



Isolation and Characterization of Bacterial Species from Respiratory Tracts of Cattle Slaughtered in Addis Ababa City, Central Ethiopia

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ABSTRACT

The present study was an attempt to isolate and identify the diverse bacteria localizing pneumonic lungs and the associated tracheas of 50 slaughtered cattle at Addis Ababa Abattoirs enterprise, central Ethiopia, in both aerobic and anaerobic environments. 158 and 135 bacterial isolate was found in aerobic and anaerobic state, respectively using primary and secondary microbiological testes. Gram positive bacteria were the dominant bacteria in both conditions. The frequency of isolation increased from trachea down to the lung in both state indicating the bacterial role in the progress of bovine pneumonia. Most prevalently isolated bacteria from both aerobics and anaerobics conditions were *Staphylococcus* species, *Bacillus* species, *Mannheimia haemolytica* and *Pasteurella multocida*. Whereas the *Streptococcus* species, *E.coli*, *Klebsiella pneumoniae*, *Actinobacillus* species, *Micrococcus* species, *Arcanobacterium*, species *Neisseriaspecies*, *Acinetobacter* species, *Corynebacterium* species, *Bordetella* species, *Pseudomonas* species, and *Rhodococcus equi* were among the bacteria isolated.

Key words: Aerobic, Anaerobic, Bacteria, Bovine, Pneumonia

INTRODUCTION

The majority of the third world countries are located in the tropics and livestock production is very important to their economy (Thornton, 2010). Cattle population has been found to be the main components of livestock production in most farming systems in Sub-Saharan Africa (McDermott and Arimi, 2002). Ethiopia is one of the Sub-Saharan Africa countries that has the largest livestock population in Africa, with an estimate population of 54 million cattle, 25.5 million sheep and 24.06 million goat (Leta and Mesele, 2014).

The livestock productivity, despite its huge population size, remains marginal. This could be due to a number of constraints including poor genetic potential, high disease incidence, malnutrition, thermal stress and poor management conditions (Radostits et al., 2000). Among all diseases, affecting the respiratory system are generally the most important in every species of domestic animals (Thomson, 1998). The lung is continuously exposed to air that contain dust, bacteria, fungi, viruses and various noxious agents and defense against these potentially harmful materials is controlled by a complex of protective mechanisms. Stress factors such as change in weather condition, transportation, poorly ventilated housing and nutrition deficiencies have a predisposing role (Jerome, 1993).

Respiratory tract infection is one of the major causes of losses of productivity in beef as well as dairy production. Cattle are more prone to respiratory tract infection than other animals due to marked physiological problems and anatomical structures that predispose the respiratory tract to infection (Radostits et al., 2000). Cattle are susceptible to retrograde drainage from pharynx as a result to pulmonary hypertension, then their lung is well lobated and well lobulated that does not permit free air ventilation into adjacent structure (Taylor et al., 2010; Thomson, 1998).

Infectious pneumopathies are commonly attributed to *Mannheimia (Pasteurella) haemolytica*, which causes the most severe damage to the lung. In addition, bacterial agents such as *Mycobacterium*, *Mycoplasma*, *Haemophilus*, *Fusobacterium* and *Actinomyces* inflict damage on the pulmonary tissues of most domestic animals (Radostitis et al., 1994). Several infectious agents are commonly isolated from the respiratory tracts of clinically sick and healthy animals (Radostits et al., 2000). The few studies conducted in Ethiopia mainly focused on the bacterial flora of pneumonic lungs

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of sheep, cattle and camel (Mamo et al., 2011; Marru et al., 2013; Mekibeb et al., 2013; Shiferaw et al., 2006; Sisay and Zerihun, 2003). However, there are no documentations available that give brief information on aerobic and anaerobic bacterial agents that are responsible for cattle pneumonia in Ethiopia.

Therefore, the present study was designed to isolate and characterize aerobically and anaerobically growing bacteria from the respiratory tract of pneumonic cattle and to compare and contrast those bacteria colonizing the two anatomical sites (trachea and lung).

MATERIALS AND METHODS

Study area

The study was conducted at Addis Ababa abattoirs enterprise, central Ethiopia, from October 2010 to June 2011. Geographically Addis Ababa is located 9^o2' N and 38^o 42'E having an elevation of 2400M asl and mean annual rainfall of 1800mm which is in bimodal pattern. The long rainy season lays from June to September (kiremt) followed by a dry season from October to February (Bega) and a short rainy season from March to May (Belg). The city has an average minimum and maximum temperature of 10.7 and 23.6^oc respectively (NMSA, 2005).

