 (9.5%)
M. haemolytica	0 (0%)	0 (0%)	0 (0%)	0 (0%)	8 (8.7%)	8 (5.4%)
B. bronchiseptica	2 (3.0%)	0 (0%)	2 (1.7%)	1 (1.8%)	4 (4.3%)	5 (3.4%)
S. aureus	3 (4.5%)	5 (10.2%)	8 (7.0%)	4 (7.5%)	15 (16.3%)	19 (12.9%)
K. pneumoniae	8 (12.1%)	1 (2.0%)	9 (7.8%)	5 (9.1%)	8 (8.7%)	13 (8.8%)
CNS	9 (13.6%)	4 (8.2%)	13 (11.3%)	2 (3.6%)	5 (5.4%)	7 (4.8%)
Bacillus spp	7 (10.6%)	3 (6.1%)	10 (8.7%)	5 (9.1%)	3 (3.3%)	8 (5.4%)
E. coli	7 (10.6%)	13 (26.5%)	20 (17.4%)	7 (12.7%)	5 (5.4%)	12 (8.2%)
Micrococcus spp	4 (6.1%)	3 (6.1%)	7 (6.1%)	4 (7.3%)	0 (0%)	4 (2.7%)
Proteus spp	0 (0%)	0 (0%)	0 (0%)	6 (10.9%)	3 (3.3%)	9 (6.1%)
Corynebacterium spp	0 (0%)	1 (2.0%)	1 (9.0%)	2 (3.6%)	10 (10.9%)	12 (8.2%)
Neisseria spp	6 (9.1%)	2 (4.1%)	8 (7.0%)	0 (0%)	0 (0%)	0 (%)
Enterococcus spp	1 (1.5%)	1 (2.0%)	2 (1.7%)	0 (0%)	0 (0%)	0 (0%)
P. aeruginosa	0 (0%)	1 (2.0%)	1 (9.0%)	1 (1.8%)	5 (5.4%)	6 (4.1%)
S. pneumoniae	0 (0%)	0 (0%)	0 (0%)	4 (7.3%)	1 (1.1%)	5 (3.4%)
S.equisubsp.zooepidemicus	7 (10.6%)	1 (2.0%)	8 (7.0%)	3 (5.5%)	1 (1.1%)	4 (2.7%)
Other *Streptococcus* spp	5 (7.6%)	2 (4.1%)	7 (6.1%)	2 (3.6%)	0 (0%)	2 (1.4%)
Unidentified spp	7 (10.6%)	11(22.4%)	18 (15.7%)	4 (7.3%)	2 (2.2%)	6 (1.4%)
Total	66(100%)	49 (100%)	115(100%)	55 (100%)	92 (100%)	147 (100%)

According to the Pearson-Chi-Square comparison, there was a statistically significant difference between the isolation rates of *S. equi subsp. equi*, *P. multocida*, *M. haemolytica*, *B. bronchiseptica*, *S. aureus* and *K. pneumonia* and the health status of the lung ($p<0.05$) but no difference was observed for the above isolates in relation to the health status of the trachea ($p>0.05$).

A total of 104 pneumonic and 79 apparently healthy live camels in addition to 28 lungs with their corresponding trachea were sampled from Asayita. Out of these, 419 bacteria were identified in which *S. aureus*, *CNS* and *bacillus* species were the leading isolates at a rate of 95 (22.7%), 56 (13.4%) and 51 (12.2%), respectively. As shown in Table 4, the majority of the pathogenic bacteria were recovered at higher proportions from this study area.

From Dubti, 87 pneumonic, 114 apparently healthy camels and 46 lungs together with their analogous trachea were sampled. The frequently isolated species were *CNS*, 104 (21.5%), *S. aureus*, 55 (11.4%) and *E. coli*, 51 (10.5%). On the other side, *M. haemolytica*, 3 (0.6%) and *P. multocida*, 4 (0.8%) were among the species isolated at lower rate from the total 484 isolates in this district (Table 4).

Table 4. Bacterial species isolated from the respiratory passageways of camels in the study areas

	Study Woreda		
Bacterial species	Asayita	Dubti	Total
CNS	56 (13.4%)	104 (21.5%)	160 (17.7%)
S. aureus	95 (22.7%)	55 (11.4%)	150 (16.6%)
Bacillus spp	51 (12.2%)	48 (9.8%)	99 (11.0%)
E. coli	26 (6.2%)	51 (10.5%)	77 (8.5%)
K. pneumoniae	17 (4.1%)	31 (6.4%)	48 (5.3%)
Neisseria spp	23 (5.5%)	13 (2.7%)	36 (4.0%)
Micrococcus spp	12 (2.9%)	19 (3.9%)	31 (3.4%)
Proteus spp	16 (3.8%)	31 (6.4%)	47 (5.2%)
Corynebacterium spp	31 (7.4%)	8 (1.7%)	39 (4.3%)
Enterococcus spp	7 (1.7%)	13 (2.7%)	20 (2.2%)
S. pneumoniae	0 (0%)	5 (1.0%)	5 (0.6%)
M. haemolytica	11 (2.6%)	3 (0.6%)	14 (1.6%)
B. bronchiseptica	9 (2.1%)	7 (1.4%)	16 (1.8%)
P. aeruginosa	4 (1.0%)	10 (2.1%)	14 (1.6%)
P. multocida	21 (5.0%)	4 (0.8%)	25 (2.8%)
S. equi subsp. equi	11 (2.6%)	6 (1.2%)	17 (1.9%)
S. equi subsp. zooepidemicus	9 (2.1%)	17 (3.5%)	26 (2.9%)
Other *streptococcus* spp	2 (0.5%)	7 (1.4%)	9 (1.0%)
Unidentified spp	18 (4.3%)	52 (10.7%)	70 (7.8%)
Total	419 (100%)	484 (100%)	903 (100%)

The Pearson-Chi-Square comparison revealed that there was a statistically significant difference between the study areas in the isolation frequency of *M. haemolytica*, *P. multocida*, *S. pneumoniae* and *S. aureus* ($p < 0.05$). Whereas, no statistically significant difference was observed for the other camel respiratory pathogens in between the study areas ($p > 0.05$).

Out of the 532 specimens collected from the nasal cavity, trachea and lung, 903 bacterial isolates were demonstrated. Among which 556 (61.57%), 277 (30.67%) and 70 (7.75%) were Gram-positive, Gram-negative and unidentified bacterial organisms, respectively. The majority of the isolates colonized all the anatomical sites investigated although to a different degree (Figure2). However, a general decrease in the isolation rate was observed as one goes down the respiratory tract.

