

Isolation and Identification of Salmonella species from Chickens in Khartoum State

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((اللهم انفعني بما علمتني و علمني بما ينفعني وزدني علما))

PREFACE

***This work was carried out at the Department of
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of Dr. Ahmed Zaki Saad and Prof. Amal Mustafa
Mohamed Ali as co-supervisor***

DEDICATION

To those who give my life.....It's meaning and it's shine

*My father's soul
My lovely mother
Brothers.....Sister
Relatives & Friends*

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ABSTRACT

This study was carried out to determine the incidence of Salmonellosis in poultry in Khartoum state. A total of 102 samples (27 intestine, 26 ovaries, 25 livers and 24 hearts) were collected from diseased birds showing signs of weakness, loss of appetite, poor growth and adherence of white chalky material at the vent region. A total of 60 blood samples were also collected for serology.

Three *Salmonella* isolates were recovered and identified. Two of them were *S. enteritidis* (isolated from intestine) and the third strain was *S. arizonae* (isolated from liver). Other bacteria were also isolated and included 8 (7.8%) *E. coli*, 17 (16.6%) *Klebsiella* spp., 21 (20.5%) *Proteus* spp., 24 (23.5%) *Pseudomonas* spp., 12 (11.7%) *Yersinia* spp. and 2 (2%) *Shigella* spp.

Collected sera from blood samples were tested against commercial stained antigen of *Salmonella pullorum* (Intervet company, Holland) using the rapid serum agglutination test (RSA). No antibodies were detected in all examined sera.

Sensitivity test of *Salmonella* and other bacterial isolates against different antibiotics was carried out using the standard disc method. *Salmonella* isolates were found sensitive to ampicillin, kanamycin, gentamycin, ciprofloxacin and chloromphenicol, and resistant to erythromycin, while moderately sensitive to doxycycline. *S. arizonae* was moderately sensitive to nitrofurantoin while *S. enteritidis* was sensitive.

Other bacterial isolates were sensitive to gentamycin and ciprofloxacin and resistance to doxycycline. The isolates showed varying degree of sensitivity to Kanamycin, erythromycin, nitrofurantoin ampicillin and choromphenicol .

بسم الله الرحمن الرحيم

ملخص الأطروحة

تتلخص اهداف هذه الدراسة فى تحديد انتشار داء السالمونيلا فى الدواجن فى ولاية الخرطوم.

تم جمع جملة 102 عينة (27 امعاء، 26 مبيض، 25 كبد و24 قلب) من دواجن مصابه تظهر اعراض من الاعياء، فقدان الشهيه، نمو ضعيف والتصاق مواد جبريه فى منطقه الشرج. بالاضافه الي جمع 60 عينه دم للاختبارات السيرولوجيه.

عزلت ثلاثه عزلات من باكتريا السالمونيلة وصنفت كالاتى: عزلتين من السالمونيلة الملهبه للامعاء وتم عزلها من الامعاء و العزلة الثالثه السالمونيلة الأريزونية وعزلت من الكبد. كما تم عزل بكتريا اخري وصنفت كالاتى: الاشريشيا القولونيه 8 (7.8%)، كلبسيلا 17 (16.6%)، بروتييس 21 (20.5%)، سيدوموناس 24 (23.5%)، يرسينيا 12 (11.7%) وشيقل 2 (2%).

المصل المفصول من عينات الدم تم اختباره ضد انتجين السالونيلة بلورم باستخدام اختبار التلازن ولم تظهر اجسام مضاده.

تم إجراء اختبار الحساسيه للسالمونيلة والعترات البكتريه الاخرى باستخدام عدده مضادات حيويه. عزلات السالمونيلة اعطت حساسيه للامبسلين، كاناميسين، جنتاميسين، سبروفلوكسين والكلورمفينيكول ومقاومه للارثرومييسين. ووجدت متوسطه الحساسيه للدوكسيسايكلين. السالمونيلة الأريزونية وجدت متوسطه الحساسيه للنايتروفيرنتوين بينما السالونيل الامعائيه حساسه له. اظهرت البكتريا الاخرى حساسيه للجنتاميسين والسبروفلوكسين بينما كانت مقاومه للدوكسيسايكلين. وهنالك درجات مختلفه للحساسيه ضد الكاناميسين و الارثرومييسين والنايترو فيرنتوين والامبسلين والكلورامفينيكول.

INTERODUCTION

Members of the family *Enterobacteriaceae* are Gram-negative, non-spore forming rods. Some of them are human and animal pathogens producing intestinal infection and food poisoning. The genera of pathogenic importance in poultry include *Salmonella* and *Escherichia* (Holt *et al.*, 1994).

Salmonella as a group of microorganisms has long been recognized as an important zoonotic pathogen of worldwide economic significance in animals, birds and man. They are intestinal bacteria which give rise to enteritis and typhoid-like disease. The early observation of the disease was made by Eberth (1880) who described the typhoid bacillus in the tissue of a dead patient, and the organisms was isolated by Salmon in 1885 (Merchant & packer 1967) and named after him.

Avain Salmonellosis is an acute or chronic disease of fowl caused by different species including *S. pullorum* (Pullorum disease), *S. gallinarum* (Fowl typhoid), *S. arizonae* (arizonae infection), *S. typhimurium*, *S. enteritidis* and others (paratyphoid infection) (Carter and Wise, 2004).

Salmonellosis in poultry resulted in continuous increase of public health problems as stated by Corrier *et al.* (1990). Contamination of poultry meat with *Salmonella* was investigated by many scientists in Sudan as well as in other countries. In the Sudan, Mamon *et al.* (1992) succeeded to isolate 21 *Salmonella enteritidis* from embryonated eggs. Yagoub and Mohamed (1987) studied the occurrence of *Salmonella* in poultry carcasses in Khartoum State; twenty three serotypes were identified and most of them were *S. mons* and *S. amek*. *S. auganda*

(Khan, 1969) and *S. sandiego* (Rahman *et al.*, 2000) were also isolated from poultry ration in the Sudan.

During 1990s, *S. enteritidis* infection increased in Europe (Nilakovic *et al.*, 1990; Pitol *et al.*, 1991). In Portugal , 57% out of 300 chicken carcasses yielded salmonellae when they were tested by swabbing method. Demonstrated serotypes were *S. enteritidis* (66%) *S. derby* (4%), *S. typhimurium* (3%) and *S. brado* (1%) (Machado and Bernardo, 1990).

The main objective of the present study was to investigate the incidence of fowl salmonellosis in Khartoum State and to determine the sensitivity of *Salmonella* isolates to most commonly used antibiotics in the Sudan.

CHAPTER ONE

LITERATURE REVIEW

1.1. *Salmonella*:

Salmon and Smith were the first to isolate *Salmonella* from pigs in (1885) (cited by Ryan and Ray, 2004). *Salmonella* is an important genus of the family *Eenterobacteriaceae*. Members of the genus are Gram-negative, facultative anaerobes and inhabit the intestinal tract of man and animals (Holt *et al.*, 1994). They may be recovered from a wide range of hosts such as poultry, swine, human, foods and from the environment. Members of the genus *Salmonella* may be pathogenic to wild or domestic animals and human (Holt *et al.*, 1994).

It is an important pathogen to the food industry and has been frequently identified as the etiological agent of food borne outbreaks (Siqueira *et al.*, 2003; Zhao *et al.*, 2001). In human the pathogenic condition of *Salmonella* include enteric fever, gastroenteritis and septicemia.

1.2. Classification:

The scientific classification of *Salmonella* was described by Hafez (2005) as follows:

Domain: *Bacteria*

Kingdom: *Monera*

Phylum: *Proteobacteria*

Class: *Gamma Protobacteria*

Order: *Enterobacteriales*

Family: *Enterobacteriaceae*

Genus: *Salmonella*

Recent advances in *Salmonella* taxonomy divide the genus into two species: *Salmonella bongori* and *Salmonella enterica* (Le Minor and Popoff, 1987). *S. bongori* contains less than 10 serovars while *S. enterica* contains more than 2500 serovars and are divided into six subspecies namely *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*.

All centers of disease control and prevention recommended that *Salmonella* species should be named by their genus and serovar e.g. *Salmonella typhi* instead of *Salmonella enterica subspecies enterica serovar Typhi*.

