Isolation, Identification and Antibiotic Sensitivity of Aerobic Bacteria Associated with Respiratory Tract Infections of Chickens in Khartoum North Area

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DEDICATION

To my Parents

With Thankfulness

To my Brothers and Sister

With great love

To my Friends and Colleagues

With Best Wishes
ACKNOWLEDGEMENTS

First of all, my thanks and praise to almighty Allah, the most beneficent, the merciful, for giving me health and strength to accomplish this work.

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# LIST OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>II</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>III</td>
</tr>
<tr>
<td>LIST OF CONTENTS</td>
<td>IV</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>VIII</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>IX</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>X</td>
</tr>
<tr>
<td>ARABIC ABSTRACT</td>
<td>XI</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER ONE LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>1.1 Poultry respiratory system</td>
<td>4</td>
</tr>
<tr>
<td>1.2 Bacteria</td>
<td>4</td>
</tr>
<tr>
<td>1.2.1 Infection via the respiratory tract</td>
<td>4</td>
</tr>
<tr>
<td>1.2.2 Bacteria of the respiratory tract</td>
<td>4</td>
</tr>
<tr>
<td>1.2.4.1 <em>Escherichia coli</em></td>
<td>4</td>
</tr>
<tr>
<td>1.2.4.2 Haemophilus</td>
<td>6</td>
</tr>
<tr>
<td>1.2.4.3 Pseudomonas</td>
<td>7</td>
</tr>
<tr>
<td>1.2.4.4 Pasteurella</td>
<td>8</td>
</tr>
<tr>
<td>1.2.4.5 Bordetella</td>
<td>9</td>
</tr>
<tr>
<td>1.2.2.6 Staphylococcus</td>
<td>9</td>
</tr>
<tr>
<td>1.2.4.6 Streptococci</td>
<td>10</td>
</tr>
<tr>
<td>1.2.4.7 Mycoplasmas</td>
<td>10</td>
</tr>
<tr>
<td>1.2.4.8 Chlamydia</td>
<td>11</td>
</tr>
<tr>
<td>1.2.4.10 Other bacteria species</td>
<td>11</td>
</tr>
</tbody>
</table>
1.3 Respiratory disease caused by fungal and viral agent 12
1.3.1 Aspergillosis 12
1.3.2 Newcastle disease 13
1.4 The avian immune system 14
1.5 The avian immune response 15
1.6 Antimicrobial agents 17
1.6.1 Antibiotics 17
1.6.2 Benefits of antimicrobial use 18
1.6.3 Disadvantages of antimicrobial use 19

CHAPTER TWO MATERIALS AND METHODS 22
2.1 Sterilization 22
2.2 Reagents and indicators 22
2.2.1 Reagents 22
2.2.1.1 Alpha-naphthol solution 22
2.2.1.2 Potassium hydroxide 23
2.2.1.3 Hydrogen peroxide 23
2.2.1.4 Methyl red 23
2.2.1.5 Tetra methyl-p-phenyl diamine dihydrochloride 23
2.2.1.6 Nitrate test reagent 23
2.2.1.7 Kovac’s reagent 23
2.2.2 Indicators 24
2.2.2.1 Andrade’s indicator 24
2.2.2.2 Bromothymol blue 24
2.2.2.3 Phenol red 24
2.2.2.4 Lead acetate paper 24
<table>
<thead>
<tr>
<th>2.2.2.5 Bromocresol purple (BDH)</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3 Collection of blood for enriched media</td>
<td>24</td>
</tr>
<tr>
<td>2.4 Preparations of media</td>
<td>25</td>
</tr>
<tr>
<td>2.4.1 Nutrient broth (Oxoid CM 1)</td>
<td>25</td>
</tr>
<tr>
<td>2.4.2 Peptone water (Oxoid CM 9)</td>
<td>25</td>
</tr>
<tr>
<td>2.4.3 Peptone water sugars (Carbohydrate fermentation medium)</td>
<td>25</td>
</tr>
<tr>
<td>2.4.4 Nutrient agar slant</td>
<td>25</td>
</tr>
<tr>
<td>2.4.5 Glucose-phosphate medium (MR-VP test medium)</td>
<td>26</td>
</tr>
<tr>
<td>2.4.6 Nutrient agar (Oxoid CM 3)</td>
<td>26</td>
</tr>
<tr>
<td>2.4.7 Blood agar (Oxoid CM 55)</td>
<td>26</td>
</tr>
<tr>
<td>2.4.9. Chocolate agar medium</td>
<td>26</td>
</tr>
<tr>
<td>2.4.9 Diagnostic sensitivity test agar (Oxoid CM 261)</td>
<td>27</td>
</tr>
<tr>
<td>2.4.10 MacConkey agar medium (Oxoid CM 7)</td>
<td>27</td>
</tr>
<tr>
<td>2.4.11 Eosin Methylene Blue Agar – EMB</td>
<td>27</td>
</tr>
<tr>
<td>2.4.11 Motility medium - Cragie tube medium</td>
<td>27</td>
</tr>
<tr>
<td>2.4.12 Hugh and Liefsons (O/F) medium</td>
<td>28</td>
</tr>
<tr>
<td>2.4.13 Simmon citrate medium (Oxoid CM 155)</td>
<td>28</td>
</tr>
<tr>
<td>2.4.14 Urea agar medium</td>
<td>28</td>
</tr>
<tr>
<td>2.5 Collection of samples</td>
<td>29</td>
</tr>
<tr>
<td>2.5.1 Sampling and culture</td>
<td>29</td>
</tr>
<tr>
<td>2.5.1.1 Nostril</td>
<td>29</td>
</tr>
<tr>
<td>2.5.1.2 Trachea</td>
<td>29</td>
</tr>
<tr>
<td>2.5.1.3 Lung</td>
<td>29</td>
</tr>
<tr>
<td>2.5.1.4 Conjunctiva</td>
<td>29</td>
</tr>
<tr>
<td>2.6 Primary culturing</td>
<td>29</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>2.7 Purification and preservation of culture</td>
<td>30</td>
</tr>
<tr>
<td>2.8 Microscopic examination</td>
<td>30</td>
</tr>
<tr>
<td>2.9 Identification of isolates</td>
<td>30</td>
</tr>
<tr>
<td>2.10 Biochemical methods for identification of isolated bacteria</td>
<td>31</td>
</tr>
<tr>
<td>2.10.1 Catalase test</td>
<td>31</td>
</tr>
<tr>
<td>2.10.2 Oxidase test</td>
<td>31</td>
</tr>
<tr>
<td>2.10.3 Oxidation fermentation (O/F) test</td>
<td>31</td>
</tr>
<tr>
<td>2.10.4 Motility test</td>
<td>31</td>
</tr>
<tr>
<td>2.10.5 Sugar fermentation test</td>
<td>32</td>
</tr>
<tr>
<td>2.10.6 Indole production test</td>
<td>32</td>
</tr>
<tr>
<td>2.10.7 Methyl red test</td>
<td>32</td>
</tr>
<tr>
<td>2.10.8 Voges-Proskure test</td>
<td>32</td>
</tr>
<tr>
<td>2.10.9 Citrate utilization test</td>
<td>32</td>
</tr>
<tr>
<td>2.10.10 Hydrogen sulphide production</td>
<td>33</td>
</tr>
<tr>
<td>2.10.11 Nitrate reduction test</td>
<td>33</td>
</tr>
<tr>
<td>2.10.12 Coagulase test</td>
<td>33</td>
</tr>
<tr>
<td>2.10.12.1 Slide coagulase test</td>
<td>33</td>
</tr>
<tr>
<td>2.10.12.2 Tube coagulase test</td>
<td>33</td>
</tr>
<tr>
<td>2.10.13 Novobiocin sensitivity test</td>
<td>33</td>
</tr>
<tr>
<td>2.11 Antibacterial sensitivity test</td>
<td>34</td>
</tr>
<tr>
<td>CHAPTER THREE RESULT</td>
<td>36</td>
</tr>
<tr>
<td>3.1.1 Bacterial isolated from nostrils</td>
<td>36</td>
</tr>
<tr>
<td>3.1.2 Bacteria isolated from conjunctiva</td>
<td>36</td>
</tr>
<tr>
<td>3.1.3 Bacteria isolates from trachea</td>
<td>37</td>
</tr>
<tr>
<td>3.1.4 Bacteria isolated from lung</td>
<td>37</td>
</tr>
</tbody>
</table>
3.2 Characters and biochemical reactions 38
3.3 Antibacterial sensitivity of the isolated bacteria 38

CHAPTER FOUR DISCUSSION 49
Conclusion and Recommendations 52
Conclusion 52
Recommendations 52
REFERENCES 53

LIST OF TABLES

<table>
<thead>
<tr>
<th>Tables</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Isolation frequency of aerobic bacteria from respiratory tract of infected chicken in Khartoum North area</td>
</tr>
<tr>
<td>2</td>
<td>Gram positive bacteria isolated from respiratory tract of infected chickens in Khartoum North area</td>
</tr>
<tr>
<td>3</td>
<td>Gram negative bacteria isolated from respiratory tract of infected chickens in Khartoum North area</td>
</tr>
<tr>
<td>4</td>
<td>Characters and biochemical reaction of gram-positive bacteria isolated from respiratory tract of infected chickens in Khartoum North area.</td>
</tr>
<tr>
<td>5</td>
<td>Characters and biochemical reactions of gram negative bacteria isolated from respiratory tract of infected chickens in Khartoum North area.</td>
</tr>
<tr>
<td>6</td>
<td>Antibacterial sensitivity of bacterial isolated from respiratory tract of infected chicken in Khartoum North area</td>
</tr>
<tr>
<td>7</td>
<td>The antibacterial sensitivity of Gram positive bacterial species isolated from respiratory of infected chickens in Khartoum North area.</td>
</tr>
<tr>
<td>8</td>
<td>The antibacterial sensitivity of Gram negative bacterial species isolated from respiratory of infected chickens in Khartoum North area.</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Growth of <em>E. coli</em> on MacConkey's agar</td>
<td>47</td>
</tr>
<tr>
<td>2</td>
<td>Growth of <em>E. coli</em> on Eosin Methylene Blue Agar</td>
<td>47</td>
</tr>
<tr>
<td>3</td>
<td>Sensitivity test of Gram-positive bacteria</td>
<td>48</td>
</tr>
</tbody>
</table>
ABSTRACT

This study was carried out in Khartoum North area to isolate and identify the bacteria associated with the respiratory tract infections of chickens.

Seventy eight samples were collected from five farms in Khartoum North area one farm in each of Alkadaro, Umalgora, Almazallat and two farms in Shambat. Samples were aseptically collected from different breeds of chickens showing clear respiratory symptoms. The samples were nasal swabs, conjunctival swabs, tracheal swabs and specimens from lungs. The sensitivity of bacteria isolated from infected chicken to antibiotics was examined.