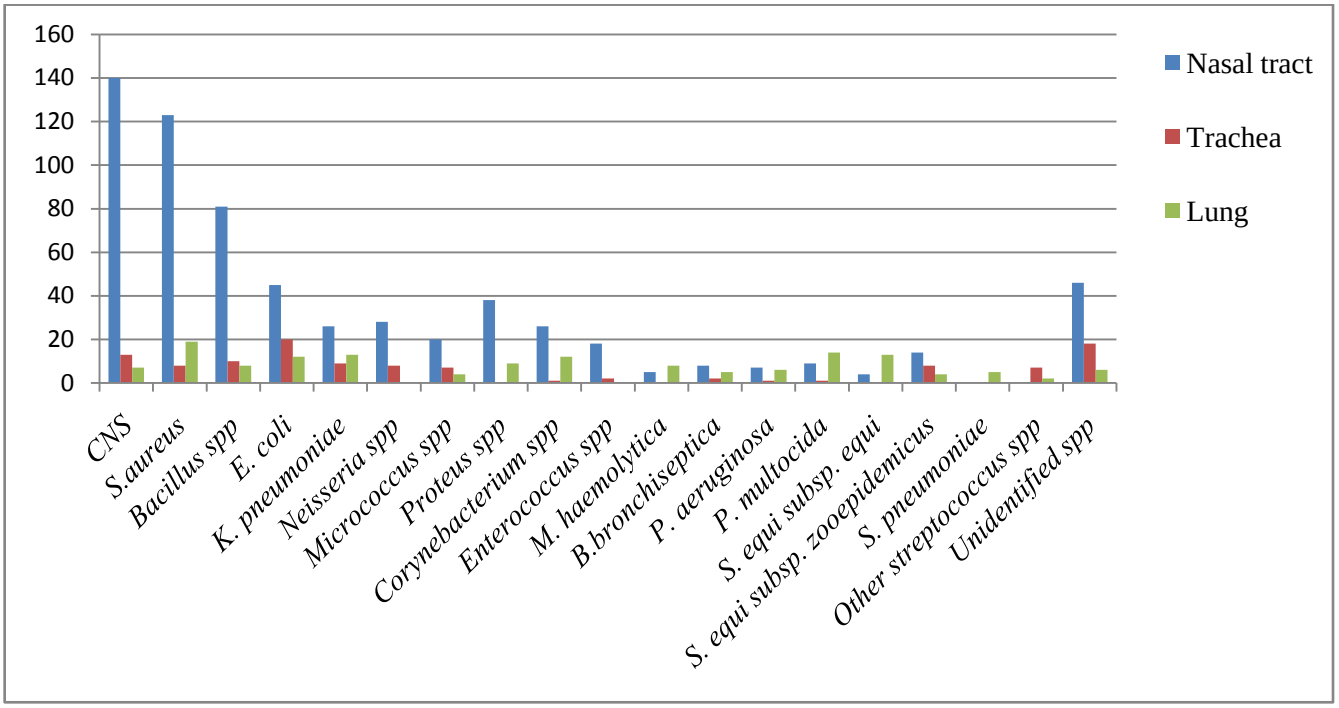

Figure 2. Relative proportions of the bacterial isolates in relation to anatomical location.

The Pearson-Chi-Square comparison indicated that there was a statistically significant difference among the anatomical sites examined with the isolation rate of the major respiratory pathogens (except *B. bronchiseptica)* ($p<0.05$).

Generally, the present study showed that the isolation frequency of the major respiratory pathogenic bacteria was higher in camels that exhibited different respiratory clinical signs than the rate obtained from the camels which did not manifest any clinical sign. Furthermore, most of the pathogenic bacteria were isolated from the pneumonic lungs in the abattoir based study. On the other side, the normal bacterial flora were more prevalent in the respiratory passage ways of apparently healthy camels than the pneumonic ones.

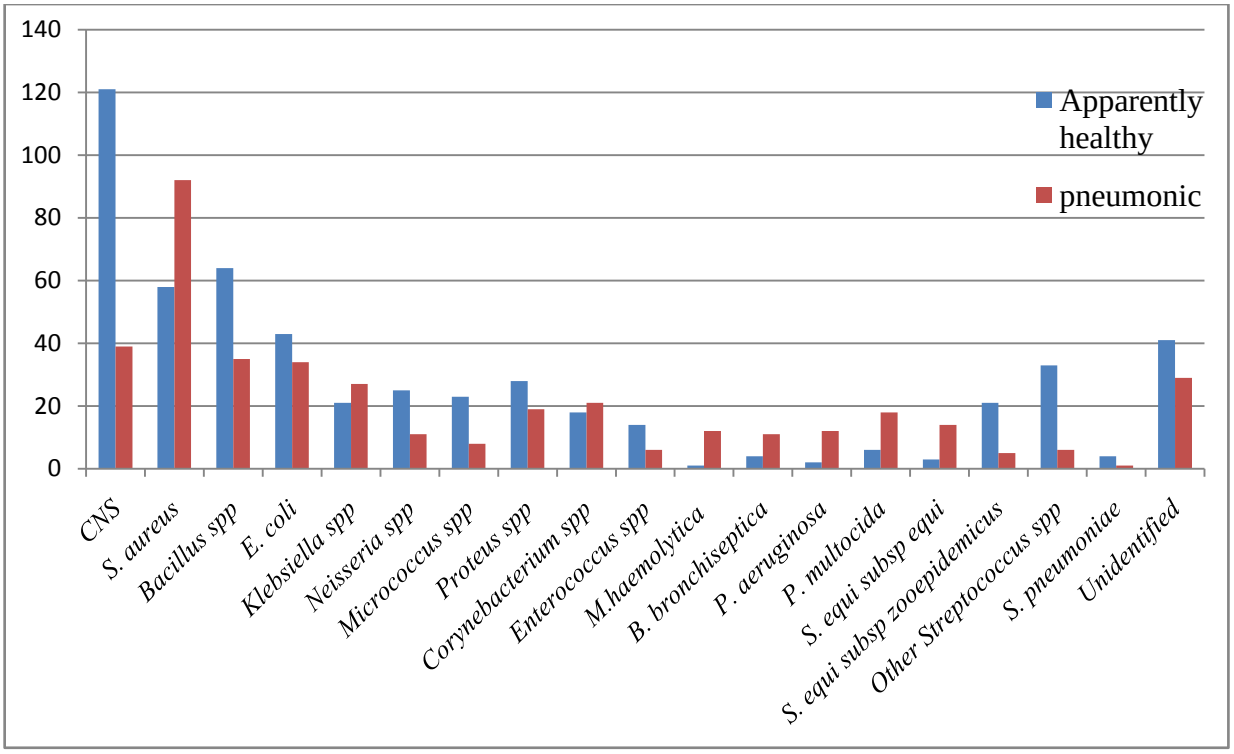

Figure 3. Bacterial species isolated from the respiratory passageways of apparently healthy and pneumonic camels.

The result of Pearson-Chi-Square comparison revealed that there was a statistically significant difference between pneumonic and apparently healthy camels with the isolation rate of the major respiratory pathogens (except *B. bronchiseptica* and *K. pneumoniae*) ($p<0.05$).

4.4. Identification of bacterial isolates using Biolog-Omnilog

Streptococcus equi subsp. equi, *streptococcus equi subsp. zooepidemicus* and *streptococcus pneumoniae* were identified using the Omnilog-Biolog identification system at the Institute of Biodiversity Conservation, Addis Ababa, Ethiopia.