Most commonly, the *Salmonella* are classified according to serology. The main division is first by the somatic (O) antigen and by the flagellar (H) antigen. (O) Antigen is of a lipopolysaccharide nature and (H) antigen of protein nature (Kauffmann White Scheme 1960).

The genus *Salmonella* can roughly be classified into 3 groups (Hafez and Jodas 2000). Group I includes highly host adapted and invasive serovars such as *S. gallinarium*, *S. polurium* in poultry and *S. typhi* in human. Group II includes non-host adapted and invasive serovars such as *S. typhimurium*, *S. arizonae* and *S. enteritidis*. Group III contains non-host adapted and non invasive serovars, most of these serovares are harmless for animals and human.

1.3. Cultural characteristics:

Salmonella are facultative anaerobic. The optimum growth temperature is 37°C, but some growth is observed in a range from about 5 to 45°C. *Salmonella* can grow within a pH range of approximately 4.0 to 9.0, with an optimum pH around 7.0 (Cruickshank, 1972).

The organisms grow in selective enrichment media such as selenite-F-broth and tetrathionate broth, and on differential plating media such as MacConkey, bismuth sulfite, and brilliant green agars.

The optimum incubation times for *Salmonella* enrichment cultures were obtained by inoculation of enrichment broth onto plating media after 24 hours incubation at 37°C, after 48 hours at 37°C, after a 3-day delayed secondary enrichment (DSE), and after a 5-day DSE procedure. Inoculations of the enrichment broth onto plating media after 24 hours incubation followed by 5-day (DSE) enable the detection of 96-98% of *Salmonella* positive samples and were the best combination of condition (Waltman *et al.*, 1993).

The *Salmonella* colonies appear with different shapes and colours on different media. On nutrient agar they appear small, smooth, circular and translucent while *S. gallinarum* colonies are blue gray. On macConky agar they are colourless, smooth, round, shiny and up to 2mm in diameter. *S. gallinarum* produce colonies larger than *S. pullorum* and have a characteristic odour. On selenite-F- broth the growth is turbid with heavy flocculent sediment. On desoxycholate citrate agar (DCA) the colonies are slightly opaque, dome shaped with central black spot. *S. pullorum* is a lactose fermenter producing pink colonies with a precipitate in surrounding media. On triple sugar iron agar (TSI) *S. pullorum* and *S. gallinarum* produce a red slant with a yellow butt that show delayed blackening from H₂S production.

1.4. Biochemical characteristics:

The genus *Salmonella* produce usually gas from glucose except *S. typhi* which ferments glucose and manitol without gas production (Cruickshank, 1972). Hydrogen sulphide is usually produced on triple sugar iron agar but some strains of *S. choleraesuis* and most stains of *S. paratyphi A* do not, and *S. arizonae* utilizes manitol.

Nitrate is reduced to nitrite and citrate is usually utilized by *Salmonella* as a carbon source (Minor, 1984). The members are urease,

indole and oxidase negative but catalase positive (Cruickshank, 1972). Sucrose, salicin and lactose are not generally fermented by *Salmonella*. However many strains of *S. arizonae* ferments lactose rapidly or slowly as well as having activity to B-galactosidase enzyme (Holt *et al.*, 1994).

1.4.1 Analytical profile index (API) for enterobacteria:

It is a multitest micromethod for the identification of members of the family *Enterobacteriaceae* and other Gram-negative bacteria.

This system utilizes a plastic strip with 20 separate compartments. Each compartment consists of a depression and a small tube that consist a specific dehydrated medium. The system has a capacity of 23 biochemical tests (Benson, 1998). It identifies members of enterobacteria to the species level.

1.5. Antigenic Structure:

The antigenic classification of *Salmonella* is based on a number of antigens namely O, H, K, M and 5 antigens.

The (O) somatic antigens are polysaccharides that associate with the body of the cell and are designated with Arabic numerals (Buxton and Frasser, 1977). These antigens are heat and alcohol stable (Kauffmann 1966). Serogroups of *Salmonellae* are defined by particular somatic antigens; most *Salmonella* isolates in poultry belong to serogroups B, C, or D.

The (H) antigens are determined by flagellar proteins and are both heat and alcohol-labile, divided in two phases 1 and 2, and designated with small letters and Arabic numerals (Williams, 1972).

The (K) antigens are capsular or envelope antigens (Kauffmann 1966). A capsular antigen (namely Vi) is discovered by Feleix and Pitt (1943). This antigen is destroyed by boiling for twenty minutes.

The (M) antigens are mucoid antigens which are found in mucoid strains of *S. paratyphi B* (Kauffmann 1966). It is polysaccharide nitrogen free and produces more than 4% glucose on hydrolysis (Birch Hirschfeld 1935). The (5) antigen is mucoid antigen and completely destroyed by heating at 120 °C and normal hydrochloric acid.

Both *S. pullorum* and *S. gallinarum* possess the (O) antigens 1, 9, and 12 (Wilson and Nordholm, 1995). Variation involving antigen 12 occurs in *S. pullorum* strains only, which contains (O) antigens 9, 12₁, 12₂, and 12₃.

1.6. Serological tests:

These are satisfactory for establishing the presence and estimating the prevalence of the infection within a flock. The tests that are readily applied include tube agglutination (TA) test, rapid serum agglutination (RSA) test, stained antigen whole blood (WB) test, and micro-agglutination (MA) test (Gast, 1997). Other serological tests include micro-antiglobulin (Coombs), immunodiffusion, haemagglutination and enzyme linked immunosorbent assay (ELISA).

The rapid serum agglutination test can be used under field conditions and the reactors can be identified immediately. Chickens can be tested at any age, although some authorities specify a minimum age of 4 months (Wray, 2000)

1.7. Pathogenicity of *Salmonella*:

Three toxins (endotoxin, enterotoxin and cytotoxin) play roles in the pathogenicity of *Salmonella*. The endotoxin produces fever (Pfeiffer, 1894; Chantemasse, 1897; Briger, 1902), the enterotoxin causes less mucosal damage in cell culture and the cytotoxin inhibits protein synthesis (Koo *et al.*, 1984).

1.8. Drug susceptibility:

An increase of *Salmonella* strains showing resistance and multiple resistances against different antibiotics have been found from isolates from poultry in recent years. Kheir El-Din *et al.* (1987) examined in vitro the sensitivity of 89 isolates of *S. gallinarum*, *S. pullorum*, *S. Virchow* and *S. newport* against 11 antibiotics. The results revealed that 70-80% of the isolates were sensitive to flumaquine and chlormphenicol, and that 38-57% were moderately sensitive to nitrofurantoin, ampicillin and neomycin and only 15-18% were weakly sensitive to lincomycin and streptomycin, but completely resistant to erythromycin, penicillin, tetracycline and trimethoprim.

Bolinski *et al.* (1988) reported that flumaquine inhibited 86% of *Salmonella* strains, followed by apramycin, ampicillin, oxytetracycline and gentamycin. The minimum inhibitory concentration of flumaquine varied between 1.0 and 5.0 µg/ml. On the other hand, Ghosh (1988) found that 36 strains of *S. virchow* were highly sensitive to gentamycin, streptomycin and kanamycin but resistant to bacitracin, penicillin, sulphaphenazole and tetracycline.

Lee *et al.* (1993) determined that 57% of 105 *Salmonella* isolated were resistant to one or more antimicrobial agent and 45% were resistant to two or more agents. Highest resistance was to tetracycline 45%, streptomycine 41%, sulfisoxazole 19% and gentamycin 10%

Jacobs *et al.* (1994) reported that 7.5% of 94 *Salmonella* isolated were resistant to nalidixic acid and flumaquine but did not to ciprofloxacin.

Roliniski *et al.* (1994) determined that 52.98% of *S. enteritidis* and *S. typhimurium* were resistant to nitrofurans, oxytetracycline, sulphonamides alone and with trimethoprim. The similar levels of

resistance (49.84%) were shown by *S. gallinarum* isolates to oxyteracycline and sulphonamides alone and with trimethoprim and only 8% were resistant to nitofurans.

Esaki *et al.* (2004) isolated 94 *Salmonella* strains of 10 serotypes from different poultry farms in Chile (broiler and layyin hens). Thirty-nine of them were resistant to flumaquine, nalidixic acid and oxolinic acid. All strains were sensitive to ciprofloxacin. The most frequent serotypes were *S. enteritidis* and *S. heidelberg*.

