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ISOLATION IDENTIFICATION AND CHARACTERIZATION OF SALMONELLA SPP. FROM POULTRY AND POULTRY PRODUCT IN KHARTOUM STATE- SUDAN

Microbiology

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ABSTRACT

Salmonella, a genus of the family Enterobacteriaceae with over 2450 species, has been responsible for diseases ranging from typhoidal salmonellosis to non- typhoidal salmonellosis. Several groups of antibiotics such as β-lactams, aminoglycosides, quinolones, cephalosporin and sulfonamides are used against Salmonella species. 27 Salmonella spp. were isolated out of 385samples from poultry carcasses, poultry products, and from poultry environment in 2019-2022. These Salmonella were identified to their exact source, and some were serogrouped, serotyped, and tested for antibiotic sensitivity. Food poultry samples tested included broilers poultry and poultry carcasses, poultry eggs, poultry water supply, poultry feed stuff, and also samples were collected from poultry organs (liver, kidney, intestine, ovaries) All specimens were collected and transfer to the food and water department at national health laboratoryfor isolation and identification of salmonella spp. Serogroups of the isolated organisms were done by API20E method then byserology test usingpolyvalent and monovalent antisera and finally confirmed by polymerase chain reaction.(PCR) which revealed 25 out of 27 isolates ofSalmonella spp.(6.6%) The percentage of isolates were describe respectively as flows from poultry carcass13(.52%), from feed stuffs 3(12%), from poultry intestine 3(12.%). from poultry water supply 3(12%), from local eggs 2 (8%) and from broiler poultry1(4.0%) The confirmingorganisms revealedasS.enteritidis,S.typhimurim,S.arizona, s.pullorum,S.chlorasuis and S.paratypi(c)lastly the isolates of salmonella species was carried out against antibiotics sensitivity test 9 different types of standardantibiotic disc use against conforming isolateswhich were found sensitive to Ceftrixone , Cefotaxime, Kanamycin, and moderate sensitive to Ciprofloxacin, Chloromphenicol, and Co-trimexazole while appear resistant to Erythromycin, Tetracycline, and Ampicillin Many Salmonella species had developed resistance to several antibiotics Some factors identified to contribute to the emergence and dissemination of antibiotic resistant-Salmonella include; miss-used of antibiotics, used of antibiotics in agriculture, unregulated sales of antibiotics, inappropriate prescription and dispensing practices, and poor hygiene practices (external or behavioral factors), A more radical approach and commitment from the policy makers in health sector to solving problems emanating from .increasing spread of resistant Salmonella in different antimicrobials. The purpose of this study was to isolate, and assess prevalence of Salmonella infection In poultry in Khartoum state from different poultry locations (farmers, broilers , factories)

Matrial And Method: A cross-sectional study was conducted in the poultry carcass of different eight factories, broiler poultry, also from water supply and feed stuff of different poultry cultivation area and lastly from poultry products (liver, kidneys, intestine, ovaries, eggs)in sites of Khartoum state in Sudan to an isolate and identify the prevalence of Salmonella infection in different type of poultry and poultry environment (including feed stuff and water supply) buffer peptone water, tetrathionate and Rappaport vassiliadisbroth are use as preenrichment and selective media forisolation and identification for salmonella spp.organism isolates of microorganism will be done by serological test using Commercial antisera and biochemical test using APIE20, andfinally conventional PCR method use as molecular confirmatory test

Results: The results showed that the maximum number percentage of different Salmonella species was recovered and which obtained as flows respectively from raw and frozen poultry (49.8%), from feed stuff (16.6%), poultry organs (12.2%),water supply (9.9%), poultry products(6.6%), broiler poultry(3.3%) In additionally six species of Salmonella were obtained from this study, namely: Salmonella typhimurium. S.enteritidis, S. arizonae, S. pullorum, S. chlorasuis, and S. paratyphi©.

Conclusion: Poultry can be one of the most important food but still zoonotic disease risks exist. Human Salmonellosis from contact with live poultry is a challenging, for health problem. Prevention requires an integrated one health approach including public health and animal health officials collaborating with feed store industry and healthcare providers, veterinarians, and backyard flock owners. To dissolving and eradicate the infection among population. Several factors such as misuse of antibiotics, use of antibiotics in agriculture, poor hygiene practices by hospitals and individuals, unregulated sales of antibiotics and genetic factors affecting potentially in infections and transmission of Salmonella organisms among community health and the efforts of collaboration with stockholder must be done to protect the public's health.

KEYWORDS

Isolation, Identification and Characterization of Salmonella spp.