4.5. Antibiotic sensitivity pattern of bacterial isolates

Antibiogram was conducted on the dominant bacteria isolated from the different parts of the respiratory passage ways of camels to evaluate the current status of the field isolates against the commonly used antimicrobial drugs. The antibiogram profile of *K. pneumoniae* strains indicated a marked susceptibility to norfloxacin, streptomycin and gentamicin with 100% inhibitory activity and resistant to ampicillin and tetracyclin with 100% resistivity (Table 5).

Out of 17 isolates of *S. equi subsp. equi*, the percentage of sensitive isolates was 52% to ampicillin, 58.8% to erythromycin, 35.3% to kanamycin, 94.1% to norfloxacin, 41.2% to tetracyclin, 29.4% to chloramphenicol, 17.6% to streptomycin, 52.9% to gentamicin, 100% to vancomycin and 47.1% to doxycyclin.

Most of the strains of *P. multocida* have shown a high level of sensitivity to gentamicin, streptomycin, norfloxacin and chloramphenicol. They were poorly sensitive to vancomycin, doxycyclin and erythromycin but the effect of ampicillin and tetracycline was challenged by these isolates (Table 5).

Streptomycin, kanamycin, gentamicin and norfloxacin were the drugs revealed a large inhibited zone of growth for most of the strains of *M. haemolytica*. On the other side vancomycin and tetracyclin were the least effective drugs (Table 5).

Norfloxacin and vancomycin were the most effective drugs against *S. aureus* with 100% growth inhibition. The bacteria resisted the action of chloramphenicol, streptomycin and ampicillin at higher degree. The sensitivity pattern of S. *pneumonia, P. aeruginosa, E. coli, Proteus and B. bronchoseptica* isolates is recorded in table 5.

Table 5. The results of antibiotic susceptibility tests of bacterial isolates from the respiratory tracts of camels (disk diffusion method).

Bacterial species	N	% of strains sensitive to the antibacterial agents									
		AMP	E	K	NOR	TE	C	S	CN	VA	DO
P. multocida	12	25	50	58.3	75	16.7	75	75	83.3	33.3	41.7
M.haemolytica	11	27.2	45.5	81.8	72.3	9.1	63.6	90.9	90.9	18.2	36.4
B. bronchoseptica	10	40	50	30	90	20	70	100	80	10	60
K. pneumoniae	11	0	72.7	9.1	100	0	18.2	100	100	9.1	81.8
S. equi subsp. equi	17	52	58.8	35.3	94.1	41.2	29.4	17.6	52.9	100	47.1
S. pneumoniae	5	0	80	0	100	20	40	80	40	100	60
S. aureus	9	33.3	77.7	66.6	100	44.4	11	22.2	88.8	100	33.3
P. aeruginosa	10	10	70	50	90	20	40	100	90	0	60
E. coli	8	50	37.5	62.5	100	25	87.5	100	100	12.5	75
Proteus	9	22.2	44.4	0	33.3	11.1	44.4	22.2	77.7	55.5	88.9

N= number of isolates tested, AMP= Ampicillin, E= Erythromycin, K= Kanamycin, NOR= Norfloxacin, TE= Tetracyclin, C= Chloramphenicol, S= Streptomycin, CN= Gentamicin, VA= Vancomycin, DO= Doxycyclin

5. DISCUSSION

It is clear that the respiratory tract of apparently healthy animals acted as a reservoir for many species and types of microorganisms. These microorganisms reached the nasal cavity either through inhalation, direct or indirect contact or during drinking. This study has shown that a wide variety of bacterial species colonizes the respiratory passageways of apparently healthy and clinically diseased camels. This is supported by several researchers who previously demonstrated diverse bacterial species from the nasal tracts of apparently healthy camels (Abdulsalam, 1999), from the nasal, tonsil, trachea and lung of apparently healthy camels (Azizollah *et al*., 2008), from nasal tract, trachea and lung of pneumonic camels (Al-Doughaym *et al*., 1999), from the normal and pneumonic lungs of camels (Abubakar *et al*., 2008) and from pneumonic lungs of camels (Al-Tarazi, 2001).

In addition to the above findings, Yimer and Asseged (2007) as well as Merga *et al*. (2006) have isolated a diversified bacterial species from the lungs of pneumonic sheep and apparently healthy caprines, respectively. The consistent isolation of these organisms from the pneumonic lungs of various species of animals might indicate their role in resulting different respiratory infections especially when the immune system of the animal is compromised by some other external factors. The normal bacterial flora of a healthy individual can be altered by several factors such as changes in the hygienic condition, environmental and climatic conditions as well as the nutritional and immunological status of the animal. Such factors would lower the resistance of the lung tissue and the existing organism most probable would get the upper hand, leading to the presentation of a variety of pathologies (Herthelius *et al*., 1989).

The pathogenic bacteria isolated during the present study period are comparable to those previously reported from the respiratory tract of apparently healthy camels (Shigidi, 1973; Azzizolah *et al.,* 2008). This may confirm the fact that the respiratory tract serves as a reservoir for a variety of microorganisms which under suitable predisposing factors invade the different parts of the tract and cause pathological lesions.

The current study revealed that the majority of the isolates colonized all the anatomical sites investigated although to a different degree (Figure 2).

However, a general decrease in the isolation rate was observed as one goes down the respiratory tract. The normal bacterial floras were more prevalent in the respiratory passage ways of apparently healthy than the pneumonic camels. This is quite suggestive of the logic that these organisms live as a commensal in the upper respiratory tract, invading the lung under conditions of stress.

Stress factors with or without viral infection have been reported to suppress the mucociliary clearance mechanism and the overall respiratory defensive system which allows the proliferation of bacterial commensals in the respiratory tract and an abrupt shift from commensal to pathogen (Brogden *et al*., 1998). Yimer and Asseged (2007) have reported analogous remark while investigating their abattoir survey of bacterial isolation from the slaughtered sheep in Dessie. Similar trend of bacterial distribution was also observed by Azzizolah *et al.* (2008), Bekelle (1999) and Al-Tarazi (2001) during their bacteriological studies on the respiratory tracts of camels.

Considering the stress of weather, disease and poor management conditions to which the camels are constantly subjected, the pathogenic role of several bacterial species that inhabit the upper respiratory tract of apparently normal camels could be enormous. Therefore, *S. equi subsp. equi*, *M. haemolytica, P.multocida, K.pneumoniae, S. aureus* and *B. bronchiseptica* are described as normal inhabitant of upper respiratory tract as well as causative agent of pneumonia after the host defense mechanism has been suppressed by primary initiating agent. In line with this, these bacteria were also isolated at higher incidence rate from the pneumonic lungs in the present investigation.