1.9. Incidence of *Salmonella* in poultry:

In India, Saikia and Patgiri (1986) isolated 28 *Salmonella* strains from 150 dead poultry, 12 of which were *S. chester*. Most strains were from liver and intestine followed by spleen and heart.

Yagoub and Mohamed (1987) isolated 58 *Salmonella* strains from 1488 samples collected from slaughtered chickens within 18 months in Khartoum North and Omdurman. Twenty three serotypes were identified, the most common of them were *S. mons* (25.6%) and *S. amek* (16.3%) and none of these serotypes had previously been isolated in the Sudan but *S. uganda* was isolated as stated by Khan (1969).

Mrden *et al.* (1987) isolated 152 *Salmonella* species from 1067 livers of dead chickens. 55 were *S. typhimurium*, 45 were *S. virchow*, 27 were *S. enteritidis*, 15 were *S. heidelberg*, 6 were *S. infantis* and 4 were *S. bredeny*.

In Netherlands during 1984-1988 the proportion of *S. enteritidis* isolates was about 12% of 3699 poultry samples (Edel and Visser, 1988).

Baumgartner *et al.* (1992) isolated 130 *Salmonella* from 945 broiler carcasses; 47 (36.2%) were *S. infantis*, 39 (30%) *S. typhimurium* and 25 (19.2%) *S. enteritidis*.

Jardy and Michard (1992) tested samples of raw poultry feed components for *Salmonella*. The most commonly isolated serovars were *S. senftenbeg*, *S. rissen*, *S. tennessee*, *S. Iandott*, *S. mbandaka*, *S. agona* and *S. havana*.

In Iraq, Al-Aboudi *et al.* (1992) sampled dead-in-shell embryo from 4 local hatcheries to investigate the causative pathogenic microorganisms. They recovered 35 isolates representing 8 bacterial genera which included *Klebsiella*, *Proteus*, *Escherichia coli*, *Staphylococcus*, *Salmonella*, *Shigella*, *Pseudomonas* and *Streptococcus*.

Orhan and Guler (1993) isolated *Salmonella* from internal organs, cloacal swabs, feed samples and eggs. These strains were identified as *S. gallinarum* (25) and *S. enteritidis* (13). While, Pan *et al.* (1993) reported the isolation of 63 strains of *S. pullorum*.

In Ankara, Bekar *et al.* (1993) reported the isolation of 116 of *Salmonella* isolates, 68 were *S. enteritidis*, 12 *S. bredeney*, 10 *S. typhimurium*, and 7 *S. gallinarum* from a total of 6238 samples of skin, liver and intestinal contents of fowls.

In Canada, Poppe (1994) reported the isolation of *S. enteritidis* from samples taken from liver, heart, gizzard, small intestine and caeca.

In Sudan, Ezdihar (1996) examined 610 samples from infected chickens and reported the isolation of 14 bacterial genera which included *Klebsiella*, *Citrobacter*, *E. coli*, *Salmonella*, *Enterobacter*, *Proteus*, *Yersinia*, *Edwardsiella*, *Serratia*, *Morganella*, *Hafnia*, *Acinetobacter* and *Shigella*.

1.10. Incidence of *Salmonella* in animal and poultry feeds:

Humans get infected by *Salmonella* spp. from a variety of human food, and 95% of salmonellosis cases were estimated to originate from food materials (Mead *et al.*, 1999; Murray, 2000). Izat *et al.*, (1991) isolated *Salmonella* spp. from 50% of retail broiler chickens, with population estimates ranging from 5 to 34 organisms per 100ml.

In a comparative study in England, Humphrey (2000) reported that 30% to 80% *Salmonella* spp. were contaminating poultry carcasses. Also, *Salmonella* spp. were isolated from alfalfa seeds, chocolate, cheddar cheese, red meat, salad, milk and vegetables (Craven *et al.*, 1975; Inami and Moler, 1999; Humphrey, 2000).

The transmission of *Salmonella* spp. to animals' feed was noted by Jones *et al.* (1982) who detected the same serotype (*S. ser. Mbandaka*) in both cattle and unopened bags of vegetable fat on the same farm site. However, Grimont *et al.*, (2000) noted that the habitat of *Salmonella* spp. is limited to the digestive tracts of animals and humans, and that its presence in other environments may be limited to faecal contamination.

Marx (1969) noted that *S. enteritidis* was isolated from field mice (*Apodemus sylvaticus*) as early as 1900. Later, Singer *et al.* (1992) isolated *S. enteritidis* again from mice (Family: *Muridae*). Other *Salmonella* spp. serovars such as *S. derby* and *S. typhimurium* were isolated from rats (Schnurrenberger *et al.*, 1968).

In birds, *Salmonella* spp. was isolated in a study from racing pigeons (Adesiyun *et al.*, 1998) but not in wild pigeons (Nielsen and Clausen, 1975). *Salmonella* spp. was isolated from wild birds such as crows and gulls (Kapperud and Rosef 1983; Devi and Murray, 1991). Evidence also exists that *Salmonella* spp. may survive in the intestinal tracts of insects (Everard *et al.*, 1979). Jones *et al.* (1991) and Kopanic *et*

al. (1994) suggested that insects may be vectors for the transmission of *Salmonella* spp.

Salmonella spp. have been isolated from poultry feed stored at 25°C after 16 months of storage (Willims and Benson, 1978). Survival and heat resistance of *Salmonella* spp. in meat, bone meat, dry milk and poultry feed is related to moisture content and relative humidity (Carlson and Snoeyenbos, 1970). The only feed ingredient that is resistant to contamination by *Salmonella* spp. is liquid animal and vegetable fat (Harris *et al.*, 1997). Fatty acids have been shown to inhibit the growth of gram negative bacteria (Khan, 1969).

1.11. Incidence of *Salmonella* in man:

In France, *Salmonella* is one of the major sources of toxin-infection in humans ([Bouvet *et al.*, 2002](#)). The incidence of human salmonellosis has increased greatly over the past 20 years and this can mostly be attributed to epidemics of *Salmonella enteritidis* in poultry in numerous countries ([Barrow *et al.*, 2003](#); [Guard-Petter, 2001](#)). The association between egg consumption and *S. enteritidis* outbreaks is a serious international economic and public health problem ([Centers for Disease Control, 2000](#) and 2003; [Guard-Petter, 2001](#); [Patrick *et al.*, 2004](#)).

Transmission to hens may originate from contaminated food or water or by contact with wild animals. But the main concern with this bacterium is the existence of silent carriers. These animals can, in turn, transmit the bacterium to their flock-mates through horizontal transmission or to their offspring by vertical transmission. However, they are difficult to distinguish from healthy animals, thus are responsible for transmission to human beings.

[Zhao et al. \(2001\)](#) and [Siqueira et al. \(2003\)](#) reported the occurrence of 1.4 million cases of human salmonellosis in the United States.

The transmission of *Salmonella* spp. is usually associated with the consumption of contaminated food ([Soumet et al., 1999](#)). However, a great number of outbreaks might be associated with contaminated water, which is known to be an important transmission route ([Furtado et al., 1998](#)).

1.12. Control and Treatment:

The administration of injectable antibiotics such as gentamycin in the hatchery played a pivotal role in controlling the spread of *S. arizonae* in turkey pouts (Shivaprasad *et al.*, 1998). Antibiotics have been used to control *S. enteritidis* infection in several experimental and commercial contexts. Treatment of chicks with polymyxin B sulphate and trimethoprim both prevent and cleared experimental infection (Goodnough and Johnson., 1991). Administration of flavophospholipol or salinomycin sodium as feed additives reduced fecal shedding (Bolder *et al.*, 1999). Provision of a competitive exclusion culture to restore a protective normal microflora after treatment with enrofloxacin reduced the isolation of *S. enteritidis* from broiler breeders and their environment (Reynolds *et al.*, 1997).

Mellory *et al.* (1989) reported that antibiotics were used effectively both as therapeutic and prophylactic agents as part control efforts for *S. enteritidis* in broiler and broiler breeder flocks in Northern Ireland.

