Sudan Universityof Science and Technology

College of Graduate Studies

Antibiotic Resistant Microflora Bacteria in Intestine of Broiler Chicken

بكتيريا الفلورا الميكروبيه المقاومه للمضادات الحيوية في امعاء الدجاج اللاحم

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DEDICATION

This work is dedicated to

MY Family

You have always pushed me to pursue my passions in the life and I'm forever indebted to you.....

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Abstract

This study was done to investigate antibiotic resistance of micro flora isolated from cloacal swabs ofhealthy broiler chicken aged 57-60 day to 9 different antibiotics.

A total of 50 cloacal swabs was collected from two farms in Omdurman county(Khartoum state) 72 aerobic bacteria were isolated, 15(21%) were Gram positive while the remaining 57(79%) were Gram negative bacteria.

Susceptibility test were done using disc diffusion method for the isolated bacteria .Only 4 Gram negative isolates were found sensitive to all antibiotics used ,the remaining 53 isolates showed different resistance to antibiotic.Higher resistance was observed in cefapime while higher sensitivity was detected in Gentamycin.

Gram positive bacteria showed different resistancepattern toantibiotics. Higher resistance was observed to cefapime and erythromycin while higher sensitivity was detected to colistin, gentamycin and amoxicillin.Multi resistance pattern was detected in 34 (47%) of isolated bacteria.

Regarding antibiotics higher sensitivity was found to colistin (75%) followed by gentamicin (53%) and ciprofloxacin (45%) while only (9%) of the isolates showed sensitivity to cefapime. Higher resistance was detected to cefapime (76%) followed by doxycycline (53%) and tetracycline (49%) while 7 (10%) isolates were found resistant to colistin

ملخص الدراسة

أجريت هذه الدراسة بغرض معرفه مقاومه البكتيريات المعوية الموجودة في الفلورا الطبيعية للمضادات الحيوية. تم اخذ مسحات شرجية من دواجن لحم سليمة ظاهريا أعمارها تتراوح بين57-60 يوم .

جمعت خمسون مسحه شرجية من مزرعتين بامدرمان ولاية الخرطوم . تم عزل 72 بكتيريا هوائيه منها15 (21%) موجبه لصبغه غرام57 (79%) سالبه لصبغه غرام.

إجري اختبار الحساسية للعينات المعزولة، أظهرت النتائج وجود أربع عينات فقط من البكتيريا السالبة لصبغه غرام حساسية لجميع المضادات الحيوية المستعملة.كما أظهرت53عزله مقاومه مختلفة للمضادات الحيوية اعلي مقاومه كانت للسيفابيم واعلي حساسية كانت للجنتامايسين.

كما أوضحت نتائج البكتيريا الموجبة لصبغه غرام، مقاومه مختلفة للمضادات الحيوية واعلي مقاومه كانت للسيفابيم والارثرومايسين والاموكسيسلين.

في جميع البكتريات المعزولة كانت هنالك 34(47%) بكتيريا لها مقاومة متعددة للمضادات الحيوية المستعملة في الدراسة.

أعلي حساسية كانت للكولستين(75%)، جنتامايسين (53%) يليه سبروفلاكساسين(45%)، (9%) من العزلات فقط كانت حساسة للسيفابيم وهي اقل حساسية سجلت.

أما اعلي مقاومه كانت للسيفابيم(76%) يليه دوكسايكلين(53%) يليه التتراسايكلين(49 %) وقد كما أظهرت 7 عينات(10%)مقاومه للكولستين.

Introduction

Poultry production provides the human population with two main products eggs and meat (Freiji, 2008).Poultry meat well fit the current consumer demand for a low fat meat with a high instauration degree of fatty acids and low sodium and cholesterol levels. Poultry meat may also be considered as functional food, which provides bioactive substances with favorable effects on human health, like conjugated Linoleic acid (CLA), Vitamins and antioxidants (Petracci and Cavani 2012)

In Sudan Poultry industry was estimated to be 5 million birds. Modern intensive poultry farming system were established in the country mainly in Khartoum State. The total number of broiler farms in Khartoum State was estimate as 619 farms. (Ministry of agriculture and animal resources. 2015)

Antibiotics in poultry production is an important tool to control outbreaks of various poultry diseases that threaten poultry industry Antibiotics were also used as prophylaxis, growth promoter and sometimes as alternatives to biosecurity (Saif *et al*., 2008).

Now a day's Antibiotics resistance is a serious global problem that facing both human and animal health. Misuse of different antibiotics in animal's leads to the emerging of resistant bacteria. Bacterial resistance to antimicrobial drugs has become an issue of increased public concern and scientific interest during the last decade. This resulted from a growing concern that the use of antimicrobial drugs in veterinary medicine and animal husbandry may compromise human health if resistant bacteria develop in animals and are transferred to humans via the food chain or the environment. While there is still no consensus on the degree to which usage of antibiotics in animals contributes to the development and dissemination of antimicrobial

resistance in human bacteria, experimental evidence and epidemiological and molecular studies point to a relationship between antimicrobial use and the emergence of resistant bacterial strains in animals, and their spread to humans, especially via the food chain.

Growth promoters are usually given in a sub therapeutic dose (which is lower than the Minimum Inhibitory Concentration MIC for pathogen). Continuing Sub-Therapeutic dose of these antibiotics is the most important factor leading to resistance to antibiotic by microorganism in poultry (Henning and Marianne, 2001).

Different international organizations such as the Food and Agriculture organization (FAO) and the Official International Des Epizootics (OIE) World Health Organization (WHO) (2004) has already given global recommendation for antimicrobial use in agriculture production.

Bacteria are one of the most diverse groups of organisms on the planet existing ubiquitously in interdependent communities in any given environment (Whitman *et al* 1998) Consequently higher organisms such as birds and mammals have had to adapt to a world full of microbes and interact with many different bacteria on daily basis resulting in symbiotic, competitive and pathogenic relationships **(**Hooper and Gordon 2001)all of these interactions are exhibited in the GI tract (Zoetendal*et al*2004.). Along the entire GI tract there is a diverse microbial community consist of bacteria, yeasts, archaea, ciliate protozoa, anaerobic fungi and bacteriophages (Mackie, 2002**)** commonly referred to as the intestinal microbiota (Guan *et al* 2003) .

Avian intestine have been considered as a reservoir of different bacteria including facultative aerobes such as enterobactericeae, lactobacillus and

streptococcus and anaerobes such as clostridia (Barnes *et al*., 1972 and Gong *et al*., 2008).

Resistant organisms from animals reenter the human and animal population through water, vegetables and food. Resistant bacteria are shed in faeces where they can share antibiotic resistance plasmid with native bacteria and may be disseminated to other animals.

The overuse and misuse of antibiotic in poultry production has lead to development of antibiotic resistance among food borne pathogens that can cause illness to human (Capita and Calleja2013). Resistance rate in almost all types of bacteria are much higher in developing than in developed countries (Collignen, 2012).

Problem statement:

Misuse of antibiotics in poultry industry in the country is a common practice leading to the development of bacterial resistance to different antibiotics. Intestinal antibiotic resistant bacteria in broiler might contaminate the whole carcasses during evisceration process and hence transmitted to human through direct or cross contamination, chicken flock reared for shawerma might exposed to more antibiotics due to it long life span (60) day in comparison to broiler chicken which considered main cause of resistant development .

Objective of study:

General objectives:

- To assess resistance of intestinal bacterial flora of broiler to certain antibiotics

Specific objectives:

- To determine aerobic bacteria in the intestinal flora of broiler chicken.
- To determine the susceptibility of isolates to commonly available antibiotics.
- To determine patterns of resistance of isolates from broiler chickens.

