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College of Applied Science

Department of Applied Biology

Prevalence and antibiotic susceptibility profiles of *campylobacter*, *staphylococcus aureus*, *salmonella* and *E.coli* from chicken carcasses

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Submitted to the College of Applied Science in partial fulfillment of the requirements for the degree Bachelors in Applied Biology

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DEDICATION

First we thank god for his blessing and to reconcile and repaid during these years that have passed us to get this stage of science , knowledge , and culture .

Dedication to ones who gave us life and grow us up ,those angles who were always my supportive .I owe them each moment of my life and praise them in every breath,our parents also our families on their continuous efforts and givin and permanent support to reach achievements .

It's with our deepest gratitude and warmest affection that we dedicate this thesis to our teachers to Mr Murad Ishnewier who has been a constant source of knowledge and inspiration, also to Dr Fawzi Razem , and Miss Arwa Mujahed . In addition, we dedicate it to our friends and colleagues who were with us within these special four years.

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ABSTRACT

Prevalence and antibiotic susceptibility profiles of *campylobacter, staphylococcus aureus, salmonella* and *E.coli* from chicken carcasses

Reham Shahateet[1], Siren Rjoob[1], Layan Daraweesh[1], &Jawaher Abu Ramoz[1]

Several studies implicated that contamination of poultry carcasses in abattoirs is considered a significant source of human infections. *Campylobacter, Salmonella, E.coli* and *Staphylococcus aureus* are among the high prevalent pathogens causing foodborne diseases.

However, due to the random and inefficient use of antibiotics in poultry, such pathogens are increasingly resistant to the clinically important antibiotics and this rising resistance is a concern for public health. This study aimed to compare the Presence and antimicrobial susceptibility of *Campylobacter*, *Salmonella*, *E. coli* and *S. aureus* species in chicken carcasses.

Four samples were collected from a poultry farms in (Dora and Hebron), Bacteria strains were isolated and recognized using selective and deferential media. Isolates were then tested for sensitivity to Azithromycin, Ampicillin, Tetracycline, Gentamicin, and Nalidixic Acid antibiotics using the Kirby-Bauer disk diffusion test with reference of the Clinical and Laboratory Standards Institute (CLSI).

Results indicates the highest level of resistance among Campylobacter (n=19) tested was to Nalidixic Acid (NA) 100%, Tetracycline and Azithromycin (AZM) 94%,It showed moderate resistance to a Gentamycin 10.5 %, and Ampicillin 26.3%

While Salmonella (n=6) bacteria showed highly 100 % resistance to AM, NA, also 67 % to AZM. While its highly sensitive to CN antibiotic.

For S. aureus (n=9), disc diffusion testing showed 100% correlation with agar dilution for (TE) and (NA), and 89% AZM. A high level of sensitivity 100% to AZM, CN and 16.6% to AM was found in the E.coli isolates (n=6) by disc, whereas 83 % of isolates were resistance to AM, 50% to NA and low level 33.3% to TE.

Overall, our study has emphasize on minimize the misuse of available antimicrobials in agriculture and medicine, this would aid to easily control and eliminate these bacteria, and to lower farmer's material losses as well the risk of its impact on humans.

Keywords: Antibiotics, Multi drug resistance, Antibiotic resistance, Campylobacter.

ABSTRACT IN ARABIC

موضوع البحث

انتشار وقابلية المضادات الحيوية ل*staphylococcus aurous, campylobacter,*

salmonella, E.coli من جثث الدجاج

أشارت دراسات عديدة أن تلوث جثث الدجاج في المذابح يعتبر مصدر هام للإصابات البشرية بالأمراض. تعد بكتيريا,*Campylobacter, Staphylococcus aureus, Salmonella, E.coli*هي من أكثر البكتيريا الممرضة المسببة الأمراض عالية الانتشار المنقولة عن طرق الغذاء للإنسان .

نظرا للاستخدام العشوائي والغير فعال للمضادات الحيوية في الدواجن ،أهم مسببات الامراض لها مقاومة عالية للمضادات الحيوية الهامة في الطب السريري وتطوير سلالات جديدة أكثر امراضية تشكل مصدر قلق للصحة العامة .

الهدف من هذه الدراسة هو مقارنة وجود *Salmonella, E. coli ,S. aureus ,Campylobacter* ومقاومتها للمضادات الحيوية في الدواجن.

تم جمع اربع عينات من مزارع الدواجن في دورا والخليل ،وتم عزل سلالات البكتيريا والتعرف عليها باستخدام (selective and deferential media)و كما تم دراسة مقاومة البكتيريا ل خمسة من المضادات الحيوية الأكثر استخدام في فلسطين Azithromycin, Ampicillin, Tetracycline, Gentamicin, Nalidixic Acid
باستخدام اختبار (Kirby-Bauer disk diffusion) مع الرجوع الى المعايير المخبرية.

أظهرت النتائج مستوى مقاومة عالية ل *Campylobacter (n=19)* التي تم دراستها ضد المضادات الحيوية Azithromycin (AZM) 94%, Tetracycline 94%,Nalidixic Acid (NA)100%. وأظهرت اقل مقاومة ضد% Gentamycin 10.52 , %Ampicillin 26.3.
بينما اظهرت (n=6) *Salmonella* مقاومة عالية ل NA, AM وأيضا AZM % 67 وحساسية عالية للمضاد الحيوي CN.

أظهر بروتوكول Disc diffusion (n=9) *S. aureus* مقاومة عالية %100ضدTEو NA, %89 AZM ،مستوى عالي من الحساسية وجدت في (n=6) *E.coli* بينما أظهرت مقاومة عالية % 83.3 ضد AM و%50ضد NA ومستوى منخفض %33.3 TE.

وبشكل عام، أكدت دراستنا على تقليل إساءة استخدام مضادات الميكروبات المتاحة في الزراعة والطب، وهذا من شأنه أن يساعد على السيطرة على هذه البكتيريا والقضاء عليها بسهولة، وخفض الخسائر المادية للمزارعين، فضلا عن مخاطر تأثيره على البشر

ABBREVIATIONS

AMP	Ampicillin
APEC	Avian pathogenic *E. coli*
GBS	Guillain–Barré syndrome
S.aureus	*Staphylococcus aureus*
E. coli	*Escherichia coli*
EHEC	enterohemorrhagic *E. coli*
HC	Hemorrhagiccolitis
AZM	Azithromycin
TE	Tetracycline
NA	Nalidixic Acid
CN	Gentamicin
STEC	Shiga toxin-producing *E. coli*
CLSI	Clinical & Laboratory Standards Institute.

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CHAPTER ONE: INTRODUCTION

1.1Food-borne diseases

Because of the low cost of production over and above the relatively cheap prices, poultry meat is very important in the consumer market inclusive the Palestinian one, But, epidemiological notice showed that the presence of pathogenic in poultry meat and its by-products remains a considerable concern. [1, 2,3]

Foodborne diseases are a significant public health challenge in Palestine; it is defined as a diseases caused by consumption of food contaminated with microorganisms or their toxins. Meat and poultry are among the leading vehicles for foodborne illnesses, most types of foodborne disease are characterized by symptoms of gastrointestinal infection. Several studies showed that contamination of Poultry carcasses with food borne pathogens is considered a significant source of human infections. Several foodborne pathogens have been reported to contaminate Poultry meat and eggs, and associated with human diseases following eating under cooking and raw products. These pathogens have emerged with resistance to one or more antimicrobial drugs, and have been responsible for a number of notable food-borne outbreaks, and reflect another emerging public health concern. The majority of these pathogens including *Capmpylobacter, Salmonella, Staphylococcus aureus and E. coli.* [4]

However, Antibiotics are often used for therapy of infected humans and animals as well as for growth promotion of animals. Due to the random misuse and inefficient use of antibiotics in poultry for prophylactic, therapeutic or performance enhancing purposes, as well as being Zoonotic pathogens (i.e., those that can be transmitted between animals and humans), Such pathogens are increasingly building resistant to the clinically important antibiotics and this rising resistance is thought to increase the risk of emergence and burden the public health globally. Its further leads to significant mortality and morbidity rate in broilers which influence economic sector through increase the cost of food production and decline the quality of products, Therefore, considerable efforts should dedicated to studying them in an effort to better prepare for them and ultimately predict when they will appear affects the economic sector .[4]

1.2 Bacterial human Pathogenic

Salmonellosis and Campylobacteriosis are among the most frequently reported foodborne diseases worldwide. The incidence of such disease cases among humans has been shown to correlate with the prevalence of their species among broiler chickens . Contamination of poultry carcasses with *Salmonella* or *Campylobacter* seems to be mostly linked to flock contamination during rearing and/or transportation to slaughter. [5, 6, 7]

Campylobacter cause food-borne enteric infection among consumers world-wide. Particularly, young humans are the most severely affected. Infection may be acquired by two pathways directly and indirectly . Direct pathway by direct contact with infected human shedders in the family environment ,while indirect pathway by consumption of undercooked poultry or red meat , unpasteurized milk , consumption of non-chlorinated and contaminated surface water or water from wells [2.3.4.5].

Campylobacter belongs to the Campylobacteraceae family. It is typically appear comma or s-shaped and motile, Gram-negative, the optimal growth of *Campylobacter* strains occurs at 42°C. Three species from *Campylobacter* were detected commonly being, *C. jejuni, C. coli and C. lari*. The optimum condition for growth requires specific selective media, and incubation at 42°C with a micro aerobic environment comprising a low level of oxygen (5% to 10%) with elevated carbon dioxide (1 % to 10%).These species of *Campylobacter* can be differentiated by biochemical characteristics and hydrogen sulphide production. [5, 6, 8].

However, Campylobacteriosis is a commonly *campylobacter* related disease that infect humans. Most people who get campylobacteriosis recover completely within two to five days. However it can results in long-term, infection which is usually caused by C. jejuni, a spiral and comma shaped bacterium that is normally inhabited cattle, swine, and birds. Clinical symptoms appears 1 to 10 days after exposure [7,8,9], and characterized by a bloody dysentery, cramps, fever and pain, as a result of tissue jejunum, ileum, and colon injuries as well destroying the mucous epithelial cells . In addition, *C. jejuni* can also infect the peripheral nervous system, enhancing a latent autoimmune effect on nerves of the legs, called "Guillain–Barré syndrome (GBS) " , in which symptoms of acute flaccid paralysis respiratory failure could be seen. Symptoms of GBS appear several weeks after diarrheal illness, it usually lasts several weeks and requires intensive medical care. Approximately one in every 1000 reported *Campylobacter* cases results in GBS. [5,6]

Furthermore, *Campylobacter* infection can lead to Reactive Arthritic; Symptoms include inflammation of the joints, eyes, or reproductive or urinary organs. On average, symptoms appear 18 days after infection. Other complications, cause appendicitis or infect specific parts of the body, including the abdominal cavity, the heart, the central nervous system, the gall bladder, the urinary tract, or the blood stream.[5,6]

Salmonella is another common food born pathogen; it is one of the causative public health problems worldwide. It can be detected in eggs, poultry and other meats, raw milk. Exponential efforts to prevent and control this disease are important because of many human cases and thousands of deaths every year.

Salmonella belongs to the *Enterobacteriaceae* family they are gram-negative, oxidize negative, non-spore forming, facultative anaerobic bacterium rod shape and motile by flagella. *Salmonella* has about 60 of O antigens and there are some unlike flagella (H) antigens. It can be divided into groups which are using specific antisera based on somatic antigens. There are two main types of *Salmonella, Salmonella Enteriditis and Salmonella Typhimurium.* [7]

Salmonella Enteriditis and *Salmonella Typhimurium* are the most important serovars that are transmitted from animals to humans; *Salmonella Enteriditis* has become the most common cause of salmonellosis in humans. It is usually transferred by contaminated food of animal sources (meat, poultry, eggs, milk) or vegetables contaminated by manure and water. [9].

Salmonellosis is an infection caused by the *Salmonella* bacteria. Usually it occurs when a person eats food contaminated with the feces of animals or humans carrying the *Salmonella* serovars . Foods that are most likely to contain *Salmonella* include raw or uncooked eggs, raw milk, contaminated water, and raw or uncooked meats. [9, 10]
When *Salmonella* is ingested, they pass through a person's stomach and colonize the small and large intestine. It then invades the intestinal mucosa and proliferates. Severity of clinical disease varies among people, but mostly it develops diarrhea, fever, vomiting, and abdominal cramps 12 to 72 hours after infection. In immune compromised individuals like infants and elderly people, it can develop further sever complications; this includes severe dehydration and inflammations to different gastrointestinal tissues and organs like appendicitis, pancreatitis, cholecystitis, cholangitis. Furthermore, some srovars like *Salmonella* serotype. Typhi can invasive

other tissues and organs through the blood circulation causing meningitis or septicemia in case of the delay of treatment. Lungs, heart, liver and spleen also being susceptible to infection, leading to pneumonia, endocarditis, hepatic and splenic respectively. [10, 11].

Food borne pathogens include, *Staphylococcus aureus*, it is considered the third largest cause of food related illness worldwide. *Staphylococcus aureus* is a bacteria related to the *Staphylococcaceae* family .It is a gram positive bacteria , round shaped , and considered as facultative anaerobic since it can live without the need of oxygen. Poultry meat and red meat are commonly reported as the most food borne pathogen vehicle. [12, 13]

Moreover, there are many foods shows as good growth medium for *Staphylococcus aureus* and its implicated in food poisoning as milk, cream , butter , hum , cheeses , sausages , canned meats , salads , cooked meals and sandwish filling.
It's the only species found in humans that produces coagulase enzyme. In solid media formed colonies are "6-8 mm" in diameter ranging from grey to deep golden yellow colonies. [12, 13].