INTRODUCTION

Salmonella is a genus of rod-shaped (bacillus) Gram-negative bacteria of the family Enterobacteriaceae. Salmonella species are non-spore-forming, predominantly motile enterobacteria with cell diameters between about 0.7 and 1.5 µm, lengths from 2 to 5 µm, and peritrichous flagella (all around the cell body). (Fabrega, A. Vila J. April 2013) They are chemotrophs, obtaining their energy from oxidation and reduction reactions using organic sources. They are also facultative anaerobes, capable of generating ATP with oxygen ("aerobically") when it is available, or when oxygen is not available, using other electron acceptors or fermentation ("an aerobically .(Fabrega, A. Vila J. April 2013)

Salmonella is the causative agent of *Salmonellosis*. *S.* typhiand *Salmonella* paratyphi are pathogenic exclusively for humans, causing systemic infections and typhoid fever, whereas salmonella enterica serovars typhimurium cause gastroenteritis (Zhang XL, Jeza VT, Pan Q. 2008)

The genus Salmonella is part of the family of Enterobacteriaceae. Its taxonomy has been revised and has the potential to confuse. The two species of Salmonella are Salmonella enterica and *Salmonella* bongori. S. enterica is the type species and is further divided into six subspecies. (Su LH, Chiu CH (2007).) that include over 2,600 serotypes (Gal-Mor O. Boyle, EC, Grasssl GA, 2014).Salmonella was named after Daniel Elmer Salmon (1850–1914), an American veterinary surgeon the subspecies are: S. enterica, S. salamae, S. arizonae, S. diarizonae, S. .houtenae, and S. indicate (Brenner, FW, et,

al July 2000) The taxonomic group contains more than 2500 serotypes (also serovars) defined on the basis of the somatic O (lipopolysaccharide) and flagellar H antigens (the Kauffman–White classification). The full name of a serotype is given as, for example, Salmonella enterica subsp. enterica serotype typhimurium, but can be abbreviated to *Salmonella* typhimurium. Further differentiation of strains to assist clinical and epidemiological investigation may be achieved by antibiotic sensitivity testing and by other molecular biology techniques such as pulsed-field gel electrophoresis, multilocus sequence typing, and, increasingly, whole genome sequencing. Historically, salmonellae have been clinically categorized as invasive (typhoidal) or noninvasive (nontyphoidal salmonellae) based on host preference and disease manifestations in humans (Okoro, CK, et, al Jan. 2002)