Chapter one

Literature review

1.1Gastrointestinal tract in chicken (GIT)

The gastrointestinal GIT tract of chicken is composed of different organs extending from the beak to the cloaca. Food picked up by beak cutter the mouth which contain glands secreting saliva and some enzymes where Digestion of food starts. The tongue pushs the feed to the back of the mouth so that it can be swallowed. The esophagus allows the passage of food from the beak to the crop, at the crop the food is moistened and softened by the mucus secretion (Dibner and Richards., 2004).In the proventriculus HCL, pepsin and mucus are secreted; digestion continues into the gizzard (Leeson and Summers, 2001)where the grinding effect of the gizzard breaks down large particles of feed. Food from the gizzard enters the small intestine as digesta. The small intestine serves an important function for nutrient digestion and absorption and overall health of the bird. There are three parts of the small intestine duodenum, jejunum and ileum. As Sklan(2001) has reported, digestive enzymes, such as trypsin, amylase and lipase, are secreted by the pancreas located in the duodenal loop and play an important role in the breakdown of complex nutrients into their simpler form for absorption. These small molecules are further broken down by "brush border" enzymes, produced in the enterocytes of the small intestine. The small intestine is lined with villi which are structures extending from the muscular layer on the intestinal wall. On the outside of each villus are microscopic structures known as microvillie. (Uni*et al*. 1995) noted that the villi folds and microvilli increase the surface area of the intestinal lining. Goblet cells which located in the outer lining of the villi produce mucin that functions as protective

mucosal coating and providing a nutrient source for the residing normal intestinal bacterial community (Geyra *et al*., 2001), the digesta then passes into the large intestine which responsible for water absorption. There are two cloacal pouches, which harbor a large population of anaerobic microbes, in numbers far greater than any other area in the GIT (Dibner and Richards ;2004).

The terminal part of the GIT is the Cloaca In this part a mixing of the digestive wastes together with wastes from the urinary system (urates)exsit. Fecal material is usually voided as digestive waste with white uric acid crystals on the outer surface. The reproductive tract also exits through cloaca but when a hen lays an egg the vagina folds over to allow the egg to leave through the vent without coming into contact with the faeces or urine (Dibner and Richards ;2004).

1.2 Development of GIT in chicken

1.2.1 Pre-Hatch Development

(Uni et al. 2003a)found that through the progression of incubation the embryo increases in body weight (BW) and the small intestine grows at a faster rate than the BW.The earliest signs of development in the GIT occur at 11 d of incubation where there is an increase in duodenal epithelial cells. At 14 to 16 d of incubation the villi start to emerge from the muscular layer with the presence of pre-villus ridges, which later develop into the mature villus (Grey, 1972). Sklan (2001) found that at 17 d of incubation the small intestines are rapidly developing and make up to 1% of the embryos BW.

Marchain and Kulka (1967) observed that, prior to hatch, pancreatic enzymes start to be produced and increase in concentration closer to the time of hatch.

1.2.2 Post-hatch development

A well-developed GIT is crucial for utilization of exogenous nutrients and proper nutrient digestion and absorption. After hatch, the chick is exposed to different factors that differ significantly from what the chick embryo encounter during incubation, such as feed, water and the type of diet. One of the most critical changes the chick experiences is the change in diet from a lipid rich yolk diet to an almost grain-based diet high in carbohydrates (Sklan, 2001). The early contact of chicks to feed helps stimulate intestinal motility and will increase the rate at which the yolk is fully absorbed through the intestine(Noy*etal*, 2001) .In addition to yolk absorption, the GIT of the chick undergoes rapid development and morphological changes immediately following hatch. And continues until 6 to 10 d of age (Sklan. 2001).

1.3 Bacterial Microflora in GIT of chicken

Different bacterial species were found in the GIT of chicken as anormal flora. Number, type and speciesof these bacteria differ from organ to other (Table1). Nutritional composition of feed will impact the intestinal flora The preferred substrate of the intestinal bacteria is carbohydrates, which the bacteria are able to ferment to produce volatile fatty acids and protein as a source of bacterial biomass which can benefit the host (Apajalahti, 2005). The host provides a food source (substrate) to the bacterial community, which can dictate the biodiversity of the intestinal bacteria. Through substrate specificity and the use of prebiotic feedings, the percentage of beneficialbacteria in the host can be increased (Sonnenburg *et al.* 2005 and Lan *et al.*2005).

The microflora population can be affected by different factor such as ingestion of various bacteriaspecies from hatchery debris, feed, and water; factors at farms such as litter management, cleaning of houses between

flocks and bio-security (Mead and Adams, 1975) through the years, the assessment of microbial populations has relied on in vitro culture techniques using selective or non-selective media. One of the main disadvantages of culture-based method is that it can only assess culturable microorganisms (Barnes *et al*., 1972; Bjerrum *et al.*, 2006). Recently, other culture independent methods that rely on molecular techniques have been developed to study bacterial populations(Gong *et al*., 2008; Amit-Romach *et al*., 2004; Bjerrum *et al*., 2006; Pissavin *et al.*, 2012) .Different bacterial species were isolated from GIT of chicken by several workers Table(1) .

Table (1) Summary of publication related to bacterial species of the chicken intestinal micro flora

Organ	Bacterial species	References
Crop	Lactobacilli *Ecoli*,Salmonella,Campylobacter	Sarra *etal*(1992)
Proventriculus	Gram+ve bacilli	Smith(1965)
Gizzard	Campylobacter,*E. coil*	Smith *et al*(2005)
Duodenum/ileum 2week old chicks	Lactobacilli, *Ecoli*,Streptococcus	Smith(1965)Barnes *etal*(1972) Jin *et al* (1997)
Ileum of adalt	Lactobacilli	Bjerrum etal (2006)
Caecum	Enterobacter,enterococus,lactobacilli, bacteriods,eubacterum ,E.coli,eubacter staphylococcus, streptococuss	Vander *etal*(2001) Jin *et al* (1997)
Caecum mature bird	*Clostridum spp*,,Spororusaspp Bifidobacteria,	Zhu *etal* (2002)
Cloacal	*E.coli,protus, vlgaris, E.hermannii,*	Vander *etal*(2001)
Ceacal of young bird	Anaerobic gram +ve, *Clostridium* Spp, lactobacilli,fusobacteruim	Habib *etal*(2007)
GIT of chicken	Staphylococcus	Sleekes(1997)Casey e*tal*(2007)
	Shigella spp	Sackey *et al*(2001)
	Salmonella spp	Michael and Lrry (2007) Madadgar *et al* (2008) Zhao *etal* (2001)
	Enterobacter	Sleekes(1997)Casey *etal*(2007)
	Enterococcus	Zhou(2007)
	Provedencia	Manos and Belas (2006) Foti *etal*(2009) Interaminense (2010)

1.4 Uses of antibiotic in broiler production

Different types of antibiotics have been used in poultry industry worldwide for three main purposes, treatment, prophylaxis and growth promoters .Antibiotics are added to poultry feed to reduce the effect of crowded environment and poor hygiene (Johnson *et al*, 2008).

1.5 Effects of antibiotics on intestinal microflora

Antibiotics in chicken are used as therapeutics, growth promoters and preventive measures Pedroso *etal* (2006) stated that broilers raised either in battery cages or in floor pens when treated with antibioticchanges in the composition of the intestinal bacterial community were observed. However, the changes in the composition of the intestinal bacterial community induced by antibiotics may be related toimprovement in growth performance. In general, the use of low doses of antibiotics decreases the number of the most susceptible bacterial communities and enhances the growth of the resistant bacteria.