Study animals

The study was conducted on 50 cattle at Addis Ababa abattoirs enterprise without referencing of age, sex of the animals at which ante mortem examination was conducted and passed for slaughter.

Study design and sampling

Fifty pneumonic lungs were selected randomly with their corresponding tracheas and sampled aseptically for microbiological examination. Samples were then kept separately provided with identification mark and transported to Addis Ababa University (AAU), School of Veterinary Medicine (SVM) microbiology laboratory.

Sample collection

Lung sample collection: Before lung and its corresponding tracheal sterile swab samples were collected, gross examination of pneumonic lung was undertaken. Lungs that were congested were assumed to be pneumonic without involving parasitic cause of pneumonia. From each pneumonic lung, lung tissue was aseptically collected. Sampling was undertaken according to the procedure recommended by Quinn et al. (1999). Lung tissues were removed and were put in a sterile screw capped universal bottle and those samples were separated, identified, and transported on ice box to AAU, SVM microbiology laboratory.

Tracheal swab samples collection: After slaughtering and separating of the offal from the carcass, the trachea of each pneumonic lung of cattle was grasped with tissue forceps and opened with sterile scalpel blade. Then, sterile cotton tipped swabs soaked with Tryptose Soya Broth (TSB) (OXOID, Hampshire, England) transport medium were inserted into the tracheal tube, and the mucus membrane was rubbed. From a trachea two swab samples were taken for aerobic and anaerobic culture. The collected tracheal swabs were putt inside test tubes containing TSB, and which were separated, identified, and transported to the microbiology laboratory. One group of tracheal swab samples were placed in a sterile anaerobic jar together with a flaming candle and the jar was closed and incubated at 37^oc for 24 hours. The other group of tracheal swab samples were placed in a rack and incubated at 37^ocfor 24 hours in an aerophilic incubator.

Bacterial isolation and characterization

Bacteriological Culturing

Lung: The lung parenchymal tissue was cut and cultured on blood agar (Oxoid, Hamshire, UK) which contain 7% sheep blood using sterile forceps. Each lung sample was inoculated into two plate containing blood agar medium, for aerobic and anaerobic culture. The anaerobic group was kept inside a sterile anaerobic jar containing flaming candle and incubated at 37^oc for 24 hours in Co₂ incubator while the aerobic group was incubated at 37^oc for 24 hours in an aerophilic incubator.

Tracheal swabs: After 24 hours of incubation in the TSB medium, both aerobically and anaerobically cultured tracheal swab samples were thoroughly agitated to aid mixing. A loop full of broth culture was taken and streaked over identified Petri-plates containing blood agar medium supplemented with 7% sheep blood (Quinn et al., 1994).

Morphological and cellular characterization: Pure culture colonies were subjected to Gram staining to study staining reaction and cellular morphology under light microscope, at 100×magnification and potassium hydroxide (KOH) test were done to avoid confusion in Gram stain reaction. Mixed colonies and Gram-negative bacteria were sub-cultured on both blood and MacConkey (Oxoid, Hamshire, UK) agar plates for further analysis. Pure cultures of single colony type, from both blood and MacConkey agar, were transferred onto nutrient agar-medium for a series of primary

tests such as Catalase , Oxidase, Motility, and Fermentative-Oxidative and secondary tests including triple sugar iron agar, citrate utilization test, methyl red test and indole test, following standard procedures described in Quinn et al. (1999).

Data analysis

Descriptive statistics were used to summarize the data generated from the study. The relative abundance of each species/genera was expressed as a percentage in comparison to the total number of isolates.

Ethical Approval

The study considered direct observation of slaughter animals in the abattoir and took appropriate samples for further microbiological experiments and no animal was subjected to suffering as a result of this study. Nevertheless, ethical approval was conducted by research ethical approval committee of Addis Ababa University, school of veterinary medicine, Ethiopia.

RESULTS

Aerobically isolated bacteria

Out of 100 specimens collected from trachea and the corresponding pneumonic lung (50 from each site), 100 (100%) harbored bacteria. In general, 158 different bacteria species were isolated from 100 infected specimens in aerobic condition. The proportion of Gram positive bacteria were dominated over Gram negative bacteria accounting about 58.8% while Gram negative were 37.97%.

