Isolation and Characterization of Salmonella SPP from Poultry and Evaluation Their Resistance to Antibiotics

عزل وتوصيف السالمونيلا SPP من الدواجن وتقييم مقاومتها للمضادات الحيوية

By: Sittna Salaheldeen M. Hussein&Dr. Sanaa Osman Yagoub Ahmed

Al Neelain University, Graduate Faculty, Khartoum, Sudan

تاريخ الاستلام: 2023/12/5 تاريخ القبول: 2023 /12/14 تاريخ النشر: 2024/1/15

الملخص:

تهدف هذه الدراسة إلى عزل وتوصيف بكتيريا السالمونيلا SPP من الدواجن وتقييم مقاومتها للمضادات الحيوية في ولاية الخرطوم. تم جمع 700 عينة من منتجات الدواجن، وزرعت العينات في أوساط الزراعة وتم تشخيص البكتيريا المعزولة عن طريق اختبار صبغة جرام واختبار الكيمياء الحيوية، كما تم إجراء اختبار الحساسية المضادة للبكتيريا. تم التأكد من جميع أنواع السالمونيلا باستخدام تفاعل البوليميراز المتسلسل. تم تعريض البكتيريا لدرجات حرارة مختلفة (50، 60، 70، 80، 90، و100) لفترات مختلفة مدتها خمس دقائق، وعشر دقائق، و15 دقيقة. تم تجميد أنواع السالمونيلا المعزولة ثم تم وضعها في درجة حرارة الغرفة ثم تم تربيتها في أجار الزيلوز ليسين. ولاحظت الدراسة أنه من بين 700 عينة، تم عزل 24 عينة (3.4%) من السالمونيلا SPPبينما كانت 676 (96.6%) غير معزولة. لاحظت الدراسة أنه من بين 200 عينة من لحم الدجاج تم عزل 8 أنواع من السالمونيلا، 5 منها (62.5%) كانت S. pullorum بينما 3 (37.5%) كانتS. entiritidis ، وذلك على أساس 100 بيضة 50 ككيس صفار و50 كقشرة بيضة، من هذه 4 S.pullorum تم عزلها. من بين 100 عينة من أيدي العمال، 2 (50%) كانت من S.pullorum و 2 (50%) كانت منS.typhi ، ولاحظت الدراسة أنه من بين 100 عينة من Aleaga كان هناك 4 معزولة وكانت 2 من .S.pullorum أظهرت الدراسة أنه من بين 100 عينة من الماء تم عزل 3 أنواع من السالمونيلا SPP وأكثرها عزلة كانت 2 S.pullorum (66.7%) تليها 1 (33.3%) .S.typhiقرص المضاد الحيوي الذي تم استخدامه هو أمبيسيلين، كوتريموكسازول، كلورامفينيكول، سيبروفلوكساسين، بيبيراسيلين، سيفوتاكسيم، أميكاسين، جنتامايسين، تتراسيكلين سيفتازيديم، كانت S.entiritidis و S.pollurumحساسة للاميكاسين والسيبروفلوكساسين والكلورامفينيكول والأمبيسيلين والسيفوتاكسيم وكانت مقاومة للأوكسازول والتتراسيكلين في نفس الوقت وكانت وسيطة للبيبيراسيلين، تم اختبار جميع العينات المعزولة بطريقة تفاعل البلمرة المتسلسل وتم التأكد من عزلة السالمونيلا.

الكلمات المفتاحية: الغذاء، الألبان، اللحوم، الطرق التقليدية، الوسائط اللونية، API، الطريقة السريعة.

