

Full Length Research Paper

Aerobic and anaerobic bacterial isolates from the respiratory tract of sheep slaughtered at Addis Ababa Abattoirs Enterprises, Central Ethiopia

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The present study was an endeavor to isolate and identify the various bacteria localizing pneumonic lungs and the associated tracheas of sheep slaughtered at Addis Ababa Abattoirs Enterprise, Central Ethiopia, in both aerobic and anaerobic conditions. A total of 60 pneumonic lungs and 60 tracheal swabs were examined bacteriologically. From all the samples collected, a total of 440 bacterial isolates (239 from the aerobic culture and 201 from the anaerobic culture) were obtained. The result of aerobic isolates include: *Staphylococcus* species (31.38%), *Pasteurella hemolytica* (29.71%), *Bacillus* species (10.04%), *Bibersteinia trehalosi* (6.69%), *Micrococcus* (3.77%), *Escherichia coli* (3.35%), *Streptococcus* species (2.51%), *Rhodococcus equi* (2.93%), *Pseudomonas* species (2.09%), *Klebsiella pneumoniae* (0.84%), *Actinobacillus* species and *Bordetella* species (1, 29%); whereas *Staphylococcus* species (26.87%), *P. hemolytica* (37.81%), *Bacillus* species (3.98%), *B. trehalosi* (10.45%), *Micrococcus* (3.48), *E. coli* (6.97%), *Streptococcus* species (0.5%), *Rhodococcus equi* (0.5%), *Klebsiella pneumoniae* (2.99%) and *Actinobacillus* species (1.49%) were among anaerobic isolates. Thus, isolation of multiple bacterial species from the respiratory tracts of pneumonic sheep in this study signifies their possible role in the involvement of respiratory diseases. Appropriate prevention and control methods should be established along with identification of the most pathogenic species by future studies.

Key words: Bacteria, lung, pneumonia, sheep, trachea.

INTRODUCTION

Ethiopia is home to various indigenous sheep breeds. From the total livestock population of the country, sheep

owns about 46%. Despite this huge resource, Ethiopian sheep productivity remains far lower than expected. The

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major biological constraints contributing to low productivity include bacterial and parasitic infections (Kaur et al., 2009; Leta and Meles, 2014; CSA, 2014-15; Fikru and Gebeyehu, 2015; Gebremeskel et al., 2017; Pawar et al., 2017; Singh et al., 2017).

The lungs are continuously exposed to air that contains dust, bacteria, fungi, viruses and various noxious agents and defense against these potentially harmful materials is controlled by a complex of protective mechanisms (Mohan et al., 2013). Stress factors such as inclement of weather, cold and stress of weaning, transportation, poorly ventilated housing and nutritional deficiencies have predisposing roles. In addition, concurrent infections with some viruses, bacteria or parasitic infestation degrade the potential of the host to combat infections (Gebremeskel et al., 2017; Gupta et al., 2009; Radostits et al., 2000).

The impact of respiratory disease is extensive and can be measured as the sum of the direct economic losses occurring due to mortality, morbidity, treatment and prevention costs. Loss of production (reduced animal performance and carcass quality) and the indirect costs such as labor, infrastructures and intangibles (Jim, 2009; Monot et al., 2015).

A number of bacterial, viral and parasitic agents participate in sheep respiratory diseases, however most important include: mycoplasma species such as *Mycoplasma ovipneumoniae*, *Mycoplasma arginini*, *Mycoplasma agalactiae* (Lin et al., 2008), lung worms particularly (*Dictyocaulus filaria*) (Borji et al., 2012) and bacteria like *Pasteurella multocida*, *Manheimia haemolytica*, *Chlamydia psittaci*, *Histophilus somni*, which can suppress the animal's immune system, allowing opportunistic microorganisms to colonize the lung and cause the disease (Angen et al., 1998; Radostits et al., 2000; Tesfaye et al., 2013; Fulton, 2009; Alemneh and Tewodros, 2016).