Most species of the genus *streptococcus* were considered potential pathogens, occur in nature, and some are commensal in the respiratory, genital and alimentary tracts and skin of animals and man (Biberstein and Zec, 1990). *Streptococcus* species have been isolated from clinically healthy camels although they were not definitely identified and characterized (Shigidi, 1973; Mahmoud *et al*., 1988; Rana *et al*., 1993). On the other hand, *Streptococcus* species such as β-haemolytic *Streptococci, S. viridans, S. pneumoniae* and *S. pyogenes* were described in active respiratory disease of camels *(*Thabet, 1994).

Yigezu *et al*. (1997) have reported the bacteria from camel lungs using biochemical tests and suggested its role in the epizootic occurring in camels in Ethiopia. In the present work, S. *equi subsp. equi was* demonstrated from the nasal tract and lungs of camels using a series of biochemical tests and finally by Biolog-Microstation identification system. The bacteria were isolated from the healthy and diseased nasal tracts at a very close amount, 0.5% and 0.8%, respectively. It was recognized at a rate of 1.8% from a normal lung but frequently encountered in the pneumonic lungs with the incidence of 13.0%. It was not identified from trachea.

In this study, the isolation of S. *equi subsp. equi* from the healthy nasal tract and lung tissue at low level can suggest that they represent the normal flora. However, it can be involved in camel pneumonia under conditions of stress, co-infection and immune suppression. Therefore, the presence of S. *equi subsp. equi* in much higher proportion in the pneumonic camel lungs can make the bacteria to be considered as one of the major factors resulting camel respiratory infections.

Streptococcus equi subsp. equi has been identified as the causative agent in equine strangles which is a disease of domestic equines of all age groups and is more severe in horses than in donkeys and mules. The agent is directly transmissible via oral or nasal routes and by indirect means through contamination of the environment (feed, water, stable) by excretions from sick horses. Regrouping of horses is usually a risk factor in a strangles outbreak (Yigezu *et al*., 1997). Although the camel rearing areas of Asayita and Dubti are uninhabited by horses, they are inhabited by donkeys and, in fact, these species are used as pack animals for transportation of agricultural products to markets and this could favor the transmission of the agent by contact.

Pasteurella species have a worldwide distribution with ample host spectrum and are commensals residing on the mucosa of the upper respiratory and intestinal tracts of animals (Quinn *et al*., 2002). All *Pasteurella* species are involved as a primary or secondary agent in resulting pneumonia when the body defense mechanisms are impaired (Quinn *et al*., 2002; Radostits *et al*., 2007).

In this investigation, *pasteurella multocida* was found frequently inhabited the nasal cavity. It was isolated from the nasal tracts of apparently healthy and pneumonic camels at a rate of 0.5% and 3.1%, respectively. The bacteria have been also isolated from the nasal tracts of clinically

diseased young camels at a higher proportion (5.6%). This is in harmony with previous reports (Mohammed, 1999; Merga *et al.*, 2006; Yimer and Asseged, 2007), indicating that the organism lives as a commensal in the upper respiratory tract, invading the lung under conditions of stress (Collier and Rossow, 1964).

The result of the abattoir survey also specified that the proportion of isolation of these bacteria from the pneumonic camel lung (10.9%) was higher than that of healthy lungs (7.3%). In addition, it was identified from the trachea of pneumonic camels at 2.0%. Abubakar *et al*. (2008) have also demonstrated *P. multocida* at a rate of 4.4% from pneumonic lungs of camels in Nigeria but failed to isolate it from normal lungs. The recovery rate of the bacteria from lung lesions in the present work is higher compared that of previous reports from camels (Abubakar *et al.*, 2008), from sheep (Merga *et al*, 2006) and from goats (Tilaye, 2010). This discrepancy might be attributed to the variation in sample size, difference in agro-ecological zones of study areas and host species diversity as well as health status of the study animals.

K. pneumoniae, a common saprophyte in the soil and respiratory tract of various animals was recovered at a rate of 9.6% and 4.5% from the nasal tracts of young and adult camels showing respiratory problems, respectively. It was also reported from nasal cavity of apparently healthy rabbits (Ajuwape and Aregbesola, 2002) and pneumonic camels (Al-Doughaym *et al*., 1999). The exploration of the abattoir survey revealed an enormously comparable frequency of *K. pneumoniae* in the pneumonic and healthy lungs. But it was found at higher proportion in the trachea of healthy camels (12.1%) than the trachea of the diseased ones (2.0%). The result described by Al-Doughaym *et al*. (1999) from the lungs of pneumonic camels, presented as 10.9%, is comparable to the present finding. They have also stated the involvement of these bacteria to the development of respiratory problems in camels.

K. Pneumoniae has been reported from the respiratory tracts of diverse animals. The bacteria inhabits the intestinal tracts of animals, fecal contamination of the environment accounts for the wide distribution of the organism, and contributes to the occurrence of opportunistic infection. Desissa *et al*. (2006) have isolated the organism from apparently healthy and pneumonic donkeys but more frequently recovered from donkeys having respiratory problems. A slightly lower figure (6.3%) has also been reported by Abubakar *et al*. from the lung lesions of camels in Nigeria in

2008. The invariable isolation of these organisms from the pneumonic lungs of various animal species might indicate their role in different respiratory syndromes.

B. bronchiseptica are commensals on the mucous membranes of the upper respiratory tract (Quinn *et al*., 2002) and infects a wide range of animals including man. The current finding indicated that the bacteria colonized the nasal tracts of both young (4.8%) and adult (1.5%) clinically diseased camels. Nesibu *et al*. (2010) have reported the bacteria at a rate of 3.5% from the pulmonary lesions of camels. The isolation rate of the bacteria from the pneumonic lungs at a rate of 4.3% is a bit higher than what has been previously reported by Nesibu *et al*. (2010). In addition to this it has been also isolated from the trachea (3.0%) and lung (1.8%) of apparently healthy camels. There are no further reports of isolation of the bacteria from respiratory tracts of camels. But the present finding together with the habitat of the bacteria and little previous reports indicate the involvement of the bacteria in the respiratory problems of camels especially when the animal is stressed.

Mannheimia haemolytica are commensals, residing in the nasopharyngeal microflora and are all capable of causing infection when the body defense mechanisms are impaired (Zamri *et al*., 2006). Their presence is mainly confined to ruminants with most adequately characterized strains originating from cattle, sheep and goats (Biberstein and Hirsh, 1999a). However, *Mannheimia haemolytica* were isolated at prominent incidence from the nasal tracts and lungs of clinically diseased camels in this study. It was identified from the nasal tract of apparently healthy young camels at a rate of 0.5% though a higher rate was reported from the clinically diseased young (3.2%) and adult (0.8%) camels.

M. haemolytica isolated from the pneumonic lungs of camels (8.7%) in this work is to a great extent higher than the report of Desisa *et al*. (2006) as well as Abubakar *et al*. (2008): who demonstrated 0.5% and 0.3% from the upper respiratory tracts of donkeys and pulmonary lung lesions of camels, respectively. Although this finding is consistent with the reports of Al-Tarazi who accounted a prevalence of 6.6% from the pneumonic lungs of camels slaughtered in northern Jordan in 2001, much higher rates have been reported from ovine pneumonic lungs (Tilaye, 2010) and lungs of apparently healthy goats (Merga *et al*., 2006). These bacteria were not isolated from the trachea and apparently normal lungs. The occurrence of *M. haemolytica* in

higher proportion in the clinically ill camels can make the bacteria to be considered as one of the major factors resulting camel respiratory disease complex.