Salmonellae are initially detected by their biochemical characteristics. Like other enterobacteriaceae, salmonella possess several O Antigens (from a total of more than 60) and different H antigen in one or both of two phases. Some salmonellae have capsular (K) antigens, referred to as VI, which may interfere with invasiveness. Agglutination tests with absorbed antisera for different O and H antigen form the basis for serologic classification of the salmonella (Jawetz, Melnick, and Adelberg's 1998).The O factor of this bacteria determines the serogroups and the H factors define the sero type (Fitzgerald C. et,al, 2003). The flagella antigens HI (phase 1) and H2 (phase 2) are encoded by flic and fLjB genes, respectively. These two genes are present at two different locations on the salmonella chromosome. However, one come of them is expressed at one time duo to face variation (McQuiston, JR . et, al, 2004). In salmonella, the genes responsible for biosynthesis of the O antigens are normally grouped together on the chromosome in a gene cluster called rfb (Luk, IMC, et al, 1993). Salmonella vary in length most species except *Salmonella* pullorum–gallinarum are motile with peritrichous flagella. *Salmonella* grow well on simple media but they almost never ferment lactose or sucrose. They form acid and sometimes gas from glucose and mannose. They usually produce HS. They survive freezing in water for long periods. *Salmonellae* are resistant to certain chemicals(eg, brilliant green, sodium terathionate, sodium dxycholate) that inhibit other enteric bacteria Such compounds are therefore useful for inclusion in media to isolate salmonella. (Jawetz, Melnick, and Adelberg's 1998). Salmonella species are facultative intracellular pathogens ;(Brenner, FW, et, al July 2000) certain serotypes causing illness. Nontyphoidal serotypes can be transferred from animal-to-human and from human-to-human. They usually invade only the gastrointestinal tract and cause *Salmonellosis*, the symptoms of which can be resolved without antibiotics. However, in sub-Saharan Africa, nontyphoidal Salmonella can be invasive and cause paratyphoid fever, which requires immediate treatment with antibiotics. Typhoidal serotypes can only be transferred from human-to-human, and can cause food-borne infection, typhoid fever, and paratyphoid fever editors, (Gillespie, SH, Hawkey PM, 2006). Typhoid fever is caused by Salmonella invading the blood stream (the typhoidal form) or in addition spreads throughout the body, invades organs, and secretes endotoxins (the septic form). This can lead to life-threatening hypovolemic shock and septic shock, and requires intensive care including antibiotic. Irresistible microbial sickness is a major cause of death in so many countries of the continent, particularly the developing countries. The aim of food safety has now been shifted by developed countries from investigative causes of food-borne diseases to proactive food, contamination prevention, and counteractive processes (Khalil AT, et ,al, 2018). Salmonella has been recognized as a harmful food and waterborne pathogen that can infect humans and animals through extreme pain and mortality. Salmonella has three different entry pathways that lead to gastrointestinal disease by infected foods (such as poultry, poultry organs and eggs) and environment litter and fertilizer, and consumption of contaminated raw fruits and vegetables (Beach, JC. Et, al 2002-Ibrahim SM, et, al 2014). The majority of human *Salmonellosis* is common to both wild and domestic animals, thus, food of animal origin is a source of *Salmonellosis*, typically between unprocessed poultry and prepared food products, i.e. through cross contamination in food catering or at home. While all *Salmonella* is a non-host-adapted serotypes that cause most of the food-borne Salmonella emerge (Zhao C, et, al 2001, Khalil AT, et ,al, 2018, Worldemariam E, et, al2005). Handled raw poultry meat naturally host bacteria, most of which provoke poultry meat deterioration. Food-borne pathogens, such as Salmonella serotypes, Campylobacter jejuni, Listeria monocytogenes, C. perfringens and S. aureus can harbor food-borne pathogens (Waldroup, Al, 1996 - Ibrahim SM, et, al 2014). As disease-related meat, the food borne disease outbreaks reported in poultry and poultry products to be ranked first and second in most countries around the world, respectively, and third in the United States (Mishu, B, et, al, 1994). Nevertheless, Salmonella infections of livestock and poultry products have been reported in Sudan Mamoun, IE, 1992 , Yagoub, SO 2009, Elsafi HH, et, al 2009). Many studies have reported Salmonella outbreaks in relation to meat or eggs from poultry). A critical manifestation in poultry industry is the vertical transmission of infections from breeding hens to poultry meat as an epidemiology of Salmonella species infections (Mishu, B, et, al, 1994, Keller, LH, et, al a1997, Gast, R. K.,et al, 2003). Regardless of the coordinated efforts for the eradication of typhoid, malnutrition and related problems caused by Salmonella, it continues a major general medical issue around the world. Because most of Salmonella diseases are derived from the ingestion of unsafe food, a possible explanation for the prevalence of safe antimicrobial Salmonella is indicated by antimicrobial specialists in the feeding of affected creatures (Antunes P,et, al,2003). In Sudan, the broiler chicken population has been estimated to be 22.5 million chicks (Khalifa Ak, 2015) and Khartoum State generates 90% of Sudan's production (Sirdar,MM, 2014,). However, the traditional sector (small farms) produced about 60% of total broiler production and the rest was provided by the new sector (companies). However, many people in Sudan rear household chicken and eat their produce (meats and eggs) locally or sell it on the local market. Therefore, the purpose of this study was to isolate, detect and assess Salmonella infection in raw and frozen carcass poultry, poultry products, and feed stuff and water supply samples from Khartoum state, Sudan.

RATIONALE

The influence of public health outcomes will be addressed regularly, one of these is *sallmonellosis.* It is a major health problem of mainly developing countries where conditions for the existence of endemic disease are still favorable in developing countries like Sudan, typhodidal and Non-typhoidal Salmonella is the public health problem and major cause of diseases because of poor hygiene and poor sewage system, so the disease could be easily transmitted via food and animal contact.

Statistical analysis of food-borne diseases in Sudan stated that 10-30% of food-borne diseases can be caused by food handlers (Nagawa B.E.et, al, 2014). In many states in Sudan, it is difficult to evaluate the situation of *sallmonellosis,* due to lack of reprehensive data. The prevalence of Salmonella serovars is not well documented, because the lack of epidemiological surveillance system and inadequacy of laboratory facilities for culture, as Salmonellae are not routinely isolated, identified, serotyped, genotyped and managed correctly, so the patients were empirically treated. On the other hand, during the last years Sudan cities exhibited a rapid increase in production and consumption of poultry meat and eggs which constitutes the important vehicles for food borne pathogens affecting consumer.

OBJECTIVES

General Objective

To isolate and identify the *Salmonella.* Spp in poultry food using ordinary culture procedure and conventional molecular procedure (PCR)

Specific Objectives:

1- To isolate and identify *Salmonella* spp. by conventional methods.
2- To determine the percentage of Salmonella species among poultry in Khartoum state
3- To determine the antimicrobial susceptibility profile of the *Salmonella spp.* isolates
4- To confirm *Salmonella spp.* serovars using 16s rRNA gene methods

MATERIALS AND METHODS

Study Design:

This study is a descriptive cross-sectional study in poultry and poultry products

Study Area:

The study was conducted at different eight factories and from different restaurant in Khartoum State ,the areas included in the study were Alarabia Factory, Alarabi Factory, Loli factory, Elwagba Factory, Elwatania Factory, Elneil Factory, Mickey factory, Elgossi Factory, shandawi restaurant ,kack restaurant and

Study Period:
The study carried out during the period from 2019-2022 in poultry for isolation and identification of salmonella spp. infection.