According to the *S.aureus* origin from animal or human and to the biochemical characteristics , it can be classified into six biotypes as follow , Humans , Non-β – hemolytic human , Avian , Bovine , Ovine , and Nonspecific .[14]
About 30 – 50 % of humans are carriers for *S.aureus* as a part of the skin normal flora , also it can be present on the mucous membranes of the upper respiratory tract , and lower urogenital tract and as transients in the digestive tract ,also it present in the oral cavity, it lives and grow in an optimal condition of (7° to 48.5 °C) temperature , with pH (4.2 to 9.3) , and sodium chloride concentration up to 15% Na Cl .These characteristics gave it the ability to grow in different kinds of foods.[14]

Incidence of *S. aureus* infection can be vary by age since in the first year of life and in the advancing ages its known in high rates , but in young adult-hood its determined with low incidence rates . Moreover the gender plays a significant role but till know the reason it is not understood as its higher in males gender, with male-to-female ratios of (1.5). Also it's associated with ethnicity; black people are with higher risks than the white ones. This is also the same for people with HIV infection. [15]

Severity of *S. aureus* infection significantly depends on a specific types of virulence factors, which are the staphylococcal enterotoxins (SEs,).

Staphylococcal enterotoxins are a short protein secreted in the medium and soluble in water and saline solutions. These SEs are highly resistant to heat, so it can be inactivated by heat treatment in the sterilization process as present in low concentrations .[15]

When food poisoning many symptoms may be recognized as abdominal cramps, nausea, vomiting, in some cases it may be followed by diarrhea, they appear rapidly from 30 min to 8 hours, and usually spontaneous remission is observed after 24 hour . [14].

It can further associated with bacteremia and endocarditis , wound infections, infections of intravascular catheters and vascular devices , skin and soft tissue infections , central nervous system infection , eye diseases , osteomyelitis and other infections of bones and joints , respiratory and urinary tracts infections and toxin mediated syndrome . [15]

Escherichia coli belong to the Enterobacteriaceae family. It is a rod-shaped, Gram-negative, facultative anaerobic bacterium that can live on a wide variety of substrates. *E. coli* uses aerobic or anaerobic respiration. In anaerobic conditions, it uses the mixed-acid fermentation, producing lactate, succinate, ethanol, acetate and carbon dioxide. Optimum growth of *E. coli* occurs at 37 °C, but some laboratory strains can multiply at temperatures up to 49 °C. *E. coli* can transfer its DNA via bacterial conjugation, transduction, or transformation allowing horizontal spreading of genetic material through an existing population [1].

Escherichia coli bacterium is common to many environments and there are over 150 different strains. Most *E. coli* strains are harmless to their hosts since it is part of the normal intestinal flora, it can benefit their hosts by producing vitamin K2, and preventing colonization of the intestine with pathogenic bacteria. But however, some strains can be highly pathogenic and may cause serious problems in immuno-compromised individuals. Pathogenic *E. coli* is associated with intestinal and extraintestinal human infections refer to pyelonephritis, cystitis, septicemia, and some strains are associated with meningitis in neonatal infants. [1, 2].

In addition, the introduction of such strains to respiratory tract can causes invasive infections, collectively known as *Colibacillosis*, which starts with severe abdominal cramp; within a few hours, it is followed by a watery diarrhea causing loss of fluids and electrolytes. Diarrhea lasts for about one day, then, intestinal sores will change

this diarrhea to bright red bloody stools. Bloody diarrhea usually lasts for 2 to 5 days. In severe cases, the disease may cause damage to the central nervous system. [1, 2, 16].

1.3 Common poultry bacterial Pathogenesis

The poultry sector is considered a vital industrial sector worldwide, poultry meat safety and quality are of major concern for human consumers. It represents a significant rich source of production of meat and eggs that supplies the basic human nutrition. Eggs contain essential elements of human nutrition, such as protein, vitamins and minerals, while poultry meat contains high amount of high-value protein, it also has a low fat, which reduce the risk of fat for human.[5]

It remains the significant public health issue concerning the contamination of poultry meat with foodborne pathogens. Contamination is associated with increasing concomitant diseases, increases mortality rate reduction in enterprise profitability and ultimately with risks to animal welfare and human health. Several pathogens have been reported to expose to poultry, particularly *campylobacter, Salmonella*, *Staphylococcus aureus* and *E. coli* are the main predominant foodborne pathogens associated with poultry and are frequently implicated in human illness. Infection of commercial poultry ducks, broilers turkeys, and chickens are among the most infected poultries. With no doubt , the misuse of antibiotics for prophylactic or performance-enhancing purposes, contributes to significant rise in the virulent of these pathogens, it develops new resistance mechanisms that leads to new mutant multi drug resistance strains that can have serious consequences for the treatment of human illness from these organism . [5]

The *campylobacter* bacteria mainly *C. jejuni, C. coli* represents the principal cause of poultry infection. It is a virulent pathogen that can invasively infect deep tissues and organs in poultry chicken. It can cause transient diarrhea associated with sub mucosalo edema, Oral infection, gastrointestinal tract, which leading to high mortality morbidity rates especially among the first week's embryos.
While cocking tends to relatively killing these pathogens, that tends to be up to 10^9 cfu / carcass, but due to its low infective dose, it is easily causing illness in humans if being eats raw poultry products. [5, 6]

Salmonella contamination of poultry carcasses is also frequently been reported. Main poultry-adapted *Salmonella* that develop disease in multi animal and human hosts

include *S .Typhimurium, S. Enteritidis* and particularly *Salmonella* Pullorum. It can cause acute septicemia, enteritis or chronic enteritis and abortions or acute gastroenteritis. *Salmonella* commonly affects chickens, but also infects turkeys, game birds, guinea fowls, sparrows. The bacterium is fairly resistant to normal climate, surviving months but is susceptible to normal disinfectants. [17]

Pullorum disease is among the most important diseases of poultry, these conditions are caused by two very closely related organisms, Salmonella enterica and *S.Typhimurium*. [17]

Chickens are the natural hosts for Salmonella Gallinarum and Salmonella Pullorum, but other birds can also be infected. In addition to chickens, Salmonella Pullorum infections can be found in many avian species including chickens, turkeys, quail, guinea fowl, pheasants, ducks, pigeons, sparrows, canaries, bullfinches and parrots; however, Pullorum disease is uncommon except in chickens, turkeys. [17]

Transmission of Pullorum disease Horizontal and vertical transmission are both important in the epidemiology Pullorum disease , Horizontal transmission occurs via the respiratory and oral routes .The incubation period is usually 4 to 6 days. [17]

Clinical Signs such as depression, weakness, somnolence, loss of appetite, drooping wings, huddling, dehydration and ruffled feathers. Labored breathing or gasping, as well as diarrhea and pasting of the vent feathers, may be seen. The droppings can be white and viscous in Pullorum disease. [17]

Pullorum disease can be sub-acute, and lameness and joint swelling may be apparent. Blindness has also been described. Birds that survive may be underweight and poorly feathered, and may not mature into productive adults. [17]

Other poultry-related pathogens include *S. aureus*; it is also responsible for causing a variety of animal diseases such as mastitis, arthritis and urinary tract infections and a prominent cause of food poisoning due to poor hygienic practices. [18]

It is often infect chickens and turkeys worldwide. Infection is usually influence by the respiratory route with an incubation period of 2-3 days. Wounds, with subsequent spread via the bloodstream to the typical sites of lesions may also be also a route of entry. *Staphylococci* cause diseases in poultry as inhabitants of skin. The associated lesion is bumble foot, which is a localized bulbous lesion of the ball of the foot that arises from the penetration of a foreign body followed by a secondary invasion by *S. aureus*. The first symptom is lameness and the swelling may not be obvious until the planter aspect of the foot is examined. Sometimes a septicemia may occur when

S.aureus enters to the circulation of birds, and it's not fatal, leads to arthritis and synovitis. In addition it causes arthritis and septicemia in turkeys, and omphalitis in chicken. [13]

Moreover, avian pathogenic *Escherichia coli* (APEC) is a large infectious agent present in the modern poultry industry worldwide. Every year, economic losses, in order of millions, due to Avian Pathogenic *E.coli* (APEC) in the poultry chain. The development of diseases caused by *E. coli* in chickens depends on the agent's
Interactions with the environment and the host under specific conditions .Ten to fifteen percent of the intestinal coliforms in chickens have a potential to be pathogenic. [1, 16].

APEC belongs to the extra-intestinal pathogenic. *E. coli* category and is associated with Colibacillosis including: respiratory tract infection, septicemia, omphalitis, enteritis, cellulitis, and swollen head syndrome, among others diseases in poultry. This infectious disease is considered to be initiated in the avian upper respiratory tract; air sacs being the first organs infected, followed by septicemia and organ colonization. [1, 16].

In broilers and hatchers chickens, swollen head syndrome is one of the common syndromes caused by APEC strains. This syndrome is responsible for mortality of 3 to 4% of total birds and for reduction in egg production of 2 to 3 % .Epidemiological data about this syndrome are not available but the lesions associated with cellulitis have a role in causing economic losses in the avian industry. [2,16].

Escherichia coli O157 and other enterohemorrhagic *E. coli* (EHEC) are a worldwide threat to public health. Mostly in developed countries, *E. coli* O157 has been recognized as a cause of serious clinical symptoms such as hemorrhagiccolitis (HC) and hemolytic uremic syndrome (HU) characterized by thrombocytopenia, hemolytic anemia،and nephropathy . Cattle are the primary reservoir, but the presence of *E. coli* O157 in poultry is of maximum significance (4.3%) and our results demonstrated that poultry carcasses and giblets may be contaminated by *E. coli*O157 with feces during evisceration and asymptomatic carriers of *E. coli* O157. The bacteria were spread via cattle faces. [3].

1.4 Bacterial transmission

Fomites are considered as indirect mechanical transmission of *Campylobacter*, movement of personnel and equipment between breeder, broiler and turkey growing farms contribute to introduction of infection if clothing, boots and equipment are contaminated with moist faecal material from a flock excreting *Campylobacter*.[5,19]

Vertical transmission of *Campylobacter* may be occur from breeding flocks to progeny via the egg, or associated with infection during incubation, handling or delivery especially by the intra-cloacal route, while horizontal transmission occurred rapidly among chicks in the hatching trays of commercial incubators and also in chick delivery boxes Abiotic transmission is facilitated on multi-age farms or where units are in close proximity. [5, 6]

Intraflock transmission is rapidly influence; studies showed an infection rate increasing from 2% on the tenth day of the growing cycle to 80% on the twentieth day based on cloacal isolation. Wild birds include passeriformes, columbiforrnes and Anseriformes, also, insects especially darkling beetles (Alphatobiusdiaperinus) and houseflies (Muscadomestica) can transmit *C. jejuni*. [5]

In addition, transmission could be influence through direct pathway by direct contact with infected human, undercooked poultry or red meat , unpasteurized milk , consumption of non-chlorinated and contaminated surface water or open water receptacles, including troughs and suspended drinkers. Storage areas work surfaces are also leading to transfer of *Campylobacter* to salads and other non-cooked foods, also improper and unhygienic procedures during storage and preparation contribute *campylobacteriosis* in catering units [5, 6]

Salmonella is mostly transferred to animals, food and environment (water, crops) by fecal shedding. Faucal or intestinal contagion of carcasses is the main resource of human foodborne infections. It is the exception when pathogen is directly transmitted into the food product, such as S. Enteritidis into eggs and sometimes other serovars into milk. Humans excrete the bacteria as animals do. Excreted bacteria infect other animals on the farm and can transmit to rodents and other wild fauna live near humans or domestic animals. [20]

In contrast, *S. aureus* is transmitted through air droplets or aerosol. Moreover, by numerous small droplets of saliva that remains suspended in air that contain the bacteria from coughing or sneezing of infected persons, also by contaminated food.

Another way is through direct contact with objects that are contaminated by the bacteria or by bites from infected persons or animals. Approximately 30% of healthy humans carry *S. aureus* in their nose, back of the throat and on their skin. [21].

E. coli O157:H7 is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Fecal contamination of water and other foods, as well as cross-contamination during food preparation (with beef and other meat products, contaminated surfaces and kitchen utensils), will also lead to infection. Examples of foods implicated in outbreaks of *E. coli* O157:H7 include undercooked hamburgers, dried cured salami, unpasteurized fresh-pressed apple cider, yogurt, and cheese made from raw milk. [3]

An increasing number of outbreaks are associated with the consumption of fruits and vegetables (including sprouts, spinach, lettuce, coleslaw, and salad) whereby contamination may be due to contact with feces from domestic or wild animals at some stage during cultivation or handling. STEC has also been isolated from bodies of water (such as ponds and streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments. Waterborne transmission has been reported, both from contaminated drinking-water and from recreational waters. [3]

Person-to-person contact is an important mode of transmission through the oral-fecal route. An asymptomatic carrier state has been reported, where individuals show no clinical signs of disease but are capable of infecting others. The duration of excretion of STEC is about 1 week or less in adults, but can be longer in children. Visiting farms and other venues where the general public might come into direct contact with farm animals has also been identified as an important risk factor for STEC infection. [3].

1.5 Bacterial reduction

The amelioration of Campylobacter contamination must be based on control during both the pre-harvest and processing components of the chain of production.