Staphylococcus aureus occurs both as commensal on skin and mucous membranes and as environmental contaminant. The bacteria reside in the upper respiratory tract and are involved in disease processes only when stress conditions prevail. Infection of *S. aureus* can be either endogenous or exogenous in origin (Quinn *et al*., 2002). The incidence of *S. aureus* at 28.0% in the nasal tracts of pneumonic camels in this study was higher than the finding of 19.2% by Al-Doughaym *et al*. (1999) and 6.3% by Yimer and Asseged (2006) from the nasal tracts of pneumonic camels and apparently healthy sheep respectively.

A much higher frequency, 100% was reported previously from the nasal tracts of rabbits by Ajuwape and Aregbesola (2002). This variation could be attributed to a feeding habit, as rabbits are coprophagous animals (Klemn, 1993) while camels are mainly browsers. But the extensive management under which the camels were kept might have also contributed to the comparatively lower nasal involvement.

In this study, *S. aureus* was the commonest bacteria in the pneumonic lungs isolated at a rate of 16.3% which is higher when compared to 10.6% reported by Al-Tarazi (2001) from lung lesions of camels. Similarly, Al-Doughaym *et al*. (1999) have reported it at a higher proportion (24.8%) from pneumonic camel lungs. The present investigation and other previous wide-ranging works have reported *S. aureus* from the nasal tract and pneumonic lungs of various animals at higher level. This suggests that the bacteria resides as a normal inhabitant of upper respiratory tract as well as a causative agent of secondary pneumonia after primary initiating agent has suppressed the defense mechanism of the animal.

Young animals with underdeveloped immunity are more susceptible to infectious diseases as usually reported in literature. Newborn camel calves that were not fed with adequate amount of colostrums are possibly susceptible to such infection (Abbas and Omer, 2005).

The people in the study districts apply some management practices to their herds. However, they still believe that the intake of colostrum will result in ill-health to newborn camel calves, thus it is common practice to deny the newborn calf access to colostrum. Mostly, camel owners either

prevent early colostrum consumption or feed the calf first with water instead of colostrums and even milk out the colostrum due to fear of calf diarrhoea. In addition, the herdsmen also keep the young camels in a confined area and provide insufficient milk supply. Such traditional practice has been as well reported from other camel rearing areas (Wilson, 1998).

Hence, the isolation of the camel respiratory pathogens at comparatively higher proportions from the young camels could be due to the denied access to colostrum, insufficient milk supply and dusty as well as confined area in which they are managed. But the extensive management under which the adult camels were kept might have also contributed to the relatively lower proportion of the camel respiratory pathogens in this age group.

The field and abattoir based study of the respiratory tracts of apparently healthy and pneumonic camels revealed a total of 903 bacterial species. Failure to isolate bacteria from 4 lung tissues having observable lesions might be due to the involvement of other pathogens such as *Mycobacterium*, anaerobic bacteria, virus, *Mycoplasma* and fungi. However, most normal lung tissues yielded one or more bacterial types even though Lopéz (2001) reported that normal lungs were ideally supposed to be sterile as the pulmonary macrophages continue to scavenge for bacterial agents in the lungs thereby making the lung free from pathogens as a defensive mechanism.

In addition to the above major bacterial pathogens, other bacterial species have been isolated at high proportions from both the apparently healthy and pneumonic camels from all of the anatomical sites examined. This signifies the fact that a number of factors are complicating the respiratory infection in camels. Despite the fact that *Micrococcus, Enterococcus, CNS, Bacillus, Proteus, Corynebacterium, E. coli, P. aeruginosa and Neisseria* species are considered non-pathogenic (Quinn *et al*., 2002), their isolation from pneumonic camel lungs by various workers (Al-Doughaym *et al*., 1999; Al-Tarazi, 2001; Abubakar *et al*., 2008; Azizollah *et al*., 2008;) indicated that they could have a role in the development of respiratory infections. Hence, the momentous role of these bacteria in camel respiratory disease complex should not be overlooked though they have not been considered as a major causative agent.

Antimicrobial drugs are important resources that must be conserved for proper use. Choosing the most effective antimicrobial agent is often difficult. A judgment should be made on the basis of *invitro* sensitivity results (Hirish, 1999; Caprioli *et al.*, 2000). Hence, antibiotic susceptibility tests were done on the representative isolates of the leading bacterial species found in the respiratory tract of camels using diverse oxoid antibiotic disks.

The present study has shown an increased level of resistance among the respiratory pathogens against the commonly used antimicrobials in respiratory tract infections (Table 5). The majority of camel respiratory pathogens were found susceptible to norfloxacin, streptomycin and gentamicin but resistant to the action of ampicillin and tetracycline. This is correlated with the studies conducted in Nigeria by El-Mahmood *et al*. (2009). In addition, a similar resistance of *S. equi subsp. equi* and *K. pneumoniae* to tetracycline was recorded (Nkang *et al*., 2009).

Fazlani *et al*. (2006) reported a high level of resistance of *M. haemolytica* and *P. Multocida* to streptomycin while the organisms were found to be susceptible to ampicillin and oxytetracycline. In this study, however, both organisms were found to be susceptible to streptomycin, while they were resistant to tetracycline and ampicillin. This represents a shift of resistance from streptomycin to tetracycline and ampicillin. This may be associated with the present lesser usage or misuse of streptomycin for animal diseases unlike tetracycline which is commonly used for most animal diseases today (Diker *et al*., 1994).

The high rate of resistance observed in many of the isolates to tetracycline and ampicillin in this study could be either because they are frequently and unnecessarily prescribed or sold over the counter in the open market and private veterinary drug shops without prescription. The people in the study areas have easy access to purchase tetracycline and ampicillin from the private pharmacies and any open market without prescription. They have also the opportunity of getting many drugs from NGO's working in livestock areas; but they have a strong desire to tetracycline. As a result, they provide health care by themselves mainly using these drugs even for the healthy camels. This probably means that the selective pressure of these commonly used antibiotics on the bacteria circulating in the community could have resulted in high frequency of resistance among the isolates. Apart from ease of accessibility, these drugs have been found to be adulterated and therefore used at a very low dosage (Allos, 2001).

Unfortunately, variations in methodology, use of different laboratory standards, quality of antibiotic discs used and choice of end points have led to such widely divergent results that the usefulness of determining antimicrobial sensitivities has been questioned. When correctly done and interpreted, antibiotic sensitivity tests are highly useful and may be of enormous help to the clinician in the treatment of infections. Therefore, there is a need for practitioners and researchers to be aware of the bacterial flora of the camels and of their antibiotic sensitivities so as to be informed of the appropriate antibiotics to be used in the course of respiratory infections and control programs.