To control this zoonosis, a number of prophylactic means have been developed. Vaccinations have a general effect and may reduce animal contamination and rate of excretion of the bacterium through the faeces ([Zhang-Barber et al., 1999](#)). Other methods aim to reduce the

introduction of the bacterium into the gut, which is based on the early implementation of an adult-type intestinal flora which competes with *S. enteritidis* ([Rabsch *et al.*, 2000](#)) or acidification of feed which deters bacterial growth. Genetic methods may also be successful in increasing resistance to systemic disease ([Bumstead and Barrow., 1988](#)) or carrier-state ([Beaumont *et al.*, 1999](#)), thus reducing the need for antibiotic treatments and the risk of antibiotic resistance.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Sampling:

2.1.1. Source of specimens:

The source of specimens were sick chickens (layers and broilers) which were selected from 11 poultry farms and institutions in Khartoum State during the period between June to August 2006.

Sites in the state from which chicken were collected include (Table 1):

- Farms in Omderman city.
- Arab Company for Life Stock Development (ACOLID) located at Gabal Aolia.
- The Clinic, Faculty of Veterinary Medicine, University of Khartoum. Khartoum North, Shambat.
- Central Veterinary Research Laboratories, Soba, Khartoum.

Ninety seven (57 broiler and 40 layers) chickens were examined. They were of different age and sex and showed signs of weakness, loss of appetite, poor growth, and adherence of chalky white material at the vent region.

2.1.2. Collection of specimens:

A total of 102 samples were collected from tissues showing prominent lesions. These samples comprised 25 livers, 24 hearts, 24 ovaries and 27 intestines. Sixty blood samples were collected for serology (Table 1).

Table (1): Origin, type and number of samples collected in the study.

Source	No. and type of organs examined			
	Ovary	Liver	Heart	intestine
1- Central Veterinary Research Laboratories, Soba (broilers)	19	13	-	11
2- Omdurman Farms (layers)	7	2	6	7
3- Arab company for life stock development (ACOLID) (layers)	-	5	16	6
4- Veterinary Clinic, U. of K. (layers)	-	5	2	3

2.1.2.1. Poultry necropsy:

The affected chickens were slaughtered and the hen was laid on its back. The legs were grasped firmly in the area of the femur and bent until the head of the femur was broken so, the two legs were laid flat on the table.

A surgical scissor was used to cut the skin until the entire ventral aspect of the body including the neck was exposed, then the abdominal wall and breast muscles were cut exposing the abdominal cavity (Calnek *et al.*, 1997).

2.1.2.2. Tissue samples:

Tissue samples (livers, hearts and ovaries) were removed using sterile scissors and forceps and placed into sterile test tubes.

2.1.2.3. Intestines samples:

Selected section of the gut were removed with sterile forceps and scissors and placed directly into sterile test tubes.

2.1.2.4. Transport and storage of samples:

All samples were placed on ice in a thermos flask immediately after collection and transported to Central Veterinary Research Laboratories at Soba, and then kept at 4°C for 18 hours in the refrigerator.

2.1.2.5. Blood samples:

Five ml of blood samples were collected from the jugular vein (during slaughtering) into sterile tubes which were kept at room temperature for 1 hour, then at 4 °C overnight. Separation of sera was achieved by centrifugation at 3000 rpm for 10 minute, and then the separated sera were poured into sterile bijoux bottles, labeled and stored frozen till examined.

2.2. Bacteriological investigation:

2.2.1. Culture media:

2.2.1.1. Liquid media:

a. Peptone water (Oxoid):

It was prepared by dissolving 50 grams of powder in 1 liter distilled water (DW), mixed well and distributed into test tubes and sterilized by autoclaving at 121 °C for 15 minutes, then stored in the refrigerator at 4 °C until used.

b. Nutrient broth (Oxoid) :

Nutrient broth was prepared by adding 13 grams to 1 liter DW, mixed well and distributed in 3 ml amounts into bijou bottles and sterilized by autoclaving at 121°C for 15 minutes, then stored at refrigerator at 4 °C until used.

c. Selenite-F-broth (Oxoid) :

Selenite broth was prepared by dissolving 4 grams of sodium biselenite in 1 liter of DW, then 19 grams of selenite broth powder were added, warmed to dissolve, mixed well and distributed into McCartney bottles each contains a portion of 5ml, then sterilized in boiling water for 10 minutes, then stored at 4 °C until used.

d. Methyl Red -Voges Proskauer medium (MR -VP) (Oxoid):

This medium was prepared by adding 15gram of powder to 1 liter of DW, mixed well, distributed into test tubes in 5ml amount and sterilized by autoclaving at 121°C for 15 minutes.

e. Nitrate broth:

1 gram KNO_3 was dissolved in 1 liter of nutrient broth (Oxoid), distributed into sterile test tubes and then sterilized at 115 °C for 20 minute.

f. Peptone water sugars:

This medium composed of peptone water and different sugars. The pH of the peptone water (900 ml) was adjusted to 7.1-7.3 before 10 ml of andradé's indicator added, then 100 ml of 10% sugar solution (glucose or sucrose or mannitol) were added to the mixture, mixed well and distributed in 2 ml amounts into sterile test tubes containing inverted Durham's tube, then sterilized by steaming for 30 minutes and stored in refrigerator at 4 °C until used.

2.2.1.2. Solid media:

a. Nutrient agar (Oxoid):

28 grams of medium were added to 1 liter of DW and boiled to dissolve completely. The medium was then sterilized by autoclaving at 121°C for 15 minutes and distributed aseptically in 15 ml amounts into sterile petri dishes. Nutrient agar slopes were also prepared and stored in refrigerator at 4 °C.

b. Triple sugar iron agar medium (TSI) (Oxoid):

Triple sugar iron agar was prepared by adding 65 grams of powder to one liter of DW, boiled to dissolve completely, mixed well, distributed in 5ml amount into McCartney bottles and sterilized by autoclaving at 121°C for 15minutes. The medium was allowed to set in a slope position about one inch butt and stored at 4 °C.

c. Desoxycholate citrate agar (Oxoid):

This medium was prepared by suspending 52 grams of powder in 1 liter of DW, boiled over flame to dissolve completely, agitated to prevent charring, and dispensed into sterile petri-dishes in portions of 15ml and stored at 4 °C.

d. MacConkey's agar (Oxoid):

The medium was prepared by adding 47 grams in 1 liter of DW, boiled to dissolve completely, sterilized by autoclaving at 121°C for 15 minutes, dispensed into sterile petri –dishes in portions of 15 ml each and stored at 4 °C.

e. Hugh and Liefson`s (O/F) medium:

The medium contain peptone, NaCl, K₂Hpo₄, agar and bromothyonol blue as an indicator. It was prepared according to Cowan and Steel (1985) by adding the solids in 1 liter of DW and boiled to dissolve completely. The pH was adjusted to 7.1 and the medium was filtered then the indicator was added followed by sterilization at 115 °C for 20 minutes. Sterile glucose solution was then added to give final concentration of 1%, mixed and distributed aseptically in 10 ml volumes into sterile test tubes of not more than 16 mm diameter.

f. Simmon`s citrate agar (Oxoid):

23 grams of powder were suspended in 1 liter of DW, boiled to dissolve completely, then sterilized by autoclaving at 121°C for 15 minutes, poured in sterile McCartney bottles and allowed to set in the slope position and stored at 4°C.

g. Diagnostic Sensitivity Test Agar (DST) (Oxoid):

This medium was prepared by suspending 40 grams of powder in 1 liter DW, boiled to dissolve completely, and then sterilized by autoclaving at 121°C for 10 minutes, dispensed in sterile petri –dishes in portions of 15 ml each.

2.2.2. Solutions and Reagents:

2.2.2.1. Normal saline solution:

This was prepared by dissolving 8.5 gram of sodium chloride in 1 liter of DW (Cowan and Steel, 1985).

2.2.2.2. Methyle Red Solution:

This solution was prepared by dissolving 0.04 gram of methyl red in 10 ml ethanol and diluted with water to 100ml.

2.2.2.3 Kovac's reagent:

This reagent was prepared for indol test. 5 gram of p-dimethyl aminobenzaldehyde was dissolved in 75 ml of amyl alcohol by warming in a water bath (50-55°C), then cooled and 25 ml of HCl was added. It was protected from light and stored at 4 °C.

2.2.2.4. Oxidase test reagent:

It was prepared by adding a loopful of tetramethyl-p-phenylenediamine dihydrochloride solution to 3 ml of DW.