Pre-harvest control of Pathogens

The improvement of *Combylopacter* contamination must be based on control during both the pre-harvest and processing components of the chain of production. In vertical transmission of Campylobacteriosis that can occur from parent flock to broiler progeny. The biosecurity precautions appropriate to breeding farms have been reviewed and incorporate both structural and operational procedures. Poultry houses should be designed and constructed to eliminate the entry of rodents and wild birds. Showering of personnel, provision of clean clothing and footwear, and placement of disinfectant boot dips. Also introduction of mechanical egg collection installation with self-cleaning belts, followed by decontamination after collection using either formalin fumigation or a phenolic disinfectant. [5, 6]

The hatchery is a potential link in the chain of transmission from breeder flock to broilers, so intensification of biosecurity procedures and decontamination should be emphasized in a control program. Rearing broilers on plastic mesh to eliminate the coprophagy offers some potential in eliminating food-borne intestinal pathogens. [5]

In contract for *Salmonella* three steps of control of *Salmonella*, usually recommended, first step is the food-producing animal regulations (pre-harvest control),Second step is the hygiene improvements during the slaughtering and additional processing of the meat (harvest control) and Third step is the final preparation of food by training and education of the food processing industries and consumers about good hygiene practices (post-harvest control) .[22]

In the one hand , *S. aureus* can be prevented by personal hygiene , keeping the wounds covered and clean , preventing incidence from working in food preparations , only using pasteurized or cooked milk and having a frequent check for cows from for mastitis , also cleaning the hospital surfaces with alcohol, quaternary ammonium or iodine compounds , and sprays for air disinfection can be used .

For *S. aureus* treatment, it can be treated with mupirocin (Bactroban) nasal gel, and daily Hibiclens skin cleanser baths. Moreover, antibacterial ointments are used for skin infection, and for skin abscesses they have to be drained, and surgical drainage is done for deep abscesses. In systemic infection, hospitalization and intravenous antibiotics are needed. Artificial heart valves and vein catheters often need to be removed or replaced. [18]

Moreover,to prevent *E. coli* O157 infections, Infection rates can be cut with better hygiene and improved patient care in hospitals, surgeries and care homes, such as ensuring staff, patients and visitors regularly wash their hands. People using insertion devices such as catheters, which are often used following surgery, can develop

infections like *E. coli* if they are not inserted properly, left in too long or if patients are not properly hydrated and going to the toilet regularly. [23]

1.6 Antibiotic susceptibility

Antibiotics are a class of antimicrobial that include anti-viral , anti-fungal , anti-bacterial drug and anti-parasitic drugs , they are chemical compounds can be synthesized naturally or artificially from microorganisms (bugs or germs such as bacteria and fungi) , these compounds has the potential to kill or slow down the bacterial growth . [24, 25]

The first antibiotic Pencillin was discovered by Alexander Fleming in 1928 from *Penicillium notatum* fungus. After that many antibiotics have discovered and tested then used in medical science. [24, 25]

There are many characteristics should be in antibiotics to be efficient when using , these include being a highly stable and well absorbed by the body tissue , having less side effects and being effective against wide range of microbes .

According to the mode action of antibiotics, it can be classified into five categories ;cell wall inhibitors are affecting cell wall thus enhance killing of cells through osmotic pressure , cell membrane inhibitors antibiotics that injure bacterial plasma membranes lead to cell death through leakage of cell contents and associated disruption of the cross-membrane potential (which essentially are ion concentration gradients) , nucleic acid synthesizes inhibitors quinolones are a key group of antibiotics that interfere with DNA synthesis by inhibiting topoisomerase, most frequently topoisomerase II (DNA gyrase), an enzyme involved in DNA replication. , protein synthesizes inhibitors a protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins, and metabolic inhibitors. [24]

By the time, antimicrobial has developed, therefore came the need to perform the antimicrobial susceptibility test as a routine. These tests have been classified into three main methods; diffusion as strokes method, Kirby-Bauer method dilution as Broth dilution, Agar dilution, in addition E-test method. These methods can be influenced by pH, moisture, effect of Thymidine or Thymine, effects of variation in divalent cations , preparation of Muller-Hinton Agar , and standardization of the inoculums .In the Kirby-Bauer the most common method use of antibiotics disks placed on the plates which are inoculated with the test organism. After incubation for

the required time, the zone of inhibition is measured for the test antibiotics, and depending on the diameter of the zone of inhibition, the organism can be classified as sensitive or resistant to the antibiotics . [24]

There are many classes of antibiotics includes ; Aminoglycosides , Cephalosporins , Fluoroquinolones , Macrolides , Penicillins , Tetracyclines . According to related studies concern of poultry infection five antibiotics were used in this study ,these are gentamicin , Tetracyclines , Azithromycin , Ampicillin , and Nalidixic acid . [25]

Gentamicin was discovered in 1963 , the brand name Garamycin , it is a type of aminoglycoside made from the bacteria Micromonosporapurpurea .It is used to treat several types of bacterial infections includes bone infections, endocarditis, pelvic inflammatory disease, meningitis, pneumonia, urinary tract infections, and sepsis among others.. It can be given intravenously, by injection into a muscle, or topically. Topical formulations may be used in burns or for infections of the outside of the eye. [26,27 ,28]

Tetracyclines are a family of antibiotics used to treat a broad spectrum of bacterial infections. It was discovered in the late 1940s and was extremely popular. The tetracycline antibiotics have a very broad spectrum of action. Tetracyclines are used to treat mild acne, Rocky Mountain spotted fever, Lyme disease, upper respiratory tract infections, urinary tract infections, sexually transmitted diseases, typhus. [24]

Azithromycin was first made in 1980; it is an antibiotic useful for the treatment of a number of bacterial infections. This includes middle ear infections, strep throat, pneumonia, traveler's diarrhea, and certain other intestinal infections. Along with other medications, it may also be used for malaria. It can be taken by mouth or intravenously with doses once per day. [29]

Ampicillin was discovered in 1958 and came into commercial use in 1961, is an antibiotic used to prevent and treat a number of bacterial infections, such as respiratory tract infections, urinary tract infections, meningitis, Salmonellosis, and endocarditis. It may also be used to prevent group *B streptococcal* infection in newborns. It is used by mouth, by injection into a muscle, or intravenously. Like all antibiotics, it is not useful for the treatment of viral infections. [30, 31, 32]

Nalidixic acid was discovered by George Lesher in the 1960s, Nalidixic acid is effective primarily against gram-negative bacteria, with minor anti-gram-positive activity. In lower concentrations, it acts in a bacteriostatic manner; that is, it inhibits growth and reproduction. In higher concentrations, it is bactericidal, meaning that it kills bacteria instead of merely inhibiting their growth It has historically been used for

treating urinary tract infections, caused, for example, by *Escherichia coli, Proteus, Shigella, Enterobacter, and Klebsiella.* [32]

During 1980s the mechanisms of antibiotic resistance in *Campylobacter*, and the prevalence of resistant strains was comprehensively reviewed. The surveillance of antimicrobial resistance to tetracycline, fluoroquinolones, sulphonamides and bacitracin (both intrinsic), streptomycin, tetracycline and penicillin.[19,33]

In *Salmonella*, according to a significant study in Brazil, antibiotics were used, some were resistant and some were sensitive tetracycline, erythromycin and novobiocin are high resistant. But ciprofloxacin, norfloxacin and gentamicin high sensitive. [34]

Acoording to recent *S. aureus* isolates antibiogram studies from different sources ,the results have shown a high resistance to tetracycline and ampicillin ,also to ciprofloxacin and sulfamethoxazole/trimethoprim antibiotics . However it's resistant against gentamicin, streptomycin with specific percentages according to each study. Moreover about 30% - 50% strains of *S. aureus* are resistant to semisynthetic penicillin, also to methicillin antibiotics, and it express a decreased susceptibility to vancomycin. [18]

It represent the first reported study of antibiotic susceptibility profile in poultry carcasses in Palestine showed high resistance levels against Tetracycline, Ampicillin, Amoxicillin, Kanamycin, Ciprofloxacin and Neomycin, while the lowest resistance levels were against Nitrofurantoin and Cephalexin. [1, 35]

CHAPTER TWO: PROBLEM STATEMENT AND OBJECTIVES

2.1 Problem Statement

In Palestine the poultry sector has a great importance especially at the economic and health levels. The poultry section is considered to be the main source of income for farmers, thus maintaining this sector and increasing its growth will contribute to the strengthening of the economy of Palestine. At the level of the health section, poultry is the main source of protein.

Due to the random misuse and inefficient use of antibiotics in poultry for prophylactic, therapeutic or performance enhancing purposes, Such pathogens are increasingly building resistant to the clinically important antibiotics and this rising resistance is thought to increase the risk of emergence and burden the public health globally.
Asia Pacific Economic Cooperation "APEC" has considered the antibiotic resistance as a pandemic feature. Therefore has come the need to find out the resistance and susceptibility of the isolated bacterial strains to the antimicrobial drugs.

In order to solve the problem of the random use of antibiotics mainly in poultry field , and the risk of developing strains of drug – resistant bacteria that have raised by the reduced amount of data about this matter , we have accomplished this study ; to apply antibiotic susceptibility results in poultry farms here in Palestine .

2.2 Objectives

This study aimed to compare the Presence and antimicrobial susceptibility of *Campylobacter*, *Salmonella*, *E. coli* and *S. aureus* species in chicken carcasses.

The results of this study also contribute to increasing farmers' awareness of the destructive effects of random use of antibiotics, as well as the publishing of these results with the Ministry of Agriculture, Veterinary Medicine and the Ministry of Health, which in turn take many safety measures.

CHAPTER THREE: MATERIAL AND METHODS

3.1 Materials

3.1.1 Samples

(1g) from poultry carcasses were collected from Dora and Hebron , from each source specific number for each bacteria were isolate as shown by table 3.1.

Table 3.1 Number of isolates, from poultry carcasses from two region Dora and Hebron.

Governates		Isolates			
		Campylobacte	*Salmonella*	*S. Aureus*	*E.coli*
Hebron		8	_	4	3
Doura	Doura 1	2	1	1	1
	Doura 2	4	2	2	2
	Doura 3	5	3	2	-
Total		19	6	9	6

3.1.2 Media

For *Campylobacter* Selective Supplement (skirrow) with Colombia Blood Agar medium was used, and prepared with specific concentrations. This media is a selective type for the isolation of *Campylobacter* species at 42 °C show Appendix 1.

For *Salmonella*, Salmonella Shigella Agar was used show Appendix 1.

For *S. aureus*, Mannitol Salt Agar shows Appendix 1.

For *E.coli*, Macconkey Agar shows Appendix 1.

3.2 Methods

3.2.1 for Campylobacter

The collected samples were mixed in an autoclave bag with (500 ml) peptone water for each sample, and then it was gently shacked for about 15 min by hand.

Columbia Blood Agar was prepared by the addition of [11.5 peptone , 2 g starch , 2.5g sodium chloride , 5g agar , 500ml D.H2O] on a bottle , then was autoclaved . Then the bottle was removed to the incubator shaker at 50 C for around 15min to offer the suitable conditions. At this time, Campylobacter supplement skirrow was prepared inside the safety cabinet [2 ml D.H2O, skirrow].

In the incubator, a 25 ml sheep blood was added gradually to the Columbia Blood Agar (fresh and free of clots, used to enrich the media), then the skirrow was added , and the mix tray was gently shacked . The prepared media was poured into the plates.

A (1: 10) dilution was done (100 µl chicken solution : 900 µl LB broth) , also another dilution (1: 100) for two samples was done { due to the high concentration of bacterial growth } (Hebron and Doura 3 samples) . A 50 µl of each diluted sample was cultured on the selective media (Columbia Blood Agar) .

A candle gar method was implemented in order to have the appropriate conditions for the *Campylobacter* growth (5% oxygen , 10 % carbon dioxide and 84-85 % nitrogen for 48hours at 42 °C) . The plates were put inside the glass bowl, then the candle was turned on and placed on the top of the plates, then the bowl was covered with the tray, till the candle was turned off a quick entrance of the oxygen was done by raising the tray just for a few seconds, then it was removed to the incubator at 42 C for two days. Show Figure 3.1.

Figure 3.1. A candle gar method was implemented in order to have the appropriate conditions for the *Campylobacter* growth (5% oxygen, 10 % carbon dioxide and 84-85 % nitrogen for 48hours at 42 °C) .

3.2.2 for bacterial species

Poultry carcasses samples were collected from two places (three from Doura, and one from Hebron). The collected samples were mixed in an autoclave bag with (500 ml) peptone water for each sample , they were gently shacked for about 15 min by hand . (1.5 ml) of each sample was put on a sterilized tube, and the rest samples were kept in a bottles in the refrigerator.

A (1:10) dilution was done (100 µl sample: 900 µl LB broth). The diluted tubes were put in the incubator shaker for an overnight (37 C) .A (50 µl) of each solution was cultured (spread) on three types of media (Macconkey, Mannitol, Salmonella Shigella (SS)) , then were kept in the incubator at (37 C) . The next day results were taken and the bacteria was grown (*E.coli* , *Staphylococcus aurous* , and *Salmonella*) . A specific labeling for the most suitable and desired colonies was done.

Then they were isolated by the loop and transferred into a sterilized tube containing (10 ml) LB broth. After that (21 tube) were kept in the incubator shaker at (37 °C)

3.2.3 Antimicrobial susceptibility

The *campylobacter* isolates were tested for antimicrobial sensitivity of Azithromycin , Ampiicillin , Gentamicin , Tetracycline , and Nalidixic Acid) on Muller Hinton Agar , by Kirby-Bauer disk diffusion technique , through which standard paper disks were laid on the medium then kept on the candle gar in the incubator at 42 °C .

Data was collected after 24h , and 48 h , and classified according to the size of zone of inhibition into three specific categories sensitive (S) , intermediate (I) , and resistant (R) To the tested antibiotics, according to the Clinical & Laboratory Standards Institute (CLSI).