1.6 Mechanisms of action of antibacterial agents

Antibiotics are mainly divided into two categories according to their action on bacterial cell bactericidal and bacteriostatic mechanism of different antibiotics family were shown intable (2)

Table (2) Types and mode of action of antibiotics

Group	Example	Modeof action
Beta-lactams	Penicillins.Cephalosporis Carbapenems.monobactams	Bactericidal inhibit cell Wallwall synthesis
Glycopeptides	Vancomycin.Teichoplanin Telavancin	Inhibit cell wall wall synthesis Bactericidal
Macrolides ketolides	Azithromycin.Telithromycin Erythromycin.clarithromycin	Inhibit proterin prosynthesis bacteriostatic
Aminoglycoside	Gentamicin.Amikacin.Tobram Netilmicin.streptomycin	Inhibit protien pin synthesis Bactericidal
Tetracyclines Glycylcyclines	Tetracycline.Tigecycline Doxycycline	Inhibit protien n synthesis Bacteriostatic
Quinolones	Ciprofloxacin,Norfloxacin Levofloxacin.moxifloxacin	Inhibit DNA gyrase Bactericidal
Lincosamides	Clinda.mycin	Inhibits protein synthesis Bactericidal/ static
Streptogramins	Quinupristin/dalfopristin	Inhibits protien ein synthesis Bactericidal
Oxalidinones	Linezolid	Inhibits protein synthesis Bacteriostatic
Polymixins	Colistin.Polymixin B	Destroys cell membrane structure Bactericidal
chloramphenico	chloramphenicol	Inhibits protein synthesis Bacteriostatic
Sulfa drugs	Sulfamethoxazoletrimehoprim	Inhibits DNA synthesis Bactericidal

1.7 Antibiotic resistance

Antimicrobial resistance is the ability of a microorganism to survive and multiply in the presence of an antimicrobial agent that would normally inhibit or kill this particular kind of organism (Microbiology model, 2011).the primary cause of antibiotic resistance is genetic mutation in the bacterial cell this gene can be transfer between bacteria by conjugation, transduction or transformation in addition most resistant genes were located in the plasmids that assist it is transfer between different bacterial species. Single bacteria may carry several resistant genes and hence known as multiresistant (Qu et al., 2008; Zhou *et al*., 2012).Evidence suggests a number of pathogens can colonize both the human and chicken GIT (Johnson et al., 2008, Johnson et al 2009; Gipp et al., 2011), providing opportunities for gene exchange. Microbes colonizing the chicken GIT are an abundant source of antibiotic resistance genes (Qu et al., 2008; Zhou et al., 2012).

1.8 Mechanism of antibiotic resistance

Antibiotic resistance can be caused by different mechanisms such as the presence of enzymatic in-activation of the agent, mutation in bacterial receptor which results in reduction of binding antibiotics ,active efflux of the antimicrobial agent,in additionresistance maycaused by unrecognized mechanisms(Tenover *et al*2004).

1.9Antibiotic resistance in normal flora in chicken

From different bacteria isolated fromnormal flora it was found that Salmonella were resistant to tetracycline,staphylococci resistance to both tetracycline and oxacillin while Enterobacteriaceae isolates were Resistane toboth tetracycline and streptomycinGouws and Brözel(2000) and Mayrhofer *etal*(2004).Abdelnoor and Sleiman(2011) reported that the majority of *E.coli*

isolates were resistant to tetracycline ciprofloxacin,enrofloxacin,gentamicin and amoxicillinand only one isolate was resistant to ceftriaxone.

1.10 chicken intestinalbacterial flora of human importance

1.10.1*Enterococcus spp*

*Enterococccus spp*are part of the gasterointestinal andgenitourinary tract of humans and animals (Murray, 1990). Due to its ability to resist adverse environmental condition this account for their ability to colonize different habitat and trigger the potential easily spread through the food chain.

1.10.2*Proteus spp*

Proteus spp were gram negative bacteria which considered one of the most frequent bacteria in normal flora in both human and animal intestine .They are one of the most pathogens encountered in the urinary infection in human Chicken intestinal tract could be a source of this bacteria,mishandling of poultry product and raw poultry carcasses is one of the most frequent causes of human infection byproteus species (Mostafa, 2013).

1.10.3***Staphylococcus*** *spp*

The genus *Staphylococcus* are one of the most prevalent pathogens in both humans and animals (Casey *et al.*, 2007) it is found in water, dust, and air, *S. Aureus* primarily colonises the mucosa of nasopharynx and skin of humans and animals (Songer & Post, 2005). *Staphylococcus aureus* is an important agent of food poisoning all over the world (Balaban & Rasooly, 2000) as well as in the respiratory and intestinal tracts (Sleekes, 1997).

The bacterium is considered to be a normal flora of the chicken (Casey*et al.*, 2007).

1.10.4*Salmonellaspp*

Salmonellosis has been considered one of the most important infectious diseases in both humans and animals (Keusch, 2002). According to several studies the prevalence of *Salmonella spp* in poultry were reported (Zahraei *et al*., 2007; Jamshidi *et al*., 2008).The widespread occurrence of *Salmonella* in natural environment and the intensive husbandry practiceused in the meat, fish and shellfish industries has been a significant problem in public health (Michael and larry 2007). The mostcommon source of human salmonellosis is food of poultry origin. (Piyush and Anju, 2008).Bacteria such as *Salmonella* sp., *Staphylococcus aureus* and *Escherichia coli*, which can be conveyed by food, cause food poisoning and other food-borne diseases (Foskett *et al*., 2003)

1.10.5*Citrobacter*spp

ThisGenusis widely distributed in soil, water, food and the intestinal tract of humans and animals. Some serotypes of *C. koseri* (*diversus*) can also beenteropathogenic causing diarrhea. The species *Citrobacter freundii* isolated from fecal samples have cytotoxic activity, thus being implicated as a cause of gastrointestinal infection. *C. koseri* cause meningitis in human neonates and mastitis in cattle, and can also be a cause of opportunistic infections in other mammals (Quinn *etal* 2011)

1.10.6*Providencia spp*

This genus can be found in various animal reservoirs, dogs, cats, cattle, sheep and poultry as well as flies, and often also in soil and sewage water(Interaminense JA2010) Several pathogeneses in different animals are related to the genus *Providencia*. In humans, these bacteria cause urinarytract infections, keratitis, dacryocystitis, conjunctivitis and endophthalmitis(Koreishi *etal* 2006). *P. stuartii* clinical strains are mostly

isolated from urinary tract infections in patients hospitalizedfor long periods of use of urinary catheters. They may also be isolated, in fewer cases, from respiratory and skin infections. The bacteria *P. stuartii* is reported as more resistant species of the family *Enterobacteriaceae* (Feyziolu *etal* 2013).

1.10.7*Escherichia coli*,

A gram negative .motile bacteria causes food poisoning and other food-borne diseases such as dysentery, diarrhea and food poisoning (Macleod and Douglas, 1999) and pneumonia, meningitis, and sore throat (Gates, 1987).It is widely distributed in the intestine of animals and forms part of the normal intestinal flora that maintains the physiology of a healthy animal(Conway and Macfarlane, 1995).Most *E. coli* strains are non-pathogenic but pathogenic strains that cause gastrointestinal illness in humans and opportunistic ones that normally affect immune compromised patients exists (Nataro and Kaper, 1998).ETEC (*enterotoxigenic E. coli)* strains are a major cause of secretory diarrhea in both humans and animals (Bern *et al*., 1992).Resietant*E. coli* has been isolated worldwide from poultry meat (Adesiji *et al*., 2011), probably due to the increased usage of antimicrobials (Miranda *et al*., 2008).

1.10.8*Shiglla spp*

Raw poultry is considered to be one of the important sources of major foodborne bacterial pathogens such as *Shigella*(Sackey *et al*., 2001).The bacteria are facultative intracellular pathogens that show a high specificity for human or primate hosts (Downes and ito 2001; Kweon, 2008). *Shigella* sp.They has also been implicated in outbreaks of food poisoning (Morton, 1993; Gracey and Collins 1994; Collins and Lyne, 2004).

Chapter two

Materials and Methods

2.1 Study area

Samples were collected from two farms in Omdurman county (Khartoum State), located at Elsalha and Al Fetiahab, which are the only farms that raised broiler chicken dedicated for shawarma(a specific type of chicken meal) during January-February 2017 .As that type of broiler were reared according to customer request.

2.2 Type of Samples

Cloacal swabs (cs) were collectedrandomly from apparently healthy broiler chicken age 57-60 days old and weight reached up to 2.5 kg. A Total of 50 cs, 25 from each farms, were collected from life birds at slaughter house in reception room. Samples were collected randomly using sterile swabs.

2.2.1 Sampling technique

Sterile cotton swabs were inserted deeply in the cloaca and then rotated 3-5 timeand gently pulled out and placed in sterile container. Swabs were kept in ice box with ice bags during transport to the lab. Samples were processed immediately after arrival.

2.3 Sterilization Methods

2.3.1 Sterilization of equipments

Glassware such as MacCarteny , Bijous and universal bottles were sterilized by autoclave at 15 pounds pressure for 15 minute at 121 ^{0}C.