Samples Criterions
Inclusion Criteria
All samples of food were in a good frozen condition or fresh samples.

Exclusion Criteria
Any sample with Offensive smell, decomposition or not matching with the specifications required for analysis was excluded.

Data Collection:
Specification data were collected by questionnaire.

Sample Size:
According to the following equation 384 samples were collected.

Statistical Analysis:
The data will be analyzed using Statistical Package for Social Science computer program (SPSS). Frequencies and means will be calculated. Chi square test will be used for comparison between demographical variables and organism. P-value less 0.05 will be considered significant.

Samples Collection
Fresh and frozen poultry carcass samples were collected from poultry factories, and from broiler poultry, water supply, feed stuff, ,and lastly from poultry organ and products during the period of 2019-2021. The original sources of the poultry and egg samples were either local farms or local houses from where they had been brought to the market. All samples were stored in sterile ice bags and forwarded to Department of food and water at national public health laboratory in Khartoum state ministry of health -Sudan.

Procedure Of Sample Collection:
Samples will be collected randomly with equal proportion from eight poultry factories, broiler poultry, poultry environment and poultry product including (liver, intestine, kidney, ovaries, and eggs) in Khartoum state.

Procedure Of Salmonella Isolation And Identification
Samples Carcass, intestine, liver, kidney, ovaries, water supply, feed stuff, and broiler samples were collected from 384 commercially poultry samples and examined. The samples were immediately transported to the laboratories in a cool thermos and were processed for culture to identification and isolation of salmonella species. Cultures Salmonella are isolated according to standard methods (ISO 6579, 1993). Twenty-five grams of each meat poultry sample were taken into a stomacher bag containing 225 mL buffered peptone water as preenrichment medium and homogenized well using a stomacher machine mixer for homogeneous tissue and from internal organs samples intestine, liver, kidney, ovaries, feed stuff ,water supply take one gram of each sample and one ml from water aseptically to 9 mL of the same preenrichment medium witch mention above. All preenrichment medium with inoculated samples were incubated for 18 hours at 37°C.and transfer One ml of each preenriched medium including poultry carcass and internal organ samples to selective medium tetrathionate novobiocin broth and incubated at 37 °C for overnight and also from the same each preenriched medium containing carcass and internal organ samples transfer 0.2 mL of each inoculums cultures to Rappaport-Vassiliadis broth and incubated at 42°C, for 24 hours. Next day subculture done from the selective medium(tetrathionate broth and Rappaport vassiliadis broth) on XLD, and MaCconkey agar plates and incubated at 37 °C for 24h. The isolates will be identified by colonial morphology of salmonella species which appear (red with black center in XLD agar) and non lactose fermenter in Macconkey agar plates, Gram stain also done to emphasis the isolation colonies. Presumptive Salmonella colonies were streaked onto sterile nutrients agar plates and were incubated for 24 hours at 37°C. The pure colonies of Salmonella isolates were sub-culture in nutrient agar slopes and incubated for 24 hours at 37°C, and then the slope were kept in the refrigerator at 4°C until it was used for biochemical testing. Purified isolates have been identified with biochemical measures using catalase test, Vogs- Proskauer (VP) test, citrate test, urease test, indole test, motility test, sugar fermentation (TSI) and methyl red test. Biotypes and serotypes will be determined by API E 20 procedure and also by polyclonal and monoclonal antisera for *salmonella species* Antibiotic susceptibility Kirby-Bauer method will be used to determine the antibiotic sensitivity of the isolate. The antibiotic will be used as follows: Ciprofloxacin, Ceftriaxone, Cefotaxime, Kanamycin, Tetracycline, Ampicillin, CO-trimexazole, Erythromycin, Chloramphenicole.

RESULTS
This study revealed 27 isolates (7.0%) out of 384 specimens. All positive isolates were examined and identified as *Salmonella* species after 24 hours incubation at 37°C and 42°C Aerobically and indicated by the presence of turbidity (table 3) subculture were done in solid media for growth recognition smooth round pink colonies with black center appear on XLD (table 3) and smooth round colorless transparent colonies on macconkey agar plate (table 3), red slant wit H_2S and yellow butt found in TSI (table 3 figure 7). Thin smear prepared with the colony from XLD, MACA for Gram's stain which revealed Gram negative bacilli pink color, small short rods shape appearance (table 3 figure 8) all the positive isolates were motile having highly speed movement under microscope (table 3) all of the isolates were found to be indol negative (Fig 5) while the MR test appear positive (fig.5) and VP test negative (fig. 5) .polyvalent and monovalent antisera were used against positive isolates by slide agglutination to determine the salmonella organism.