The collected samples showed bacterial growth in 64 (82.1 %) samples and yielded 89 (114.1 %) isolates. Fifty two (66.7 %) of isolates were found to be Gram positive bacteria and the remaining 37 (47.4 %) isolates were Gram negative bacteria. The result of sensitivity test showed variable results, some showed high sensitivity while others showed resistance. The most effective antibacterial drug was Gentamicin (85.7%) but the lowest effective drug was Lincomycin (12.5%).

The result of this study described the bacterial respiratory diseases as the one of the constrains to poultry production in Khartoum North area.
مستخلص الأطروحة

أجريت هذه الدراسة في منطقة الخرطوم بحري وذلك بغرض عزل ومن ثم التعرف على البكتريا التي لها علاقة بإصابات الجهاز التنفسي في الدجاج.

جمعت 78 عينة من خمس مزارع في منطقة الخرطوم شمال أحد هذه المزارع تقع في الكندر وأخرى في أم القرى وكذلك واحدة في المظلات بينما تقع مزرعتان في شمبات وذلك من دجاج مصاب تظهر عليه بوضوح أعراض إصابة الجهاز التنفسي. أخذت العينات من المنخر، ملتحمة العين، القصبة العينية، وقسطة من الرئة.

وأيضاً أجرى اختبار الحساسية للمضادات الحيوية لبعض الأنواع البكتيرية التي عزلت.

العينات المجموعة أظهرت نمو في (82.1%) 64 عينة والتي أنتجت (114.1%) 89 عزلة. منها 52 (66.7%) عزلة موجبة الجرام بينما ال (37%) المتبقية سلبية الجرام.

نتيجة اختبار الحساسية أظهرت نتائج متنوعة فبعض الأنواع أظهرت حساسية عالية والبعض أظهر مقاومة للمضادات البكتيرية لكن نستطيع القول إن أكثر المضادات فعالية هو الجنثاميسين (85.7%) بينما أقلها فعاليه كان اللينكوميسين (12.5%).

نتائج هذه الدراسة وصفت أمراض الجهاز التنفسي كأحد الميوقات لصناعة الدواجن في منطقة الخرطوم بحري.
INTRODUCTION

Poultry are kept worldwide and they play a significant role in economic cycle of the communities. This role is accelerated by the comparative efficiency of poultry in conversion of cereal feed to protein and to their adaptability to intensive management.

The value of poultry industry to the economic and social communities is often reflected in the attention paid to the factor which may adversely affect the industry. One of the most important factors affecting poultry industry is diseases. They have devastating effects particularly in intensive system of production.

Most of the important poultry diseases are of worldwide occurrence (Gordan and Jordan, 1982) however some diseases are restricted to certain areas due to the presence of vectors or other factors. The number of birds kept in one unit and rearing of different age groups in the same farm may be predisposing factors to the occurrence of the diseases and this may lead to a heavy economic losses.

Diseases of the respiratory tract are often complex with anatomy, management, environment and nutrition, all playing a role (Nighot et al., 2002) and they are caused by wide range of pathogens of bacterial, viral, mycoplasmal or fungal origins. They play a significant role in death and losses in poultry industry. Any respiratory disease has a direct negative impact on the commercial parameter of poultry industry like weight gain, egg production or live ability and these causes considerable losses. Two main factors contribute to the sensitivity of these diseases in chicken; these are anatomy and physiology of the respiratory system and complex nature of the respiratory disease. The clinical picture of the respiratory diseases is
usually complicated when other diseases are involved. The severity and lesions of the respiratory diseases are sometime due mainly to the secondary invaders.

Poultry industry in the Sudan showed a significant development in the last decade. The number of large scale farming increased steadily, and the industry became more specialized in its intensive form and it covered the production of chicken in addition to meat and egg production. This study was carried out:

1) To isolate and identify bacteria which associated with respiratory tract infection of chickens.

2) To examine antibiotics susceptibility for some of bacterial isolates.
CHAPTER ONE
LITERATURE REVIEW

1.1 Poultry respiratory system:

The poultry respiratory system is composed of nostrils situated at the base of the bill, leading to the nasal cavity, the larynx, trachea, syrinx, lung and the air sacs (Getty, 1975). The main functions of the nasal cavity are smelling, filtration of air borne particles and humidification of inspired air, while the larynx main functions are prevention of foreign bodies’ entry, opening of the air ways during inspiration, aiding in swallowing and modulation of voice. The nasal cavity continues in to the long trachea, which divides before entering in to the lung. The comparatively long trachea offers pathogens easy access to an area where they can cause infection because of the high volume and low respiratory frequency. The syrinx gives rise to the left and right bronchi, each primary bronchus gives rise to four secondary bronchi, and the secondary bronchi give off numerous para-bronchi where gaseous exchange takes place. The lung in chicken is a flattened nearly rectangular structure lining the roof of the cranial end of the celom (Getty, 1975). One of the important features of avian lungs is the efficient gas exchange system which helps the bird to maintain oxygen pressure, even during limited ventilation (Nighot et al., 2002).

The air sacs are found in the thorax and the anterior positions (Getty, 1975). During inspiration, the volume of the air sacs increases and the pressure inside the air sacs decreases and vice versa during expiration. The presence of the air sacs connected to para-bronchi and occupying most of the inner body cavities are a crucial factor. A pathogen entering the nasal cavity can travel through both thorax and abdomen to close proximity to the head of femur bone.
1.2 Bacteria:

The bacteria are a group of single celled microorganisms with prokaryotic configuration. The prokaryotic cells nuclear material is not enveloped by a membrane. The bacterial cells are prokaryotes.

The bacteria have three architectural region; the appendages in the form of flagella and pili; a cell envelope consisting of a capsule, cell wall and plasma membrane; cytoplasmic region that contains the cell genome (DNA) and ribosome and various sort of inclusions. Most of cellular reaction incidental to life can be traced back to the activities of these structural components (Quinn, 2002).

All animals have what is called a normal flora, it consists of bacteria Mycoplasmas, viruses and fungi that live in or upon the normal animal without producing disease, included in this normal flora are a number of potential pathogens (Carter, 1986).

1.2.1 Infection via the respiratory tract:

The infection can be acquired by direct contact, as a result of inhalation of contaminated air. The organisms are trapped on the moist mucous membrane of the nasal pharynx and lower respiratory tract, so this is the way that diseases enter in to the mucous membrane such as Pasteurella (Tomas, 1983).

1.2.2 Bacteria of the respiratory tract:

The respiratory tract of poultry is infected by a wide range of bacteria, these include the following:-

1.2.2.1 Escherichia coli:

*Escherichia coli* are a Gram-negative flagellated rod, motile, non spore-formig bacteria (Sojka and Garnaghan, 1961). Species of this genus are widely distributed in nature and constitute a part of digestive flora of
mammals and bird. Pathogenic *E. coli* differ from the non-pathogenic *E. coli* by the presence of virulence factors organized in clusters in the chromosome or plasmid. According to the large variation in DNA content and to the difference in the distribution of genomic location (insertion site) of different virulence determination (Puente and Finlay, 2001). Some serotype cause specific disease in poultry known as colibacillosis which is a complex syndrome characterized by multiple organ lesions with air sac sacculitis and associated pericarditis, others cause disease under certain condition (Buxton and Fraser, 1977), (Swayne et al. 1998) and some act as secondary invaders. The organism adversely affects avian species through infection of blood, respiratory tract and soft tissue. The organism has also been isolated from an outbreak of respiratory disease (Chu, 1958) and from different sites of the respiratory tract of normal chicken. Elnasri (1997) isolated the organism from infra-orbital sinus and trachea. Secondary infection commonly occurs as complication with *Mycoplasma gallisepticum* infection. *E. coli* infection caused by a single agent occurs rarely. *E. coli* often infects respiratory tract of bird concurrently with various combination of infectious bronchitis viruses, Newcastle disease viruses, including vaccine strain; and Mycoplasms. Transmissions can be through inhalation, contamination of drinking water or feed, contamination of reproductive system and egg shell surface (Zahida, 2004).

*Escherichia coli* associated with respiratory infection in chickens has also been reported (Elsukhon *et al.*, 2002). Tracheitis, exudative pneumonia, pleuritis, air sacculitis, pericarditis, sinusitis characterize the infection (Canal *et al.*, 2005).
The primary routes of invasion by the organism are the respiratory system and the gastrointestinal tract. Lisons occur firstly in the respiratory tract through inhalation and in the mucus membranes in the case of intestinal infection; local lesions develop into systemic infections (Zahida, 2004). The symptoms vary with the different types of infections; in the acute septicemia form mortality may begin suddenly and progress rapidly. The common signs are restless with ruffled feathers indications of fever, also symptoms of labouredly breathing, occasional coughing and rales (Sojka and Carnavan, 1961). Mouline (1983) found that \( E.\ coli \) was a predominant organism of the tracheal flora. \( E.\ coli \) was isolated from the lung and air sac (Price et al., 1975, Malokwa et al., 1987; and Rajashekar et al., 1998) and from sinues (Eisa and Alnasri, 1985). Linzitto et al. (1988) isolated the organism from cases of infectious coryza.

The Annual Reports of the Sudan Veterinary Service (1948-1958). showed the presence of fowl coryza and \( E.\ coli \) infections since 1948. The importance of this disease is due to difficulty of prevention and control because of it's resistance to a wide range of antibiotics and due to the large number of varying serogroups involved in field outbreaks (Abdellah, 2003).

1.2.2.2 Haemophilus:

De Bleich (1932) was the first to isolate the causative agent of infectious coryza and named the organism \( Heamoglobinophilus\ coryza\ gallinarum \) (Yamamoto, 1991; Linzitto et al., 1988).

Bacteriological studies indicated that a number of organisms are associated with infectious coryza, these include \( Heamophilus\ avium\), Streptococcus, Staphylococcus, \( Escherichia\ coli\), \( Pasteurella\ multocide\), \( Psedomonus\ aeruginosa\), \( Pasteurella\ gallinarum\) and \( Mycoplasma\ gallisepticum\) (Yamamoto and Mutsumoto, 1970; Kojiuchida et al., 1991).
Bacteriological examination of infectious coryza infected chickens in Sudan shows the disease is caused by *Haemophilus gallinarum* (Shigidi, 1971). The disease is usually transmitted through drinking water contaminated with infective nasal exudates (Page, 1962). Infection may also occur by contact and by air-borne infected dust or droplet. Infectious coryza is an acute or chronic disease of upper respiratory tract of poultry caused by *Haemophilus* group of bacteria. These are heterogeneous group of small Gram-negative, aerobic bacilli, non motile and non spore-forming (Gordan and Jordan, 1982) requiring enriched media for culturing and growth. The organism is classified according to the X factor (hemin) and V factor (nicotinamide adenine dinucleotide) (Eliot and Lewis, 1934) also Narita *et al.* (1978) and Black and Reid (1982) confirmed this finding. Two species are named: *Haemophilus gallinarum* and *Haemophilus paragalinarum* (required V factor). These two species are identical in growth characteristics and ability to produce the diseases (Rimler, 1979).