Petri dishes, graduated pipettes, flask, test tubes, glass bottles and forceps were sterilized in the hot air oven at 160 ^{0}C for one hour. Loops and forceps were sterilized byflaming while used.

2.3.2 Sterilization of culture media and solutions

MacConkey agar, nutrientagar, Muller hinton agar, Blood agar base, mannitol salt agar .nutrient broth, urease media,citrate, TSImedia, peptonewater and normalsaline were sterilized in autoclave. Hugh and leifson (OF) medium was sterilized in autoclave at 10 pound for 10 minutesat 115 ^{o}C.Carbohydrate media were sterilized by steaming for 30 minutes for three following days.

2.4 Preparation of media

2.4.1 Solid media

2.4.1.1. MacConkey's Agar (Oxoid –England CM0115)

The dehydrated form consists of Lactose, peptone, bilesalt sodium chloride ,agar and neutral red. It was prepared according to the manufacturer's instruction by dissolving 52 g of the powder in one liter of distilled water sterilized and then distributed as 20ml amounts in sterile petridishes.

2.4.1.2 Nutrient agar (oxoid - England C M0003)

The medium is composed of lab lemco powder,yeast,peptone,sodium chloride and agar it was prepared according to manufactures instruction by dissolving 28g of dehydrated media in one liter of distilled water then sterilized and distributed in 20ml amounts in sterile petridishes.

2.4.1.3 Muller Hinton agar (Oxoid-England CM0337)

The media consist of Beef dehydrate infusion, casein hydrolysate, starch and agar. the medium was prepared according to the manufactures instruction by dissolving 38g of dehydrate powder in one liter of distilled water, sterilized and then poured in 20ml amounts in sterile petridishes.

2.4.1.4 Muller Hinton agar(Himedia india MP173)

Mueller-hinton agar medium is the only susceptibility test medium that has been recommended by the National Committee for Clinical Laboratory standard (NCCL 2001). It was prepared by dissolving 38g in one liter of distilled water then poured in 20ml amount in sterile petridishes.

2.4.1.5 Blood agar (Oxoid- England CM0271)

This medium is composed of blood agar base (Oxoid) and defibrinated sheep blood. The blood agar base contained lab-lemco powder peptone,sodium chloride and agar .it was prepared according to the manufacturers instruction by dissolving 40g of the base medium in one litre of distilled water, then sterilized and cooled to 50^0C. Sterile defibrinated sheep blood was added aseptically at the rate of 7% the media was then distributed as 20 ml amounts in sterile petridishes.

2.4.1.6 Mannitol Salt Agar (Oxoid-CM85)

A selective medium prepared according to the recommendation of chapman[1] for the isolation of presumptive pathogenic staphylococci.The media composed of lab-lemco powder, peptone, mannitol, sodium chloride, phenol red, and agar. The media was prepared by suspending 111g in 1litre of distilled water and bring to the boil to dissolve completely. Sterilized by autoclaving at 121 ^{0}C for 15 minutes.

2.4.1.7 Urease medium

The medium is composed of peptone, glucose, sodium chloride, di-soduim phosphate,potassium dihydrogen phosphate,phenol red and agar.The medium was prepared by dissolving 2.4g in95ml distilled water in a water bath at 100 ^{0}C,then cooled to 50 ^{0}C and aseptically one ampoule of sterile urea solution was added, mixed well,distributed in 10ml amounts into sterile containers and allowed to settle in the slope position. Positive reaction is indicated by change of color to red.

2.4.1.8 Citrate medium

Simmons citrate agar contained magnesium sulphate, ammonium dihydrogen phosphate, sodium ammonium phosphate, sodium citrate tribasic, sodium chloride ,bromothymol blue and agar.Medium was prepared by suspending 23g in 1 liter of distill water, Brought to the boil to dissolve combletely and Sterilized by autoclaving at 121 ^{0}C for 15 minutes.

2.4.1.9 Triple sugar iron agar(TSIA)

The medium contains lab-lemco powder,yeast extract, peptone,sodium chloride, lactose, sucrose, dextrose, ferric ,sodium thiosulphate, phenol red, agar. Suspend 65g of the dehydrated powder were suspended in 1litre of distilled water, boiled to dissolve completely,Mixed well ,distributed and sterilize by autoclaving at 121 0C for 15 minutes. A composite medium for the differentiation of enterobacteriaceae according to their ability to ferment lactose, sucrose and dextrose and producdion hydrogen sulphide (H_2S).

2.4.2 Semisolid media

2.4.2.1 Hugh and leifsons media (OF)

The medium contained dipotassium hydrogen phosphate, peptone, sodium chloride, agar and 1.5ml of 0.2%aqueous solution of bromocresol purple.the media was prepared according to (Barrow and Felt ham 2003) by dissolving the solids in one liter of distilled water adjust the PH to 7.1 then the indicator was added sterilized by autoclaving at 115 ^{0}C for 20 minutes sterile solution of the glucose was added aseptically to give final concentration of 1 % the medium was then dispense into 10 ml volume in sterile tubes.

2.4.3 Liquid media

2.4.3.1 Peptone water (Oxoid Code L37)

This medium contains peptone and sodium chloride it was prepared according to(Barrow and Felt ham 2003) by dissolving 10 gram of peptone and 5g sodium chloride in 1 liter of distilled water the medium was distributed in 5 ml amount in bijoui bottles and sterilized by autoclaving at 121^{0}C for 5 minutes and then refrigerated at 4^{0}C until used .

2.4.3.2 Carbohydrate fermentation media

Medium was prepared according to(Barrow and Felt ham2003). 900 ml of peptone water was prepared and its pH adjusted to 7.2-7.4 then 10 ml of bromocresol purple (0.2gram bromocesol purple to 100 distilled water was added and the medium was then sterilized . The sugar solution was prepared by dissolving 10 g of sugar in 90 ml sterilize distilled water these amount was added aseptically to the sterile peptone water and indicator .it was then distributed into 5 ml amount in sterile bijou bottle with durhums tubes and sterilized thecolor of media was purple .

2.4.3.3 Nutrient Broth

It was prepared by dissolving 13 grams of dry medium to one liter of distilled water. Sterilized and distributed in 5 ml bijou bottles, store at 4^0C until use.

2.4.4 Solutions and Reagents

2.4.4.1 Hydrogen peroxide (H_2O_2)

This was obtained from the (Bell sons –England).It was prepared as 3% aqueous solution for catalase test.

2.4.4.2 Tetramethyl-P phenylen-Dimino Dihydrochloride

It was obtained from Lab Tech Chemical-Italy .It was prepared as 1% aquoes solution and used for oxidase test.

2.4.4.3 Kovacs Reagent

This reagent contained P-dimethyl aminobenzaldehyde amyl alcohol and concentrated hydrochloric acid .It was prepared as described by(Barrow and Felt ham 2003) dissolving the aldehyde in the alcohol by heating in water bath.it was then cooled and the acid was added the reagent was stored at 4 ^{0}C in dark bottle. untill used for the indole test.

2.4.4.4 Normal saline

It was prepared by suspending 8.5 grams sodium chloride in liter ofdistilled water sterilized and distributed in glass container.

2.4.5 Antibiotic susceptibility test

2.4.5.1Antibiotics discs

Commercial antibiotic disc (Hi media,India) was used in this study type and concentrations of antibiotic were shown inTable (3).

2.4.5.2 Turbidity Standards (Mc Farland)

McFarland 0.5 (Ba SO4) turbidity standard supplied by bio Merieux (France) were used to adjusted turbidity of the culture.

2.4.6 Culture Methods

2.4.6.1 Primary culture

Fresh cloacal swabs were spread onto nutrient agar and Macconkey agar plates and incubated aerobically at 37^0C for 24 hours.

2.4.6.2 Sub-culturing of primary isolates

A part of typical and well isolated colony was picked with sterile wire loop and streaked onto the surface of a fresh plate and incubated aerobically at 37^0C for 24 hours.

2.4.6.3 Examination of culture

All cultures on solid media were examined with the naked eye for growth, colonial morphology and change in the media.