The total positive isolates were confirmed by conventional PCR method which reveled 25 isolates as salmonella species and two isolated of the total positive were remained negative gene of salmonella species.(Table3)The isolated results revealed as follows. Thirteen positive of salmonella spp. isolated from poultry carcasses out of total samples. Nothing isolated from poultry liver, ovaries, kidneys, and one isolated of broiler and three isolated from intestine two were isolated from poultry eggs including external shell eggs and internal shell also Three were isolated from feeding stuffs and lastly three were isolated from water supply(Table2).. The total positive isolates were collected from different locations and sources. The prevalence percentage of Salmonella was the highest among poultry carcass 13(52%) and were identified as S. enteritidis, S. typhimurium, S. arizona. S. chlorasuis followed by feed stuff 3(12.0 %) were identified as S. enteritidis, S. typhimurium, and from poultry intestine 3(12.0%) S. typimurium, S. pullorum, S. paratypi© from water supply3(12%) were identified as S. enteritidis, S. typhimurium from local eggs 2(8.0%)were identified as S. enteritidis and from broiler poultry1(4.0%) identified as S. enteritidis (table 2). the confirmed isolated carried out against antibiotic sensitivity test which revealed the highest sensitivity of antimicrobial were Ceftriaxone, Cefotaxime , Kanamycin and moderate sensitive to Ciprofloxacin, Co-trimexazole, Chloramphenicol while resistant to Tetracycline Ampicillin ,Erythromycin (table 4, fig 4).

Table 1: Result Of Isolated Salmonella Species Done By PCR Method.

Isolated organism	No of positive isolate	No of percentage
s.enteritidis	10	2.6 %
s.typhimurium	11	2.8%
s.arizona	1	0.3 %
S.pullorum	1	0.3%
s.chlorasuis	1	0.3 %
s.paratyphi(C)	1	0.3 %
Total	25	6.6 %

Salmonella typhimurium revealed high number of the total positive isolates 11(2.8%) followed by salmonella enteritidis 10(2.6%) while salmonella Arizona , S. pullorum, S.chlorasuis, and S.paratyphi© found in equal low number respectively 1(0.3%)

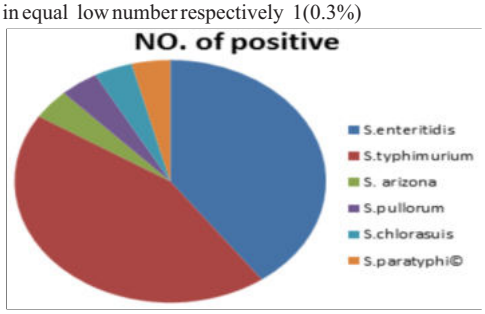

Figure 1: Result Of Isolated Salmonella Species Done By API E20 Method

Salmonella typhimurium show's deep brown wide area while salmonella enteritidis appear with blue color area but salmonella Arizona , S. pullorum, S.chlorasuis, and S.paratyphi© found in equal low number respectively.

Table 2: Location Area Of Isolated Microorganism And Percentage

Organism	Carcass	Feed stuffs	Water supply	Liver	Poultry egg	Ovaries	Kidney	Broiler	intestine
S. enteritidis	5.0 (20%)	1.0 (4.0%)	1.0(4.0%)	zero(0.%)	2.0(8.0%)	zer(0%)	Zero(%)	1.0(4.0%)	Zero(0%)
S.typhimurium	6 (24%)	2.0(8.0%)	2.0(8.0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	1.0(4.0%)
S. arizona	1.0 (4.0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)
S. pullorum	0 (0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	1.0(4.0%)
S. chlorasuis	1.0 (4.0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)
S. paratypi©	0 (0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	Zero(0%)	1.0(4.0%)

Table 2: S. typhimurium showed high number and percentage in carcass samples followed by S. enteritidis, S. arizona and S. chlorasuis, respectively. In feed stuff samples the high number and percentage found in S. typhimurium followed by S. enteritidis while the high number and percentage in water supply samples are S. typhimurium and S,enteritidis. And in poultry eggs samples we found only S. enteritidis. In broiler samples we found low number and percentage of S. eneritidis . While S.typhimurium, S.pullorum and S. paratyphi © found in equal number and percentage in intestine samples. but no organism isolated from liver, ovaries and kidney samples.

The salmonella species were found in high number and percentage in poultry carcass 13(52%) And equal number and percentage in food stuff, water supply, intestine respectively 3(12%) while in poultry egg 2(8.0%) and in broiler 1(4.0%) but nothing isolates from liver, kidney, ovaries.