Salmonella, S.aureus and *E.coli* isolates were tested for the same antibiotics, but on Muller Hinton Agar and Mannitol Agar, and the plates were kept in the incubator at (37 °C). Moreover, Data was collected after 24h, and 48 h, and the same classification was done.

Table 3.2 : Reference zone of inhibition of antibiotics used in this study according to the Clinical & Laboratory Standards Institute (CLSI).[36]

Antibiotic(Disc identifier)	Disk concentration	Inhibition zone diameter to nearest mm		
		Resistant ≤	Intermediate	Susceptible ≥
Azithromycin	15µg	12	-	13
Ampiicillin	10µ g	13	14–16	17
Gentamicin	10µg	12	13 – 14	15
Tetracycline	30µg	11	12 – 14	15
Nalidixic Acid	30µg	13	14 – 18	19

CHAPTER FOUR: RESULTS

4.1 Antibiotic susceptibility testing

Antibiotic test results were taken for each type of bacteria , *Campylobacter* , *Salmonella* , *S.aureus* , *E.coli* at two different periods 24 – 48 h , as shown in table (4.1 , 4.2 , 4.3 , 4.4, 4.5, 4.6, 4.7, 4.8) according to the Clinical & Laboratory Standards Institute (CLSI) in table (3.2).

For *Campylobacter* the (nineteen isolates) have shown a highest sensitivity for Ampicillin 57.89 % as it can be used for poultry treatment , but limiting the use of other antibiotics since it has expressed a 100 % resistance for Nalidixic Acid , followed by Tetracycline , Azithromycin 94.73 % , and an intermediate susceptibility for Ampicillin , Gentamcin 21 % , 15.78 % respectively (Table 4.1, 4.8) .

Moreover, the six *Salmonella* isolates have shown 100 % sensitivity for Gentamicin, also a 100 % resistance for Ampicillin, Tetracycline, and Nalidixic Acid, and only an intermediate susceptibility for Azithromycin 16.66 %. (Table 4.3, 4.6)

In addition , *S.aureus* nine isolates have expressed a 88.88 % sensitivity for Ampicillin , and 55.55 % for Gentamicin , a 100 % resistance for Tetracycline and Nalidixic Acid , also a 89 % for Azithromycin , and an intermediate susceptibility 22.22 % for Gentamcin . (Table 4.4, 4.7)

For the six isolates of *E.coli* , they were sensitive 100 % for Azithromycin and Gentamicin , 83.33 % Ampicillin , also 83.33 % resistance for Tetracycline , and have expressed a 16.66 % intermediate susceptibility for Tetracycline and Nalidixic Acid . (Table 4.2, 4.5).

Summary for Resistance, intermediate and sensitivity results of *Campylobacter* , *Salmonella* , *S.aureus* , and *E.coli* species against 5 types of antibiotic at 24 and 48 hr are shown in tables with simple diagrams . Summary for resistance results at 24 hr have shown in table (4.9) and figure (4.1), while results at 48hr have shown in table (4.12) and figure (4.4).

Sensitivity percentage for *Campylobacter*, *Salmonella*, *S.aureus* , and *E.coli* species at 24 hr have shown in table (4.10) and figure (4.2), while results at 48hr have shown in table (4.13) and figure (4.3). Also, Intermediate results at 24 hr have shown in table

(4.11) and figure (4.3), while results at 48hr have shown in table (4.14) and figure (4.6).

The isolated *campylobacter* samples gives an indication that they doesn't cause an illness or any kind of infection in poultry as they are lower than the standard safety level that is up to 10^9 cfu/g , since the calculations of the collected samples are 5.16 x 10^6 , 1.6 x 10^6 , 1 x 10^6 , and 4 x 10^5 for Doura 1 , Doura 2 , Doura 3 and Hebron respectively .

Table 4.1: Summary of Antibiotic sensitivity results for *Campylobacter* at 24 hr.

Antibiotic	Disk content (µg)	*Campylobacter* (n=6)			Percentage Of *Campylobacter*		
		S	I	R	S	I	R
Azithromycin(AZM)	15 µg	0	0	19	0	0	100%
Ampicillin(AM)	10 µg	10	2	7	25.6 %	10.5 %	36.84%
Gentamicin (CN)	10µg	7	5	7	36.8 %	26.3 %	36.84%
Tetracycline(TE)	30µg	1	0	18	5.26 %	0	94.73%
Nalidixic Acid (NA)	30µg	0	0	19	0	0	100%

Table 4.2 : Summary of Antibiotic sensitivity results for *E.coli* at 24 hr.

Antibiotic	Disk content (µg)	*E.coli* (n=6)			Percentage Of *E.coli*		
		S	I	R	S	I	R
Azithromycin(AZM)	15 µg	6	0	0	100%	0	0
Ampicillin(AM)	10 µg	1	0	5	16.66%	0	83.33%
Gentamicin (CN)	10µg	6	0	0	100%	0	0
Tetracycline(TE)	30µg	1	0	5	16.66%	0	83.33%
Nalidixic Acid (NA)	30µg	2	0	4	33.33%	0	66.66%

Table 4.3 : Summary of Antibiotic sensitivity results for *Salmonella* at 24 hr.

Antibiotic	Disk content (µg)	*Salmonella* (n=6)			Percentage Of *Salmonella*		
		S	I	R	S	I	R
Azithromycin(AZM)	15 µg	2	0	4	33.33%	0	66.66%
Ampicillin(AM)	10 µg	0	0	6	0	0	100%
Gentamicin (CN)	10µg	5	1	0	83.33%	16.66%	0
Tetracycline(TE)	30µg	0	1	5	0	16.66%	83.33%
Nalidixic Acid (NA)	30µg	0	0	6	0	0	100%

Table 4.4 : Summary of Antibiotic sensitivity results for *S.aureus* at 24 hr.

Antibiotic	Disk content (µg)	*S.aureus* (n=6)			Percentage Of *S.aureus*		
		S	I	R	S	I	R
Azithromycin(AZM)	15 µg	1	0	8	11.11%	0	88.88%
Ampicillin(AM)	10 µg	8	0	1	88.88%	0	11.11%
Gentamicin (CN)	10µg	5	2	2	55.55%	22.22%	22.22%
Tetracycline(TE)	30µg	0	0	9	0	0	100%
Nalidixic Acid (NA)	30µg	0	0	9	0	0	100%

Table 4.5:Summary of Antibiotic sensitivity results for *E.coli* at 48hr

Antibiotic	Disk content (µg)	*E.coli* (n=6)			Percentage Of *E.coli*		
		S	I	R	S	I	R
Azithromycin(AZM)	15 µg	6	0	0	100%	0	0
Ampicillin(AM)	10 µg	1	0	5	16.66%	0	83.33%
Gentamicin (CN)	10µg	6	0	0	100%	0	0
Tetracycline(TE)	30µg	1	3	2	16.66%	50%	33.33%
Nalidixic Acid (NA)	30µg	2	1	3	33.33%	16.66%	50%

Table 4.6 : Summary of Antibiotic sensitivity results for *Salmonella* at 48 hr.

Antibiotic	Disk content (µg)	*Salmonella (n=6)*			Percentage Of *Salmonella*		
		S	I	R	S	I	R
Azithromycin(AZM)	15 µg	2	0	4	33.33%	0	66.66%
Ampicillin(AM)	10 µg	0	0	4	0	0	100%
Gentamicin (CN)	10µg	6	0	0	100%	0	0
Tetracycline(TE)	30µg	0	1	5	0	16.66%	83.33%
Nalidixic Acid (NA)	30µg	0	0	6	0	0	100%

Table 4.7 : Summary of Antibiotic sensitivity results for *S. aureus* at 48 hr

Antibiotic	Disk content (µg)	*S. aureus (n=9)*			Percentage Of *S. aureus*		
		S	I	R	S	I	R
Azithromycin(AZM)	15 µg	1	0	8	11.11%	0	88.88%
Ampicillin(AM)	10 µg	8	0	1	88.88%	0	11.11%
Gentamicin (CN)	10µg	5	2	2	55.55%	22.22%	22.22%
Tetracycline(TE)	30µg	0	0	9	0	0	100%
Nalidixic Acid (NA)	30µg	0	0	9	0	0	100%

Table 4.8 : Summary of Antibiotic sensitivity results for *campylobacter* at 48 hr

Antibiotic	Disk content (µg)	*campylobacter(n=19)*			Percentage Of *Campylobacter*		
		S	I	R	S	I	R
Azithromycin(AZM)	15 µg	1	0	18	5.26%	0	94.7%
Ampicillin(AM)	10 µg	11	2	6	57.89%	10.52%	31.57%
Gentamicin (CN)	10µg	1	0	18	36.84%	26.31%	36.84%
Tetracycline(TE)	30µg	7	5	7	5.26	0	94.73%
Nalidixic Acid (NA)	30µg	0	0	19	0	0	100%

Table 4.9 Resistance results of *Campylobacter* , *Salmonella* , *S.aureus* , and *E.coli* species at 24.

	Resistance Percentage				
Bacteria	**Azithromycin (15µg)**	**Ampiicillin (10µg)**	**Gentamicin (10 µg)**	**Tetracycline (30 µg)**	**Nalidixic Acid (30 µg)**
***Campylobacter* (n=19)**	**100%**	**36.84%**	**36.84%**	**94.73%**	**100%**
***Salmonella* (n=6)**	**66.66%**	**100%**	**0**	**83.33%**	**100%**
***S.aureus* (n=9)**	**88.88%**	**11.11%**	**22.22%**	**100%**	**100%**
***E.coli* (n=6)**	**0**	**83.33%**	**0**	**83.33%**	**66.66%**

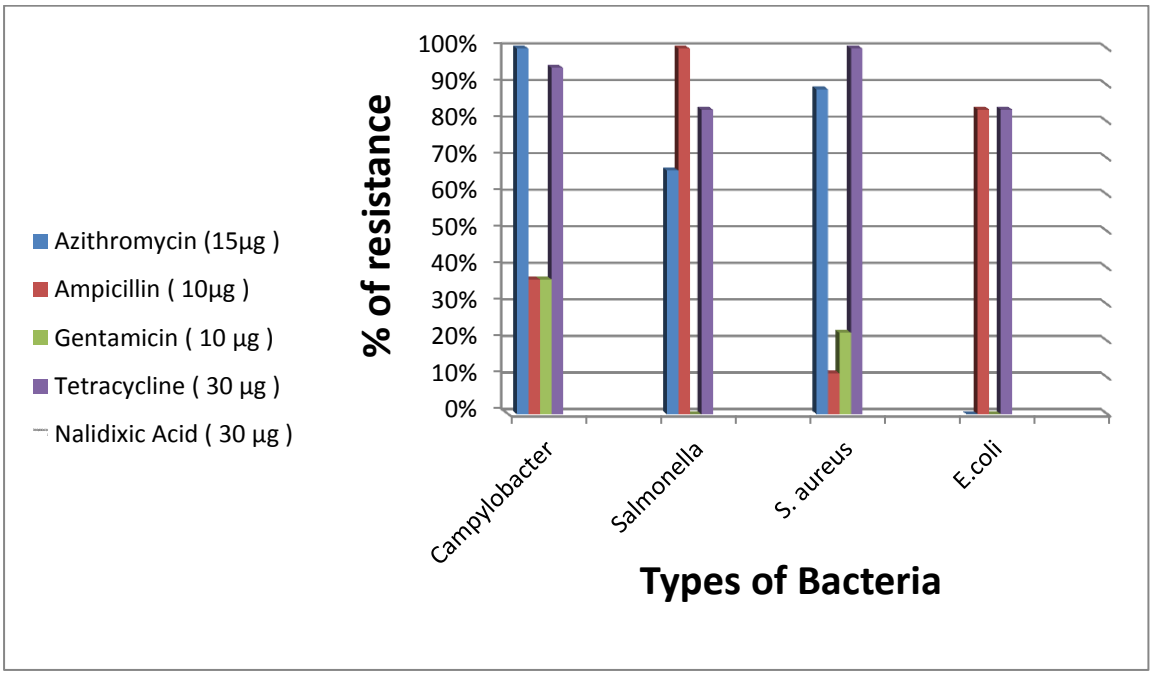

Figure 4.1. Diagram of resistance percentage for *Campylobacter*, *Salmonella*, *S.aureus* , and *E.coli* species against 5 types of antibiotic at 24 hr.