2.4.6.4 Purification of culture

It was done by sub culturing a part of typically isolated colony on the corresponding medium .This process was repeated twice, subcultures were check for purity by examining stained smear. Pure culture were then stored at 4 ^{0}C till used.

Table (3) Types and concentration of antibiotics

Type	Concentration per disk
Gentamycin	10 mcg
Ciprofloxacin	5 mcg
Doxycycline	30 mcg
Cefapime	30 mcg
Amoxicillin	25 mcg
Colistin	10 mcg
Norofloxacin	10 mcg
Erythromycin	15 mcg
Tetracycline	30 mcg

2.4.6.5 Identification of Bacteria

Purified cultures were identified according to the criteria outlined by (Barrow and Felt ham 2003) which include reaction to gram stain, shape, motility, colonial characterization and biochemical tests.

2.4.7 Primary test

2.4.7.1 Gram stain

This was done according to(Barrow and Felt ham 2003) smear was prepared by emilsifing part of a colony on clean ,grease free slide the smear was allowed to dry in air then fixed by gentle flaming. Fixed smear was Stained with crystal violet (5%) for 1-2 minutes ,Staining with loughols iodine for 1-3 minute.Smear was washed with tape water,Decoloraized with 95% alcohol for 15 second then Washed with tape water Counter Stain with carbol fuchsin for 2-3 minutes then Washed with tape water, Blot dry and examine underthe microscope 100 X oil lens.

2.4.7.2 Oxidase test

This test was performed by growing the organism in the media free from glucose and nitrate a filter paper impregnated with oxidase reagent (himedia) was placed in a Petri dish , part of the colony was picked with sterile glass rods and smeared across the filter paper development of dark purple color indicated positive reaction .

2.4.7.3 Catalase test

A drop of catalase reagent H_2 O_2 (3%) was placed on clean slide part of colony was picked by sterile loop and mixed with the drop, production of gas immediately or after few second indicated positive reaction.

2.4.7.4 Carbohydrate O/F

Duplicate tubes of Hugh and liefsion media were inoculated by stabbing with straight wire loop .the medium in one tube was covered by a layer of sterile soft paraffin oil to the depth of 1cm then incubated at 37C^{o} for 24h .Media were examined daily for up 14 days change in color to yellow in both tubes indicated a fermentative organism while change in uncovered tube only indicate the bacteria was oxidative .No color change in both tubes indicated negative results.

2.4.7.5 Motility

It was examined in the semisolid O/F media. Growth of the bacteria in the inoculated area indicate non motile bacteria while the spread of inoculated bacteria throughout the media indicate motile bacteria.

2.4.7.6 Glucose fermentation

In this test media were inoculated by stabbing with straight loop then incubated at 37C^{o} for 24h. Media were examined daily for up 14 days change in color to yellow that indicated a fermentative organism while change to yellow and release of gas in Durham tube indicate that bacteria was formative with gas production .

2.4.8 Secondary tests

2.4.8.1. Urease test

Pure culture of organism was inoculated in the surface of a urease agar slope and incubated at37C^{o} for 24h. Positive reaction organism changed medium to purple-red, negative bacteria cannot change color.

2.4.8.2 Citrate

Pure culture of organism was inoculated in the surface of a Simmons citrate agarslope, incubated at37C^{o} for 48h. Positive reaction changed medium color from green to bright blue.In negative bacteria medium remains unchanged.

2.4.8.3 Triple sugar iron agar

Pick a single colony and inoculated in the surface of a slope Triple sugar iron agar to tested and incubated at37C^{o} for 48h. Positive reaction organism changed medium colure to black color other colure indicated negative reaction.

2.4.8.4 Monnitol salt agar

It is used to differentiate between staphylococcus spices; pink colonies indicated *staphylococcus epidermis*, yellow colonies. *Staphylococcus aureus.*

2.4.8.5 Hemolysis in Blood Agar

Hemolytic bacteria shown clear zone round colonies. The test was performed for identification of staphylococcus.

2.4.8.6 API 20 E (Analytical Profile Index Biomerioux ,Frace)

It was performed according to the manufacture instructions foidentification of enterobacteriaceae and other non- fastidious gram negative rods. Tests include O-Nitro phenyl-D-galactopyranosidase(ONPG),aArgininеDihydrolase(ADH),LysineDecarboxylase(LDC),Ornithine,Decarboxylase(ODC),Citrate(CIT),hydrogensulphide (H2S), Urease (URE), Tryptophan Deaminase (TDA), Indole test(IND),VogesPrskauertest(VP),Gelatinliquifacationtest(GEL),
Glucose (GLU), Nitratereduction, Mannitol, Insitol, Sorbitol,

Rhaminose, Sucrose, Melibiose, Amygdalin, Arabinose. Result of the test was readmanually.

2.4.9 Antimicrobial susceptibility test

2.4.9.1 Disc diffusion methods

This test was performed according to National Committee for Clinical Laboratory Standard (NCCLS, 2001).the top of the single and well isolated colony was touched with sterile loop then transfer into 2 ml normal saline. The turbidity was then adjusted to a 0.5 McFarland standard.

Sterile cotton swab was dipped into the adjusted suspension. The swab firmly pressed against theinsideof the tube above the fluid level.

The soaked swab was then spread evenly over the entire surface of the plate of Muller hinton agar media.The plates were then allowed to dry for 3 -5 minutes antibiotic impregnated disc were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed downonto the agar to ensure complete contact with the agar surface, even distribution of the disc and minimum distance of 24 mm form center were insuredwithin 15 minute the plate were inverted and incubated at 37C.After 24h the plates were examined and diameter of the zone of complete inhibition to the nears whole mm were measured.