All susceptible birds in a flock show clinical signs of the disease within few weeks. They include depression, seromucoid nasal discharge, conjunctivitis, facial oedema, swollen wattles and rales, appetite and production are reduced, resulting in inferior food conversion ratio in broilers and reduced egg production in layers. In avian host *Haemophilus gallinarum* was involved in respiratory disease complex (Hafez, 2002).

1.2.2.3 *Pseudomonas:*

Gram-negative, rod shape, motile, aerobic and non spore-forming. This organism is distributed widely in nature and found in soil and water. *Pseudomonas aeruginosa* is associated with infection in man and animals (Merchant and Packer, 1967). *Pseudomonas aeruginosa* is not only responsible for embryonic mortality but also for mortality in chicken and
heavy losses of broilers (Valadae, 1961: Saad et al., 1981: Andreev et al., 1982 and Bapat et al., 1985). The pathogenic effect of *Pseudomonas aeruginosa* in chicken was reported by Markaryan (1975) and Mrden et al. (1988) also this organism was isolated from infectious coryza cases by Linzitto et al. (1988) and Elnasri (1997). *Pseudomonas pyocyanae* cause septicemia in young chicks (Banerji and Ray 1969).

The species of this organism were significant in mixed infection particularly with Streptococci and Staphylococci (Carter, 1986).

1.2.2.4 *Pasteurella*:

Avian pasteurellosis is an infectious disease caused by certain related bacteria which are *Pasteurella multocida*, *Yersinia pseudotuberculosis* and *Pasteurella gallinarum*.

*Pasteurella* is a Gram-negative, non motile, non spore-forming, rod shape bacterium and shows bipolarity when stained with Gimsa (Jordan, 1986). All species of Pasteurella (exept *Pasteurella urase*) occurs as a commensall in upper respiratory tract and digestive flora of animals (Carter, 1986).

Ibrahim (1995) isolated *Pasteurella multocida* from different species of animals in Sudan on basis of their morphology, culture, biochemical characteristics and serology. *Pasteurella multocida* and *Pasteurella gallinarum* were isolated by Linzitto et al., (1988).

The organism is pathogenic to a wide range of animal species. In poultry Pasteurella causes an acute disease known as fowl cholera; the infection may be acquired by contact, inhalation or ingestion Linzitto et al., (1988).
Respiratory infection is the most serious disease affecting poultry and causes heavy economic losses in the poultry industry worldwide. In avian host, several microorganisms of the genus Pasteurella (*P. multocida*, *P. gallinarum*, *P. haemolytica* and *P. anatipestifer* are involved in respiratory disease complex (Hafez, 2002).

1.2.2.5 **Bordetella:**

Bordetella is heterogenous group of Gram-negative, small, rod shaped and oxidase-positive bacteria. The disease is of an upper respiratory tract, named as Turkey coryza which affected bird and the milder form of the disease affected the broilers (Jordan, 1986). It was first described in the USA and reported in many countries. The causative agent is *Bordetella avium*. There is considerable variation in virulence among strains, it is relatively resistant to heat and can probably survive on farm premises and it is susceptible to the common disinfectant at recommended concentrations and to direct sunlight. The severity of the disease may be greatly influenced by other pathogens, such as *E. coli*, Newcastle disease virus, *Pasteurella multocida*, *Mycoplasma gallicpticum* as well as a number of management faults such as overcrowding, excessive atmospheric ammonia, cold and high humidity (Kersters *et al*., 1984). Hafiz (2002) demonstrated that *Bordetella avium* was involved in respiratory disease complex and causes heavy economic losses in the poultry industry worldwide.

1.2.2.6 **Staphylococcus:**

Staphylococci are spherical Gram-positive bacteria, non motile, non spore-forming, non capsulated and usually arranged in grape-like irregular clusters (Jawetz *et al*, 1990, Geo *et al*., 1998). Staphylococci present in the upper respiratory tract and on other epithelial surfaces of all warm blooded animals, some strains are pathogenic others are nonpathogenic (Bibersein *et
al., 1974). Pigmentation produced by staphylococcus is varying from white to deep yellow (Jawets et al., 1990). Golden pigmentation is produced by many strains especially with extended incubation (Songer, 2000). Coagulase positive Staphylococcus produces colonies surrounded by yellow zones while non pathogenic produces purple colonies (Saeed, 1995).

Staphylococcus can some time produce air sac infection but it is associated with chronic arthritis, pyogenic infection and abscesses formation (Buxton and Fraser, 1977). Linzitto et al., (1988) reported that Staphylococcus was isolated from cases of infectious coryza. Elnasri (1997) isolated staphylococcus from trachea and air sac. A study concerning in respiratory diseases in commercial broiler flock. Bacteriologic examinations resulted in the isolation of E. coli and Staphylococcus spp. (Georgiades et al., 2001).

1.2.2.7 Streptococci:

Streptococci are Gram-positive, non spore-forming cocci occurring in pairs or chains (Jordan, 1986). They are usually found on the skin and mucous membrane of the upper respiratory tract, the organism causes pneumonia in chicken (Merchant and Packer. 1967). The organism was isolated and considered one of the organisms associated with infectious coryza.

1.2.2.8 Mycoplasmas:

Jordan (1986) stated that there are many species of the genus mycoplasma, some of them are of economic importance to the poultry industry. Such as Mycoplasma gallisepticum which causes disease in chicken and turkey. Mycoplasma synoviae causes synovitis and respiratory infection in chicken and turkey. Mycoplasma meleagridis causes respiratory disease in turkey. Many species of Mycoplasmas are nonpathogenic. Yamamoto and Matsumoto (1979) isolated Mycoplasma gallisepticum from
cases of infectious coryza. In the Sudan suspicion of Mycoplasma infection in poultry was based on clinical manifestations and confirmed by serological testing (Harbi et al., 1975; Elhassan et al., 1989). In eastern Sudan many clinical cases of respiratory tract and joint infection were observed. The prevalence of Mycoplasma synoviae and Mycoplasma gallisepticum in poultry farms in eastern Sudan was proved serologically in flocks showing clinical respiratory signs (Salim and Mohammed, 1993). Recently, it was demonstrated using in vitro assays that the avian pathogen Mycoplasma gallisepticum is able to invade nonphagocytic cells. It was also shown that this mycoplasma can survive and multiply intracellularly for at least 48 h and that this cell invasion capacity contributes to the systemic spread of M. gallisepticum from the respiratory tract to the inner organs (Vogl et al., 2008).

1.2.2.9 Chlamydia:

Chlamydiosis is a disease of man, birds, and other animals. The Chlamydia form a well defined group of organism and with worldwide distribution, they are associated with many different diseases in many species of birds and animals. The infection can be source of serious economic loss to the poultry industry. The signs of the disease are serous or purulent exudates from eyes and nostrils accompanied by loss of appetite and in-activity. A common feature is diarrhoea: respiratory distress and hyperthermia are also observed. Egg production is severely affected and drops rapidly (Jordan, 1986).

1.2.2.10 Other bacterial species:

One of most important bacteria is enterobacteria (Carter, 1986). Enterobacteria are of worldwide distribution: many of them are part of the normal flora of the intestinal tract. Some species are free living occurring on
the soil and water e.g. *Yersinia pseudotuberculosis*, *Shigella* and *Klebsiella spp* mainly *K. pneumoniae* as causal agent of serious and sometimes fatal infections in both man and animals has been seen at increasing rates in the recent years. Elhassan and Elsanosi (2002) isolated *K. pneumoniae* subspecies *ozaenae* from lung, intestines, liver, ovaries, and eyes of chicken. *Ornithobacterium rhinotracheal* has recently been identified as a pathogen causing respiratory tract infections in poultry and other birds (Vandamme *et al*., 1994; Chin *et al*., 2003). Tracheitis, exudative pneumonia, pleuritis, air sacculitis, pericarditis, sinusitis, characterize the infection (Zorman-Rojs *et al*., 2000). Canal *et al*. (2005) isolated *Ornithobacterium rhinotracheale* from chicken, turkeys, quails, ducks, geese, ostriches, guinea fowl, pheasants, rooks and pigeons. There were reports of *O. rhinotracheale* infections in the United States, Germany, South Africa, The Netherlands, France, Israel, Belgium, Hungary, Japan, the United Kingdom, Turkey, Canada, Jordan and Brazil (Travers, 1996; Joubert *et al*., 1999; Van Empel and Hafez, 1999; Chin *et al*., 2003).

1.3 Respiratory disease caused by fungal and viral agents:

1.3.1 Aspergillosis:

Aspergillosis is a respiratory tract infection caused by members of the genus Aspergillus, of which *A. fumigatus* is the primary species responsible for infections in wild birds. Aspergillosis is not contagious, and it may be an acute, rapidly fatal disease or a more chronic disease. Both forms of the disease are commonly seen in free-ranging birds, but the acute form is generally responsible for large-scale mortality events in adult birds and for brooder pneumonia in hatching birds. Friend and Franson (1999-2001). Fungi are of greatest importance in causing disease in poultry do so enter by tissue invasion and damage or by producing toxin. The respiratory tract is
commonly infected by fungi. Infections are most frequently due to Aspergillus species. Respiratory aspergillosis is common mismanagement problem in commercial and backyard poultry. Organisms culture from affected organ are \textit{Aspergillus fumigatus}, \textit{A. flavus}, \textit{A. nigar}, \textit{A. glaucus} and \textit{A. terreus}. Chicken and duck are highly susceptible to infection. Aspergilosis is produce by inhalation of spores from infected eggs that are opened during incubation or hatching. It is also produced by inhalation of spores from contaminate feed of poultry house litter. Airborne conidia come to rest on conjunctiva, nasal, trachea, parabronchial and air sacs. Infected poultry flocks exhibit a biphasic mortality pattern, also lesions are found in the respiratory tract including particularly the trachea, bronchi, lung and air sac (Jordan \textit{et al.}, 2001).