The predominant species among the aerobic isolates were the *Staphylococcus* species with proportion of 27.85% frequently isolated from the lungs (58%) and the trachea (30%) followed by *Bacillus* species (17.18%), *Pasteurella multocida* (13.29%) and *Mannheimia haemolytica* which accounted for 10.13%. On the other hand, *Arcanobacterium* and *Acinetobacter* species (0.63% each) were among the least encountered bacterial genera in aerobic state.

As shown in table 1, the majority of the isolates colonized both trachea and descended to the lung with the exception of *Rhodococcus equi*, *Pseudomonas*, *Micrococcus*, *Bordetella*, *Acinetobacter* and *Arcanobacterium* species which were absent from tracheas but were isolated from the corresponding lungs, whereas *Klebsiella pneumonia* absent from lung but isolated from trachea. Among Gram negative bacteria the most frequently isolated and the least encountered bacteria were the *P. multocida* and *Acinetobacter* species respectively.

Table 1. Aerobically isolated bacteria from trachea and pneumonic lung of cattle slaughtered in Addis Ababa abattoirs enterprises, central Ethiopia from October 2010 to June 2011

Bacterial isolates	Trachea	Lung	Frequency	Proportion
Gram positive bacteria	No (%)	No (%)		
<i>Streptococcus</i> species	3(6)	5(10)	8	5.06
<i>Bacillus</i> species	8(16)	19(38)	27	17.18
<i>Staphylococcus</i> species	15(30)	29(58)	44	27.85
<i>Micrococcus</i>	0(0)	7(14)	7	4.43
<i>Rhodococcus equi</i>	0(0)	6(12)	6	3.88
<i>Arcanobacterium</i> species	0(0)	1(2)	1	0.63
Gram negative bacteria				
<i>Actinobacillus</i> species	2 (4)	3(6)	5	3.25
<i>Escherichia coli</i>	4(8)	4(8)	8	5.06
<i>Klebsiella pneumonia</i>	2(4)	0(0)	2	1.27
<i>Mannheimia haemolytica</i>	8(16)	8(16)	16	10.13
<i>Pasteurella multocida</i>	14(28)	7(14)	21	13.29
<i>Bordetella</i> species	0(0)	4(8)	4	2.53
<i>Pseudomonas aeruginosa</i>	0(0)	3(6)	3	1.98
<i>Acinetobacter</i> species	0(0)	1(2)	1	0.63
Unidentified bacteria	1(2)	4(8)	5	3.16
Total	57	101	158	100

Anaerobically isolated bacteria

From the anaerobically cultured samples, strictly anaerobic bacteria were not isolated. Most of the bacteria were similar with aerobically isolated bacteria indicating that the bacteria were facultative anaerobes. Most frequently anaerobically isolated bacterial species were *Staphylococcus species* (35.56%), *Bacillus species* (21.48%) *Mannheimia haemolytica* (11.8%) and *Pasteurella multocida* (10.37%), these results are similar with that of the aerobically isolated bacteria except the percentage and frequency. *Arcanobacterium species*, *Neisseria species* and *Klebsiella pneumoniae* (0.74% each) were the least bacteria isolated from the anaerobic condition.

Similar with the aerobic isolates, as shown in table 2 most of the anaerobically isolated bacteria colonized the lung and bacteria that did not exist in the trachea were isolated from the lung including *Micrococcus species*, *Klebsiella Pneumoniae*, *Arcanobacterium*, *Neisseria* and *Corynebacterium species*. Like that of the aerobically isolated bacteria Gram positive bacteria dominated (65.2%) while Gram negative bacteria accounted for 33.33% in anaerobic situation.

In general, there was a general increment in the isolation rate as one goes down from the trachea toward the lung with the highest and lowest infection rate being the lung and trachea, respectively in both aerobic and anaerobic conditions. Most of the bacteria that were isolated aerobically have a larger frequency of isolation in anaerobic condition.

Table 2. Anaerobically isolated bacteria from trachea and pneumonic lung of cattle slaughtered in Addis Ababa abattoirs enterprises, central Ethiopia from October 2010 to June 2011

Bacterial isolates	Trachea	Lung	Frequency	Proportion
Gram positive bacteria	No (%)	No (%)		
<i>Streptococcus species</i>	1(2)	3(6)	4	2.96
<i>Bacillus species</i>	12(24)	17(34)	29	21.48
<i>Staphylococcus species</i>	15(30)	33(66)	48	35.56
<i>Micrococcus species</i>	0(0)	3(6)	3	2.22
<i>Arcanobacterium species</i>	0(0)	1(2)	1	0.74
<i>Corynebacterium species</i>	0(0)	3(6)	3	2.22
Gram negative bacteria				
<i>Escherichia coli</i>	11(22)	2(4)	13	9.63
<i>Klebsiella pneumoniae</i>	0(0)	1(2)	1	0.74
<i>Mannheimia haemolytica</i>	15(30)	1(2)	16	11.85
<i>Pasteurella multocida</i>	8(16)	6(12)	14	10.37
<i>Neisseria species</i>	0(0)	1(2)	1	0.74
Unidentified bacteria	2(4)	0(0)	2	1.48
Total	64	71	135	100