Table 4.10 Sensitivity results of *Campylobacter, Salmonella, S.aureus* , and *E.coli* species at 24 :

	Sensitivity Percentage				
Bacteria	**Azithromycin (15µg)**	**Ampiicillin (10µg)**	**Gentamicin (10 µg)**	**Tetracycline (30 µg)**	**Nalidixic Acid (30 µg)**
Campylobacter (n=19)	**0**	**25.63%**	**36.84%**	**5.26%**	**0**
Salmonella (n=6)	**33.33%**	**0**	**83.33%**	**0**	**0**
S.aureus (n=9)	**11.11%**	**88.88%**	**55.55%**	**0**	**0**
E.coli (n=6)	**100%**	**16.66%**	**100%**	**16.66%**	**33.33%**

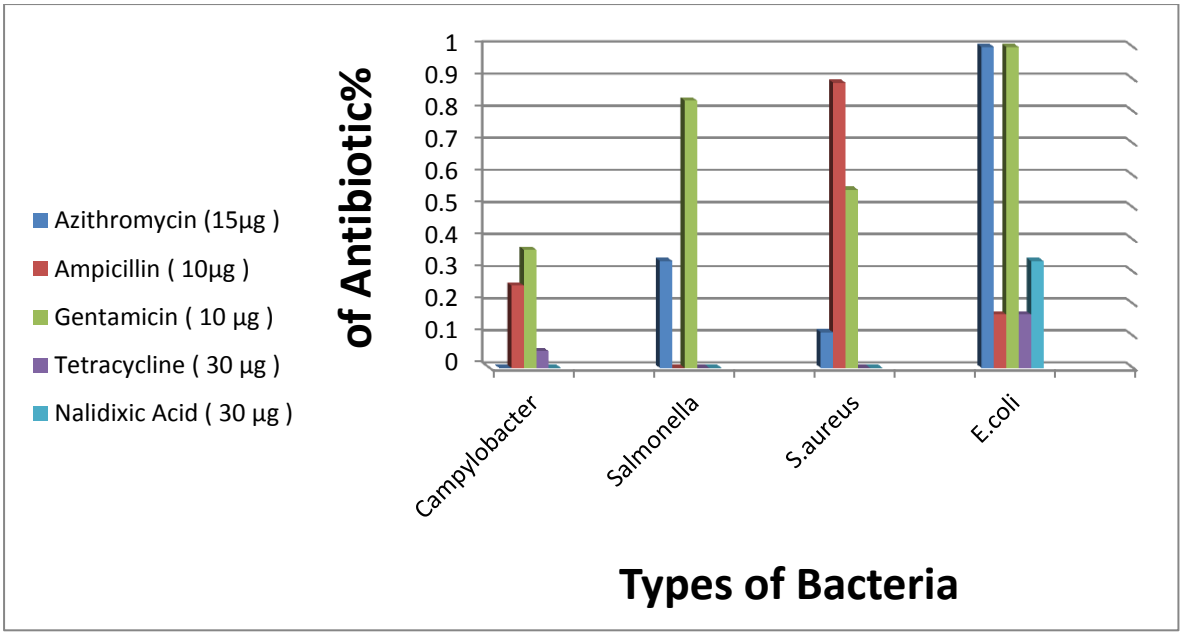

Fig (4.2): Diagram of sensitivity percentage for *Campylobacter, Salmonella, S.aureus* , and *E.coli* species against 5 types of antibiotic at 24 hr.

4.11 Intermediate results of *Campylobacter, Salmonella, S.aureus* , and *E.coli* species at 24 :

	Intermediate Percentage				
Bacteria	**Azithromycin (15µg)**	**Ampiicillin (10µg)**	**Gentamicin (10 µg)**	**Tetracycline (30 µg)**	**Nalidixic Acid (30 µg)**
***Campylobacter* (n=19)**	**0**	**10.52%**	**26.31%**	**0**	**0**
***Salmonella* (n=6)**	**0**	**0**	**0**	**0**	**0**
***S.aureus* (n=9)**	**0**	**0**	**16.66%**	**16.66%**	**0**
***E.coli* (n=6)**	**0**	**0**	**22.22%**	**0**	**0**

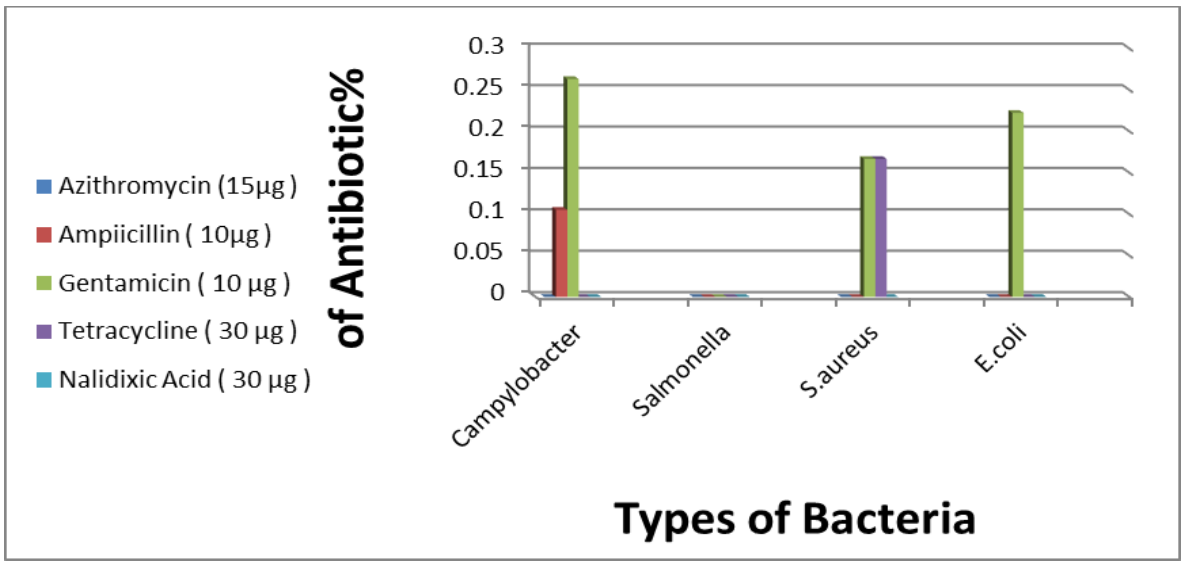

Fig (4.3): Diagram of intermediate percentage for *Campylobacter, Salmonella, S.aureus* , and *E.coli* species against 5 types of antibiotic at 24 hr.

4.12 Resistance results of *Campylobacter, Salmonella, S.aureus* , and *E.coli* species at 48 :

	% Resistance				
Bacteria	**Azithromycin (15µg)**	**Ampiicillin (10µg)**	**Gentamicin (10 µg)**	**Tetracycline (30 µg)**	**Nalidixic Acid (30 µg)**
***Campylobacter* (n=19)**	**94.7%**	**31.57%**	**36.84%**	**94.73%**	**100%**
***Salmonella* (n=6)**	**66.66%**	**100%**	**0**	**83.33%**	**100%**
***S.aureus* (n=9)**	**88.88%**	**11.11%**	**22.22%**	**100%**	**100%**
***E.coli* (n=6)**	**0**	**83.33%**	**0**	**33.33%**	**50%**

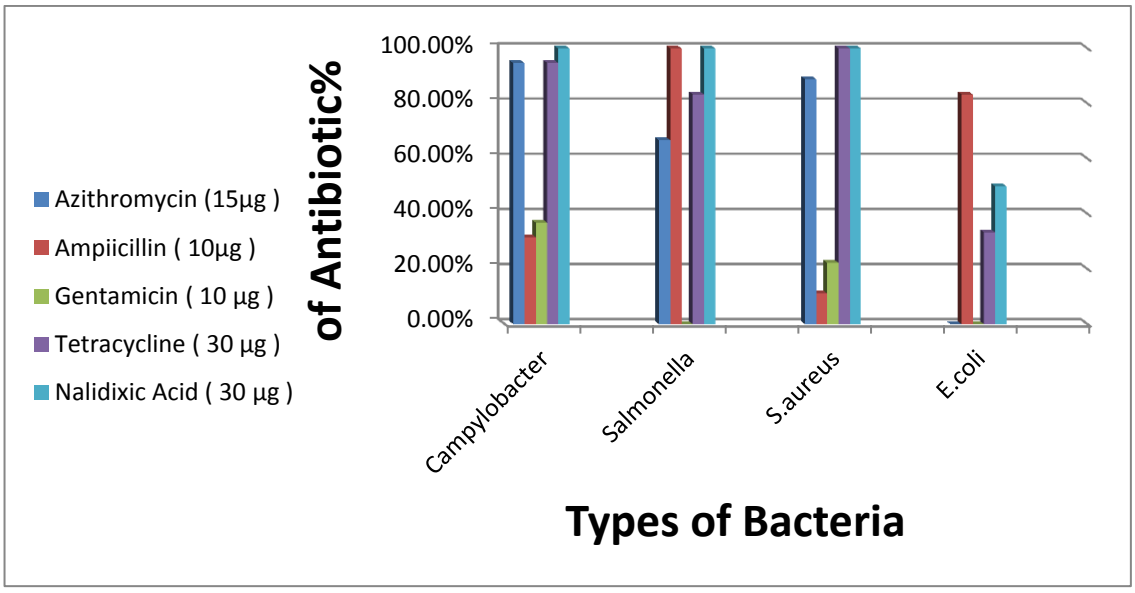

Fig (4.4): Diagram of resistance percentage for *Campylobacter, Salmonella, S.aureus* , and *E.coli* species against 5 types of antibiotic at 48 hr.

4.13 Sensitivity results of *Campylobacter, Salmonella, S.aureus* , and *E.coli* species at 48 :

Bacteria	% Sensitivity				
	Azithromycin (15µg)	Ampiicillin (10µg)	Gentamicin (10 µg)	Tetracycline (30 µg)	Nalidixic Acid (30 µg)
Campylobacter **(n=19)**	**5.26%**	**57.89%**	**36.84%**	**5.26**	**0**
Salmonella **(n=6)**	**33.33%**	**0**	**100%**	**0**	**0**
S.aureus **(n=9)**	**11.11%**	**88.88%**	**55.55%**	**0**	**0**
E.coli **(n=6)**	**100%**	**16.66%**	**100%**	**16.66%**	**33.33%**

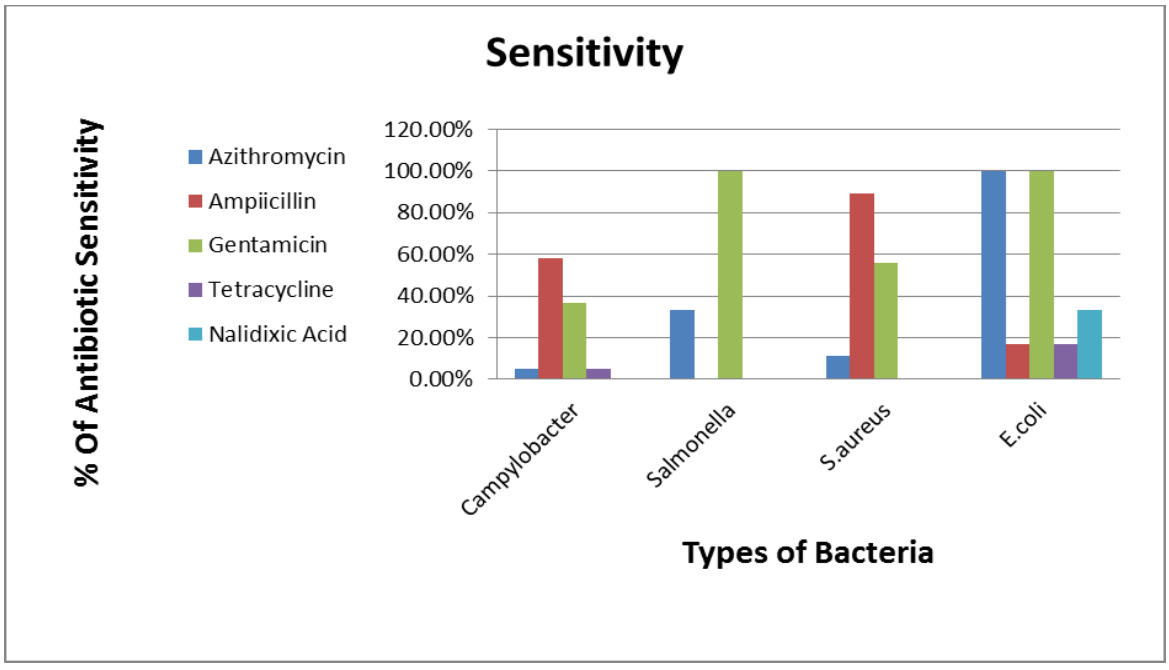

Fig (4.5): Diagram of sensitivity percentage for *Campylobacter, Salmonella* , *S.aureus* , and *E.coli* species against 5 types of antibiotic at 48 hr.

4.14 Intermediate results of *Campylobacter, Salmonella, S.aureus* , and *E.coli* species at 48 :

Bacteria	% Intermediate				
	Azithromycin (15µg)	Ampiicillin (10µg)	Gentamicin (10 µg)	Tetracycline (30 µg)	Nalidixic Acid (30 µg)
Campylobacter **(n=19)**	**0**	**10.52%**	**26.31%**	**0**	**0**
Salmonella **(n=6)**	**0**	**0**	**0**	**16.66%**	**0**
S.aureus **(n=9)**	**0**	**0**	**22.22%**	**0**	**0**
E.coli **(n=6)**	**0**	**0**	**0**	**50%**	**16.66%**

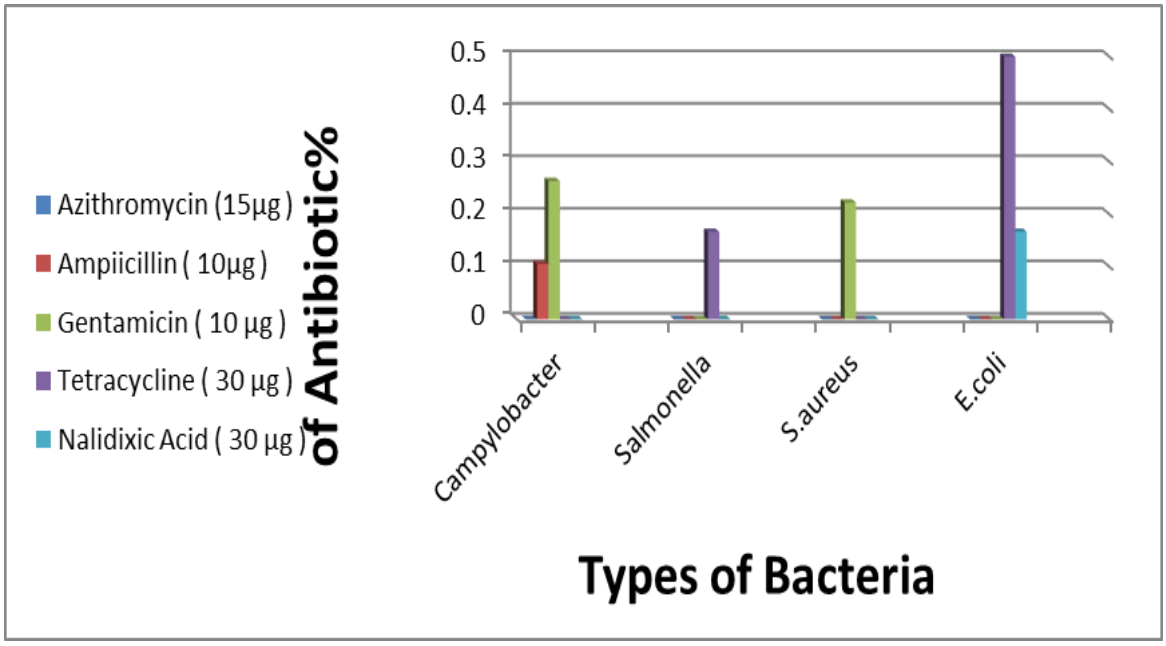

Fig (4.6): Diagram of intermediate percentage for *Campylobacter, Salmonella, S.aureus* , and *E.coli* species against 5 types of antibiotic at 48 hr

susceptibility were examined by measuring the zone of inhibition that gives an indication if the antibiotic has a sensitive , intermediate or resistance action on the grown bacteria , the following figure (4.7 , 4.8 , 4.9 , 4.10)are some examples of the plates .