\textbf{1.3.2 Newcastle disease:}

Newcastle disease is highly contagious disease that affects chickens and other birds. The virulence of some strains of the virus makes the disease a serious problem in many countries (Zein \textit{et al.}, 2001). Newcastle disease still constitutes a major hazard to the poultry industry in Sudan (Tabidi \textit{et al.}, 1998). Elhussien \textit{et al.} (1996) classified Newcastle as the most important poultry disease and still remain the major killing disease. Outbreaks of the disease have been reported regularly from different regions of the country (Elhussien \textit{et al.}, 1996). The disease causes heavy losses due to death of birds, drop of egg production and retardation of the growth. Acute respiratory tract infections are of paramount importance in the poultry industry. Avian influenza virus (AIV), infectious bronchitis virus (IBV), Newcastle disease virus (NDV), avian pneumovirus (APV), and \textit{Mycoplasma gallisepticum} (MG) have been recognized as the most important pathogens in poultry (Roussan \textit{et al.}, 2008).
1.4 The avian immune system:

The anatomical basis of the immune system of the chicken is the lymphoid tissue which has both central and peripheral components. Central parts consist of two structures which are the multilobed thymus and the bursa of fabricius. The peripheral component is the lymphoid tissue which include the spleen, caecal tonsils, bone marrow, and aggregates of lymphoid cells in various organs and tissue. Avian lymphoid cells rapidly infiltrate sites of antigenic stimulation throughout the body so that even in normal birds lymphoid aggregates found in tissue such as the nasal passage and upper respiratory tract, oesophagus and intestinal tract of the chicken (Gordan and Jordan, 1982). The lymphoid foci which are normally found in organs like the proventriclus and pancreas typically consist of diffuse unencapsulated masses of small lymphocytes and germinal centers of variable size consisting of B lymphocytes, dendritic cells and macrophages. The main immunological function of the thymus and bursa is to generate the lymphocytes (Gordan and Jordan, 1982). The bursa drive B lymphocytes while the thymus drive T lymphocytes.

Antigen-specific protection of mucosae in the upper airway is achieved mainly using the humeral immune system (Brandtzaeg, 1995; Phalipon et al., 2002) through the production and secretion of polymeric IgA and Ig M (Brandtzaeg et al., 1997). Secretary Ig performs immune exclusion by inhibiting the uptake of soluble antigens and by blocking adhesion and invasion of epithelia by micro-organisms (Avakian and Ley, 1993; Snoeck et al., 2006). As discussed above, numerous B cells and ASC are present in the head-associated lymphoid tissues and throughout the respiratory tract of avian species, and Immunoglobulins have been detected. Secreted immunoglobulins in the respiratory tract are produced by bursa-derived cells.
(Lam and Lin, 1984), primarily of the Ig A isotype, although Ig G and Ig M antibodies are also found (Russell, 1993). Comparable results were obtained in *M. gallisepticum*-infected birds which developed antigen-specific IgA, IgM and IgG responses in washings of the upper and lower respiratory tract (Yagihashi and Tajima, 1986). Functional in vitro assays demonstrated that these antibodies were protective but that protection did not correlate with the IgA titres in the washing, indicating that secreted IgM and IgG are also of relevance in mucosal defence (Avakian and Ley, 1993). Intratracheal infection with *M. gallisepticum* induces the accumulation of IgG, IgA, B cells and plasma cells in the lamina propria. In contrast, vaccination prior to infection induced the formation of lymphoid follicles and of strongly elevated numbers of antigen-specific ASC as measured by an enzyme-linked immunospot (ELISPOT) technique. It also leads to a significantly higher proportion of *M. gallisepticum*-specific IgA and IgG antibodies in tracheal washings (Javed et al., 2005). While IgA is most probably produced locally and secreted as a polymeric antibody, IgG antibodies may be locally secreted or transudated from the serum as suggested by several studies (Toro et al., 1993; Suresh and Arp, 1995; Javed et al., 2005). Finally, induction of antigen-specific IgA and IgG antibodies in tear fluid and lower respiratory tract lavage samples have been demonstrated in IBV-vaccinated birds (Toro and Fernandez, 1994; Thompson et al., 1997) and was induced equally well by antigen delivery through ocular instillation, spray or drinking water application (Toro et al., 1997).

1.5 The avian immune response:

The response to microbial infection involves interaction of both innate and acquired immunity. The development of the immunity involves both
T&B cells and eliminating infection. However this varies from disease to another, as an example the antibodies is the major protective factor in Newcastle disease and influenza infection while in Marek's disease and fowl pox; the cell mediated reactions are the most important. The avian respiratory tract is lined with local lymphoid tissue throughout its length. This protects the respiratory system by attempting to eliminate the pathogen, as well as invoking a general immune system components take part in the immune response. Various immunosuppressive agents hamper the functioning of the immune mechanism, making the birds more susceptible to respiratory challenge. The flocks suffering from immunosuppressant disease never attain optimum immunity in spite of vaccination against various diseases (Nighot et al., 2002).

Nutrition also has its effect; various dietary components play a role in the immune response of birds. Generally a higher level of nutrients is required to optimize the immune response than for growth, e.g. methionine, vitamins C and K. Imbalance of sodium and chloride can affect broiler immunity and high chloride levels may reduce immune response if sodium levels are not raised accordingly. Selenium and vitamin E are important for the protection and regeneration of tissues. As an integral part of biochemical substances involved in tissue healing, zinc is an essential nutrient. Vitamin A and C help to maintain epithelial integrity. The amino acid make-up of the protein source also influences the immune response. The protein analysis solely on nitrogen basis may not give correct idea about amino acid components, the balancing of which is essential to develop an optimum immune response.

In conclusion, respiratory disease is precipitated when the natural defenses and immunity of the bird is challenged by infectious or non
infectious causes, which mostly accompany one another. Intensive poultry farming puts additional pressure on the respiratory system, which therefore needs protection from pathogenic agents (Nighot et al., 2002).

1.6 Antimicrobial agents:

Antimicrobial agents may be defined as those substances that interfere with the growth and activity of microorganism generally, the term denotes inhibition of microbial growth (biostatic) and or destruction (biocidal). Such terms as antibacterial or antifungal are frequently employed to refer to activities against specific group of microorganisms (Pelczar et al., 1977).

The modern era of antimicrobial effects began with the work of the German physician Paul Ehrlich (1824 – 1915). In 1929, Flemings discovered the powerful bactericidal activity of Penicillin, and Domagk's in 1935 the synthetic chemicals sulfonamides with broad-spectrum activity.

In the early 1940, Penicillin was isolated, purified and injected into experimental animals. The rapid isolation of streptomycin, chloramphenicol and tetracycline soon followed and by 1950s these and several other antibiotics were in clinical usage (Carter, 1986).

1.6.1 Antibiotics:

Antibiotics are low molecular weight substances that are produced as a secondary metabolites by certain groups of microorganisms specially streptomycyes, bacillus and a few molds (pencillium and cephalosporium) that are inhabitants of soils. Antibiotics may have a cidal (killing) effects or a static (inhibitory) effects on a range of microbes. The ranges of bacteria or other microorganisms that affected by a certain antibiotics is expressed as its spectrum of action. Antibiotics effective against a wide range of Gram positive and Gram negative bacteria are said to be broad spectrum, if effective mainly against Gram-positive or Gram-negative bacteria they are
called narrow spectrum, if effective against a single organism or disease, they are referred to as limited spectrum (Prescott et al., 2002).

I.6.2 Benefits of antimicrobial use:

Early warnings have been made since the 1940s regarding the subtherapeutic use of antibiotics that might expose microbes to non-lethal quantities of antibiotics, making them resistant (Fleming, 1945). The food-animal industry is ostensibly ignoring this admonition by daily use of subtherapeutical antimicrobial doses in animal feeds. Although this common practice is increasingly controversial, it is hard to disregard the potential benefits of antibiotic use in food-animal production, for both animal and human health. Antimicrobial prophylaxis prevents diseases and decreases microbial competition for nutrients in the animal's GI tract. Furthermore, sick animals are treated with adequate medication, reducing their microbial load and thus, enhancing their growth performances and general health status. This allows better economic returns to producers and increases consumers confidence in consuming food products produced from those animals. On the other hand, antibiotics significantly reduce the threat to human health related to foodborne pathogens such as Campylobacter, Salmonella, hemorrhagic *Escherichia coli* O157: H7, *Listeria monocytogenes* and *Clostridium* (NRC, 1999).

Use of antibiotics to eliminate transferable bacteria from food producing animals is a measure of food security that protects consumers from exposure to life threatening diseases, thus preserving their good health status. Most importantly, this measure also could reduce infiltration of pathogenic bacteria into the environment, mainly through manure application. Runoff water, especially after heavy rains, could contaminate wells and other community water systems as happened in Walkerton in
2000. This deadly waterborne outbreak resulted from entry of *Escherichia coli* O157:H7 and *Campylobacter* spp. from neighbouring farms into the town water supply (Clark *et al.*, 2003). Crops, fruit trees and other commodities are commonly sprayed with antimicrobials to prevent microbial spoilage, thus contributing in controlling environmental contamination (Khachatourians, 1998).

The benefits for animal health and welfare and for human health are undeniable. However, there are concerns arising about the appropriateness of the use of antibiotics in food animal production. Some of these concerns include antibiotic residues and the emergence of antibiotic resistant pathogens (Khachatourians, 1998; Conly, 2002; Bywater, 2005).

### I.6.3 Disadvantages of antimicrobial use:

Any drug has a connotation of toxicity, especially when it and/or its metabolites and absorbed from the intestines. Antimicrobials used in food-animal production are not an exception to this rule. Residues from drugs used in food animal production could be ingested either directly through animal tissues or products, or indirectly through the environment (Samanidou and Evaggelopoulou, 2008). Antibiotic residue consumption can induce adverse effects such as toxicities, allergies and infection by disease-causing bacteria that are antibiotic-resistant. Antimicrobials and their metabolites can concentrate in animal tissue leading to toxicities that could be manifested by teratogenic or carcinogenic effects. Consequently, the US FDA has strict regulations: (no proven carcinogen should be considered suitable for use as a food additive in any amount) (Committee on Drug Use in Food Animals Panel on Animal Health, Food Safety, and Public Health, 1999); Canada also follows similar regulations (Health Canada 2002). Therefore antimicrobials, that are systemic and thus absorbed from
intestines in significant amounts (e.g. tetracycline, penicillin, erythromycin and lincomycin), supplied to animals through feed or water, fall under regulatory withdrawal periods. Non systemic antimicrobials, which are not absorbed or very slightly absorbed from the intestine, do not require any removal period and can be administered to animals until slaughter (e.g. bacitracin, virginiamycin, bambermycin, neomycin, tylosin, novobiocin and streptomycin) (Health Canada, 2002).

Allergic reactions are less frequently reported in the literature (Health Canada, 2002). Few cases are related to the consumption of milk containing penicillin residues although there is no enough evidence to prove that antimicrobials used in animal food provoke allergic reactions in humans (Borrie and Barret, 1961; Wicher et al., 1969; Barton, 2000). Heat treatment during further processing could degrade the residue epitopes and reduce the potential for allergic reactions (NRC, 1999). Although strict control exerted by regulatory agencies strongly contributed to limit the risks of food-animal tainting by antimicrobial residues, antibiotic resistant bacteria still could contaminate animal carcasses and enter the food chain.