Camel respiratory infection is a complex, multifactoral, communicable, economically important and frequently emerging problem in Ethiopia in general and in Afar in particular. Therefore, a combination of all rounded and definitive diagnostic methods, effective vaccines including the newly emerging and re-emerging pathogenic strains, efficient and properly targeted antimicrobial agents as well as valuable managemental practices should be considered as an integrated strategy to control this overwhelming problem.

Generally, studying the bacterial flora in health and pneumonic status of camels as well as identifying the dynamics of bacterial species associated with respiratory diseases of these animals under the ecology of Ethiopia is very important. This may help one in identifying bacterial pathogens involved in pneumonia of camels and also identifying emerging and re-emerging bacterial pathogens during changes in climate, evolution of commensal bacteria and changes associated with the host body defense system.

6. CONCLUSION AND RECOMMENDATIONS

The present study and other previous works in this area pointed up that respiratory infection is considered as the major cause of morbidity and mortality in camels and is frequently emerging in Ethiopia. It is a multifactoral process typically involving predisposing factors and opportunistic bacterial pathogens which are commensally resident in the respiratory passage ways of susceptible camels.

A variety of bacterial species have been associated with respiratory disease problems in camels. In the present investigation, *P. multocida, M. haemolytica, K. pneumoniae, S. aureus and B. bronchiseptica* have been isolated in much higher proportions from the nasal swabs and lungs of pneumonic than the apparently healthy camels. This is quite suggestive that these organisms are potentially pathogenic agents which can pose a threat to camel pneumonia together with other internal and external stress factors. Furthermore, S. *equi subsp. equi,* S. *equi subsp. zooepidemicus* and *S. pneumoniae* were isolated from the respiratory tract of both clinically healthy and pneumonic camels using the Omnilog-Biolog identification system. But S. *equi subsp. equi* was more frequently recovered from lungs with different lesions than the nasal tracts and normal lungs. The presence of S. *equi subsp. equi* at higher rate in the pneumonic camel lungs can, therefore, make the bacteria to be considered as one of the major pathogens causing camel respiratory infections in Ethiopia.

The antibiogram conducted in the current work has shown an increased level of resistance among the camel respiratory pathogens against the commonly used antibiotic drugs. The majority strains of *P. multocida, M. haemolytica, K. pneumoniae* and *B. bronchiseptica* showed multidrug resistance pattern to the routinely used drugs including tetracycline and ampicillin. These bacteria were sensitive to norfloxacin, streptomycin and gentamicin. *S. equi subsp. equi* isolates were resistant to most of the antibiotics demonstrated but revealed a marked susceptibility to Vancomycin with 100% inhibitory activity.

Therefore, based on the above concluding remarks the following points are recommended:

- Generally, co-infection with other microorganisms is the most familiar predisposing factor for RDC in camels. Hence, further studies should be carried out to look in the role of viruses, mycoplasma, fungi and other parasites as primary or secondary causes of camel pneumonia.

- In all of the cases, regarding disease, prevention is a priority. Thus, a polyvalent and effective vaccine which includes the antigenic component of *S. equi subsp. equi* jointly with the other major respiratory pathogens should be developed to control this distressing disease in camels.

- The present study could not exceed to investigate the pathogenesis of *S. equi subsp. equi* caused pneumonia due to the limitation of time and financial issues. Therefore, a further research should be conducted to determine the serotypes involved, magnitude and pathogenicity of this bacterium in camel pneumonia.

- In consideration of the most effective *invitro* antibacterial agents against the major camel respiratory pathogens, gentamicin and norfloxacin should be the drugs of choice in controlling pneumonia in camels. In addition to this awareness creation through extension works should be carried out as the society in the study areas has a strong desire to ampicillin and tetracycline.

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8. ANNEXES

Annex 1. Colony characteristics recording format

Colony Characteristics on the pure culture media									
NO	Sample code	Shape	Edge	Pigmentation of growth	Haemolysis	Surface	Size	Opacity	Remark

Annex 2. Staining reaction and cellular morphology recording draft

NO	Sample code	Staining reaction	Shape	Size	Arrangement	Staining	Pleomorphism	Remark

Annex 3. Primary identification tests verification set-up

No	Sample code	Gram Rxn	Size & Shape	Arrangement	Growth on MacConkey			Catalase	Oxidase	O-F	Motility	Genera
					NO	Yes						
						LF	NLF					

Annex 4. The main differential characteristics of the Gram-positive Cocci (Quinn *et al.*, 2002)

	Coagulase	Catalase	Oxidase	O-F(Glucose)	Hemolysis	Bacitracin susceptibility	Frazolidon susceptibility	
Pathogenic *Staphylococci*	+	+	-	F	+(-)	R	S	
Non-Pathogenic *Staphylococci*	-	+	-	F	-(+)	R	S	
Enterococci	-	-	-	F	(+)	R	S	
Streptococci	-	-	-	F	(+)	R	S	
Micrococci	-	+	+	O	-(+)	S	R	

+ = Positive, - = Negative, (+) = Some strains positive, (-) = Some strains negative, F = fermentative, O= Oxidative, R= resistance, S= Susceptible. (Susptible to bacitracin= Zone10-25mm, Susptible to fyrazolidon= Zone15-35mm)

Annex 5. Antibiotic susceptibility test registration set-up using Oxoid antibiotic disks

No	Bacterial isolate	Antibiotic Disk used	Disk Code	Potency	Inhibited growth zone in mm	Interpretation

Annex 6. Sample (Nasal swab) collection layout

NO	Sample code	Age	Sex	Health status	Woreda	Kebelle

Annex 7. Primary biochemical identification tests used

Gram's Stain (Quinn *et al*., 2002)

Principle : Gram staining is a bacteriological laboratory technique used to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the physical properties of their cell walls.

Procedure: A thin smear was made & allowed to dry on air. The smear was fixed by passing through the Bunsen flame 3-4 times. Then the film was flooded with crystal violet for 1 minute. The crystal violet was washed off with water & drained. The slide was flooded with iodine solution for 2 minutes. The iodine was washed off with water & the smear was let dry. The film was decolorized with 95% ethanol alcohol for about 15-20 seconds and washed with water. Then the smear was counter stain with safranin for 1 minute, washed with water, blot dried and finally observed via microscope using oil immersion.

Catalase Test (Quinn *et al*., 2002**)**

Principle: catalase is an enzyme that converts hydrogen peroxide into water and oxygen. The bacteria that contain this enzyme are usually aerobic or facultative anaerobes. A positive reaction is indicated by a continuous bubble formation when the hydrogen peroxide is introduced into bacterial colonies.

Procedure: A loop full of bacterial growth was taken from the nutrient agar and placed on a clean microscope slide. A drop of 3% H_2O_2 was added. Then an effervescence of oxygen gas within few seconds indicates a positive reaction.

Oxidase Test (Quinn *et al*., 2002**)**

Principle: Cytochromes, found principally in organisms that can utilize free oxygen, are heme-containing proteins involved with oxidative phosphorylation. The enzyme cytochrome oxidase catalyzes the oxidation of cytochrome C, transferring the electrons to the terminal electron acceptor, oxygen. The oxydase reagent tetramethyl-p-phenylenediamine reacts with the oxidized cytochrome C producing a dark purple color.