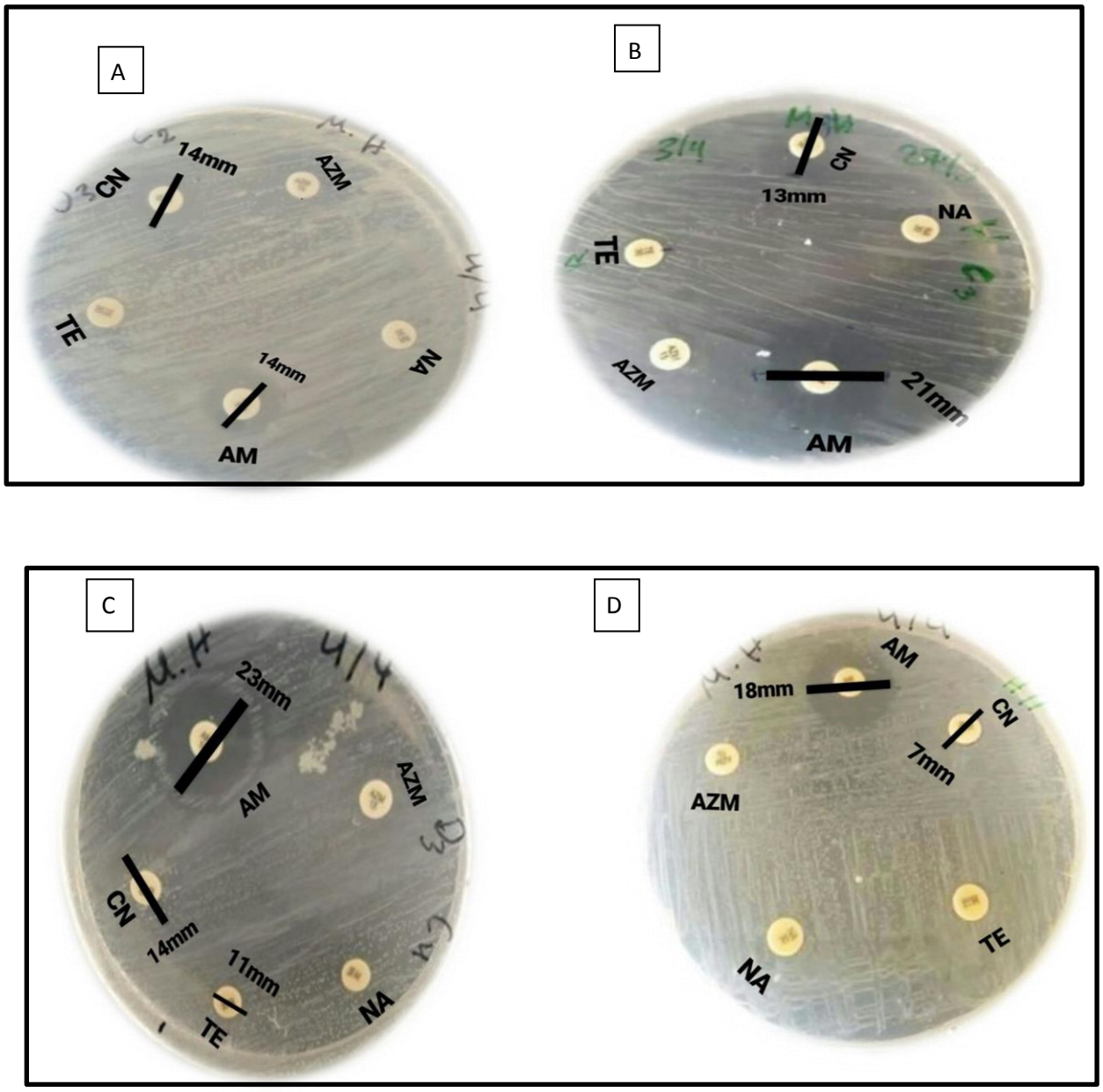

Figure 4.7. Plates showing antibiotic resistance of isolates from the chicken carcass of *campylobacter* (100µl) tested for resistance to each of five different antibiotics. The clear zones around each disc are the zones of inhibition that indicate the extent of the test organism's inability to survive in the presence of the test antibiotic**. A.** Campylobacter resistant to Azithromycin *(AZM 15),* Tetracycline*(TE 30),*and Nalidixic Acid*(NA 30).*Note *Campylobacter* Intermediate to Ampicillin*(AM 10)* ,and Gentamicin(CN 10),Zone of inhibition around AM ,CN 14 mm **.B.** Campylobacter resistant to Azithromycin *(AZM 15),* Tetracycline*(TE 30),and* Nalidixic Acid(NA 30).Note Campylobacter only sensitive to Ampicillin(AM 10) , Zone of inhibition around AM 21mm . and Intermediate to Gentamicin(CN 10),Zone of inhibition around CN 13 mm.**.C.** Campylobacter resistant *to* Azithromycin (AZM 15), Tetracycline(TE 30),and Nalidixic Acid(NA 30).Note Campylobacter only sensitive to Ampicillin(AM 10) , Zone of inhibition around AM 23mm . and Intermediate to Gentamicin(CN 10),Zone of inhibition around CN 14 mm**. D.** *Campylobacter* resistant to Azithromycin (AZM 15), Tetracycline(TE 30) Gentamicin(CN 10) ,and Nalidixic Acid(NA 30).Note *Campylobacter* only sensitive to Ampicillin(AM 10) , Zone of inhibition around AM 18mm .

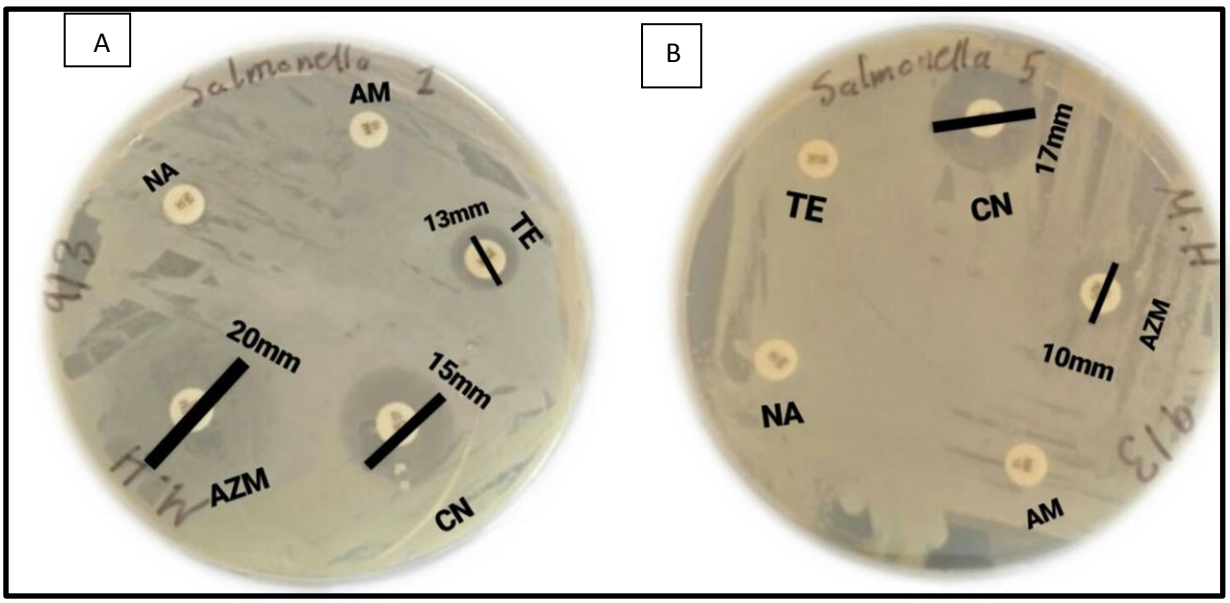

Figure 4.8. Plates showing antibiotic resistance of isolates from the chicken carcass of *Salmonella* (100µl) tested for resistance to each of five different antibiotics. The clear zones around each disc are the zones of inhibition that indicate the extent of the test organism's inability to survive in the presence of the test antibiotic ***.A. Salmonella*** sensitive to Azithromycin (AZM 15),*and* Gentamicin(CN 10) . Zone of inhibition around AZM 20 mm. Note Salmonella resistance to Ampicillin(AM 10) , Tetracycline(TE 30),and Nalidixic Acid(NA 30***).B. Salmonella*** only sensitive to *Gentamicin(CN 10)* . Zone of inhibition around *CN 15 mm. Note Salmonella* resistance to Ampicillin(AM 10*)* , Tetracycline*(TE 30),* Azithromycin *(AZM 15)and* Nalidixic Acid*(NA 30)***.**

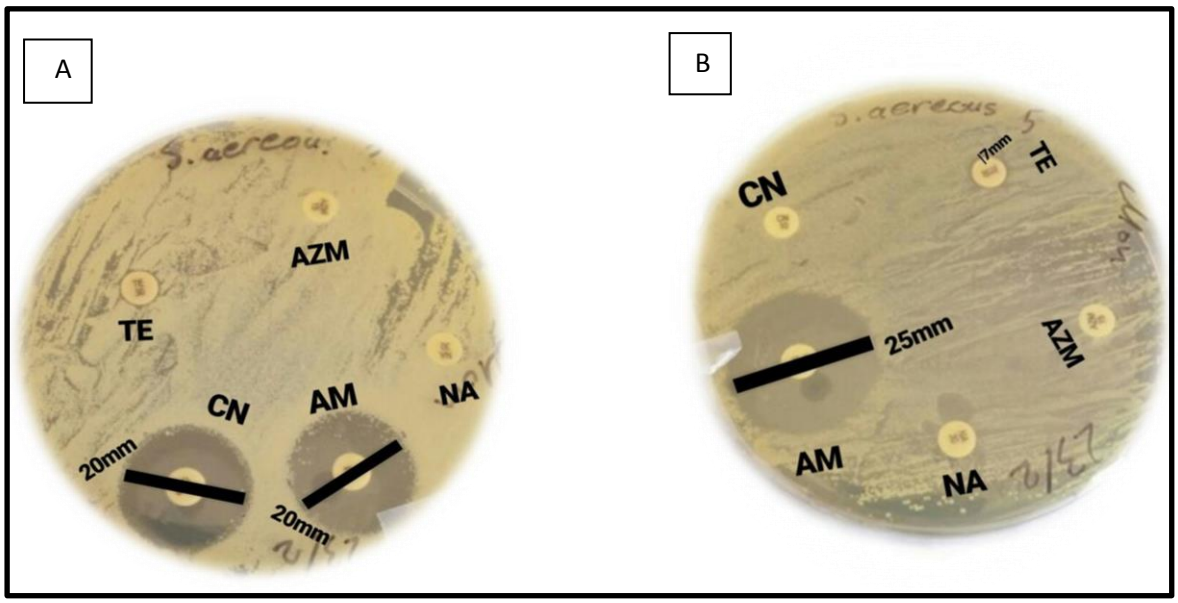

Figure 4.9. Plates showing antibiotic resistance of isolates from the chicken carcass of *Staphylococcus aureus* (100µl) tested for resistance to each of five different antibiotics. The clear zones around each disc are the zones of inhibition that indicate the extent of the test organism's inability to survive in the presence of the test antibiotic ***.A. Staphylococcus aureus*** sensitive to Ampicillin(AM 10) , *and* Gentamicin(CN 10) . Zone of inhibition around AM, and CN 20 mm. Note *Staphylococcus aureus* resistance *to* Tetracycline*(TE 30),* Azithromycin *(AZM 15), and* Nalidixic Acid*(NA 30)***.B. *Staphylococcus aureus*** sensitive only to Ampicillin(AM 10) , Zone of inhibition around AM 25 mm. *Note Staphylococcus aureus* resistance to Tetracycline(TE 30), Azithromycin (AZM 15), Gentamicin*(*CN 10) , and Nalidixic Acid*(*NA 30***).**