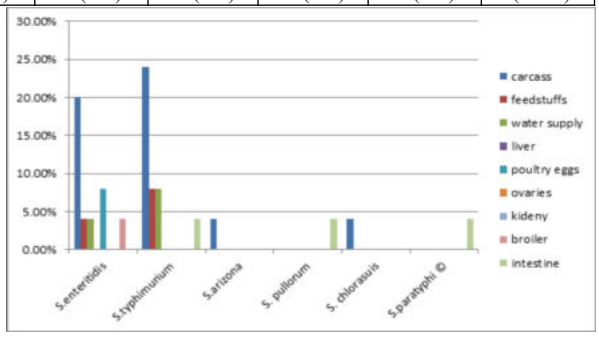

Figure 2: Location Area Of Isolated Microorganism And Percentage

Table 3: Detection Of Salmonella Species On Culture, Staining And Morphological Characters

NO. of sample	Media used	Change in broth	Culture morphology	Staining by gram stain	Motility by hanging drop	Biochemical test	NO. Positive isolated salmonella	Confirmed positive isolated salmonella by PCR	Percentage of total positive
384 samples Sources of isolates samples carcass, feed stuff, water supply, Broiler, poultry egg, poultry organs	Tetrathionat broth	Turbid			motile		27	25	6.6%
	Rappaport-Vassiliadis broth	turbid							
	XLD		Smooth, round Red colony with black centre	Gram negative bacilli and short rod shape					
	Mac A.		smooth, transparent, colorless colonies (NLF)						
	TSI A					Red slope with H_2S and yellow butt			
	Peptone water					No change of color ring after kovacs reagent			
	Mr-vp media					Red color in MR test and no change color in VP test			
	Citrate					blue color developed in positive test			

Table 4: Result Of Antibiotic Test Among Salmonella Species

oraganisms	Cefotaxime	Ampicillin	Kanamycin	Ciprofloxacin	Tetracycline	Ceftriaxone	Chloramphnicol	Co-Trimexazole	Erthromycin
S.enteritdis	S	R	S	S	R	S	M	R	R
S.typhimurium	S	R	S	M	R	S	M	R	R
S.arizona	S	R	S	S	R	S	M	M	M
S.pullorum	S	R	S	R	R	S	M	S	R
S. paratyphi ©	S	M	S	S	R	S	R	S	R
S. choleasuis	S	R	M	R	R	S	M	M	R

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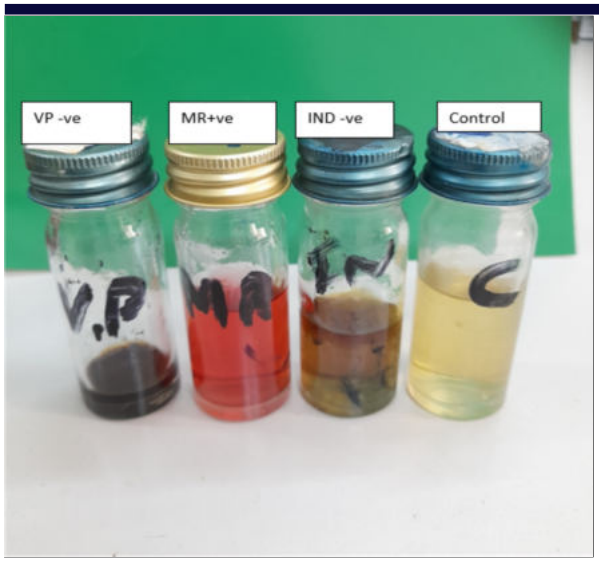

Figure 5: Biochemical Test: From Left To Right Shows VP Negative, MR Positive Change In Red Color, IND Negative No Change In Ring Color.

Figure 6: Citrate Test From left to right shows green color negative test, and blue color in positive test

Figure 7: KIA test growth of salmonella serovars shows red slop with H_2S with gas

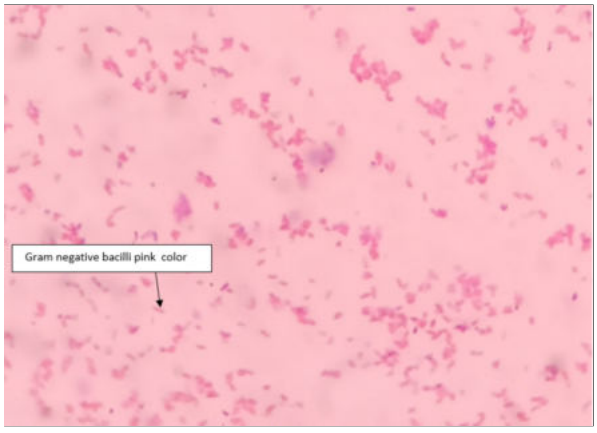

Fig. 8: Showing Salmonella Serovars Gram Negative Small Bacilli On Gram Staining (100X).