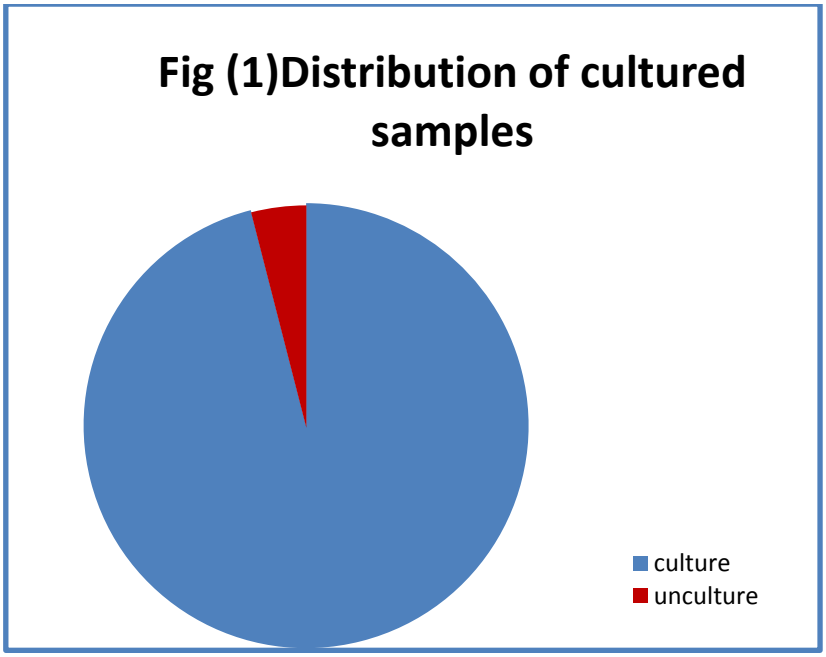
Chapter three

Result

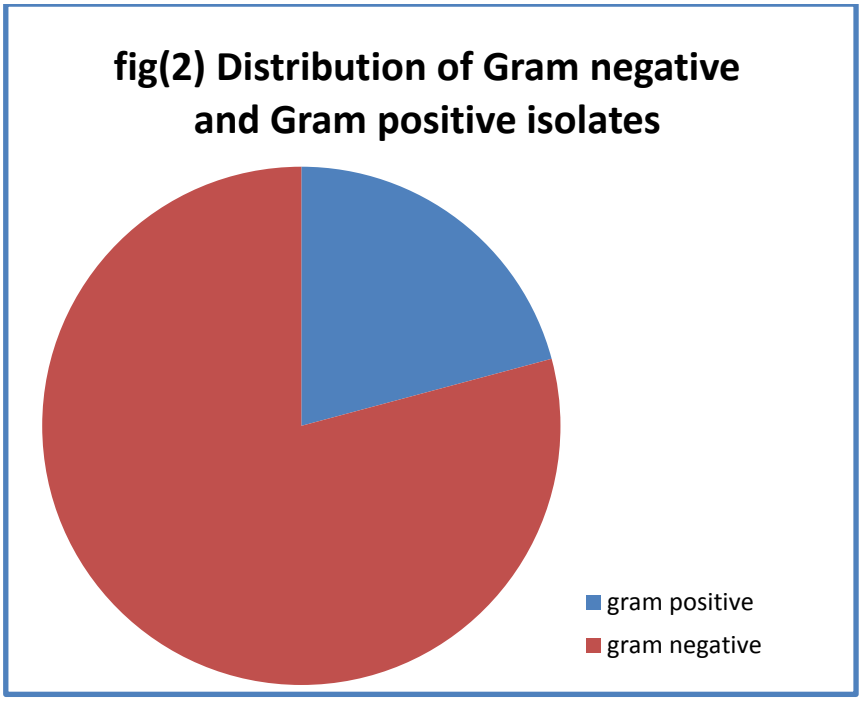
3.1 Isolation of bacteria

During this study a total of 72 aerobic bacteria were isolated from 50 cloacal swabs collected from57- 60days old broiler chickens.

Isolates were identified using conventional biochemical tests and API20E for enterobacterceae, according to this investigation both Gram negative and Gram positive bacterial species were isolated from 48 samples, however 2 sample were cultured negative for bacterial growth fig (1).



A total of 15(21%)Gram positive and 57(79%)Gram negative isolates were recovered fig (2).



3.1.1 Gram negative isolates

Total of7 Gram negative bacterial species were isolated from the cloaca of healthy broiler chickens species and percentage were shown in(table 4), about 8Gram negative isolates were designated as unidentified according to result of the standard lab tests. (Barrow and Felt ham 2003) used for identification of bacteria according to gram stain shape, motility, oxidase, catalase and O/F in addition to other biochemical tests.

Proteus and Escherichia species were the most prevalent isolates while the shigella was the least recovered one.

Table (4) Distribution of Gram negative bacterial species isolated

Bacterial species	No. of isolate
Escherichia spp	10(18%)
Citrobacter spp	7(12%)
Proteus spp	24(42%)
Enterobacter spp	3(5%)
Salmonella spp	2(4%)
Providence spp	2(4%)
Shigella spp	1(2%)
Unidentified spp	8(14%)

3.1.2 Gram positive isolates

Two Gram positive bacterial species were isolated from the cloacal swab of healthy broiler chickens, seven Gram positive isolates were designated as unidentified accordingto (Barrow and Felt han 2003) the standard lab test used for identification of bacterial result of gram stain shape, motility, oxides, cataloes and O/F in addition to different biochemical tests, type and percentage were shown in table (5).

Table (5) Distribution of Gram positive bacterial isolates

Bacterial speci	No. of isolate	
Staphylococcus	7(47%)	4(57%) *Staphylococcus epidermidis*
		3(43%) *Staphylococcus aurus*
Enterococcus	1(7%)	
Unidentified	7(46%)	

3.2 Antibiotic susceptibility test

A total of nine antibiotics were used to detect the antibiotic susceptibility of bacteria isolated during this study. Result showed that 64 isolates were resistant to at least one antibiotic while 4 isolates were found sensitive to all antibiotics used.

3.2.1 Susceptibility of Gram negative isolates

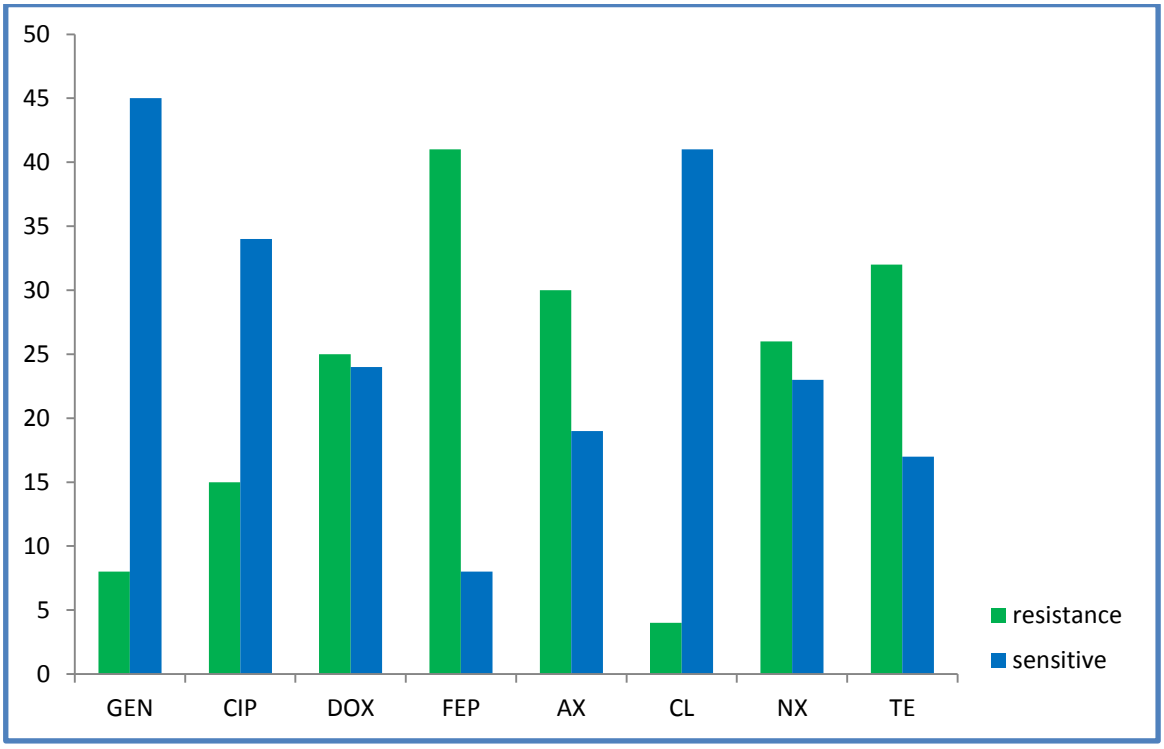
Forty nine isolates were tested against nine antibiotics to detect their resistance pattern, only 4 isolates were found sensitive to all antibiotic used including 2 salmonella and 2 *E.coli* isolates. Resistance pattern of Gram negative isolates were shown in table (6). Higher resistance was observed to cefapime while higher sensitivity was detected to Gentamycin as shown in fig (3).

Table (6) Antibiotic resistance of gram negative isolates

Antibiotic	*E.coli*	*Citrobactar*	*proteus*	*entero*	*salmonella*	*providincia*	*shigella*	unidentified
Gentamycin	10%	29%	18%	33%	0%	50%	0%	0%
Ciprofloxacin	40%	57%	18%	33%	0%	50%	0%	25%
Doxycycline	20%	86%	50%	66%	0%	100%	66%	63%
Cefapime	50%	86%	100%	100%	0%	100%	100%	100%
Amoxicillin	60%	57%	50%	100%	0%	100%	100%	25%
Colistane	10%	0%	19%	0%	0%	0%	10%	0%
Norofloxacin	40%	14%	38%	33%	0%	50%	100%	25%
Erythromycin	NT	NT	NT	NT	NT	NT	NT	NT
Tetracycline	60%	85%	50%	100%	0%	100%	0%	75%

NT: not tested

Fig (3) Distribution of susceptibility of Gram negative isolates to each antibiotic