DISCUSSION

The present study recognized both aerobically and anaerobically growing bacteria from the respiratory tract of 50 cattle slaughtered at Addis Ababa abattoirs enterprises. A total of 158 bacteria were isolated from both tracheas and lungs aerobically. The isolation of Gram positive bacteria in higher proportion than the corresponding Gram negative bacteria in this study agrees with studies of few workers such as Esra et al. (2009) who isolated from apparently healthy and unhealthy nasal cavities of Holstein cattle (65.68%).

Esra and Hakan (2010) isolated these aerobic bacterial floras from the nasal cavity in healthy Anatolian water buffalo calves in Turkey, Yimer and Asseged (2007) identified aerobic bacteria from pneumonic lung from sheep. GutDelete et al. (2009) reported aerobically similar bacteria from the upper respiratory tract of diseased donkey. Jenberie et al. (2012) isolated and identified similar bacteria from pneumonic lung of camels. The invariable isolation of those organisms from pneumonic lungs of various animal species may indicate their significance in different respiratory system problems in different animals.

In parallel with the aerobic isolates, all the bacteria recovered from the anaerobic culture were facultative anaerobes and were similar to those bacteria isolated from aerobic condition. A total of 135 facultative bacteria were isolated and in contrast with the aerobically isolated, the frequency of isolation of some of these bacteria increased comparing to aerobically isolated bacteria. In line with this study Chirino-Trejo et al. (1983) reported similar facultative bacteria from pneumonic lung of cattle in Canada. In the present study unlike to Chirino-Trejo et al. (1983), strictly anaerobic bacteria were not isolated.

The higher isolation rate in this study is probably associated with the anatomy and physiology of bovine lung. Bovine lung is highly lobated and lobulated whereas small ruminants' lung is highly lobated and poorly lobulated. This structure of bovine lung doesn't allow collateral ventilation (Thomson, 1998). Moreover, bovine lung is prone to respiratory hypertension due to retrograde drainage from the pharynx and it has a low tidal volume and relatively small size that predispose it to respiratory infection (Taylor et al., 2010).

Among the total isolates, the *Staphylococcus* species were the predominant bacteria in both conditions and have high proportions in the lung and trachea. In agreement with this study *Staphylococcus* species were isolated and reported in higher proportion in the previous few studies aerobically. Esra et al. (2009) isolated these bacteria from unhealthy Holstein cattle nasal cavities with high frequency and percentage (33.3%). The dominance of these bacteria is also in line with the study conducted by Megra et al. (2006) who isolated aerobic bacteria flora of the respiratory passage of healthy goats. These indicate there is a likelihood of association between these bacteria and pneumonic syndrome of lung. The bacteria are commensally living in the mucous membrane of the upper respiratory tract of animals and are opportunistic pathogens (Quinn et al., 1994).

Bacillus species were the second abundant bacteria harvested with higher frequency in both situations. Different workers at different times isolated these species of bacteria with variable rates of isolation aerobically. It was isolated by Esra et al. (2009) (16.70%) from unhealthy Holstein cattle, Chirino and Prescott (1982) were unable to isolate these bacteria anaerobically. The high localization of these bacteria in the lung may play an important role in the progress of bovine pneumonia.

In the present study, *M. haemolytica* and *P. multocida* isolated with a relatively higher proportion and the result is similar to the report obtained from Garedew et al. (2010) who isolated these bacteria from Maedi-Visna affected sheep in Ethiopia with the rate of 7.89% and 7.02% of *M. haemolytica* and *P. multocida*, respectively. While anaerobically, *M. haemolytica* and *P. multocida* were reported by Chirino and Prescott (1982) at the rate of 13.68% and 18.94%, respectively. These bacteria are commensally living in the upper respiratory tract and all *Pasteurella* species are probably extracellular parasites with various stresses, including concurrent viral infections predisposing to infections as in shipping fever. They play a part in shipping fever and cause pneumonia as a primary or a secondary etiology. *M. haemolytica* produces a soluble cytotoxin (leukotoxin) that has a role in breaching the lung's primary defense mechanism by its action on the alveolar macrophage and other leukocytes of ruminants (Quinn et al., 1999).