Therefore, objectives this study is to identify most bacterial pathogens involved in pneumonic lung of sheep as well to compare and contrast the types of isolated bacterial species in different sites of the respiratory system both in aerobic and anaerobic environments.

MATERIALS AND METHODS

Study area

The study was conducted from October 2010 to April 2011 at Addis Ababa abattoir enterprises, central Ethiopia. Geographically Addis Ababa is located 9°2' N and 38° 42'E having elevation of 2400 above sea level (a.s.l) and mean annual rainfall of 1800 mm. The city has average minimum and maximum annual temperature of 10.7 and 23.6°C, respectively (NMSA, 2005; Jury and Chris, 2013).

Study animals and sampling strategy

The study was conducted on 60 randomly selected sheep lungs with pneumonic lesions and tracheal swabs slaughtered at Addis

Ababa Abattoirs Enterprises. Samples were kept separately and transported to the School of Veterinary Medicine, Microbiology Laboratory of Addis Ababa University in a cool box containing ice pack at 4°C.

Sample collection

Tracheal swabs

Samples were taken with sterile cotton swabs moistened with tryptose soya broth from the trachea of sheep. Two swabs were introduced directly into the trachea of slaughtered sheep and rubbed smoothly against the mucosa in a circular motion. The swabs were allowed to remain in contact with the secretions for up to 1 min, and the two swabs collected from each sheep were kept in a tryptose soya broth transport medium and transported to the laboratory (Lees et al., 1990; OIE, 2008).

Pneumonic lung tissue

Samples of pneumonic lung tissue were collected at post mortem for microbial culture. Each piece of tissue was placed in a fully labeled separate sterile screw capped universal bottle. Containers were fully labeled with the date, tissue and sterile instruments (knife, scalpel, forceps and scissors) were used for collecting specimens for microbiological cultures. After collection and transportation to the laboratory, the samples were processed immediately (Gebremeskel et al., 2017).

Bacteriological sample processing

Culturing the tracheal swabs

The broth culture samples were incubated overnight under aerobic and anaerobic conditions, respectively. After 24 h of incubation the samples were thoroughly agitated, mixed and a loop of broth cultures was taken and streaked over labeled Petri plates containing blood agar base supplemented with 7% sheep blood as described by Quinn et al. (1994).

Culturing the lung tissues

The outer surfaces of the lungs were first seared with a heated spatula, followed by cutting and mincing of the inner surface of the lungs using sterile scissors and forceps, and then transferred to sterile Petri dish. The minced interior part of the lungs were further incised with sterile scalpel blade, then printed on the blood agar and streaked with wire loop. All bacteriological procedures were conducted in a level two biological safety cabinet.

Cultural characterization and bacteriological examination

The growths of typical colonies on blood agar were characterized based on the presence or absence of hemolysis, the type of hemolysis and general appearance of the colonies (color, shape, size, consistency etc.). On MacConkey agar, the colonies were examined for the presence or absence of growth, general appearance and ability to ferment lactose (Sharma and Adlakha, 1996; Alemneh and Tewodros, 2016). All cultures were incubated under aerobic and anaerobic conditions at 37°C for 24 to 48 h.

Isolation and Identification

Single colony type from pure cultures on blood agar was transferred

to nutrient agar for a series of primary and secondary biochemical tests. Primary tests such as Grams staining, motility, catalase, oxidase, and oxidative-fermentative (O-F) tests were conducted. In addition, secondary biochemical tests including indole, methyl red, and citrate utilization tests were performed for further confirmation of the isolates. General procedures for isolation and identification of Gram positive and Gram negative bacteria were as described by Carter (1984) and Quinn et al. (1994).

Data analysis

Descriptive statistics was performed to analyze the data obtained from the study. The number 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 abattoirs and took appropriate samples for further microbiological examination. As a result of this study, no animal was subjected to suffer. Nevertheless, ethical approval was conducted by Research Ethical Approval Committee of Addis Ababa University, School of Veterinary Medicine, Ethiopia.