The results shows high sensitive to cefotaxime and Ceftriaxone followed by Kanamycine and moderate sensitive to Ciprofloxacin, chloramphnicol, Co- trimexazole and resistant to Ampicillin, tetracycline, Erthromycin

Antibiotics And Sensitivity Tests

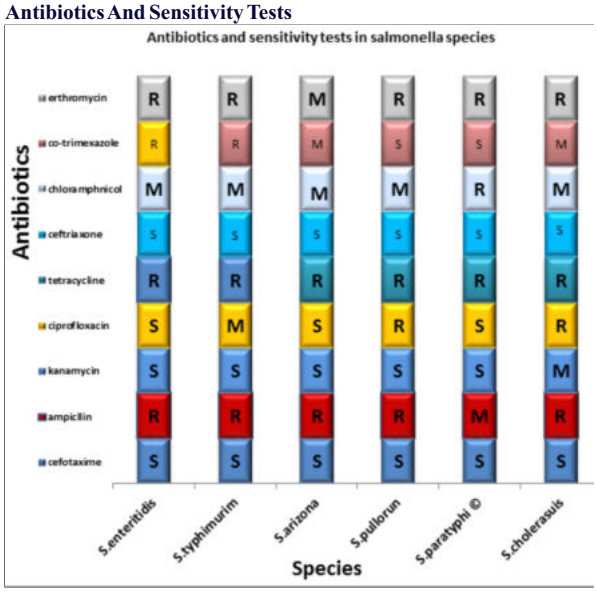

Figure 4: Most of the salmonella species were sensitive to Ceftriaxone, Cefotaxime, Kanamycin, and moderate sensitive to Ciprofloxacin, chloramphnicol, Co- trimexazole and resistant to Ampicillin, tetracycline, Erthromycin

DISCUSSION

Different Salmonella serotypes in different serogroups were isolated from the samples sources witch responsible for salmonella infection among population in this study the result of total percentage of salmonella organism in total sample collection is 6.6 % the major serogroups isolated in this study with the heights prevalence in the samples is salmonella typhimurium followed by the others erogroup salmonella Enteritidis which is in agreement with other reports. The incidence of reported *Salmonella* infections in humans in 2009 in European Union countries was reported to be 108,614 cases. The European Union reports that 23.7 percent of every 100.000 people have *Salmonella* cases. It has been found that 56% of the cases reported in 2009 are in the Czech Republic, Germany, England and Poland (Grimont**,** P.A.D. and F.X. Weill, (2007).).

The different species of Salmonella were isolated. from poultry contamination was still partial higher than that recorded by (Yagoub SO. 2010) and Elsafi HH.et al.2009) and lower than (El Hussein et al.,et, al,2016) which were, 6.2%, 3.4%, 9.2% In addition, our findings were lower percentage than reports from other countries, such as Nepal 14.5% (Grimont**,** P.A.D. and F.X. Weill, (2007) Canada 14% (Arsenault J, et, al, 2007), and South Africa 19.2% (Van Nierop W, et, al 2005). And Turkey 12% (Hoelzer K, et, al, 2011). Many countries have shown a higher percentage of salmonella infection than this study in humans, food, and animals such as 73.3% in Egypt (ELHussein AR, 2016). 68.2% in Ethiopia, 51.2% in Argentina, 25.9% in Korea, and 72% in Thailand (Cardinale E,et, al, 2003) in my opinion this variation occur duo to the environmental atmosphere which become very hot most of the year. The most important sources of salmonella infection in Khartoum state usually occur in poultry and also may be happened by the other food which already contaminated by human feces this fact reported by the other study carried out in isolation and identification of *Salmonella.* Species by *(*Roy, P.,et, al,2002) Which mention the initial survey has provided useful information about the status of salmonellosis in Khartoum, Sudan. We have demonstrated that *Salmonella* was isolated mostly from humans followed by chicken. *Salmonella* was isolated from different sources with an overall prevalence of 9.2%, which is comparable to the isolation frequency (11.99%).

Some of the common *Salmonella* serotypes isolated are *Salmonella, Salmonella*. typhimurium *Salmonella* Enteritidis known as nontyphodial salmonella which causes *Salmonellosis diseases* among population. The manifest symptoms appear as Gastroenteritis lead to continues diarrhea, sometimes vomiting, and fever, and the other symptom may be Bacteremia and septicemia which remain one of the public health problem in Sudan and I agreement with the other report by(Maria T. et, al, 2020) which they reported that the Nontyphoidal