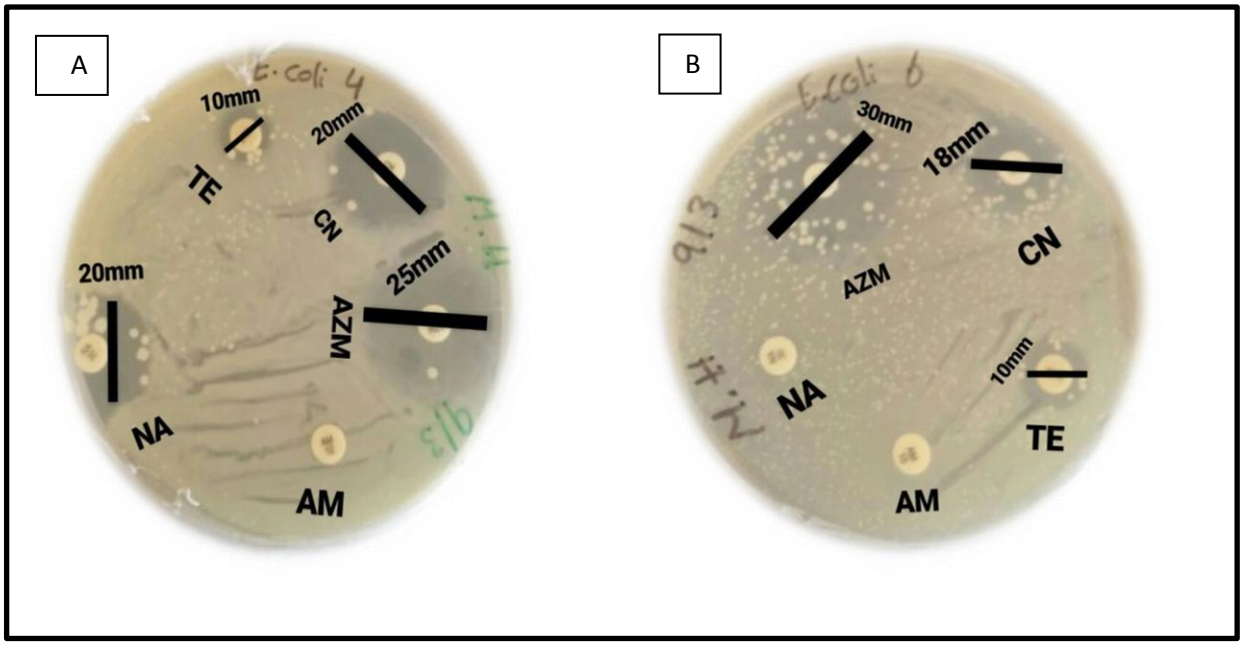

Figure 4.10. **Plates showing antibiotic resistance of isolates from the chicken carcass of *Escherichia coli* (100µl) tested for resistance to each of five different antibiotics.** The clear zones around each disc are the zones of inhibition that indicate the extent of the test organism's inability to survive in the presence of the test antibiotic **.*A. Escherichia coli*** sensitive to *Azithromycin* (AZM 15), *Nalidixic Acid*(NA 30**)** *and Gentamicin*(CN 10) . Zone of inhibition around AZM 25 mm , and NA, CN 20 mm. Note *Escherichia coli* resistance to *Tetracycline*(TE 30) , and *Ampicillin*(AM 10) **. B. *Escherichia coli*** sensitive to *Azithromycin* (AZM 15), *and Gentamicin*(CN 10) . Zone of inhibition around AZM 30 mm , and, CN 18 mm. Note *Escherichia coli* resistance to *Tetracycline*(TE 30) , *Ampicillin*(AM 10) and *Nalidixic Acid*(NA*).*

CHAPTER FIVE: DISCUSSION

5.1 Bacterial Pathogenesis

This study show the prevalence of *campylobacter, salmonella ,staphylococcus aureus* ,and *E. coli* and comparison of antibiotic susceptibility .These bacteria present the main causative of food poisoning in Palestine .

The control of these bacteria and antibiotic resistance are closely related to each other. It means, the control of these bacteria in animal, animals' food, environment and humans is the best possible way for control of antibiotic resistance.[37]

The main reservoir of these bacteria is the poultry's intestine .These bacteria have been associated with the consumption of uncooked meat and meat products, unpasteurized dairy products, and feces-contaminated vegetables and water. To prevent these bacterial infections, hygiene rules must be tightly observed.[1]

However, due to the random and inefficient use of antibiotics in poultry, it may increase the risk of development of drug-resistant bacteria strains, and this rising resistance is a concern for public health. Therefore, suitable selection for antibiotics and spread awareness among farmers.

5.2 Samples Identification

Samples were collected from a poultry farms in (Dora and Hebron), then diluted and cultured .After that they were put in a suitable environment to grow these bacteria for isolation . 40 colonies were isolated from total samples, 19 colonies isolation from *campylobacter,* 6 colonies isolation from *Salmonella,* 9 colonies isolation from *Staphylococcus aureus*, and 6 colonies isolation from *E.coli.*

5.3 Media characteristic

Campylobacter Selective Supplement (skirrow) with Colombia Blood Agar medium was prepared with specific concentrations. This media is a selective type for the isolation of *Campylobacter* species at 42 °C.

Campylobacter Selective Supplement (skirrow) contains three specific types of antibiotics. Vacomycin, bacteriostatic, and Polymyxin B prevent growth all bacteria except *campylobacter* .Sheep blood were added gradually to the Columbia Blood Agar as a food for *Campylobacter*.

Candle gar method was implemented in order to have the appropriate conditions for the *Campylobacter* growth (5% oxygen, 10 % carbon dioxide and 84-85 % nitrogen for 48hours at 42 °C). [38]

Muller Hinton Agar (500 ml) is considered as a nonselective and non-differential media , contain starch (for toxic absorption and mediating the antibiotic diffusion) . It was used for the isolation of pathogenic *Neisseria* species , but now it is commonly used for the susceptibility testing of microorganism by the Kirby-Bauer disk diffusion technique. [39]

Macconkey Agar (500 ml) is a selective and differential media used for the isolation differentiation of *E.coli* species with red/pink color, also for *Salmonella* and *Shigella* lactose non-fermenting strains that appears colorless and transparent .*E.coli* ferment lactose forming lactic acid .The crystal violet and bile salts inhibit the growth of gram-positive organisms which allows for the selection and isolation of gram-negative bacteria. Enteric bacteria that have the ability to ferment lactose can be detected using the carbohydrate lactose, and the pH indicator neutral red. [40]

Mannitol Salt Agar (500 ml) used for *S.aureus* isolation and differentiation , expressing yellow colonies with yellow zones . *S. aureus* ferments the mannitol, producing an acid, which changes the indicator from red to yellow. Those *Staphylococci* that do not ferment mannitol show a purple, pink or red zone around the colonies. [41]

Salmonella Shigella Agar (500 ml), that is moderately selective and differential medium for *Salmonella* and *Shigella* species . *Salmonella* species appears by colorless colonies, since it doesn’t ferment lactose, but it produces hydrogen sulfide gas . That

make it black center .However , *Shigella* species colonies are colorless without fermenting lactose or producing hydrogen sulfide gas . [42]

5.4 Campylobacter safety level

The safety level of presence campylobacter in food $10^{\wedge 7}$ cfu/g [4] . Our result the number of organisms present per unit sample approximately $10^{\wedge 6}$ cfu/g not exceed $10^{\wedge 7}$ cfu/g , mean below level normally associated with food poisoning, but the Campylobacter infectious dose is thought to be very low (<500 bacterial cells) . Moreover, the infective dose appears to be particularly low for children. From the few data available from outbreaks, it has been concluded that 100 cfu or levels of 10 cells of C. jejuni per 100 ml in contaminated milk was sufficient to cause disease in children

5.5 Antibiotic susceptibility

These antibiotics are considered as the drugs of choice for the treatment of human gastroenteritis infections, so the increased resistance of such strains poses a public health problem .

Isolates were then tested for sensitivity to Azithromycin , Ampicillin , Tetracycline, Gentamicin , and Nalidixic Acid antibiotics using the Kirby-Bauer disk diffusion test with reference of the Clinical and Laboratory Standards Institute (CLSI).

Results indicates the highest level of resistance among Campylobacter (n=19) tested was to Nalidixic Acid (NA) 100%, Tetracycline and Azithromycin (AZM) 94%,It showed moderate resistance to a Gentamycin 10.5 %, and Ampicillin 26.3%

While Salmonella (n=6) bacteria showed highly 100 % resistance to AM, NA, also 67 % to AZM. While its highly sensitive to CN antibiotic.

For S. aureus (n=9), disc diffusion testing showed 100% correlation with agar dilution for (TE) and (NA), and 89% AZM. A high level of sensitivity 100% to AZM, CN and 16.6% to AM was found in the E.coli isolates (n=6) by disc, whereas 83 % of isolates were resistance to AM, 50% to NA and low level 33.3% to TE.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

This study *Campylobacter, salmonella, staphylococcus aureus, and E. coli* show the prevalence and comparison of antibiotic susceptibility, to detect most sensitive and resistance of antibiotic for bacteria. These Bacteria the main cause of food poisining in human and animal that can transmit mainly by chicken carseses and others .The main reservoir of these bacteria is the poultry's intestine.

6.2 Recommendations

We recommend farmers to use Gentamycin, and Ampicillin to treatment infection of *Campylobacter* .While Salmonella using CN antibiotic. For S. aureus using CN and AM antibiotics .while E.coli using AZM, CN and AM..

Therefore, programs are recommended to increase farmer's awareness about the devastating effects of antibiotic misuse. In addition, the authorities must take a responsible role through imposition a set of regulations to ensure safe poultry products.

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APPENDIX

Appendix 1: All Media were prepared with ultrapure 500 ml water.

Name	Reagents
Colombia Blood Agar Base	11.5 Mg/L Peptone , 2 Mg/L Starch ,2.5Mg/L Sodium Chloride and 5Mg/L Agar.
Campylobacter Selective Supplement (skirrow)	10 Mg/L Vacomycin, 5 Mg/L Trimethoprim and 2500 IU Polymyxin B.
Muller Hinton Agar	1 Mg/L beef heart , 8.75Mg/L casein hydrolysate , 0.75Mg/L starch and 8.5Mg/L agar.
Macconkey Agar	8.5Mg/LPeptone,1.5Mg/L Proteose peptone,5Mg/L Lactose,0.75Mg/L Bile salts,2,5Mg/L Sodium chloride,0.015Mg/L Neutral red,0.0005Mg/L Crystal violet and 6.75Mg/L Agar.
Mannitol Salt Agar	2.5Mg/L enzymatic digest of casein ,2.5Mg/L enzymatic digest of animal tissue,0,5Mg/L, beef extract,5Mg/L D-mannitol,37.5Mg/L sodium chloride ,0.0125Mg/L phenol red and 7.5Mg/L agar.

Salmonella Shigella Agar	2.5Mg/LProteose peptone,5Mg/L Lactose,4.25Mg/L Bile salts mixture,4.25Mg/L, 4.25Mg/L Sodium citrate , 4.25Mg/L Sodium thiosulphate ,0.5Mg/L Ferric citrate,0.000165 Mg/L Brilliant green,0.0125Mg/L Neutral red and 6.75 Agar.
LB Browth	5 Mg/L Trypton , 5Mg/L NacL , and 2.5Mg/L Yeast extract

Appendix 2: Antibiotic susceptibility first results for the five isolates after 24 h.

Test / report Group	Zone of inhibition	Zone diameter Interpretive criteria (nearest whole mm)		
		S	I	R
Azithromycin(AZM), (15µg) Disk Content				
S.aureus (1)	No			R
S.aureus (2)	23 mm	S		
S.aureus (3)	No			R
S.aureus (4)	No			R
S.aureus (5)	No			R
S.aureus (6)	No			R
S.aureus (7)	8 mm			R
S.aureus (8)	9mm			R
S.aureus (9)	No			R
E.Coli (1)	23mm	S		
E.Coli (2)	30mm	S		
E.Coli (3)	24mm	S		
E.Coli (4)	25mm	S		
E.Coli (5)	22mm	S		
E.Coli (6)	30mm	S		
Salmonella(1)	17mm	S		
Salmonella(2)	No			R
Salmonella(3)	No			R
Salmonella(4)	No			R
Salmonella(5)	10mm			R
Salmonella(6)	19mm	S		
Ampicillin(AM),(10 µg) Disk Content				
S.aureus (1)	24mm	S		
S.aureus (2)	No			R
S.aureus (3)	20mm	S		
S.aureus (4)	24mm	S		
S.aureus (5)	24mm	S		
S.aureus (6)	19mm	S		
S.aureus (7)	19mm	S		
S.aureus (8)	22mm	S		
S.aureus (9)	24mm	S		
E.Coli (1)	No			R
E.Coli (2)	No			R
E.Coli (3)	No			R
E.Coli (4)	No			R
E.Coli (5)	34mm	S		
E.Coli (6)	No			R
Salmonella(1)	No			R
Salmonella(2)	No			R
Salmonella(3)	No			R
Salmonella(4)	No			R