RESULTS

The predominant species among the aerobic isolates were *Staphylococcus* species (31.38%), followed by *M. haemolytica* (29.71%), *Bacillus* species (10.04%), *B. trehalosi* (6.69%), *Micrococcus* species (3.77%), *E. coli* (3.35%), *Rhodococcus equi* (2.93%), and *Pseudomonas* species (2.09%). On the other hand *Streptococcus*, *Bordetella*, *Klebsiella* and *Actinobacillus* were among the least encountered bacterial genera as indicated by Figure 1.

The majority of the isolates (aerobic and anaerobic) colonize the two anatomical sites investigated with *Streptococcus*, *Rhodococcus equi*, *Pseudomonas*, and *Actinobacillus* as exceptions which were not seen in trachea. However, a general increase in the isolation rate was observed as one that goes down the respiratory tract. In a nutshell, Gram positive bacteria were the predominant species inhabiting the respiratory tract in aerobic condition, whereas Gram negative bacteria predominate in anaerobic conditions.

A total of 191 facultative bacteria were isolated in anaerobic condition with Gram negative bacteria as the dominant isolates. The isolated bacteria with their isolation rate include *M. haemolytica* (37.81%), *Staphylococcus* species (26.87%), *B. trehalosi* (10.45%), *E. coli* (6.97%), *Bacillus* species (3.98%), *Micrococcus* species (3.48%), *K. pneumoniae* (2.99%), *Actinobacillus* species (1.49%), *Streptococcus* species (0.5%) and *Rhodococcus equi* (0.5%) as denoted by Figure 2.

DISCUSSION

The present study assessed the frequency and type of

aerobic and facultative anaerobic bacteria isolated from ovine pneumonic lungs and their associated trachea at Addis Ababa Abattoir Enterprises, Central Ethiopia among which several of them were isolated. A number of workers such as Gebremeskel et al. (2017), Akloul and Mohammed (2016), Sarker et al. (2016), Porter et al. (1994), Al Sultan (1995), Barbour et al. (1997), Almeida et al. (1986), Okolo (1985), Esra et al. (2009) and Richard et al. (1986) have isolated similar bacteria from pneumonic lungs of domestic animals. The invariable isolation of those organisms from pneumonic lungs of various animal species may indicate their significance in various respiratory syndromes in different animal species (Megra et al., 2006).

Among 239 isolates, *Staphylococcus* species were the dominant bacteria having high proportion accounts (23.25 and 35.95%) of the trachea and lung isolates, respectively. In agreement to this study *Staphylococcus* species were isolated and reported in high proportion by few studies such as Alley (1975) and Queen et al. (1994). Other investigations includes that by Esra et al. (2009) who isolated *Staphylococcus* spp. from unhealthy *Holstein* cattle nasal cavities with high frequency and percentage, and Asaduzzaman et al. (2013) who reported on *Staphylococcus* species from Black Bengal goat in Bangladesh. These indicate that there is a probability 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 (Rashid et al., 2014; Quinn et al., 1994).

Bacillus species has been described as ubiquitous microbes found in nature as normal micro-flora. Thus, their role in the pathogenesis of respiratory infections is thought to be insignificant (Carter et al., 1995; Garedew et al., 2010). A high proportion of *Bacillus* species was obtained mainly from the lungs in this study. Other studies reported similar data such as Asaduzzaman et al. (2013), Garedew et al. (2010), Rajiv et al. (2000) and Shigidi (1973) from various domestic animals.

Micrococcus species is known primarily to be a normal flora of the respiratory tract. However, in most of the infections along with other pathogens, it may flare up and act as a secondary invader (Akloul and Mohammed, 2016; Carter, 1984). Our finding indicated that only 3.77% of *Micrococcus* species were isolated from ovine pneumonic lungs, with similar studies found to have agreed with present results such as Marru et al. (2013) and Esra et al. (2009).