Numerous studies have eliminated any doubt that antibiotic-resistant bacteria could be transferred from food animals to humans (FSIS 1997; Wegener et al. 1999; Poppe et al., 2006). Commensal pathogens such as Salmonella, entero-hemorrhagic E. coli could exchange or receive multiple antibiotic resistance genes from non pathogenic carriers such as generic E. coli. (Schwarz et al., 2006; Zhao et al., 2006). Young children, elderly and immuno-compromised people are the most at risk during infection by such pathogens. They pay for what they are usually not directly responsible for and sometimes, they have to pay with their own lives. Seven pathogens were recognized by the Center for Disease Control and Prevention (CDC) as the
most common cause of foodborne illnesses. Campylobacter is the most frequently isolated foodborne bacterium (49.4%), followed by Salmonella (27.4%), Shigella (15.7%), *E. coli* O157:H7 (4.2%), Yersinia (1.7%), Listeria (1%) and Vibrio (0.6%). They constitute a serious health risk due to the fact that they are easily transferable, thus making them difficult to control (FSIS, 1997).
CHAPTER TWO
MATERIALS AND METHODS

2.1 Sterilization:

a. Flaming:
It was used to sterilize glass slides, cover slips, needles and scalpels.

b. Red heat:
It was used to sterile wire loop, points and searing spatulas by holding them over Bunsen burner flame until they became red-hot.

c. Hot air oven:
It was used to sterilize glass wares such as test tubes, graduated pipettes, flasks, forceps and cotton swabs. The holding period was one hour and oven temperature was 160 °C.

d. Steaming at 100 °C:
Repeated steaming (Tyndallization) was used for sterilization of sugars and media that could not be autoclaved without deterioration effect to their constituents. It was carried out as described by Barrow and Feltham (1993).

e. Moist heat (autoclave):
Autoclaving at 121°C (15lb/ inch²) for 15 minutes was used for sterilization of media and plastic wares.
Autoclaving at 115°C (10lb/ inch²) for 10 minutes was used for sterilization of some media such as sugars containing media.

2.2 Reagents and indicators:

2.2.1 Reagents:

2.2.1.1 Alpha-naphthol solution:
Alpha-naphthol is a product of British Drug House (BDH); London. This
reagent was prepared as 5% aqueous solution for Voges Proskauer (VP) test.

2.2.1.2 Potassium hydroxide:
It was used for Voges Proskauer test and prepared as 40 % aqueous solution.

2.2.1.3 Hydrogen peroxide:
This reagent was obtained from Agropharm Limited Buckingham. It was prepared as 3% aqueous solution, and it was used for catalase test.

2.2.1.4 Methyl red:
It was prepared by dissolving 0.04 g methyl red in 40 ml ethanol. The volume was made to 100 ml with distilled water. It was used for methyl red test (MR).

2.2.1.5 Tetra methyl-p-phenyl diamine dihydrochloride:
This was obtained from Hopkin and William; London. It was prepared in a concentration of 3% aqueous solution and was used for oxidase test.

2.2.1.6 Nitrate test reagent:
Nitrate test reagent was consisting of two solutions which were prepared according to Barrow and Feltham (1993). Solution A was composed of 0.33% sulphanilic acid dissolved by gentle heating in 5N-acetic acid. Solution B was composed of 0.6% dimethyl amine-alpha-naphthylamine dissolved by gentle heating in 5N-acetic acid. It was used for nitrate reduction test.

2.2.1.7 Kovac’s reagent:
This reagent composed of para-dimethylaminobenzaldehyde, amyl alcohol and concentrated hydrochloric acid. It was prepared as described by Barrow and Feltham (1993) by dissolving the aldehyde in the alcohol by heating in water bath, it was then cooled and the acid was added carefully. The reagent was stored at 4 ºC for later use in indole test.
2.2.2 Indicators:

2.2.2.1 Andrade’s indicator:
It composed of 5 g acid fuchin, 1 L distilled water and 150 ml N-NaOH. The acid fuchin was dissolved in distilled water, and then the alkali solution was added and mixed. They solution was allowed to stand at room temperature for 24 h with frequent shaking until the color changed from red to brown.

2.2.2.2 Bromothymol blue:
Bromothymol blue was obtained from BDH. The solution was prepared by dissolving 0.2 g of bromothymol blue powder in 100 ml distilled water.

2.2.2.3 Phenol red:
Phenol red was obtained from Hopkins and William ltd, London. It was prepared as 0.2% aqueous solution.

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

2.2.2.5 Bromocresol purple (BDH):
Bromocresol purple indicator was prepared by dissolving 0.2 g of the powder in 100 ml distilled water.

2.3 Collection of blood for enriched media:
Blood for enriched media was collected aseptically into sterile flask containing glass beads by veinopuncture of jugular vein of healthy sheep kept for this purpose. The blood was defibrinated by shaking the flask after collection. The defibrinated sheep blood was used for preparing blood agar medium.
2.4 Preparations of media:

2.4.1 Nutrient broth (Oxoid CM 1):
The medium was prepared by adding 13 g of nutrient broth powder to 1 L of distilled water and well mixed. The pH was adjusted to 7.4. The mixture was distributed in 5 ml volumes into clean bottles, and then sterilized by autoclaving at 121°C (15 lb/inch²) for 15 minutes.

2.4.2 Peptone (Oxoid CM 9) water:
This medium was prepared by dissolving 10 g peptone and 5 g sodium chloride in 1 L of distilled water. The mixture was distributed in 5 ml volumes into clean bottles and sterilized by autoclaving at 121° C (15 lb/ inch²) for 15 minutes.

2.4.3 Peptone water sugars (Carbohydrate fermentation medium):
Peptone water sugar medium was prepared according to Barrow and Feltham (1993). It contained 900 ml peptone water, 10 ml Andrade’s indicator, 15 g sugar and 90 ml distilled water. The pH of peptone water was adjusting to 7.1-7.3 before the addition of Andrade’s indicator. The complete medium was well mixed, then distributed in portions of 2 ml into clean test tubes containing inverted Durham’s tube. The medium was autoclaving at 115 °C (10 lb/inch²) for 20 minutes. The carbohydrates examined were glucose, sucrose, lactose, fructose, maltose, mannitol, xylose, sorbitol and salicin.

2.4.4 Nutrient agar (Oxoid CM 3) slant:
This was prepared by adding 28 g of nutrient agar to 1 L of distilled water and dissolved by boiling. The pH was adjusted to 7.4. The prepared medium was distributed in 10 ml volume into clean bottles, sterilized by autoclaving at 121 °C (15 lb/inch²) for 15 minutes and left to solidify in inclined position.
2.4.5 Glucose-phosphate medium (MR-VP test medium):
This medium was prepared according to Barrow and Feltham (1993) by adding 5 g peptone and 5 g phosphate buffer to 1 L distilled water, then dissolved by steaming and filtered. The pH was adjusted to 7.5, 5 g of glucose were added and then well mixed. The complete medium was distributed into clean test tube in 10 ml amount. The medium was sterilized by autoclaving at 115 °C (10 lb/inch²) for 15 minutes.

2.4.6 Nutrient agar (Oxoid CM 3):
This was prepared by adding 28 g of nutrient agar to 1 L of distilled water and dissolved by boiling. The pH was adjusted to 7.4, and then sterilized by autoclaving at 121°C (15 lb/inch²) for 15 minutes. The prepared medium was distributed in 20 ml volume into sterile Petri dishes. The poured plates were allowed to solidify on flat surface.

2.4.7 Blood agar (Oxoid CM 55):
This was prepared according to Barrow and Feltham (1993) by suspending 40 g of blood agar base in 900 ml of distilled water and dissolved by boiling. The mixture was sterilized by autoclaving at 121°C (15 lb/inch²) for 15 minutes and cooled down to about 50 °C, then defibrinated sheep blood was added aseptically to make a final concentration of 10%. The prepared medium was mixed gently and distributed in 20 ml volumes into sterile Petri dishes. The poured plates were allowed to solidify on leveled surface.

2.4.9. Chocolate agar medium:
This medium was prepared according to Barrow and Feltham (1993) by dissolving 40 g of blood agar base (Oxiod) in 1 L distilled water. The medium was sterilized by autoclaving at 121 °C (15 lb/inch²), then cooled to 75-80 °C in water bath and 5% sterile defibrinated sheep blood was added
with frequent mixing until the medium possessed a chocolate color. The prepared medium was distributed in 20 ml amounts in sterile Petri dishes. The poured plates were allowed to solidify on leveled surface.

2.4.9 Diagnostic sensitivity test agar (Oxoid CM 261):
This medium was prepared as described by Barrow and Feltham (1993). It composed of peptone, veal infusion solid, dextrose, sodium chloride, disodium phosphate, sodium acetate, adenine sulphate, guanine hydrochloride, uricil, xanthine and ion agar. Forty grams of medium was dissolved by boiling in 1 L of distilled water. The pH was adjusted to 7.4; and sterilized by autoclaving at 121 °C (15 Ib/inch²) for 15 minutes. The sterilized medium was distributed in 20 ml volumes into sterile Petri dishes. The poured plates were allowed to solidify on leveled surface.

2.4.10 MacConkey agar medium (Oxoid CM 7):
Fifty two grams of MacConkey’s agar were dissolved in 1 L distilled water. The pH was adjust to 7.4, sterilized by autoclaving at 121 °C (15 Ib/inch²) for 15 minutes and then distributed in 20 ml volumes into sterile Petri dishes. The poured Petri dishes were allowed to solidify on flat surface.

2.4.11 Eosin Methylene Blue Agar – EMB:
Ten grams of peptone (Oxoid), 10 g of lactose, 2 g of dipotassium hydrogen phosphate, 0.4 g of eosin, 0.065 g of methylene blue and 15 g of agar No.3 (Oxoid) were added to 1 L of distilled water. The pH was adjust to 6.8, sterilized by autoclaving at 121 °C (15 Ib/inch²) for 15 minutes and then distributed in 20 ml volumes into sterile Petri dishes. The poured Petri dishes were allowed to solidify on flat surface.

2.4.11 Motility medium - Cragie tube medium:
Thirteen grams of dehydrated nutrient broth (Oxoid CM 1) were added to 5 g of Oxoid agar No.1 and dissolved in 1 L of distilled water. The
pH was adjusted to 7.4. The prepared medium was distributed in 5 ml volumes into clean test tube which containing appropriate Cragie tubes and then sterilized by autoclaving at 121 °C (15 lb/inch²) for 15 minutes.

2.4.12 Hugh and Liefsons (O/F) medium:
This medium was prepared as described by Barrow and Feltham (1993). Two grams of peptone powder, 5 g of sodium chloride, 0.3 g of potassium hypophosphate and 3 g of agar were added to 1 L of distilled water. The pH was adjusted 7.1 and the indicator bromocresol purple was added. The complete medium was distributed into test tubes in 5 ml amount. The medium was sterilized by autoclaving at 115 °C for 10 minutes.