2.2.2.5. Potassium Hydroxide Solution (KOH):

This solution was prepared by dissolving 40 gram of pure potassium hydroxide in 100 ml DW.

2.2.2.6. Andrade's Indicator:

This was prepared according to Baker and Silverton (1980) by dissolving 5 gram of acid fuchsin powder in 1 litre of DW, and then 150 ml of NaOH was added to the solution mixed and allowed to stand at room temperature for 24 hours.

2.2.3. Sterilization procedures:

2.2.3.1. Hot air oven:

Glassware (flasks, test tubes, pipettes and petri dishes) and metal instruments (scissors and forceps) were sterilized in hot air oven at 160°C for 2 hours.

2.2.3.2. Autoclaving:

Culture media and discarded cultures were sterilized by autoclaving at 121 °C for 20 minutes while glassware with plastic covers were autoclaved at 121°C for 15 minutes.

2.2.3.3. Disinfectants and antiseptics:

70% alcohol was used to disinfect the surfaces of benches before and after use.

2.2.3.4. U.V. light:

It was used to sterilize the vacuum of media pouring room and laminar-flow cabinets.

2.2.4. Cultivation of Samples:

2.2.4.1. Inoculation of enrichment medium:

a. Tissues:

The whole organ (livers, hearts and ovaries) was incised by sterile scalpel and placed into sterile McCartney bottles containing 10 ml selenite-f-broth and the culture was incubated aerobically at 37°C for 24 hours.

b. Intestinal samples:

The samples were prepared by using sterile forceps and a piece of the intestine was inoculated into McCartney bottles containing selenite-f-broth and incubated aerobically at 37°C for 24 hours.

2.2.4.2. Inoculation of plates:

A loop of the inoculated selenite-f-broth was streaked on a plate of macConkey's agar and incubated aerobically at 37 °C for 24 hours.

2.2.4.3. Subculture of primary isolates:

Non-lactose fermenter colonies were further picked up with a sterile loop and spread on desoxycholate citrate agar medium and incubated aerobically at 37°C for 48 hours.

2.2.4.4. Purification and storage of isolates:

Both lactose fermenter and non-lactose fermenter colonies were purified by repeated subculture on nutrient agar. Pure isolates were stored on nutrient agar slopes in the refrigerator at 4 °C.

2.2.5. Identification of isolates:

Identification of purified isolates was performed according to Cowan and Steel (1985).

2.2.5.1 Microscopic Examination:

a- Gram's stain:

Smears were prepared from the culture by emulsifying a part of a colony in a drop of normal saline on a glass slide, dried and fixed by heating. Then the slides were flooded by crystal violet for 1 minute and then washed with tap water. Iodine solution was applied for 1 minute, and then the slide was washed with tap water. The smear was then decolorized with few drops of acetone for seconds and washed immediately with water. Then the smear was flooded with dilute carbol fuchsin for 30 seconds and washed with tap water. Slides were then blotted with filter paper and examined under oil immersion lens. Gram-positive bacterial cells appeared violet in colour while that of Gram-negative bacteria appeared red.

2.2.5.2. Biochemical tests for identification of bacteria:

a. Oxidase Test:

The test was carried out according to Cruickshank (1972). Strips of filter paper were soaked in 10% solution of tetramethyle -p- phenylene diamine dihydrochloride in a petri dish and then left to dry. Then a fresh young test culture, on nutrient agar, was picked up with a sterile glass rod and streaked on that filter paper. A dark purple colour that developed within five to ten seconds was considered positive reaction.

b. Catalase Test:

Catalase test was carried out according to Cowan and Steel (1985). A drop of 3% aqueous solution of hydrogen peroxide was placed on a clean microscope slide. A colony of test culture, on nutrient agar was then placed on the hydrogen peroxide drop. The test was considered positive when gas bubbles appeared on the surface of the culture material.

c. Sugar Fermentation Test:

The peptone water sugar was inoculated with test culture. The tube was then incubated at 37°C and examined for up to 2 days. Acid production was indicated by appearance of reddish colour, whereas gas production was indicated by development of an empty space in the Durham's tube.

d. Oxidation -Fermentation (O/F) Test:

The test was made by growing the test culture in two tubes of Hugh and Liefson's medium. A layer of soft paraffin was added to one tube to a depth of about 1 cm. Both tubes were incubated at 37°C and examined daily. Oxidizer organisms showed acid production in the upper part of medium in the open tube only; fermenters showed acid production in the paraffin-covered tube and at the bottom in the open tube.

e. Motility test:

The test culture was incubated on nutrient broth at 37°C for 6 hours, then motility test was done by using hanging-drop technique.

f. Urease Test:

Suspected *Salmonella* colonies were streaked on urea agar slope, incubated at 37°C for 2 days. A positive reaction was indicated by a change of colour to pink.

g. Indole Test:

The test culture was inoculated into peptone water medium and incubated at 37°C for 48 hours. 1 ml of Kovacs's reagent was run down to the side of the tube. A pink ring which appeared on the surface within 1 minute indicated positive reaction.

h. Methyl Red (MR) Test:

The test organism was inoculated in glucose phosphate peptone water, incubated at 37°C for 2 days. Five drops of methyl red reagent were added. A positive reaction was indicated by appearance of a red colour.

i. Voges Proskauer(VP) reaction:

The test organism was inoculated in glucose phosphate peptone water, then 3 ml of 5% alcoholic solution of α -naphthol and 1ml of 40% KOH aqueous solution was added. A positive reaction was indicated by development of bright pink colour within 30 minutes.

j. Citrate utilization:

An isolated colony from nutrient agar was picked up with a straight wire, then inoculated in simmon's citrate agar and incubated at 37°C and examined daily. A positive test was indicated by change of colour from green to blue.

k. Nitrate Reduction:

The test culture was inoculated into nitrate broth, then incubated at 37°C for 2 days, 1 ml of solution A (sulphanilic acid) was added to the test culture followed by 1 ml of solution B (a-naphthylamine). A positive reaction was indicated by a development of red colour. If the result was negative, zinc dust was added. A red colour indicated the presence of nitrate which was reduced by zinc to nitrite.

l. Hydrogen sulphide (H₂S) Production:

The test culture was inoculated by stabbing the butt and streaking the slope of triple sugar iron agar in McCartney bottles and incubated at 37°C for 2 days. A positive reaction was indicated by development of a black colour.

2.2.6. API for enterobacteria:

The identification of *Salmonella* isolates were further confirmed using analytical profile index (API 20E).

2.2.6.1. Analytical Profile Index (API):

The API 20E (Analytab products, U.S.A) was used to confirm the identification of *Salmonella* isolates. The strip consists of 20 compartment each contains different dehydrated medium. These compartments were inoculated with the test bacteria in saline suspension. After incubation for 18-24 hours, test reagents were added to some compartments. The reactions of all compartments were recorded and results were compared with that of standard table (provided with the kit) for the identification of the isolates to the species level.

2.2.7. Antibiotic sensitivity test:

Sensitivity of representative isolates to a number of antibiotics (table 2) was determined by the standard disk diffusion method (Buxton

and Fraser, 1977). Two plates of nutrient agar were used for each isolate to test 8 different antibiotics. Colonies from each isolate were emulsified in 2 ml nutrient broth, shaken thoroughly to obtain a homogeneous suspension of the test culture. The plates were then flooded with the bacterial dilution suspension, tipped in different directions to cover the whole surface with the suspension. Excess fluid was aspirated and the plates were left to dry for 30 minutes.

The antibiotic disks were placed on the agar medium about 2 cm from each other and from the plate rim using sterile forceps. The plates were then incubated at 37°C and examined after 24 hours for zones of inhibition which were measured in mm. The isolates were described as resistance, intermediate and sensitive to different antibiotics according to (Bauer *et al.*, 1966) (Table 3).

2.2.8. Serological tests:

2.2.8.1 Rapid serum agglutination test:

Using a clean white tile marked into squares of about 3x3 cm, 1 drop of commercial stained antigen of *S. pullorum* (Intervet Company, Holland) was placed in the center of each square, then an equal size drop of fresh serum from chickens was placed next to a drop of antigen, then the two drops were mixed using a fine glass rod. A gentle rocking motion was used to keep the drops agitated for up to 2 minutes. Both positive and negative controls from Central Veterinary Research Laboratories centre, soba were included in the test.