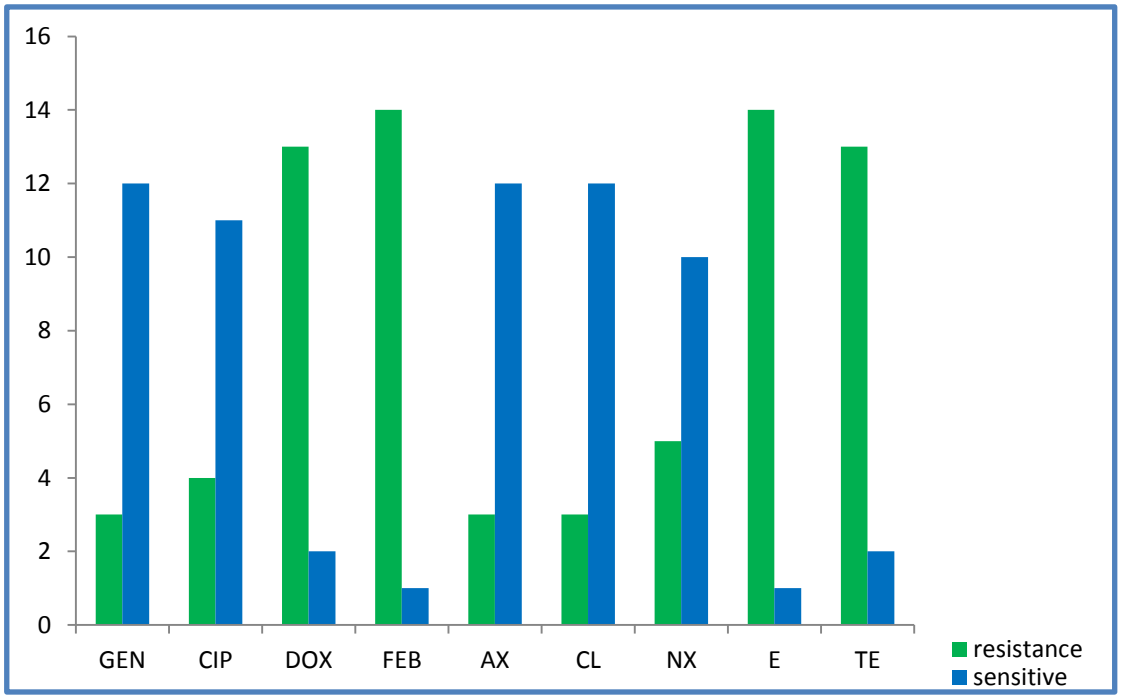
3.2.2 Susceptibility of Gram positive isolates

Fifteen Gram positive isolates were recovered during this study. All isolates showed resistance to at least one antibiotic, none was found sensitive to all of antibiotics used .Different patterns were observed table (7). Regarding type of antibiotic higher resistance was observed to cefapime and erythromycin while higher sensitivity was detected to colistin, gentamycin and amoxicillin that shown in fig (4).

Table (7) Antibiotic resistant pattern of Gram positive isolates

antibiotic	*Staphylococcus*	*Enterococcus*	Unidentified
Gentamycin	%0	%100	%29
Ciprofloxacin	%14	%100	%29
Doxycycline	%100	%100	%71
Cefapine	%100	%100	%86
Amoxicillin	%14	%0	%29
ColistIn	%0	%100	%29
Norofloxacin	%43	%0	%29
Erythromycin	%100	%100	%100
Tetracycline	%100	%100	%71

Fig (4) Distribution of susceptibility of Gram positive isolateS to each antibiotic



3.2.3 Multi resistance patterns

3.2.3.1 Multi resistance of Gram negative isolates

Thirty four isolates showed multi resistance pattern to antibiotics as shown in table (8).

Table (8) Multi-resistance pattern of Gram negative isolates

No. of Antibiotic (n=9)	No. of resistant isolates
3	6
4	13
5	4
6	4
7	3
8	4
9	0

3.2.3.2 Multi resistance of Gram positive isolates

Thirteen gram positive isolates showed multi- resistance, their distribution is shown in table (9).

Table (9) Multi resistance pattern of Gram positive isolate

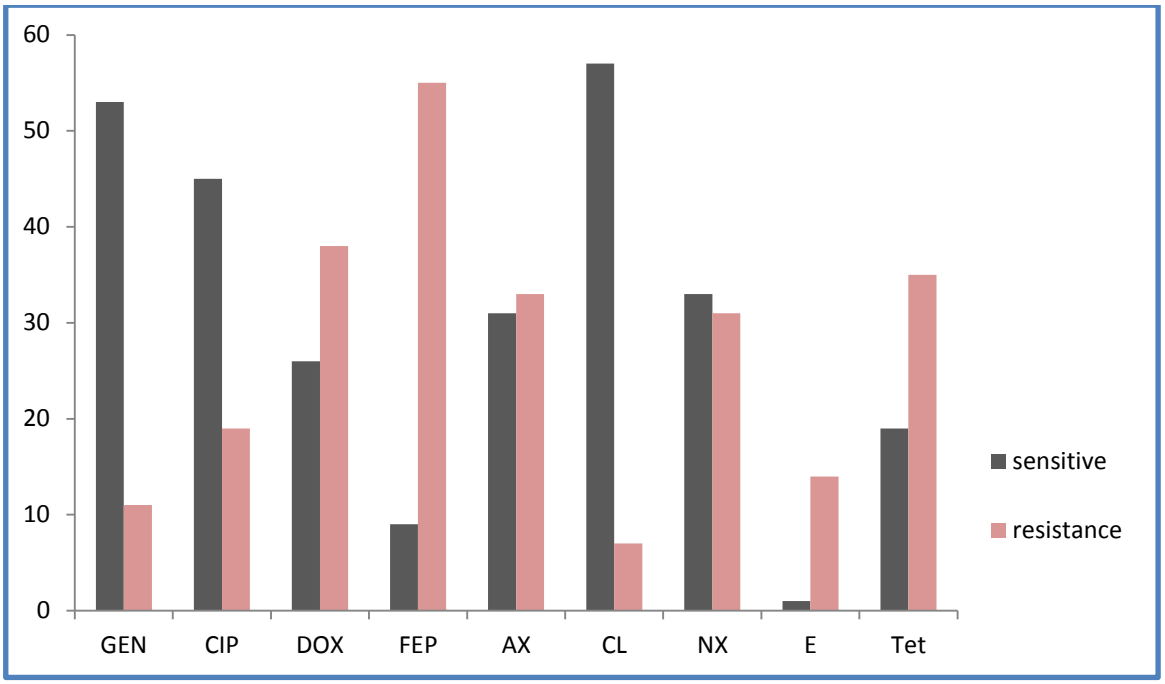
No. of Antibiotic (n=9)	No. of resistant isolate
3	0
4	4
5	6
6	0
7	2
8	1
9	0

3.2.4 over all resistance of isolates

Result showed that higher antibiotic sensitivity was found to colistin (75%) followed by gentamicin (53%) and ciprofloxacin (45%) while (9%) of the isolates shown sensitivity to cefpime.

Higher resistance was detected to cefpime 55 followed by doxycycline 38 and tetracycline 35 while 7 isolates were found resistant to colistin fig (5**).**

Fig(5) Distribution of overall susceptibility of isolates to each antibiotic



Chapter four

Discussion

In this study samples were taken from broiler chickens that reared for shawerma(57-60 days), in Sudan poultry owners used antibiotics during all the rearing period of chicken for prevention, and that habit considered one of the misuse of antibiotics. This type of chicken is exposed to more doses of antibiotic than normal broiler which might increase the ability of bacteria to be resistant. During this study a total of 50 samples were examined 48 were cultured positive while 2(4%) samples were cultured negative, this might be due to the presence of unculturable bacteria. about 40 to 80% of the intestinal bacteria are uncultivable (Sarma-Rupavtarm et al., 2004).

Recently molecular techniques have been developed to completely study bacterial populations of chicken GIT (Gong et al., 2002; Pissavin *et al.*, 2012).

Isolates were recovered from GIT of apparently healthy broiler chicken included*e.coli,protus*spp , Shigella, Salmonella,Citrobacter Enterobacter Provedinvcia Stahylococucc spp Snterococus. Other bacteria isolated from GIT of Broiler chicken were not reported during this study such as *E hermanni* and *Streptococuss spp*. Nutritional composition of feed and thus local availability of raw materials as well as seasonal changes will impact the intestinal flora (Bjerrum *et al.*, 2006).