Procedure: A solution of 1% tetramethyl-p-phenylenediamine dihydrochloride was used to moisten a piece of filter paper placed in a clean petridish. The test bacteria were streaked across the reagents placed on the filter paper using a sterile stick. A development of dark purple color along the streak line with in 10 seconds indicates a positive reaction.

Oxidation-Fermentation (O-F) Test (Quinn *et al*., 2002**)**

Principle: Bacteria utilize glucose and other carbohydrates by various metabolic pathways. Some use oxidative routes but others involve fermentation reactions. The oxidation-fermentation test, also known as the "oxferm" test, is used to determine which route is used. The medium is semi-solid & usually contains glucose as the test agar & bromothymol blue as the PH indicator.

Procedure: O-F base medium was prepared & allowed to cool to 50^{o}c. 100 ml of sterile glucose solution (10% glucose) was added in to 1000 ml of O-F base medium for a final concentration of 1% glucose & then dispended in to sterile tubes. Two tubes of the O-f medium were heated in a beaker of boiling water immediately before use to drive off any dissolved oxygen & the tubes were cooled rapidly under cold running water. Both tubes were inoculated with the same unknown bacteria using a straight needle, stabbing the medium 3-4 times half way to the bottom of the tube. One tube of each pair was layered with sterile wax to a depth of 1cm & the tubes were incubated at 37^{o}c & examined daily for about 2 weeks.

Motility Test (Quinn *et al*., 2002**)**

Procedure: SIM medium was prepared to evaluate the motility nature of the bacteria. The medium was stab inoculated using a straight wire. The tubes were incubated at 37^{o}c for 24-48 hrs & checked for motility. A diffused growth throughout the medium indicates a motile bacteria but the growth of non motile bacteria was confined to a stab line. While reading the result, the tubes were hold against a good light & the inoculated tubes were compared with the uninoculated tube as a control.

Annex 8. Secondary Biochemical Identification Tests used

Methyl Red (MR) Test (Quinn *et al*., 2002)

Principle: It is a quantitative test for acid production (mainly used in the identification of Enterobacteriaceae), requiring positive organisms to produce strong acids (lactic, acetic &formic) from glucose through the mixed acid fermentation pathway. Since many species of the Enterobacteriaceae may produce sufficient quantities of strong acid that can be detected by methyl red indicator during the initial phases of incubation, only organisms that maintain this low

PH after prolonged incubation(48-72 hrs) overcoming the PH buffer system of the medium, can be called methyl red positive.

Procedure: MR-VP broth was inoculated with the pure culture of test organism & then incubated at 37^{o}c for 48 hrs. Then about 5 drops of MR solution was added in to the media. A positive result was indicated by the production of red color and negative result was indicated by yellow color in the test.

Indole Test (Quinn *et al*., 2002)

Principle: The Indole Test is based on the formation of a red color complex when indole reacts with aldehyde group of p-dimethyl aminobenzaldehyde. This is the active chemical in Kovac's reagent. Indole positive bacteria posses an enzyme tryptophanase which converts tryptophan to indole.

Procedure: SIM medium was stab inoculated with the test bacterium using straight wire & then incubated at 37^{o}c for 24 hrs. Then 0.3 ml of Kovac's reagent was added & let stood for about 1 minute. The formation of dark red ring indicates a positive reaction but a negative reaction forms a yellow ring.

Citrate Utilization Test (Quinn *et al*., 2002)

Principle: Certain bacteria can obtain energy in a manner other than the fermentation of carbohydrate by utilizing citrate as a sole of Carbon source. The measurement of these characteristics is important in the identification of the Entrobactericeae.

Procedure: The slant surface of Simmons citrate agar was inoculated with a pure colony & then incubated at 37^{o}c for 24-48hrs. A positive test was indicated by the development of a deep blue color within 24-48 hrs, indicating that the test organism was able to utilize the citrate contained in the medium with the production of alkaline products. The color remained green in a negative reaction.

Coagulase Test

Slide Coagulase Test (Quinn *et al*., 2002)

Principle: The bound coagulase is also known as clumping factor. It cross-links the α and β chain of fibrinogen in plasma to form fibrin clot that deposits on the cell wall. As a result, individual coccus sticks to each other and clumping is observed.

Procedure: A drop of distilled water was placed on a clean glass slide. The water drop was emulsified with the test organism by using wire loop. The test suspension was treated with a drop of rabbit plasma and mixed well. The slide was rocked gently for about 10 seconds. Macroscopic clumping observed in the plasma within 5-10 seconds was taken as positive. Some strains of *S.aureus* may not produce bound coagulase, and such strains must be identified by tube coagulase test.

Annex 9. Microbial Medias used for the isolation & characterization of the bacteria

Blood Agar (Oxide, Hampshire, England)

Formula in grams per liter: Lab-Lemco powder---10.0; Meat peptone---10.0; Sodium chloride---5.0; bacteriological agar---15.0 & the final PH is 7, 3± 0, 2 at 25^{o}c.

Preparation: 40 grams of the medium was suspended in to 1000 ml of distilled water. The mixture was allowed to stand for about 5 minutes until a uniform suspension was obtained. The medium was heated with gentle agitation & then sterilized at 121^{o}c for 15 minutes. Finally the medium was cooled to 45-50^{o}c, 5-10% sterile defibrinated sheep blood was added, homogenized & poured in to sterile petridishes.

MacConkey Agar (Oxide, Hampshire, England**)**

Formula in grams per liter: peptone---20.0; Lactose---10.0; agar---12.0; Bile salts---5.0; Neutral red---0.075 and the final PH is 7, 1± at 25 ^{o}c.

Preparation: 50 Grams of the medium was suspended in to 1000 ml of distilled water. The mixture was mixed well by boiling until a uniform suspension was obtained. It was sterilized in the autoclave at 121^{o}C for 15 minutes & was let cooled to 45^{o}C in a water bath. Finally it was

poured in to sterile petridishes, allowed to solidify & placed upside down to avoid excessive moisture.

Sulfide Indole and Motility (SIM) media (Oxide, Hampshire, England**)**

Formula in grams per liter: Casien peptone…20,00; meat peptone…6,10; Ferric ammonium sulfate…0,20; Sodium Thiosulfate …0,20; bacteriological agar…3,50 & the final PH is 7,3± 0,2 at25 ^{o}c.

Preparation: 30 Gram of SIM basal Medium was measured & mixed in 1000 ml of distilled water in a flask & let dissolved. The solution was autoclaved at 121^{o}C for 15 minutes to sterilize. It was taken out from the autoclave & allowed to coll. Finally the medium was poured in to sterile tubes, cooled & placed at +4 ^{o}c for preservation.

O-F basal Medium (Oxide, Hampshire, England)

Formula in grams per liter: Casien peptone---2.0; Sodium chloride---5.0; Dipotassium phosphate---0.30; Bromothymol Blue…0.80; Bacteriological agar…2.0 & the final PH is 6.8 ± 0.2 at 25 ^{o}c.