Table (2): Antibacterial used in antibiotic sensitivity test

Antibacterial	Code	Conc / disk
Ampicillin	Difco	10µg
Kanamycin	Difco	30µg
Gentamycin	Oxoid	10µg
chloramphenico	Oxoid	30µg
Erythromycin	Oxoid	10µg
Ciprofloxacin	Difco	5µg
Nitro furantoin	Difco	100µg
Doxcillin	Difco	30µg

Table (3): Standard zone of inhibition to different antibiotics

Antimicrobial agent	Disk potency	Zone of inhibition (Diameter in mm)		
		Resistant	Intermediate	Sensitive
1- Ampicillin Am	10 µg	12 or less	13 – 14	15 or more
2- Kanamycin K	30 µg	13 or less	14– 17	18 or more
3- Gentamycin Gn	10 µg	12 or less	13 – 14	15 or more
4- Cipro floxacin CIP	5 µg	15 or less	16 – 20	21 or more
5- Erythromycin E	30 µg	13 or less	14 – 22	23 or more
6- Nitro Furantoin F	100 µg	14 or less	15 – 16	17 or more
7- Chloram phenicol C	30 µg	12 or less	13 – 14	15 or more
8- Doxycyelin Dox	30 µg	12 or less	13 – 15	16 or more

CHAPTER THREE

RESULTS

3.1. Isolation of bacteria:

A total of 102 samples were subjected to bacteriological examination. Eighty-seven Gram-negative bacteria were isolated from 102 samples; the remained 15 samples showed no bacterial growth. The isolated bacteria belonged to 7 genera which included *Pseudomonas* species (24), *Proteus* species (21), *Klebsiella* species (17), *Yersinia* species (12), *Escherichia coli* (8), *Salmonella* species (3) and *Shigella* species (2) (Table 4, Figure 1).

3.2. Sites of isolation:

Two isolates of *Salmonella enteritidis* were recovered from intestines from Arab Company for Life Stock Development, while one isolate of *Salmonella arizonae* was isolated from the liver from Omdurman farms and no isolates were obtained from Central Veterinary Research Laboratories or University Veterinary Clinic (Table 5).

3.3. Properties of *Salmonella*:

3.3.1 Cultural properties:

3.3.1.1 Growth in liquid media:

Growth in selenite-F-broth was detected by brown precipitate in the medium after 24 hours of incubation at 37°C.

Growth in nutrient broth and peptone water was indicated by the formation of turbidity and slight white sediment after 24 hours of incubation at 37°C.

3.3.1.2 Growth on solid media:

On nutrient agar *Salmonella* colonies were moderately large (2-4 mm), circular with smooth surface and grayish-white in colour after 24 hours at 37°C (Figure 2).

On MacConkey agar all colonies were colourless (non-lactose fermenter), smooth, round, shiny and up to 3 mm in diameter after 24 hours at 37°C (Figure 3).

Growth on desoxycholate citrate agar showed slight opaque dome-shaped colonies measured (2-4 mm) with central black spots (indicated production of H₂S) surround by a zone of clearance after 48 hours at 37°C (Figure 4).

On triple sugar iron agar *Salmonella* colonies produced hydrogen sulfide which was indicated by black discoloration, gas production causes bubbles in the agar, and pH change was indicated by production of red colour in the slant (Figure 5).

3.3.1.3 Motility:

Salmonella isolates were found motile under the light microscope after 6-hours of incubation on nutrient broth at 37°C.

3.3.2. Microscopic properties:

All *Salmonella* isolates were Gram-negative, short rods occurred singly or in groups.

3.3.3. Biochemical reactions:

Salmonella isolates were oxidase and urease negative. They produced gas from glucose and mannitol, while sucrose, salicin and lactose were not fermented. Hydrogen sulphide was produced by the isolates (Table 6).

The identification of *Salmonella* isolates was confirmed by API 20E (Table 7).

3.4. Serological identification:

Using commercial stained antigen of *S. Pullorum*, separated hen sera were test for antibodies using rapid serum agglutination test (RSA). No antibodies were detected in all investigated sera.

3.5. Sensitivity to Antibiotics:

Sensitivity test to the 3 *Salmonella* isolates against eight antibiotics was carried out. They were found sensitive to ampicillin, kanamycin, gentamycin, ciprofloxacin, chloromphenicol and resistant to erythromycin, while moderately sensitive to doxycycline. *S. arizonae* was moderatly sensitive to nitroferniton while *S. enteritidis* was sensitive.

2 isolates of *E coli*, 2 isolates of *Proteus* and 3 isolates of *Pseudomonus* were found sensitive to gentamycin and ciprofloxacin and resistance to doxycycline. They showed varying degree of sensitivity and resistance to other tested antibacterials (Table 8, figure 6).

Table (4): Isolated bacteria from different chicken samples in the present study.

bacteria	No. of isolates	Rate of isolation
<i>Pseudomonas spp.</i>	24	23.53%
<i>Proteus spp.</i>	21	20.59%
<i>Klebsiella spp.</i>	17	16.67%
<i>Yersinia spp.</i>	12	11.74%
<i>E. coli</i>	8	7.8%
<i>Salmonella spp.</i>	3	2.94%
<i>Shigella spp.</i>	2	2%
No growth	15	14.71%

Table (5): Isolated *Salmonella* from different chicken samples and areas in Khartoum State.

Source	No. and organs from which <i>Salmonella</i> was isolated		No. of <i>Salmonella</i> Isolated	Species of <i>Salmonella</i> Isolated
	Liver	intestine		
1- Omdurman farms	1	-	1	<i>S. arizonae</i>
2- Arab company for life stock development (ACOLID)	-	2	2	<i>S. enteritidis</i>