Streptococcal are resident flora of the upper respiratory tract mucous membrane and commonly associated with suppuration and abscess formation (Quinn et al., 1994; Obasi et al., 2001). Although streptococci have not been associated with respiratory problems in ruminants, they are well known pathogens in humans, equine and camels. In this study, isolation of these bacteria might be an indication of its involvements as opportunistic

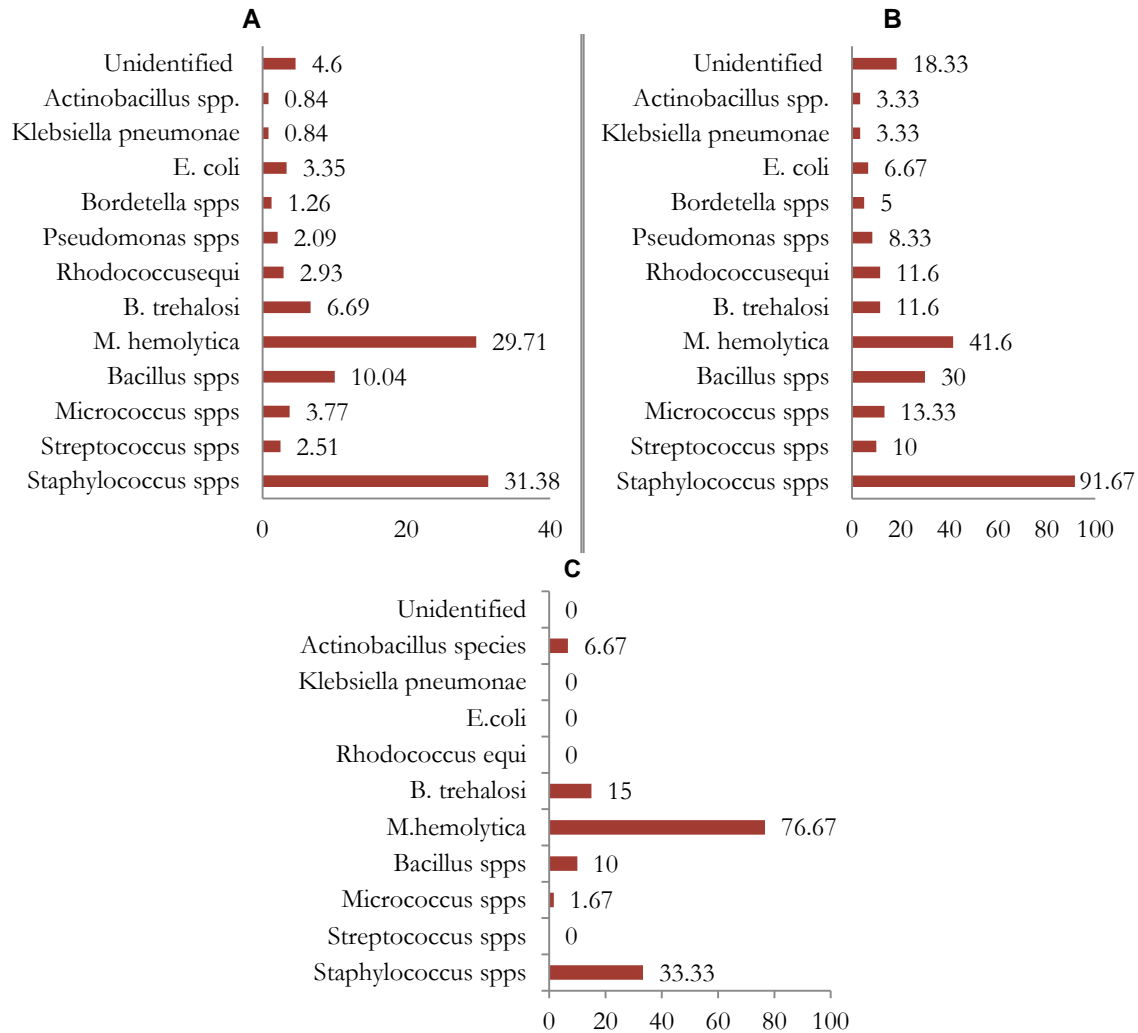


Figure 1. Bacterial isolates (%) under aerobic condition from respiratory tract of sheep slaughtered at Addis Ababa Abattoirs Enterprises. (A) Overall proportion of bacterial isolates; (B) Bacterial Isolates from lung; (C) Bacterial isolates from trachea.

pathogens in sheep pneumonia. *M. haemolytica*, is a normal flora of the upper respiratory tract and may play a secondary role after the primary initiating agent suppressed the host's defense mechanisms, and favors the multiplication of *Pasteurella* species leading to bronchopneumonia (Aiello and May, 1998; Buxton and Frazer, 1977). Stress factors with or without viral infections interact to suppress the host defense mechanisms which allow the proliferation of commensal bacteria in the respiratory tract of animals (Baker, 1998).