2.4.13 Simmon citrate medium (Oxoid CM 155):
This medium contained sodium ammonium phosphate, ammonium dihydrogen phosphate, magnesium sulphate, sodium citrate, sodium chloride, bromothymol blue as indicator and agar NO.3 (Oxoid L 13). The medium was obtained from Oxoid (Ltd). It was prepared according to manufacture instruction by dissolving 17 g of powder in 1 L of distilled water. The prepared medium was distributed in 10 ml volume into clean bottles, sterilized by autoclaving at 121 °C (15 lb/inch²) for 15 minutes and left to solidify in inclined position.

2.4.14 Urea agar medium:
This medium was obtained from Oxoid (Ltd). It contained peptone, dextrose, disodium phosphate, sodium chloride, potassium dihydrogen phosphate, agar and phenol red. It was prepared according to manufacture instructions by dissolving 2.4 g in 95 ml of distilled water and dissolved by boiling. The prepared medium was sterilized by autoclaving at 121°C for 15 minutes, cooled to 50 °C, and then 5 ml of sterilized 40% urea solution (Oxoid SR 20) were added under aseptic condition. The medium was
distributed in 5 ml volumes into sterile bottles and left to solidify in inclined position.

2.5 Collection of samples:
A total of 78 samples were collected from sick chickens with clinical symptoms of respiratory tract diseases. These symptoms include mucoid or serous nasal discharge, sneezing, lacrimation, conjunctivitis and facial swelling. The samples were collected from the common breeds raised in Khartoum North area. All samples were collected from farms where chickens are vaccinated against Newcastle disease and fowl pox.

2.5.1 Sampling and culture:
2.5.1.1 Nostril:
Sterile cotton wool swabs were used for sampling the nostril of live chicken.

2.5.1.2 Trachea:
Sterile cotton wool swabs were used for taking samples from the inside of trachea of recently slaughtered chicken.

2.5.1.3 Lung:
The lung of recently slaughtered chicken was cut into pieces with sterile scalpel and small piece was taken by sterile forceps.

2.5.1.4 Conjunctiva:
The eyes of the dead chickens were opened with sterile forcep and sterile cotton swab was used for sampling conjunctival sac.

2.6 Primary culturing:
Nasal, tracheal, conjunctival swabs and cut piece of lung were inoculated into nutrient broth and incubated overnight at 37 °C. The collected samples which were inoculated into a nutrient broth and incubated overnight at 37 °C were subcultured onto a blood agar,
MacConkey’s agar.
The inoculated plates were incubated for 24-48 h at 37 ºC. The colonies characteristics were observed. Smears were made from each type of colony, stained by Gram’s Method and examined under light microscope for cell morphology, cell arrangement and staining reaction.

2.7 Purification and preservation of culture:
Purification of culture was done by sub-culturing part of typical well separated colony on the corresponding medium. The process was repeated several times. The purity of the culture was checked by examining stained smear. Pure culture was then inoculated into nutrient agar slant medium and incubated overnight at 37 ºC. The pure culture was then stored at 4 ºC for studying cultures and biochemical characteristics and sensitivity of the isolates.

2.8 Microscopic examination:
Smears were made from each types of colony on primary cultures and from purified colonies. Then fixed by heating and stained by Gram stain method according to Barrow and Feltham (1993) and examined microscopically under oil immersion lens. The smear was examined for cell morphology, cell arrangement and stained reaction.

2.9 Identification of isolates:
The purified isolates were identified according to criteria described by Barrow and Feltham (1993). This included staining reaction, cell morphology, growth condition, colonial characteristics on different media, haemolysis on blood agar and biochemical characteristics.
2.10 Biochemical methods for identification of isolated bacteria:
All biochemical tests were performed as described by Barrow and Feltham (1993). They included:

2.10.1 Catalase test:
A drop of 3% H₂O₂ was placed on clean slide and colony of test culture on nutrient agar was picked by glass rod and added to the drop of H₂O₂. Positive reaction was indicated by evolution of gas (air bubbles).

2.10.2 Oxidase test:
Strip of filter paper was soaked in 1% solution of tetramethyl-p-phenylene diamine dihydrochloride and dried in hot air oven and then placed on clean glass slide by sterile forceps. A fresh test culture put on the filter paper strip. If a purple color developed within 5-10 second, the reaction was considered positive.

2.10.3 Oxidation fermentation (O/F) test:
Duplicate tubes of Hugh and Liefsons medium were inoculated by stabbing with straight wire. One of the tubes was sealed by layer of sterile soft paraffin oil to protect it from air; both inoculated tubes were incubated at 37 °C and examined daily for a period of fourteen days. Yellow color in open tube indicated oxidative reaction, yellow color in both tubes indicated fermentation reaction. Blue color in the open tube and green in the sealed tube indicated production of alkali.

2.10.4 Motility test:
Motility medium was inoculated by stabbing with straight wire into the center of the Cragie tube and then incubated at 37 °C for 24 h. The organism was considered motile if there was turbidity in the medium in and outside the Cragie tube while the growth of non motile organism confined
inside Cragie tube.

2.10.5 Sugar fermentation test:
Carbohydrate medium was inoculated with test culture then incubated at 37 ºC and examined daily for 7 days. The acid production was indicated by change in color to pink and gas production was indicated by presence of empty space in Durham’s tubes.

2.10.6 Indole production test:
The test culture was inoculated into peptone water and incubated at 37 ºC for 48 h. One ml Kovac’s reagent was added to the tube. The appearance of a pink color in the reagent layer within a minute indicated positive reaction.

2.10.7 Methyl red test:
The test culture was inoculated into glucose phosphate medium and then incubated at 37 ºC for 48 h. Two drops of methyl red reagent were added and shaken well. Red color indicated positive reaction. Yellow or orange color indicated negative reaction.

2.10.8 Voges-Proskure test:
The test culture was inoculated into glucose phosphate medium and incubated at 37º C for 48 h. One ml of culture medium was transferred aseptically into sterile test tubes and then 0.6 ml of 5% alpha-naphthol solution was added, followed by 0.2 of 40% KOH aqueous solution. The test tube was shaken well and kept at slant position for 1 h. Positive reaction was indicated by strong red color.

2.10.9 Citrate utilization test:
The test culture was inoculated onto Simmon’s citrate medium, then incubated at 37 ºC and examined daily for 7 days. Blue color indicated positive reaction.
2.10.10 Hydrogen sulphide production:
A tube of peptone water was inoculated by tested organism and lead acetate paper was inserted between the cotton plug and the tube, then incubated at 37 °C and examined daily for a week. Blacken of the paper indicated H₂S production.

2.10.11 Nitrate reduction test:
The nitrate broth was inoculated lightly and incubated for up to five days and 1 ml of reagent (A) Sulphanilic acid was added followed by 1 ml of reagent (B) α-naphthylene amine. A deep red colour indicated that nitrate has been reduced. To the tubes not showing a red colour within 5 minutes, powdered zinc was added and allowed to stand. Red colour indicated negative reaction.

2.10.12 Coagulase test:
2.10.12.1 Slide coagulase test:
A colony of tested culture was placed on a clean glass slide, emulsified in a drop of normal saline and then a loop-full of human plasma was added to bacterial suspension. Appearance of coarse microscopically visible clump was recorded as positive result.

2.10.12.2 Tube coagulase test:
To 0.5 ml of 1:10 dilution human plasma in normal, 0.1 ml of an 18-24 hours old broth culture of test organism was added, then incubated in water bath at 37 °C and examined after 4-6 h for coagulation. Definite clot formation indicated positive result.

2.10.13 Novobiocin sensitivity test:
The standard disc diffusion method was used to examine the sensitivity of the test organism to Novobiocin. Five milligrams Novobiocin
sensitivity disc (Oxoid, LTd) was used. A plate of DST medium was dried in oven at 40 °C for 20 minutes, and then 1 ml of diluted suspension of the test organism was poured onto the surface of the medium in the plate. Excess suspension was drawn off and the plate was allowed to dry at room temperature for 30 minutes. The Novobiocin disc was gently applied on the plate using sterile forceps. Then the plate was incubated at 37 °C for 24 h. The test organism was reported as sensitive if there was a zone of growth inhibition around the disc. The zone of growth inhibition around the disc was measured in millimeters.

2.11 Antibacterial sensitivity test:

The sensitivity of isolates to antibacterial drugs agents was determined by disc diffusion technique. The isolates were cultured in peptone water and incubated at 37 °C for 2 h. Petri dishes containing diagnostic sensitivity test (DST) agar medium were put in the incubator at 37 °C for 30 minutes to dry and then inoculated with 1 ml volume of the culture. The inoculated culture was evenly distributed by rotation, the excess inoculums were withdrawn by sterile microtitter pipette and the plate was left to dry at room temperature for 15 minutes. Commercially prepared antibiotic discs of Plasmatic laboratory were placed on surface of the medium by sterile forceps and pressed gently to insure good contact with the surface of the culture medium. The plates were then incubated at 37 °C for 24-48 h. The sensitivity of the isolates was examined to the following antibacterial drugs: Ampicillin (20 mcg), Co-trimaxazole (25 mcg), Cephalexin (30 mcg), Tetracycline (25 mcg), Cefotaxime (30 mcg), Ciprofloxacin (5 mcg), pefloxacin (10 mcg), Ofloxacin (5 mcg), Cloxacillin (1 mcg), Roxythromycin (15 mcg), Lincomycin (2 mcg), Gentamycin (10 mcg), Peperacillin\Tazobactam (100\10 mcg), Chloramphenicol (30 mcg),
Ceftizoxime (30 mcg) and Amikacin (30 mcg).
The test organism was considered sensitive if there was a zone of inhibition of 10 mm or more around the disc according to manufacture’s instructions.
CHAPTER THREE

RESULTS

3.1.1 Bacterial isolated from nostrils:
The number of samples collected from nostrils was 42.
Out of these, 34 (80.95%) samples gave positive growth and they yielded 55 (130.95%) isolates, while the remaining 8 (19.05%) samples did not show any growth. The 55 (130.95%) bacteria comprised both Gram positive and Gram negative bacteria. Thirty four (80.95%) isolates were found to be Gram positive while the other 21 (50%) isolates were Gram negative. The 34 isolates of Gram positive bacteria were 7 (16.7%) \textit{Staphylococcus aureus}, 4 (9.5%) \textit{Staphylococcus gallinarum}, 2 (4.8%) \textit{Staphylococcus epidermidis} 2 (4.8%) \textit{Staphylococcus auricularis}, 6 (14.3%) \textit{Bacillus cereus}, 2 (4.8%) \textit{Bacillus lentus}, 1 (2.4%) \textit{Bacillus pantothenticus}, 3 (7.1%) \textit{Micrococcus roseus}, 3 (7.1%) \textit{Micrococcus sedentarius}, 2 (4.8%) \textit{Micrococcus lylae} and 2 (4.8%) \textit{Streptococcus lentus}.
The 21 isolates of Gram negative bacteria were 6 (14.3%) \textit{E.coli}, Fig (1) & (2) demonstrated growth of \textit{E. coli} on MacConkey’s agar & Eosin Methylene blue agar medium consecutively. 3 (7.1%) \textit{Escherichia hermanii}, 2 (4.8%) \textit{Klebsiella ozaenae}, 1 (2.4%) \textit{Klebsiella pneumoniae}, 1 (2.4%) \textit{Klebsiella aerogenes}, 3 (7.1%) \textit{Citrobacter koseri}, 3 (7.1%) \textit{Acenitobacter calcoaceticus} and 2 (4.8%) \textit{Pseudomonas areuginosa}.