Preparation: 9.8 Gram of the medium was suspended in 1000 ml of distilled water. The mixture was heated with frequent agitation to dissolve fully and autoclaved at 121^{o}c for 15 minutes. 100 ml of 10% sterile glucose solution (any suitable sugar can be used) was added in to 1000 ml of O-F base medium for a final concentration of 1% glucose. Then the solution was mixed and aseptically dispended in to sterile tubes (about 5 ml per tube).

Simmons' Citrate Agar (Oxide, Hampshire, England)

Formula in grams per liter: Ammonium Dihydrogen Phosphate…1.0; Dipotassium phosphate…1,00; Sodium chloride…5.0; Sodium citrate…2.0; Magnesium Sulfate…0.20; Bromthymol blue…0.08; Bacteriological agar…15.0 and the final PH is 6,8 ± 0.2 at 25^{o}c.

Preparation: 24.3gram of the powder was measured & suspended in 1000 ml of distilled water in a flask & let dissolved by boiling. The solution was autoclaved at 121^{o}C for 15 minutes to sterilize. The Medium was taken out from the autoclave & allowed to coll. Finally the medium

was poured in to sterile test tubes & cooled in a slanted position (The medium can also be sterilized by dispensing it in to tubes).

Tryptone Soya Broth (Oxide, Hampshire, England)

Formula in grams per liter: Pancreatic Digest of Casein ---17.0; Papaic digests of Soybean Meal ---3.0; Sodium Chloride --- 5.0; Dipotassium Phosphate ---2.5; Glucose---2.5; Final pH: 7.3 ± 0.2 at 25°C.

Preparation: 30 g of the medium was dissolved in 1 liter of distilled water. The solution was mixed thoroughly & sterilized at 121°c for 15 minutes. It was allowed to cool to 45-50°C and finally dispensed into sterile culture tubes.

Biolog Universal Growth (BUG) (Biolog, California, USA**)**

Preparation: 57 grams of BUG agar was added into 1,000 ml distilled water then gently boiled while stirring to dissolve the agar and other components. It was let Cooled and the PH was measured and adjusted to a final PH of 7.3 ± 1. Then it was sterilized by autoclaving at 15 pounds of pressure at 121^{O}C for 15 minutes finally cooled to 45-50^{O}C and dispended into sterile petri dishes.

Edwards Medium (Oxide, Hampshire, England)

Formula in grams per liter: Lab-Lemco powder---10.0; Peptone--- 10.0; Aesculin---1.0; Sodium chloride---5.0; Crystal violet--- 0.0013; Thallous sulphate--- 0.33; Agar---15.0 and the final PH is 7.4 ± 0.2 at 25^{O}C.

Preparation: 41 grams of the medium was suspended in to 1000 ml of distilled water. The mixture was allowed to stand for about 5 minutes until a uniform suspension was obtained. The medium was heated with gentle agitation & then sterilized at 115^{o}c for 20 minutes. Finally the medium was cooled to 45-50^{o}c, 5-7% sterile defibrinated sheep blood was added, homogenized & poured in to sterile petridishes.

Muller –Hinton agar (Oxide, Hampshire, England)

Formula in grams per liter: Beef dehydrated infusion from---300.0; Casein hydrolysate---17.5; Starch---1.5; Agar---17.0 and the final PH is 7.3 ± 0.1 at 25^{O}C.

Preparation: 38 gram of the medium was suspended in to 1000 ml of distilled water. The mixture was mixed well by boiling until a uniform suspension was obtained. It was sterilized in the autoclave at 121^{o}C for 15 minutes. It was let cooled to 45^{o}C. Finally the medium was poured in to sterile Petri plates, allowed to solidify and placed at +4 ^{o}c upside down to avoid excessive moisture.

Annex 10. Morphological and biochemical characteristics of the bacterial isolates

Staphylococcus species were found being Gram positive, cocci, mostly forming clusters resembling bunches of grapes but few occurred in pairs. They grow on sheep blood agar (Oxide, Hampshire, England), nutrient agar (Oxide, Hampshire, England), Manitol salt agar (Oxide, Hampshire, England) but did not on MacConkey agar (Oxide, Hampshire, England). The colonies of *Staphylococcus* species were observed as white, opaque but some resembled golden yellow and some species formed haemolysis on blood agar. The bacterial cells were Catalase positive, oxidase negative, fermentative and non motile.

The colonies of *Streptococcus* species were recognized as small, white, translucent and shiny which have exhibited various degrees of haemolysis on blood agar (Oxide, Hampshire, England). The isolates were found being Gram-positive cocci often occurring in chains of variable length. *Streptococci* species were coagulase negative, non motile, catalase and oxidase negative as well as fermentative on the O-F test. They did not hydrolyze aesculin.

P. multocida cells were identified as Gram negative, very small and round ended bacilli; arranged singly, in pairs or in small bundles. Better growth of the species was obtained when 7% sheep blood was added in to the blood agar basal media (Oxide, Hampshire, England). The grown colonies were moderate in size, round, grayish-yellow, translucent and non-hemolytic with no growth on MacConkey agar (Oxide, Hampshire, England). *P. multocida* isolates were non motile, catalase and oxidase positive. They fermented glucose, sucrose, manitol, manose, and sorbitol

without gas being produced. The isolates also fermented maltose and trehalose and were indole and H_2S positive but negative for urea, methyl red (MR) and citrate reactions.

Mannheimia haemolytica isolates were Gram negative, short and evenly stained rods and coccobacilli. The colonies on blood agar were circular, glistering and surrounded by a narrow zone of haemolysis. In addition, the isolates grew on MacConkey agar (Oxide, Hampshire, England) as pink to red colonies and were non motile, catalase and oxidase positive. They fermented sorbitol, maltose, glucose and sucrose but did not ferment trehalose; indole was not produced, H_2S was not formed and urea was not decomposed by these isolates.

Bordetella species were isolated as Gram-negative, small rods (some were coccobacilli), catalase and oxidase positive, unreactive on O-F test. The isolates did not ferment glucose, maltose, sucrose and sorbitol. They grow on MacConkey (Oxide, Hampshire, England) without fermenting lactose.

Klebsiella pneumoniae were identified as Gram negative straight rods. The bacteria was non-motile, indole negative, H_2S negative, oxidase negative, catalase positive, fermentative and appeared pale pink on MacConkey agar (Oxide, Hampshire, England).

9. SIGNED DECLARATION SHEET

I, the undersigned, confidentially declare that this thesis is the result of my own work and none of the work has been previously submitted either in whole or in part, to any institution anywhere for the award of any academic degree, diploma or certificate. All sources of materials used for this thesis have been duly acknowledged.

Name: Mu'uz Gebru Sahle Signature: _____

Place: Addis Ababa University, College of Veterinary Medicine and Agriculture

Date of submission: June, 2012

This thesis has been submitted for examination with our approval as university advisors.

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