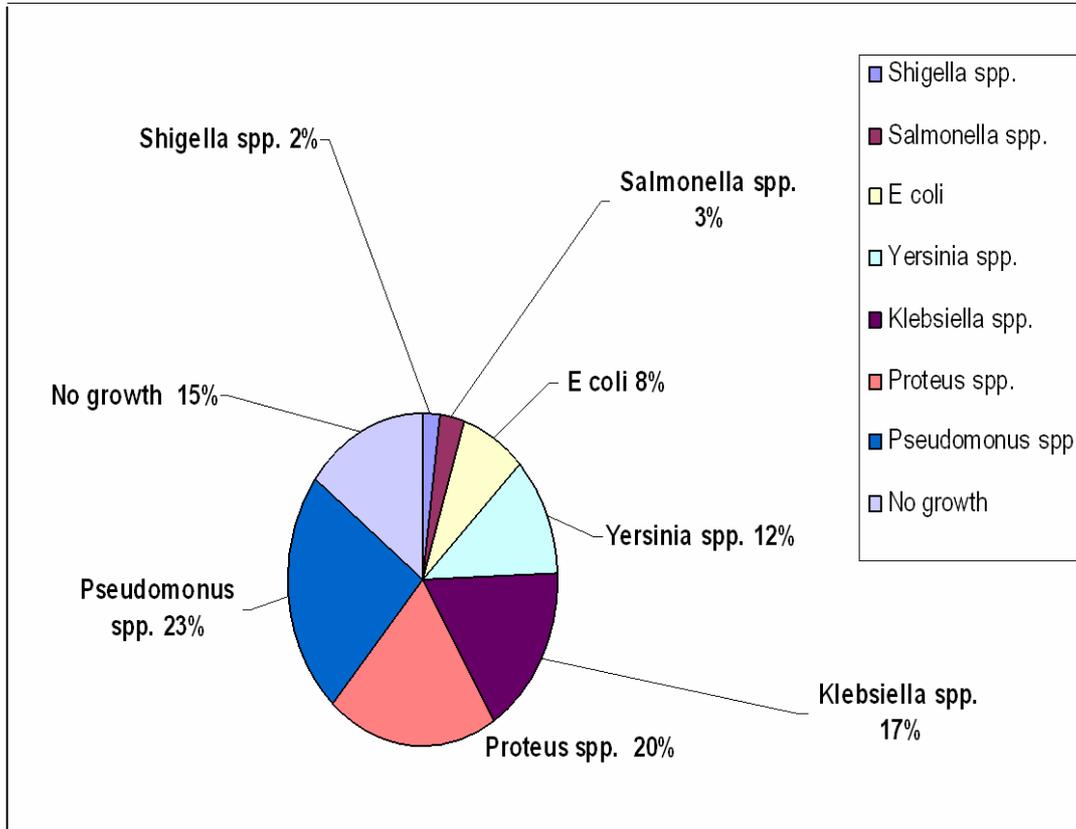


Fig (1): Percentage of isolated bacteria from chicken in the study



Fig 2: *Salmonella* colonies on nutrient agar after 24hr incubation at 37°C

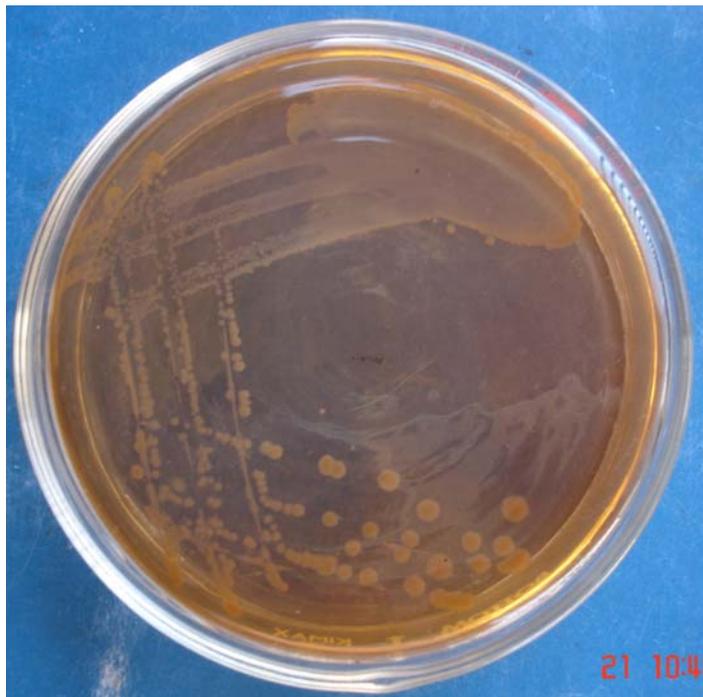


Fig 3: *Salmonella* colonies on macConkey agar after 24hr incubation at 37°C



Fig 4: *Salmonella* colonies on desoxycholate citrate agar (DCA) after 24hr incubation at 37°C



Fig 5: *Salmonella* on triple sugar iron agar (TSI) after 24hr incubation at 37°C

Table (6): Biochemical reactions of bacteria isolated in the study.

Bacteria Isolate	Biochemical Reactions												
	Motility	Oxidase test	Urease test	indole test	Glucose	Manitol	Lactose	Sucrose	Salicin	H ₂ S	Citrate	VP	MR
<i>S. enteritidis</i>	+	-	-	+	+	+	-	-	-	+	+	-	+
<i>S. arizanae</i>	+	-	-	-	+	+	-	-	-	+	+	-	+
<i>E coli</i>	+	-	-	+	+	+	-	-	+	-	-	-	+
<i>Pseudomonas</i>	+	+	-	-	+	-	-	-	-	-	+	+	-
<i>Klebsiella</i>	-	-	+	-	+	+	+	+	+	-	+	-	+
<i>Yersinia</i>	-	-	-	-	+	+	-	+	-	-	-	+	-
<i>Shigella</i>	-	-	-	-	+	+	-	+	-	-	-	-	+
<i>Proteus</i>	+	-	+	+	+	-	-	-	-	-	+	-	+

(+) Positive

(-) Negative

Table (7): Analytical Profile Index (API) for *Salmonella* isolates.

Bacterial isolates	Tests												
	ONPG	ADH	LDC	ODC	CIT	H ₂ S	URE	TDA	IND	VP	GEL	GLU	MAN
<i>S. enteritidis</i>	-	+	+	+	+	+	-	-	-	-	+	+	+
<i>S. arizonae</i>	+	+	+	+	-	+	-	-	-	-	+	+	+

Bacterial isolates	Tests												
	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	NO ₂	N ₂	MOB	McC	OF-F
<i>S. enteritidis</i>	+	+	+	-	+	-	+	-	+	+	+	+	+
<i>S. arizonae</i>	-	+	+	-	+	-	+	-	+	+	+	+	+

ONPG (Ortho Nitrophenyle-BD-Galacto pyranosidase) **ADH** (Arginine Dihydrolase) **LDC** (Lysine De carboxylase)
CIT (Citrate utilization) **URE** (Urease) **TDA** (Tryptophane De Aminase) **IND** (Indole production)
GEL (Geltinase) **GLU** (Glucose) **MAN** (Manitol) **INO** (Inositol) **SOR** (Sorbitol) **RAH** (Rhamnose)
SAC (SaCcharose) **MEL** (Melibiose) **AMY** (Amygdalin) **ARA** (Arabinose)
OX (Oxidase) **MOB** (Motility) **McC** (Growth on maCconkey)

Table (8): Sensitivity of the isolates to different antibiotics.

Name of bacteria	Ampicillin 10 µg	Kanamycin 30 µg	Getamicin 10 µg	Cipro floxacin 5 µg	Erythomycin 30 µg	Nitrofurantoin 100 µg	Chloram phenicol 3 µg	Doxi- cycline 30 µg
<i>S. arizonae</i>	S	S	S	S	R	IN	S	S
<i>S. enteritidis</i>	S	S	S	S	R	S	S	IN
<i>Proteus</i>	S	S	S	S	R	R	S	R
<i>E. coli</i>	S	R	S	S	IN	IN	S	R
<i>Pseudomonas</i>	R	R	S	S	R	R	R	R