Despite their few proportion, *Streptococcal* species were isolated from the lungs and tracheas of pneumonic cattle and the result observed in the present study is in agreement with Obasiet al. (2010) who reported an isolation rate of 8.2% from caprine pneumonic lungs and Chirino and Prescott (1982) obtained related results of 6.31% from each aerobic and anaerobic culture of pneumonic cattle. These bacteria are resident flora of the upper respiratory tract mucous membrane and are commonly associated with suppuration and abscess formation (Quinn et al., 1994).

The isolation of *E. coli* in this investigation is in agreement with Esra et al. (2009) who isolated the bacteria from unhealthy Holstein nasal cavities at the rate of 10%. Unlike to this study the isolation of Chirino and Prescott (1982) has a relatively higher isolation rate (11.5% and 20%) of aerobic and anaerobic isolate, respectively from pneumonic cattle in Ontario, Canada. The isolation of this bacterium in this study correlates with the natural habitants of *E. coli* which can survive in faecal particles, dust and water for weeks and month (Quinn et al., 1999). The finding of *E. coli* from pneumonic lungs suggests that *E. coli* is considered as a secondary invader in the bovine pneumonia and contribute to the pneumonic progress.

Micrococcus species, which are the normal flora of respiratory tract, were isolated and the obtained result coincided with that of Esra et al. (2009) who isolated *Micrococcus* species with rate of 3.33% from unhealthy Holstein cattle nasal cavities. *Micrococcus* spp. are assumed to be non-pathogenic (Carter, 1984). However, in most of the infections along with other pathogens, it may flare up and act as a secondary invader.

Corynebacterium species were isolated anaerobically from this study. In the previous time these bacteria were isolated by Chirino and Prescott (1982) in similar condition but with relatively high isolation rate (26.9%). According to Quinn et al. (1994) these are pyogenic bacteria be able to cause a variety of suppurative condition.

Among the total aerobically isolated bacteria, *Rhodococcus equi* was also reported. This is supported by a report by Nesibu et al. (2010), who isolated this bacterial spp. with a rate of 5.3%. Since *R. equi* is an inhabitant of soil and intestinal tracts of animals, it can replicate at warm temperatures in soil enriched with faeces of herbivores (Quinn et al., 2002) and likely to find this bacteria in the respiratory tract of animals. The bacteria are known to cause suppurative foal pneumonia and suppurative disease formation in domestic animals (Thomson, 1998).

Among the least aerobically isolated bacteria in this study were *Acinetobacter* and *Arcanobacterium* species having an equal isolation rate. Whereas *Klebsiellapneumoniae*, *Arcanobacterium* and *Neisseria* species are the least bacterial isolate anaerobically. *Arcanobacterium* species are present on the mucous membrane of the host animals, often in the oral cavity and nasopharynx. The infection of these bacteria is endogenous and most of the species cause pyogranulomatous reaction in animal tissues and the isolation of this bacterium from the lower and upper respiratory

tract indicates its role as a secondary invader in low respiratory tract infection (Quinn et al., 1999). Whereas, *Acinetobacter* species are commonly found in soil, sewage, water, food and milk and are part of the normal flora of human and animal and can cause nosocomial infections especially in immunocompromised patients (Quinn et al., 1994). On the other hand, *Klebsiella pneumoniae* is an inhabitant of the intestinal tract of animals as well as oil and saw dust, faecal contamination of the environment accounts wide distribution of the organism and contribute to the occurrence of opportunistic infection (Quinn et al., 1999).

CONCLUSION

Although they are normal flora in the upper respiratory tract, they may descend and involve as a primary and a secondary etiological agent in the progress of bovine pneumonia and bovine respiratory disease complex. Risk factors like suffocation, shipping, immunity compromise, malnutrition, other respiratory diseases complication and inappropriate management of animals provide ideal opportunities for these bacteria to cause respiratory diseases and aggravation. Therefore, appropriate prevention methods should be established and identifying the most pathogenic species guarantee future studies.

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Competing interest

The authors have not declared any conflict of interest

Author's contributions

Addis Kassahun Gebremeskel carried out the sample collection, experiments, analysis and writing; Addisu Awukew Yegoraw and Biruk Tesfaye Birhanu collect samples; Tesfaye Sisay Tesema designed the experiments; Shewangzaw Addisu Mekuria conducted edition.

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