In this study fifteen isolates were determine as unidentified according to Barrow and Feltham (2003).

In the present study interestingly *Salmonella spp* are the only isolated bacteria that found sensitive to all antibiotic used, this finding disagree with Gouws and Brözel(2000) and Mayrhofer *etal* (2004) who found that Salmonella were resistant to tetracycline.

While 65% of isolates showed multi resistance pattern. Higher resistance was detected to cefapime 55 (76%) followed by doxycycline 38(53%) and tetracycline35 (49%) on the other hand Higher sensitivity was detected to colistin7 (10%) followed bygentamicin and ciprofloxacin.

During this study enterococcus was found resistant to gentamycin and erythromycin this is in accordance with Miroslav*et aL* (2012) who found resistant to both gentamycin and erythromycin.

In this study higher prevalence of bacteria isolated was proteus species (42%) this is in agreement with the previous study carried out in Iran (54%) reported by Mostafa(2013). *proteus species* showed different resistant pattern,with higher resistant to cefapime and lower resistant to gentamicin. Different results were reported by (Mostafa 2013) where all isolate were found sensitive to gentamycin and higher resistant to doxycycline while lower resistant was reported to ceftriaxone.

In the current study isolated *staphylococcus spp* showed 100% resistant to erythromycin and 0% to gentamycin this results agreed with Suleiman *et al* (2013) in Nigeria, resistance to ciprofloxacin and cefapime was found higher than those reported in the Nigerian study .

Prevalence of commensal *E.coli* was on line with the finding reported by (Ghanbarpour *et al*. 2011and Mishra *et al* 2002). Higher prevalence of *E. coli* was observed by Derakhshantar and Ghanbarpour (2002). On the other hand Majalija*et al* (2010) in Uganda and Islam *et al* (2014) in Bangladesh showed lower prevalence. This might be due to different environmental and managemental condition, food habit and mixed infection with other microbes(Islam *et al*, 2014).resistant pattern of commensals e coli of poultry carriedout in the country (Salwa 2015) showed nearly similar results to

cefepime , norofloxacin and tetracycline while lower resistant was detected against gentamycin and doxycycline and higher in ciprofloxacin.

During this study only one shigella isolate was recovered it was found resistant to tetracycline,cefapime and amoxicillin In a research conducted by Sackey *et al*., (2001), in Ghana all examined chicken samples were positive for *Shigella*.

Conclusion

The importance of this study come from that the resistant bacteria might transmit to human during the process of chicken in slaughter houses and through contaminated carcass to consumer and the resistant bacteria can share the normal flora of human with this resistant gene.In addition These resistant bacteria may contaminate vegetables with chicken fecal materials (marrog) that commonly used as fertilizer without any previous treatment which is a common habit in Sudan and these materials was consider an important secondary product in the farm.

Shawerma is one of the fast meal that cooked bulk and the inner part usually exposed to lower temperature that increased possibility of presence of resistance bacteria and hence transmission to consumer.

Recommendation

1.Control used of antibiotic and used antibiotic substitutes and bio security as first line defined against disease.

2.Drug control and legislations concerning sales of antimicrobials in the country.

3.Prepare biosecurity measure in slaughterhouse to prevent of contamination in chicken carcasses.

4.Awareness of workers on chicken industry about the risk of resistant bacteria.

5.Treatment of chicken waste before used to reduce the bacterial load.

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Appendix

Appendix (1) Antibiotic susceptibility of gram negative isolates

No	Bacterial spp	Gen	Cip	Dox	Fep	Ax	Cl	Nx	E	Tet
1	Escherichia	S	R	R	R	R	R	R	NT	R
2	Escherichia	S	S	S	S	R	S	S	NT	R
3	Escherichia	S	S	S	R	R	S	S	NT	S
4	Escherichia	S	S	S	S	S	S	S	NT	S
5	Escherichia	S	S	S	S	S	S	R	NT	R
6	Escherichia	S	S	S	R	S	S	S	NT	S
7	Escherichia	R	R	S	R	R	S	S	NT	R
8	Escherichia	S	R	S	S	R	S	R	NT	R
9	Escherichia	S	R	R	R	R	S	R	NT	R
10	Escherichia	S	S	S	S	S	S	S	NT	S
11	Citrobacter	S	R	R	R	R	S	S	NT	R
12	Citrobacter	R	R	R	R	R	S	S	NT	R
13	Citrobacter	S	R	S	R	S	S	S	NT	S
14	Citrobacter	S	S	R	R	S	S	S	NT	R
15	Citrobacter	S	S	R	R	R	S	S	NT	R
16	Citrobacter	R	R	R	S	R	S	S	NT	R
17	Citrobacter	S	S	R	R	S	S	R	NT	R
18	Proteus	R	S	R	R	R	S	S	NT	R
19	Proteus	S	S	R	R	R	S	S	NT	R
20	Proteus	S	R	S	R	R	S	R	NT	S
21	Proteus	S	S	S	R	S	R	R	NT	S
22	Proteus	S	S	S	R	S	S	S	NT	S
23	Proteus	R	S	R	R	R	S	S	NT	S
24	Proteus	S	S	S	R	S	S	S	NT	S
25	Proteus	R	R	R	R	R	S	R	NT	R
26	Proteus	S	S	S	R	S	R	R	NT	S
27	Proteus	S	S	R	R	R	S	S	NT	R
28	Proteus	S	S	S	R	R	S	S	NT	R
29	Proteus	S	R	R	R	R	R	R	NT	R
30	Proteus	S	S	R	R	S	S	S	NT	R
31	Proteus	S	S	R	R	S	S	R	NT	R
32	Proteus	S	S	S	R	S	S	S	NT	S
33	Proteus	S	S	S	R	S	S	S	NT	S

34	enterobacter	S	S	R	R	R	S	S	NT	R
35	enterobacter	R	R	R	R	R	S	R	NT	R
36	enterobacter	S	S	S	R	R	S	S	NT	R
37	Salmonella	S	S	S	S	S	S	S	NT	S
38	Salmonella	S	S	S	S	S	S	S	NT	S
39	providence	R	S	R	R	R	S	S	NT	R
40	Providence	S	R	R	R	R	S	R	NT	R
41	Shigella	S	S	S	R	R	S	S	NT	R
42	Unidentified g-ve	S	S	R	R	R	S	S	NT	R
43	Unidentified g-ve	S	R	S	R	S	S	S	NT	S
44	Unidentified g-ve	S	S	R	R	S	S	S	NT	R
45	Unidentified g-ve	S	S	S	R	S	S	R	NT	R
46	Unidentified g-ve	S	S	R	R	R	S	S	NT	R
47	Unidentified g-ve	S	S	S	R	S	S	S	NT	R
48	Unidentified g-ve	S	S	R	R	S	S	S	NT	S
49	Unidentified g-ve	S	R	R	R	S	S	R	NT	R

NT: not tested

Appendix (2) Antibiotic susceptibilitypattern of Gram positive isolates

No	Bacterial spp	Gen	Cip	Dox	Fep	Ax	Cl	Nx	E	Ter
1	Staphylococcus	S	S	R	R	S	S	S	R	R
2	Staphylococcus	S	S	R	R	R	S	S	R	R
3	staphylococcus	S	S	R	R	S	S	R	S	R
4	staphylococcus	S	S	R	R	S	S	R	R	R
5	staphylococcus	S	S	R	R	S	S	R	R	R
6	staphylococcus	S	R	R	R	S	S	S	R	R
7	staphylococcus	S	S	R	R	S	S	S	R	R
8	Enterococcus	R	R	R	R	S	R	S	R	R
9	Unidentified g+ve	R	R	R	R	R	S	R	R	R
10	Unidentified g+ve	R	R	R	S	R	S	R	R	R
11	Unidentified g+ve	S	S	S	R	S	S	S	R	S
12	Unidentified g+ve	S	S	R	R	S	S	S	R	R
13	Unidentified g+ve	S	S	R	R	S	R	S	R	R
14	Unidentified g+ve	S	S	R	R	S	R	S	R	R
15	Unidentified g+ve	S	S	S	R	S	S	S	R	S