Salmonella(5)	No			R
Salmonella(6)	No			R
Gentamicin (CN), (10µg) Disk Content				
S.aureus (1)	13mm		I	
S.aureus (2)	17mm	S		
S.aureus (3)	20mm	S		
S.aureus (4)	17mm	S		
S.aureus (5)	No			R
S.aureus (6)	14mm		I	
S.aureus (7)	16mm	S		
S.aureus (8)	18mm	S		
S.aureus (9)	No			R
E.Coli (1)	25mm	S		
E.Coli (2)	21mm	S		
E.Coli (3)	22mm	S		
E.Coli (4)	20mm	S		
E.Coli (5)	20mm	S		
E.Coli (6)	18mm	S		
Salmonella(1)	13mm		I	
Salmonella(2)	15mm	S		
Salmonella(3)	16mm	S		
Salmonella(4)	18mm	S		
Salmonella(5)	17mm	S		
Salmonella(6)	16mm	S		
Tetracycline(TE), (30µg) Disk Content				
S.aureus (1)	No			R
S.aureus (2)	No			R
S.aureus (3)	No			R
S.aureus (4)	No			R
S.aureus (5)	7mm			R
S.aureus (6)	No			
S.aureus (7)	7mm			R
S.aureus (8)	8mm			R
S.aureus (9)	9mm			R
E.Coli (1)	15mm	S		
E.Coli (2)	10mm			R
E.Coli (3)	No			R
E.Coli (4)	10mm			R
E.Coli (5)	10mm			R
E.Coli (6)	10mm			R
Salmonella(1)	14mm		I	
Salmonella(2)	No			R
Salmonella(3)	No			R
Salmonella(4)	No			R
Salmonella(5)	No			R
Salmonella(6)	No			R
Nalidixic Acid (NA), (30µg) Disk Content				
S.aureus (1)	No			R
S.aureus (2)	No			R

S.aureus (3)	No			R
S.aureus (4)	No			R
S.aureus (5)	No			R
S.aureus (6)	No			R
S.aureus (7)	No			R
S.aureus (8)	No			R
S.aureus (9)	No			R
E.Coli (1)	24mm	S		
E.Coli (2)	9mm			R
E.Coli (3)	10mm			R
E.Coli (4)	20mm	S		
E.Coli (5)	3mm			R
E.Coli (6)	No			R
Salmonella(1)	No			R
Salmonella(2)	No			R
Salmonella(3)	No			R
Salmonella(4)	No			R
Salmonella(5)	No			R
Salmonella(6)	No			R

Appendix 3: Antibiotic susceptibility first results for the *campylobacter* isolates from Dura 1 after 24 h

Test / report Group	Disk content	Zone diameter Interpretive criteria (nearest whole mm)		
		S	I	R
Azithromycin(AZM), (15µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	No			R
C 4	No			R
Ampicillin(AM),(10 µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	14cm		I	
C 4	11 mm			R
Gentamicin (CN), (10µg) Disk Content				
C 1	15 mm	S		
C 2	17 mm	S		
C 3	12 mm			R
C 4	12 mm			R
Tetracycline(TE), (30µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	No			R
C 4	No			R
Nalidixic Acid (NA), (30µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	No			R
C 4	No			R

Appendix 4: Antibiotic susceptibility first results for the *campylobacter* isolates from Dura 2 after 24 h

Test / report Group	Disk content	Zone diameter Interpretive criteria (nearest whole mm)		
		S	I	R
Azithromycin(AZM), (15µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	No			R
C 4	No			R
C 5	No			R
Ampicillin(AM),(10 µg) Disk Content				
C 1	23 mm	S		
C 2	12 mm			R
C 3	21 mm	S		
C 4	18 mm	S		
C 5	17 mm	S		
Gentamicin (CN), (10µg) Disk Content				
C 1	No			R
C 2	14 mm		I	
C 3	19 mm	S		
C 4	14 mm		I	
C 5	17 mm	S		
Tetracycline(TE), (30µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	No			R
C 4	10 mm			R
C 5	No			R
Nalidixic Acid (NA), (30µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	No			R
C 4	No			R
C 5	No			R

Appendix 5: Antibiotic susceptibility first results for the *campylobacter* isolates from Dura 3 after 24 h

Test / report group	Zone of inhibition	Zone diameter Interpretive criteria (nearest whole mm)		
		S	**I**	**R**
Azithromycin(AZM), (15µg) Disk Content				
C1	No			R
C2	No			R
Ampicillin(AM),(10 µg) Disk Content				
C1	27 mm	S		
C2	26 mm	S		
Gentamicin (CN), (10µg) Disk Content				
C1	13 mm		I	
C2	15 mm	S		
Tetracycline(TE), (30µg) Disk Content				
C1	No			R
C2	20cm	S		
Nalidixic Acid (NA), (30µg) Disk Content				
C1	No			R
C2	No			R

Appendix 6: Antibiotic susceptibility first results for the *campylobacter* isolates from Hebron after 24 h.

Test / report Group	Disk content	Zone diameter Interpretive criteria (nearest whole mm)		
		S	I	R
Azithromycin(AZM), (15µg) Disk Content				
H 1	No			R
H 2	No			R
H 3	No			R
H 4	No			R
H 5	No			R
H 6	11 mm			R
H 7	No			R
H 8	No			R
Ampicillin(AM),(10 µg) Disk Content				
H 1	No			R
H 2	21 mm	S		
H 3	14 mm		I	
H 4	22 mm	S		
H 5	12 mm			R
H 6	17 mm	S		
H 7	No			R
H 8	18 mm	S		
Gentamicin (CN), (10µg) Disk Content				
H 1	13 mm		I	
H 2	15 mm	S		
H 3	No			R
H 4	15 mm	S		
H 5	9 mm			R
H 6	No			R
H 7	14 mm		I	
H 8	7 mm			R
Tetracycline(TE), (30µg) Disk Content				
H 1	No			R
H 2	No			R
H 3	No			R
H 4	No			R
H 5	8 mm			R
H 6	No			R
H 7	No			R
H 8	No			R
Nalidixic Acid (NA), (30µg) Disk Content				
H 1	No			R
H 2	No			R
H 3	No			R
H 4	No			R
H 5	No			R
H 6	No			R
H 7	No			R
H 8	No			R

Appendix 7: Antibiotic susceptibility 2nd results for the five isolates after 48 h.

Test / report Group	Zone of inhibition	Zone diameter Interpretive criteria (nearest whole mm)		
		S	I	R
Azithromycin(AZM), (15µg) Disk Content				
S.aureus (1)	No			R
S.aureus (2)	23mm	S		
S.aureus (3)	No			R
S.aureus (4)	No			R
S.aureus (5)	No			R
S.aureus (6)	No			R
S.aureus (7)	9mm			R
S.aureus (8)	9mm			R
S.aureus (9)	No			R
E.Coli (1)	26mm	S		
E.Coli (2)	32mm	S		
E.Coli (3)	31mm	S		
E.Coli (4)	30mm	S		
E.Coli (5)	29mm	S		
E.Coli (6)	32mm	S		
Salmonella(1)	20mm	S		
Salmonella(2)	No			R
Salmonella(3)	No			R
Salmonella(4)	No			R
Salmonella(5)	10mm			R
Salmonella(6)	21mm	S		
Ampicillin(AM),(10 µg) Disk Content				
S.aureus (1)	27mm	S		
S.aureus (2)	No			R
S.aureus (3)	20mm	S		
S.aureus (4)	26mm	S		
S.aureus (5)	25mm	S		
S.aureus (6)	20mm	S		
S.aureus (7)	21mm	S		
S.aureus (8)	23mm	S		
S.aureus (9)	24mm	S		
E.Coli (1)	No			R
E.Coli (2)	No			R
E.Coli (3)	No			R
E.Coli (4)	No			R
E.Coli (5)	34mm	S		
E.Coli (6)	No			R
Salmonella(1)	No			R

Salmonella(2)	No			R
Salmonella(3)	No			R
Salmonella(4)	No			R
Salmonella(5)	No			R
Salmonella(6)	No			R
Gentamicin (CN), (10µg) Disk Content				
S.aureus (1)	14mm		I	
S.aureus (2)	19mm	S		
S.aureus (3)	21mm	S		
S.aureus (4)	18mm	S		
S.aureus (5)	No			R
S.aureus (6)	14mm		I	
S.aureus (7)	17mm	S		
S.aureus (8)	19mm	S		
S.aureus (9)	No			R
E.Coli (1)	25mm	S		
E.Coli (2)	23mm	S		
E.Coli (3)	24mm	S		
E.Coli (4)	24mm	S		
E.Coli (5)	23mm	S		
E.Coli (6)	18mm	S		
Salmonella(1)	15mm	S		
Salmonella(2)	17mm	S		
Salmonella(3)	20mm	S		
Salmonella(4)	20mm	S		
Salmonella(5)	18mm	S		
Salmonella(6)	16mm	S		
Tetracycline(TE), (30µg) Disk Content				
S.aureus (1)	7mm			R
S.aureus (2)	No			R
S.aureus (3)	7mm			R
S.aureus (4)	No			R
S.aureus (5)	7mm			R
S.aureus (6)	No			R
S.aureus (7)	8mm			R
S.aureus (8)	8mm			R
S.aureus (9)	9mm			R
E.Coli (1)	15mm	S		
E.Coli (2)	12mm		I	
E.Coli (3)	No			R
E.Coli (4)	10mm			R
E.Coli (5)	12mm		I	
E.Coli (6)	14mm		I	
Salmonella(1)	14mm		I	
Salmonella(2)	No			R
Salmonella(3)	No			R
Salmonella(4)	No			R
Salmonella(5)	No			R
Salmonella(6)	No			R

Nalidixic Acid (NA), (30µg) Disk Content				
S.aureus (1)	7mm			R
S.aureus (2)	No			R
S.aureus (3)	No			R
S.aureus (4)	No			R
S.aureus (5)	No			R
S.aureus (6)	No			R
S.aureus (7)	No			R
S.aureus (8)	No			R
S.aureus (9)	No			R
E.Coli (1)	27mm	S		
E.Coli (2)	10mm			R
E.Coli (3)	14mm		I	
E.Coli (4)	20mm	S		
E.Coli (5)	3mm			R
E.Coli (6)	No			R
Salmonella(1)	No			R
Salmonella(2)	No			R
Salmonella(3)	No			R
Salmonella(4)	No			R
Salmonella(5)	No			R
Salmonella(6)	No			R

Appendix 8: Antibiotic susceptibility 2nd results for the *campylobacter* isolates from Dura 1 after 48 hr.

Test / report Group	Disk content	Zone diameter Interpretive criteria (nearest whole mm)		
		S	I	R
Azithromycin(AZM), (15µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	No			R
C 4	No			R
Ampicillin(AM),(10 µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	14mm		I	
C 4	11 mm			R
Gentamicin (CN), (10µg) Disk Content				
C 1	15 mm	S		
C 2	17 mm	S		
C 3	12 mm			R
C 4	12 mm			R
Tetracycline(TE), (30µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	No			R
C 4	No			R
Nalidixic Acid (NA), (30µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	No			R
C 4	No			R

Appendix 9: Antibiotic susceptibility 2nd results for the *campylobacter* isolates from Dura 2 after 48 hr.

Test / repor Group	Zone of inhibition	Zone diameter Interpretive criteria (nearest whole mm)		
		S	I	R
Azithromycin(AZM), (15µg) Disk Content				
C1	No			R
C2	No			R
Ampicillin(AM),(10 µg) Disk Content				
C1	27 mm	S		
C2	26 mm	S		
Gentamicin (CN), (10µg) Disk Content				
C1	13 mm		I	
C2	16 mm	S		
Tetracycline(TE), (30µg) Disk Content				
C1	No			R
C2	20mm	S		
Nalidixic Acid (NA), (30µg) Disk Content				
C1	No			R
C2	No			R

Appendix 10: Antibiotic susceptibility 2nd results for the *campylobacter* isolates from Dura 3 after 48 h.

Test / report group	Disk content	Zone diameter Interpretive criteria (nearest whole mm)		
		S	I	R
Azithromycin(AZM), (15µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	No			R
C 4	No			R
C 5	No			R
Ampicillin(AM),(10 µg) Disk Content				
C 1	26 mm	S		
C 2	14 mm		I	
C 3	21 mm	S		
C 4	23 mm	S		
C 5	17 mm	S		
Gentamicin (CN), (10µg) Disk Content				
C 1	No			R
C 2	14 mm		I	
C 3	19 mm	S		
C 4	14 mm		I	
C 5	17 mm	S		
Tetracycline(TE), (30µg) Disk Content				
C 1	8mm			R
C 2	No			R
C 3	7mm			R
C 4	11 mm			R
C 5	No			R
Nalidixic Acid (NA), (30µg) Disk Content				
C 1	No			R
C 2	No			R
C 3	7mm			R
C 4	No			R
C 5	No			R

Appendix 11: Antibiotic susceptibility first results for the *campylobacter* isolates from Hebron after 48 h.

Test / report Group	Disk content	Zone diameter Interpretive criteria (nearest whole mm)		
		S	I	R
Azithromycin(AZM), (15µg) Disk Content				
H 1	No			R
H 3	No			R
H 4	No			R
H 7	No			R
H 8	No			R
H 9	17 mm	S		
H 10	No			R
H 11	No			R
Ampicillin(AM),(10 µg) Disk Content				
H 1	No			R
H 3	21 mm	S		
H 4	21 mm	S		
H 7	22 mm	S		
H 8	12 mm			R
H 9	19 mm	S		
H 10	No			R
H 11	18 mm	S		
Gentamicin (CN), (10µg) Disk Content				
H 1	13 mm		I	
H 3	16 mm	S		
H 4	8mm			R
H 7	15 mm	S		
H 8	9 mm			R
H 9	No			R
H 10	14 mm		I	
H 11	7 mm			R
Tetracycline(TE), (30µg) Disk Content				
H 1	No			R
H 3	No			R
H 4	8mm			R
H 7	No			R
H 8	8 mm			R
H 9	No			R
H 10	No			R
H 11	No			R
Nalidixic Acid (NA), (30µg) Disk Content				
H 1	No			R
H 3	No			R
H 4	No			R
H 7	No			R
H 8	No			R
H 9	No			R
H 10	No			R
H 11	No			R