Salmonella infections are common and remain a significant public health problem in the US. Many serotypes of *Salmonella* have been given names and are referred to informally as if they were separate species even though they are not. Most nontyphoiial Salmonella species infection are caused by *Salmonella*. Enteric subspecies enteric serotype .Enteritidis, and *Salmonella*. typhimurium, Highest percentage of *Salmonella* species in current studies of samples were *Salmonella* Enteritides and *Salmonella* typhimurium while Salmonella arizona, Salmonella cholerasuis, and *Salmonella* pullorum,*Salmonella* paratyphi© were isolated in lower percentage of the total samples in Khartoum state which are responsible for typhoidal diseases cases among population . The large number type of salmonella isolated appear in Fresh and frozen carcasses samples and the other large number of the total isolates sequentially were found in the feed stuffs, water supply and from poultry intestine samples flowed by poultry eggs and lastly from poultry broiler and nothing isolates of the liver, ovaries and kidney the types of isolated salmonella species are sensitive to Ceftrixone and Cefotaxime and kanamycin and resistant to tetracycline, Erthromycin and Ampicillin. And moderate sensitive to the Co-trimexazole, ciprofloxacin and Chloromenphnicol (fig) .this result agreement with the other report which conducted in Dubai United Arab Emirates showed 87.8% resistance of Salmonella towards tetracycline, which is consistent with our study (Waldroup AL.(1996). another study it was determined that 77% Salmonella in poultry feed is resistance to tetracycline, which is much closer to the present study findings (Mishu B**,** et, al, 1994)As in contrast a study revealed that Salmonella isolated from poultry chicken showed 52.4% sensitivity to Ceftriaxone (Mishu B**,**et, al, 1994). These variations in results may be due to changes in geographic condition.

The transmission of salmonella species in poultry and poultry products according to this study I think the most important thing of transmission of the infection usually occur duo to the bad sanitation of water supply and feed stuffs which are often contaminated by air atmosphere and from remaining feces of the other animals and humans when they are live near surround the backyard of poultry. From this study the obtained result of salmonella species infection in poultry and poultry products it is reasonable results comparing to the high data result carried out in Khartoum state which have been reported as the highest contamination level by Total Viable Counts caused by Salmonella spp. (11.11 %) at DE feathering reported by (Umar Abdal-rahman, 2016)

Some researchers mentioned reported papers about the highest percentage of *salmonella* infection in poultry carcasses and poultry product in Khartoum state. Actually I suggest that the high percentage occur due to the sampling strategy and geographical area. In this study samples were obtained at the various stages of processing (from slaughter to retailing at the supermarkets)and some environmental factors such as air, feces, and contact surfaces were also play potential in the role of cross-contamination. Thus, the focus of this study was to determine the contamination sites and to trek the dissemination of *Salmonella* during slaughtering and processing. Besides reporting a high prevalence of *Salmonella* in poultry and their processing environments, our results also suggested that frequent contamination of *Salmonella* occurs along the processing line. The previous studies as reported by other researchers only focused on the prevalence of *Salmonella* in chicken carcasses or chicken cuts and chicken by-products such as organs (livers, intestines, ovaries, kidneys, and eggs) This could explain the low prevalence of *Salmonella* spp in our study which focused on the prevalence of *Salmonella* at different stages of processing of poultry in factories and in local supermarket. I think the incidence of *Salmonella* infection occurs from processing of factories and supermarkets due to cross contamination under unhygienic conditions led to the amplification of contamination of the carcass from various stages along the processing continuum. All this factors play an important role. The bad hygiene and bad sanitation also effected potentially in this percentage and in the prevalence of salmonella organism. In my opinion this results were not dangerous according to this study but still began one of the most important risk infection which causes many different problems among the population if its ignoring specially in the food poisoning which lead some times to the mortality of patient.

CONCLUSION

Poultry can be one of the most important food but still zoontic disease risks exist. Human *Salmonellosis* from contact with live poultry the most common species in this study are salmonella typhimurium and *Salmonella* enteritidis is a challenging, yet largely preventable, public health problem. Prevention requires an integrated One Health approach including public health and animal health officials collaborating with the mail-order hatchery industry, feed store industry, healthcare providers, veterinarians, and backyard flock owners. to prevention public health problem of salmonella infection. Spreading of human Salmonella infections linked to live poultry have been reported and are increasing in frequency in recent years in part due to the increasing popularity of backyard flock ownership; the continued occurrence of these preventable outbreaks highlights always needed for enhanced prevention efforts to protect the public's health. Several factors such as misuse of antibiotics, use of antibiotics in agriculture, poor hygiene practices by hospitals and individuals, unregulated sales of antibiotics and genetic factors, such as plasmids, integrin, transposons, etc., contribute to selective pressure on antibiotics and resistance gene transfer, respectively, in Salmonella species. This has led to the emergence and spread of resistance in this microorganism and resultant therapeutic failures. Potential restriction in the use of antibiotics in agriculture, production of modified drugs, and use of combined therapy future plans against MDR bacterial strains.

Competing Interests

The authors declare that they have no competing interest

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Procedure Of Sample Collection:

Samples will be collected randomly with equal proportion from eight poultry factories, poultry product, and from poultry environment in Khartoum state.

Laboratory Procedures:

384 samples of food will be collected in wide mouth sterile containers with adjust atmosphere centigrade condition from poultry carcass. Poultry product poultry environment which mention before. Different quantity of different samples will be collected from each sample and transported to the reference laboratory of food and water microbiology department, at NPHL in Khartoum state -Sudan

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