3.1.2 Bacteria isolated from conjunctiva:
The samples collected from conjunctiva were 3. Two samples showed a positive growth while the third was found negative. The 2 positive samples gave three isolates. One (33.3%) isolate was Gram positive bacteria which
was *Bacillus alvei*.

The other two isolates were Gram negative bacteria, 1 (33.3%) was *Acenitobacter woffii* and 1 (33.3%) was *Klebsiella ozaenae*.

### 3.1.3 Bacteria isolates from trachea:

Twenty five samples were collected from trachea. Twenty two samples showed positive growth while the other 3 samples were found negative. The 22 samples gave 24 isolates. Thirteen isolates were Gram positive bacteria, 2 (8 %) *Staphylococcus aureus*, 2 (8 %) *Staphylococcus epidermidis*, 1 (7.7%) *Staphylococcus chromogen*, 1 (4 %) *Staphylococcus epidermidis*, 1 (4 %) *Bacillus cereus*, 2 (8 %) *Bacillus pantothenticus*, 2 (8 %) *Micrococcus roseus*, 1 (4 %) *Micrococcus lylae* and 1 (4 %) *Streptococcus pneumoniae*.

The other 11 isolates were Gram negative bacteria, 4 (16 %) isolates were *E.coli*, 2 (8 %) isolates of *Escherichia hermanii*, 2 (8 %) *Klebsiella ozaenae*, 2 (8 %) *Citrobacter koseri* and 1 (4 %) *Acenitobacter woffii*.

### 3.1.4 Bacteria isolated from lungs:

The 8 samples collected from lung gave 6 positive samples. The six positive samples gave 7 isolates. Four isolates were Gram positive bacteria, 2 (25 %) isolates were *Staphylococcus capitis*, 1 (12.5%) *Bacillus thuringiensis* and 1 (12.5%) *Streptococcus pneumoniae*.

The other 3 were Gram negative, 1 (12.5%) *E.coli*, 1 (12.5%) *Citrobacter koseri* and 1 (12.5%) *Pseudomonas maltophilia*. Frequency of bacterial isolates from infected organs is shown in table (1). Number & % of Gram +ve & -ve isolates isolated from different organs are demonstrated in tables (2) & (3).
3.2 **Characters and biochemical reactions:**
Characters and biochemical reactions of Gram-positive and Gram-negative bacterial isolates showed in tables (4) & (5).

3.3 **Antibacterial sensitivity of the isolated bacteria:**
Table (6) summarized the number and percentage of sensitive isolates to antibacterial examined. Fig. (3) demonstrated sensitivity test of Gram-positive bacteria. The isolates were highly sensitive to Gentamicin (85.7%) followed by Ofloxacin (78.6%), Chloramphenicol (75%), Co-Trimoxazole (71.4%), Amikacin (66.7%), Piperacillin/Tazobactam (58.3%), pefloxacin (50%), Cefotaxime (46.4%), Cloxacillin (43.75%) Ampicillin (42.9%), Ceftizoxime (41.7%), Ciprofloxacin (32.1%), Cephalexin (31.25%), Tetracycline (28.6%), Roxythromycin (18.75%) while the Lincomycin (12.5%) showed the least inhibition to the growth. Detailed results of the No. of the sensitive and resistant bacterial spp. Are shown in table 7 & 8.
Table (1) Isolation frequency of aerobic bacteria from respiratory tract of infected chicken in Khartoum North area.

<table>
<thead>
<tr>
<th>Samples source</th>
<th>No. of samples examined</th>
<th>Total No. of isolates (%)</th>
<th>No. of Gram positive isolates (%)</th>
<th>No. of Gram negative isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nostrils</td>
<td>42</td>
<td>55 (130.95%)</td>
<td>34 (80.95%)</td>
<td>21 (50%)</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>3</td>
<td>3 (100%)</td>
<td>1 (33.33%)</td>
<td>2 (66.67%)</td>
</tr>
<tr>
<td>Trachea</td>
<td>25</td>
<td>24 (96%)</td>
<td>13 (52%)</td>
<td>11 (44%)</td>
</tr>
<tr>
<td>Lung</td>
<td>8</td>
<td>7 (87.5%)</td>
<td>4 (50%)</td>
<td>3 (37.5%)</td>
</tr>
</tbody>
</table>
Table (2) Gram positive bacteria isolated from respiratory tract of infected chickens in Khartoum North area.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number (%) of isolates from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nostrils n = 42</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>7 (16.7%) - 2 (8 %)</td>
</tr>
<tr>
<td><strong>Staphylococcus gallinarum</strong></td>
<td>4 (9.5%) -</td>
</tr>
<tr>
<td><strong>Staphylococcus auricularis</strong></td>
<td>2 (4.8%) -</td>
</tr>
<tr>
<td><strong>Staphylococcus epeidermidis</strong></td>
<td>2 (4.8%) 2 (8 %)</td>
</tr>
<tr>
<td><strong>Staphylococcus chromogen</strong></td>
<td>- 1 (4 %) -</td>
</tr>
<tr>
<td><strong>Staphylococcus capitis</strong></td>
<td>- - 2 (25 %)</td>
</tr>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td>6 (14.3%) -</td>
</tr>
<tr>
<td><strong>Bacillus lentus</strong></td>
<td>2 (4.8%) -</td>
</tr>
<tr>
<td><strong>Bacillus alvei</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus pantothenicus</strong></td>
<td>1 (2.4%) 2 (8 %)</td>
</tr>
<tr>
<td><strong>Bacillus thuringiensis</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Micrococcus sedentarius</strong></td>
<td>3 (7.1%)</td>
</tr>
<tr>
<td><strong>Micrococcus roseus</strong></td>
<td>3 (7.1%) 2 (8 %)</td>
</tr>
<tr>
<td><strong>Micrococcus lylae</strong></td>
<td>2 (4.8%) 1 (4 %)</td>
</tr>
<tr>
<td><strong>Streptococcus pneumoniae</strong></td>
<td>- - 1 (4 %) 1 (12.5%)</td>
</tr>
<tr>
<td><strong>Streptococcus lentus</strong></td>
<td>2 (4.8%) -</td>
</tr>
</tbody>
</table>
Table (3) Gram negative bacteria isolated from respiratory tract of infected chickens in Khartoum North area.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Nostrils (n = 42)</th>
<th>Conjunctiva (n = 3)</th>
<th>Trachea (n = 25)</th>
<th>Lung (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>6 (14.3%)</td>
<td>-</td>
<td>4 (16 %)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td><em>Escherichia hermanii</em></td>
<td>3 (7.1%)</td>
<td>-</td>
<td>2 (8 %)</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella ozaene</em></td>
<td>2 (4.8%)</td>
<td>1 (33.3%)</td>
<td>2 (8 %)</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>1 (2.4%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella aerogenes</em></td>
<td>1 (2.4%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>2 (4.8%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas maltophilia</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td><em>Acinetobacter calcoaceticus</em></td>
<td>3 (7.1%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Acinetobacter woffii</em></td>
<td>-</td>
<td>1 (33.3%)</td>
<td>1 (4 %)</td>
<td>-</td>
</tr>
<tr>
<td><em>Cetrobacter koseri</em></td>
<td>3 (7.1%)</td>
<td>2 (8 %)</td>
<td>1 (12.5%)</td>
<td>-</td>
</tr>
</tbody>
</table>
Table (4) Characters and biochemical reaction of Gram-positive bacteria isolated from respiratory tract of infected chickens in Khartoum North area.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>+ + - F - NT + + + - + + + +</td>
</tr>
<tr>
<td>Staphylococcus gallinarum</td>
<td>+ + - F - NT - + - + + - + +</td>
</tr>
<tr>
<td>Staphylococcus auricularis</td>
<td>+ + - F - NT - + - - - - - -</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>+ + - F - NT - + + - - + + + +</td>
</tr>
<tr>
<td>Staphylococcus chromogenes</td>
<td>+ + - F - NT - + + - - + + + +</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>+ + + + - + C NT - - - - + - +</td>
</tr>
<tr>
<td>Bacillus lentus</td>
<td>+ + + F + C NT - - - - - - - -</td>
</tr>
<tr>
<td>Bacillus alvei</td>
<td>+ + + + - + C NT - - - - - - -</td>
</tr>
<tr>
<td>Bacillus pantothenicus</td>
<td>+ + + F + T NT - - - - - - - +</td>
</tr>
<tr>
<td>Bacillus thuringiensis</td>
<td>+ + + + - + C NT - - - - - - + +</td>
</tr>
<tr>
<td>Micrococcus sedentarius</td>
<td>- + + O _ NT NT - - - NT - NT -</td>
</tr>
<tr>
<td>Micrococcus roseus</td>
<td>+ + + + - - NT NT - NT NT NT - NT +</td>
</tr>
<tr>
<td>Micrococcus lylae</td>
<td>- + + O - NT NT - NT NT NT NT - NT -</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>+ - - F - NT NT + + NT - - NT -</td>
</tr>
<tr>
<td>Streptococcus lentus</td>
<td>+ - - F - NT NT + NT + + NT -</td>
</tr>
</tbody>
</table>

+ = Positive reaction, _ = Negative reaction, F = Fermentative, O = Oxidative, NT = Not tested, C = Central spore, T = Terminal spore.

1 = Glucose, 2 = Catalase, 3 = Oxidase, 4 = O:F, 5 = Motility, 6 = Spore forming, 7 = Coagulase, 8 = Sucrose, 9 = Lactose, 10 = Xylose, 11 = Manitol, 12 = VP, 13 = Urease, 14 = Nitrate.
Table (5) Characters and biochemical reactions of Gram negative bacteria isolated from respiratory tract of infected chickens in Khartoum North area.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Characters</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<th>5</th>
<th>6</th>
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<th>13</th>
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</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>-</td>
</tr>
<tr>
<td>E.coli hermanii</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Klebsella ozaenae</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Klebsella aerogenes</td>
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<td>Klebsella pneumonia</td>
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<td>F</td>
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<td>Pseudomonas aeruginosa</td>
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<tr>
<td>Pseudomonas maltophilia</td>
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<td>+</td>
<td>_</td>
<td>_</td>
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<tr>
<td>Acinetobacter calcoaceticus</td>
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<td>Acinetobacter woffii</td>
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<td>+</td>
<td>_</td>
<td>O</td>
<td>_</td>
<td>_</td>
<td>_</td>
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<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>NT</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Citrobacter koseri</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>F</td>
<td>+</td>
<td>+</td>
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<td>_</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Keys:** + = Positive reaction, _ = Negative reaction, F = Fermentative, O = Oxidative, NT = Not tested.