Conclusion

This study revealed that the isolation of multiple bacterial species from the respiratory tracts of pneumonic sheep signifies their possible role in the involvement of

respiratory disease complex. However, viruses and mycoplasma species are expected to reside in the respiratory tract. Therefore, the extent of the impact of respiratory diseases on sheep production and a complete understanding of the respiratory microbial flora, both culturable and unculturable condition give future research warranty.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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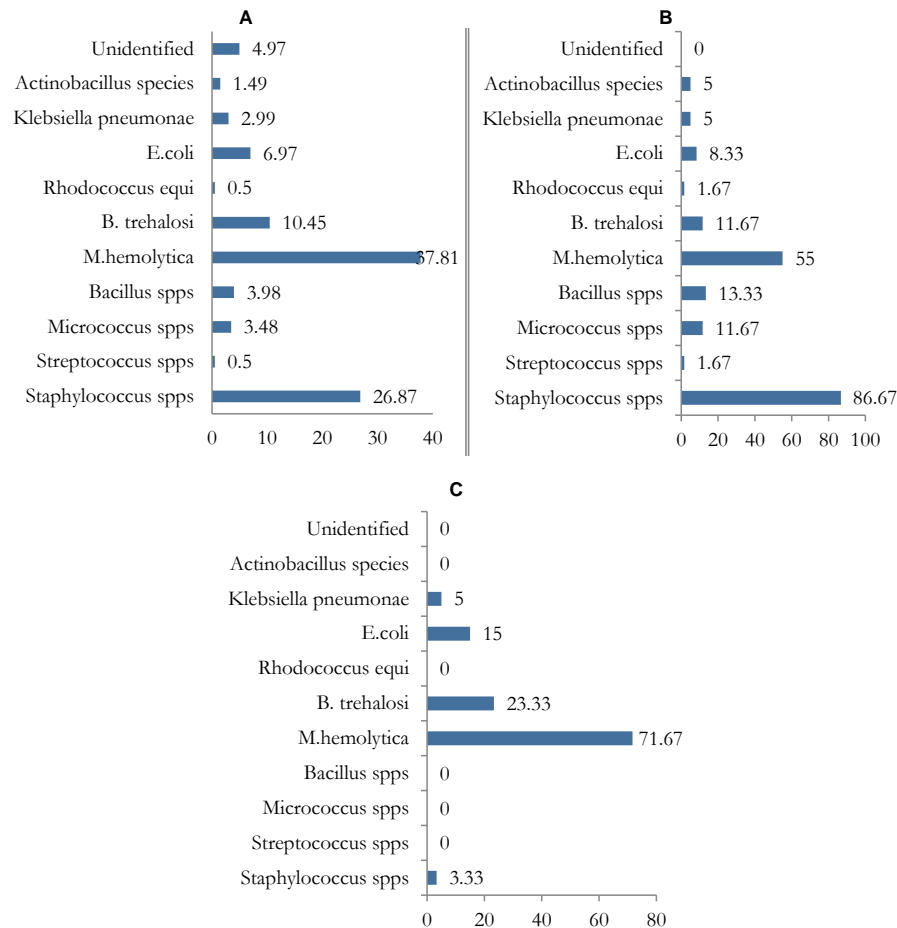


Figure 2. Bacterial isolates (%) under anaerobic condition from the respiratory tract of sheep slaughtered at Addis Ababa Abattoirs Enterprises. (A) Overall proportion of bacterial isolates; (B) Bacterial Isolates from lung; (C) Bacterial isolates from trachea.

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