(S) Sensitive

(R) resistance

(IN) intermediate

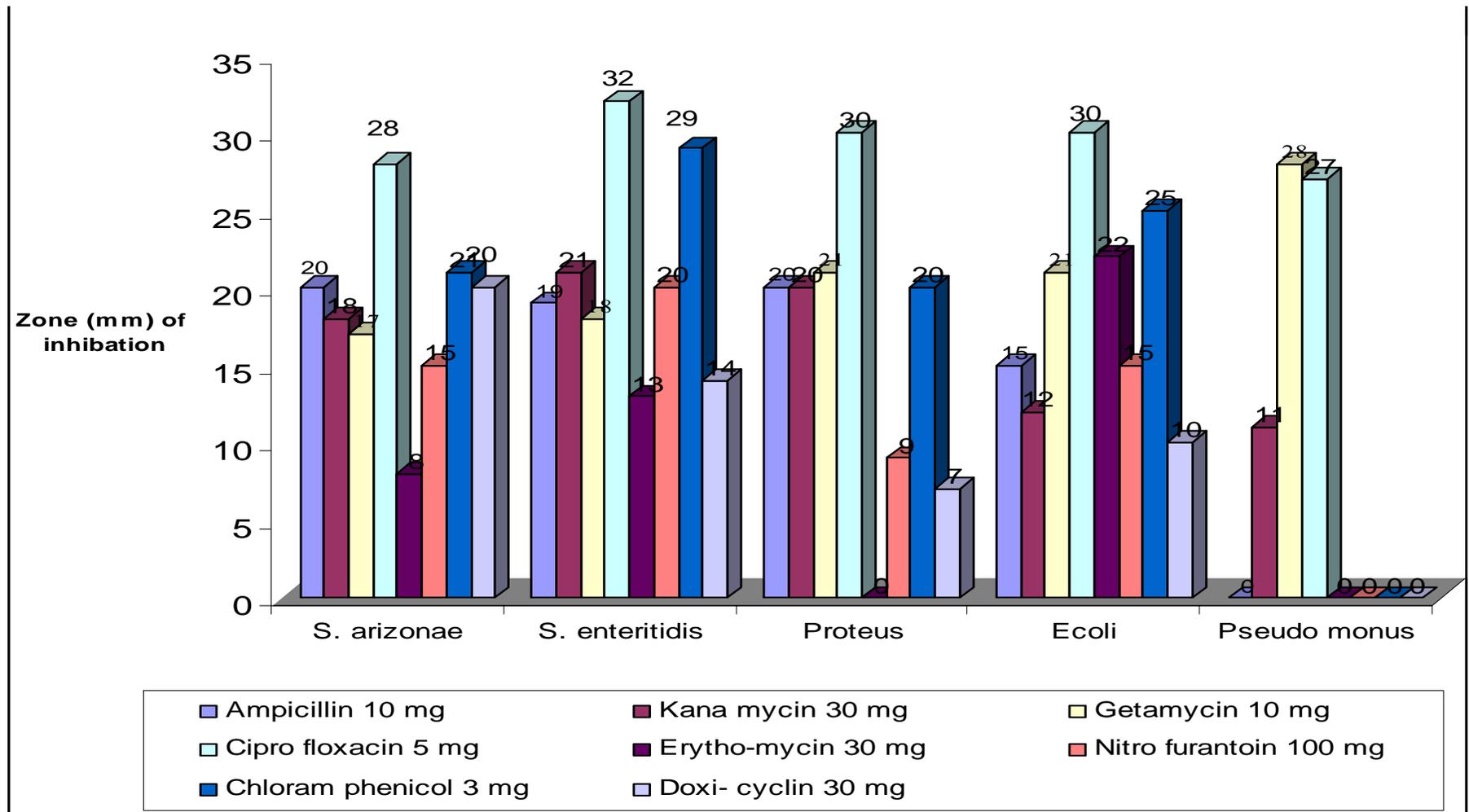


Fig (6): Sensitivity of isolates to different antibiotics

CHAPTER FOUR

DISCUSSION

Members of the family *Enterobacteriaceae* are gram-negative, non spore forming rods. Some of them are human and animal pathogens which produce intestinal infection and food poisoning. The genera of importance in poultry disease include *Salmonella* and *Escherichia* (Holt *et al.*, 1994).

Salmonellosis is a major public health concern and continues to have a serious economic importance in the poultry industry in all countries (Morales and McDowell, 1999). Broilers meat and raw poultry products are considered to be a reservoir of infection to human where *Salmonella* food poisoning in human is often associated with the consumption of poultry products (Coyle *et al.*, 1988; Lin *et al.*, 1988 and Olsen *et al.*, 2000).

The present study was conducted to investigate the incidence of salmonellosis in chicken showing clinical signs of enteritis in Khartoum State. *Salmonella* were isolated together with other bacterial genera such as *Pseudomonas*, *Klebsiella*, *Yersinea*, *Escherichia-coli*, *Proteus* and *Shigella*. Although all collected samples in the study were cultured first in selenite-F-broth, Gram-negative bacteria other than *Salmonella* were isolated. This can be explained by the fact that selenite-F-broth enriches the growth of *Salmonella* and *Shiegella* but don't kill other enteric bacteria which under other conditions (subculture on macConkey agar) will grow.

The isolation result was similar to that of other studies. In Iraq, Al-Aboudi *et al.* (1992) isolated 8 bacterial genera namely *Pseudomonus*, *Kelbsiella*, *Yersinea*, *Escherichia coli*, *Proteus*, *Shigella* and *Salmonella* in addition he found also *Streptococcus* and *staphylococcus* from dead-in - shell embryo. In the Sudan, Ezdihar (1996) isolated *Klebsiella*, *Yersina*,

Escherichia coli, *Proteus*, *Shigella* and *Salmonella* together with other bacteria from infected chickens.

In this study *Pseudomonas* represented the most dominant isolate and counted for 23.8%, followed by *Proteus* (20.5%), *Klebsiella* (16.6%), *Yersinia* (11.7%), *Escherichia-coli* (7.8%), *Salmonella* (2.9%) and lastly *shigella* about (1.9%).

The rate of isolation of *Salmonella* (2.9%) in this study was comparable to that reported in other studies. Yagoub and Mohamed (1987) examined 1488 samples in the Sudan and isolated 58 *Salmonella* which comprised 3.9% of the total isolates. In another study, Ezdihar (1996) examined 610 samples from poultry in the Sudan and isolated 45 *Salmonella* which counted for 7.4% of the total isolates. The later study showed higher isolation rate compared to the finding of this study and that might be attributed to the large difference in the number of samples collected in both studies.

Salmonella was isolated only from samples obtained from Arab Company for Life Stock Development (ACOLID) and from Omdurman farms. It was not isolated however, from the Central Veterinary Research Laboratories (Soba) and the University Clinic (Khartoum north). This finding didn't indicate that salmonellosis was not present in Soba and Khartoum North due to the small number of collected samples. On the other hand it confirms the presence of the disease in area from which *Salmonella* was isolated. In ACOLID Company the system of management is a close system, hence large numbers of birds were kept together and that might help vertical and horizontal transmtion of this type of bacteria. On the other hand, the system of management in Omdurman farms was not as good as that of ACOLID. Accordingly chicken flocks are more susceptible to enteric infection including salmonellosis due to poor hygiene

Among the examined internal organs, the highest rate of isolation was from the intestines followed by the liver. These results agree with Gast and Beard (1989) who stated that *Salmonella* were recovered most often from the intestinal tract. Moreover, Calnek *et al.* (1997) stated that caeca, caecal tonsils, caecal contents, liver and spleen are the sites more likely to offer the maximum probability of recovering Salmonellae.

S. enteritidis is the most important serovar in poultry flocks and recently it was of high occurrence worldwide (Pitol *et al.*, 1991). Phillips and Optiz (1995) showed that *S. enteritidis* could attach to the granule cells in the preovulatory membrane and subsequently infected the ovum during the ovulation. On the other hand, *S. enteritidis* had the ability to penetrate eggs through shell pores and caused egg infections.

In the present study 2 isolates of *Salmonella enteritidis* were recovered, our finding confirmed previous records (Mamon *et al.*, 1992) that *S. enteritidis* was detected in Khartoum State. As long as the Sudan depends on importation of chicken it could have been come with infected imported flocks.

From the viewpoint of public health, human salmonellosis was reported to increase recently in France and the United States due to *S. enteritidis* ([Barrow *et al.*, 2003](#)). It was reported to cause food poisoning due to consumption of undercooked egg dishes (Quinn, 2002). Isolation of this bacterium from some farms in Khartoum State represents a real threat to the public health.

S. arizonae was widely distributed in nature in a variety of avian, mammalian and reptile species (Cambre *et al.*, 1980). The variety of infection sources in the nature will expose hen flocks to infection. *S. arizonae* was reported to cause arizonae infection in chickens (Carter and Wise, 2004).

All investigated chicken serum samples were negative for antibodies against *Salmonella Pullorum* antigen. Although the number of serum samples was few, the negative result confirmed the isolation trails as no *S. Pullorum* was isolated in this study and other previous studies in the Sudan.

The antibiotic sensitivity test was carried out for *Salmonella* isolates and only representative isolates from the other recovered bacteria due to the fact that this study was directed mainly towards salmonellosis. All strains of *Salmonella* were sensitive to ampicillin, chloramphenicol, kanamycin, gentamycin, and ciprofloxacin, and resistance to erythromycin. However, *S. arizonae* was moderately sensitive to nitrofurantoin and *S. enteritidis* was sensitive. While *S. arizonae* was sensitive to doxycyclin and *S. enteritidis* was moderately sensitive.

The sensitivity of the two isolated Salmonellae was in agreement with the finding of Ezdihar (1996) who reported 100% sensitivity of *Salmonella* isolates to these different antibiotics. Similar results were obtained also by Kaluzewski *et al.*, (1988).

In general, *Salmonella* is the most important agent implicated in outbreaks of foodborne disease around the world (Lacey, 1993). Effective control or eradication programs for salmonellosis depend on good management system, identification of carrier birds and accurate medication.

Conclusion:

The following points were concluded from the present study:

- Bacterial enteritis of chicken in Khartoum State was caused mostly by different members of the family *Enterobacteriaceae* among which *Salmonella* counted for 2.9% of isolates.

- The isolated *Salmonella* include *S. arizonae* and *S. enteritidis*. Both species were found sensitive to ampicillin, chloramphenicol, kanamycin, gentamycin, and ciprofloxacin, and resistance to erythromycin.

- *S. pullorum* was not isolated and no antibodies against it were detected in chicken sera collected from Khartoum State.

Recommendations:

- Further studies are needed to investigate the relation between fowl salmonellosis and public health in Khartoum State.

- The application of quick diagnostic procedures (e.g. PCR) are needed to trace sources of infection and help in quick diagnosis.

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