1 = Glucose, 2 = Catalase, 3 = Oxidase, 4 = O\'F, 5 = Motility, 6 = Sucrose, 7 = Lactose, 8 = Maltose, 9 = Salicin, 10 = Sorbitol, 11 = Xylose, 12 = Urease, 13 = VP, 14 = H2S, 15 = Indole, 16 = Citrate.
Table (6) Antibacterial sensitivity of bacterial isolated from respiratory tract of infected chicken in Khartoum North area.

<table>
<thead>
<tr>
<th>Antimicrobial drug</th>
<th>No. of bacterial isolates examined</th>
<th>No. of sensitive isolates (percentage)</th>
<th>No. of resistant isolates (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>28</td>
<td>12 (42.9%)</td>
<td>16 (57.1%)</td>
</tr>
<tr>
<td>Co-Trimoxazole</td>
<td>28</td>
<td>20 (71.4%)</td>
<td>8 (28.6%)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>28</td>
<td>13 (46.4%)</td>
<td>15 (53.6%)</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>12</td>
<td>7 (58.3%)</td>
<td>5 (41.7%)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>12</td>
<td>9 (75%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>28</td>
<td>9 (32.1%)</td>
<td>19 (67.9%)</td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>12</td>
<td>5 (41.7%)</td>
<td>7 (58.3%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>28</td>
<td>8 (28.6%)</td>
<td>20 (71.4%)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>28</td>
<td>22 (78.6%)</td>
<td>6 (21.4%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>28</td>
<td>24 (85.7%)</td>
<td>4 (14.3%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>12</td>
<td>8 (66.7%)</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>28</td>
<td>14 (50%)</td>
<td>14 (50%)</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>16</td>
<td>5 (31.25%)</td>
<td>11 (68.75%)</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>16</td>
<td>7 (43.75%)</td>
<td>9 (56.25%)</td>
</tr>
<tr>
<td>Roxythromycin</td>
<td>16</td>
<td>3 (18.75%)</td>
<td>13 (81.25%)</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>16</td>
<td>2 (12.5%)</td>
<td>14 (87.5%)</td>
</tr>
</tbody>
</table>
Table (7) The antibacterial sensitivity of Gram positive bacterial species isolated from respiratory of infected chickens in Khartoum North area.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No. of examined isolates</th>
<th>No. of isolates inhibited by antibacterial drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AS</td>
<td>BA</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>6</td>
<td>3(++)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3</td>
<td>2(+)</td>
</tr>
<tr>
<td>Staphylococcus gallinarum</td>
<td>3</td>
<td>2(+)</td>
</tr>
<tr>
<td>Staphylococcus epedermidis</td>
<td>2</td>
<td>2(-)</td>
</tr>
<tr>
<td>Streptococcus lentus</td>
<td>2</td>
<td>2(++)</td>
</tr>
</tbody>
</table>

(AS) Ampicillin/Sulbactam (20 mcg), (BA) Co-Trimoxazole (25mcg), (PR) Cephalexin (30mcg), (TE) Tetracycline (30 mcg), (CF) Cefotaxime (30 mcg), (CP) Ciprofloxacin (5 mcg), (PF) Pefloxacin (10mcg), (OF) Ofloxacin (5mcg), (CX) Cloxacillin (1mcg), (RF) Roxythromycin (15mcg), (LM) Lincomycin (2 mcg) and Gentamicin (10 mcg)

Inhibition zone: (++++) = 25 mm, (+++) = 20 mm, (+++) = 15, (+) = 10 mm, (-) no inhibition.
**Table (8) The antibacterial sensitivity of Gram negative bacterial species isolated from respiratory of infected chickens in Khartoum North area.**

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No. of examined isolates</th>
<th>No. of isolates inhibited by antibacterial drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AS</td>
<td>BA</td>
</tr>
<tr>
<td>E. coli</td>
<td>7</td>
<td>2(+)</td>
</tr>
<tr>
<td>Citrobacter koseri</td>
<td>2</td>
<td>2(-)</td>
</tr>
<tr>
<td>Acinetobacter woffii</td>
<td>2</td>
<td>1(+)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>1</td>
<td>1(-)</td>
</tr>
</tbody>
</table>

(AS) Ampicillin/Sulbactam (20 mcg), (BA) Co-Trimoxazole (25mcg), (CF) Cefotaxime (30 mcg) (TZP) Piperacilin/Tazobactam (100/10 mcg), (CH) Chloramphenicol (30 mcg), (CP) Ciprofloxacin (5 mcg), (CI) Ceftizoxime (30 mcg), (TE) Tetracycline (30 mcg), (10mcg), (OF) Ofloxacin (5mcg), Gentamicin (10 mcg) (AK) Amikacin (30 mcg), and (PF) Pefloxacin.

Inhibition zone: (++++) = 25 mm, (+++) = 20 mm, (++) = 15, (+) = 10 mm, (-) no inhibition.
Fig. 1 : Growth of *E. coli* on MacConkey's agar

Fig. 2 : Growth of *E. coli* on Eosin Methylene Blue Agar
Fig. 3 Sensitivity test of Gram-positive bacteria
CHAPTER FOUR
DISCUSSION

This study was carried out to isolate and identify aerobic bacteria infecting respiratory tract of chickens in Khartoum North area. Seventy eight samples were collected from infected chickens and cultured. In this work the bacterial isolates were obtained from nostril, conjunctiva, trachea and lung. Fourteen samples did not show any bacterial growth despite of clear respiratory symptoms, this phenomenon may be due to Mycoplasma or viral infections. Sixty four samples showed bacterial growth and gave 89 isolates. 

*Pseudomonas* species were isolated from nostrils of infected chickens. This agrees with other studies which reported the isolation of *Pseudomonas* species from nostrils of infected chickens (Valadae, 1961; Saad *et al*., 1981; Andreev *et al*., 1982; Bapat *et al*., 1985 and Mrden, 1988). In sudan *Pseudomonas aeruginosa* was isolated from cases of substantial deaths among young chickens (Elnasry, 1997) and Mohamed *et al*. (1996). In this study *E. coli* was isolated from nostrils, trachea and lung of infected chickens. Several authors reported the isolation of *E. coli* from respiratory tract of infected chickens (Price *et al*., 1957; Sojka *et al*., 1961; Mac Martin, 1962; Khogali, 1970; Mouline, 1983; Eisa and Elnasry, 1985; Mahgoub, 1986; Linzitto *et al*., 1988; Malokwa *et al*., 1987; and Elnasry, 1997). Also other study confirms isolation *E. coli* from lung (Hofstad *et al*., 1978; Rajashekar *et al*., 1998; and Abdellah, 2003). Zahida (2004) described the respiratory tract as the primary route of invasion by *E. coli*. The lesions and symptoms of respiratory infection may be aggravated when secondary *E. coli* invasion occurred. Sanikbi (1987) and Seethe (1988)
reported the complication of infectious coryza with *E. coli* which led to chronic disease. History of recent Newcastle disease vaccination or ammonia pollution was not ruled out as predisposing factors to *E. coli* infection (Bakhiet *et al*., 1990). History of infectious bursal disease might have had a role in *E. coli* infections (Pages *et al*., 1985). Nighot (2002) reported that faulty management and lack of routine vaccination against some viral diseases may lead to activation of commensally living bacterial forming the normal flora challenges the natural immunity and defense mechanism.

*Staphylococcus* species were isolated from nostril, trachea and lung of infected chickens in this study also *Staphylococcus* species was isolated from respiratory tract infection (Bibersein *et al*., 1974; Linzitto *et al*., 1988; and Elnasry 1997).

In the present investigation *Streptococcus* species were isolated from respiratory tract of infected chickens this is in agreement with the findings of Linzitto *et al*., (1988).

The antibacterial sensitivity of the isolated bacteria obtained in this study to antibacterial drugs was variable. In the present study, bacteria isolated showed resistance to many antibiotic commonly used for treatment of bacterial diseases in animals.

Members of enterbacteriaceae isolated in this study showed very high resistance to Ampicillin and Tetracycline; this result is partially similar to that reported by Mohamed (2005) who reported that, the enterbacteriaceae species isolated from mastitic cows, goats milk, infected equine uterus and infected humans were completely resistant to Ampicillin. *E.coli* isolated in this study was found sensitive to Gentamicin (100%). This finding agrees with that of Ahmed (2006), who reported that *E. coli* isolated from calf
feaces was completely sensitive to Gentamicin and in agreement with that of Orden (2000) who found that *E. coli* strains isolated from diary calves affected by neonatal diarrhea were susceptible (89-95%) to Gentamicin. *Staphylococcus epidermidis* examined in this study showed high resistance to Ampicillin, this finding agrees with Forbes *et al.* (1998) who reported that Staphylococci are Gram-positive bacteria that most commonly produce B-lactamase and approximately 90% or more of clinical isolates are resistant to Penicillin and Ampicillin as a result of the enzyme production. This antibiotics resistance can be attributed to many factors, the extensive use of antibiotics often without prescription from qualified veterinarians, the animal owners usually use the drugs with subdosing and incomplete duration of the treatment and they use one type of drug for a long period. Also use of broad spectrum antibiotics without proper isolation of the causative agent and drug sensitivity testing is a real cause of resistance.
Conclusions and Recommendations

Conclusions:

The result of the present study demonstrated that:-

1. Both Gram-positive and Gram-negative bacterial pathogens were isolated from respiratory tract of infected chickens.
2. Mycoplasma and pathogens other than bacteria could be a cause of respiratory tract infection of chicken as 14 (17.9%) of samples collected from infected chickens did not show bacterial growth.
3. Respiratory tract bacterial infections could be an important constrain in poultry industry in Khartoum state.
4. Many bacterial isolates were found sensitive to Gentamicin (85.7%), Ofloxacin (78.6%) and Chloramphenicol (75%).
5. Antibiotic drug resistance may be due to use of the drugs with sub dosing and incomplete duration of the treatment or extensive use of these antibiotics also use one type of drug for a long period.

Recommendations:

From results and discussion of this study, the following recommendations are suggested.

1. The high prevalence of *E. coli* associated with infection of chickens needs further study.
2. The future studies should be considered to minimize the spread of bacteria respiratory diseases.
3. The role of the others pathogens such as mycoplasmas and viruses
should be studied.

4 Sensitivity test of bacterial isolates should be conducted using more variable types of antibacterial drugs to determine the most effective drugs those kill or inhibit the bacterial growth.
REFERENCES