Abstract

This is study aim to conduct to Isolation and characterization of Salmonella SPP from poultry and evaluation their resistance to antibiotics in Khartoum state. a total of 700 samples was collected from poultry product, samples was cultivated on culture media and the isolated bacteria was identified by gram stain and biochemical test, also antibacterial sensitivity test was proceeded. All species of Salmonella was confirmed using Polymerase chain reaction. The bacteria were exposed to different temperatures (50, 60, 70, 80, 90, and100) for different periods of five minutes, ten minutes and 15 minutes. The isolated Salmonella species were frozen and then placed at room temperature and then cultured in xylose lysine agar. The study observed that among 700 samples, out of these samples 24 (3.4%) Salmonella SPP was isolated while 676 (96.6%) were non-isolated. The study observed that out of 200 Chicken meat sample 8 Salmonella species was isolated 5 (62.5%) was S.pullorum while 3(37.5%) was S.entiritidis, based on 100 Eggs 50 as yolk sac and 50 as egg shell, out of these 4 S.pullorum was isolated. Out of 100 Worker's Hands samples 2(50%) was S.pullorum and 2(50%) was S.typhi, the study observed that out of 100 Aleaga samples there were 4 isolated and it's was 2 S.pullorum. The study showed that out of 100 samples of water 3 Salmonella SPP was isolated and most isolated was 2 (66.7%) S.pullorum followed by 1(33.3%) S.typhi, The antibiotics disc that been used was ampicillin, co-trimoxazole, Chloramphenicol, Ciprofloxacin, piperacillin, cefotaxime, Amikacin, Gentamycin, tetracycline Ceftazidime, S.entiritidis and S.pollurum was sensitive to Amikacin, Ciprofloxacin Chloramphenicol and ampicillin, cefotaxime and was resistant to co-trim oxazole and tetracycline and it's was intermediate for piperacillin. All isolated sample was test by polymerase chain reaction and the isolated Salmonella was confirm.

Key words: food, Dairy, Meats, traditional methods, chromogenic media, API, rapid method.

1. Introduction

Salmonella is a genus of bacteria that is commonly found in the intestines of animals and birds. It is a major cause of foodborne illness, with poultry being one of the primary sources of contamination. In order to prevent the spread of Salmonella and reduce the risk of infection, it is important to isolate and characterize the bacteria and evaluate their resistance to antibiotics.

Isolation of Salmonella from poultry involves the collection of samples from various sources such as feces, feathers, and internal organs. These samples are then culture in selective media that promote the growth of Salmonella while inhibiting the growth of other bacteria. After incubation, colonies that resemble Salmonella are further test using biochemical and serological methods to confirm their identity.

Characterization of Salmonella involves determining its serotype, which is base on the presence of specific antigens on the bacterial surface. This information is important for epidemiological purposes as it helps to track the source of infection and identify potential outbreaks. Additionally, characterization also involves testing the bacteria for the presence of virulence factors, which are responsible for the ability of Salmonella to cause disease.

Evaluation of antibiotic resistance in Salmonella is crucial for determining the most effective treatment options. Antibiotic resistance occurs when bacteria develop mechanisms to survive the effects of antibiotics, rendering them ineffective. This can be due to genetic mutations or the acquisition of resistance genes from other bacteria. By testing Salmonella, isolates against a panel of antibiotics, their susceptibility or resistance can be determined. The emergence of antibiotic-resistant Salmonella is a growing concern, as it limits the treatment options available for infected individuals. It is therefore important to monitor the prevalence of antibiotic resistance in Salmonella and respond appropriately to prevent its spread. This includes implementing strict biosecurity measures on poultry farms, promoting responsible antibiotic use in animal production, and improving hygiene practices during food processing, the isolation and characterization of Salmonella from poultry and the evaluation of their resistance to antibiotics are essential for understanding the epidemiology of Salmonella infections and guiding effective treatment strategies. By implementing these measures, we can reduce the risk of Salmonella contamination in poultry products and protect public health.

Objectives:

General objective:

The study was conducted to Isolation and characterization of Salmonella spp. from poultry and evaluation their resistance to antibiotics in Khartoum state.

Specific objective:

1. To isolate and molecular characterization of Salmonella species among poultry product in Khartoum state.
2. Isolation and identification of Salmonella SPP associated with poultry
3. Determination of the source of infection. (Environment, feeds, workers, slaughter houses)
4. Counting the number of the Salmonella SPP in samples.

2. Literature review

Fresh poultry and meat are greatly putrefiable because of their enriched nutrient component, water activity (0.98 to 0.99), and near neutral pH (5.5 to 6.5), which is the optimal environmental condition for Salmonella (Acuff, 2005). Furthermore, poultry and meat could be contaminated by bacteria present in dirt and fecal material associated with slaughter and evisceration areas. In the presence of a contaminated carrier during processing, the quality of the final product could be threatened by Salmonella. According to the Centers for Disease Control and Prevention (CDC), nontyphoidal salmonellosis causes about 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths in the USA every year (CDC, 2019). In Korea, a large outbreak of S. Thompson infections caused by contaminated eggs led to 3516 patients hospitalized in 2019 (MFDS, 2019). According to a survey conducted by the Korean Ministry of Agriculture, Food and Rural Affairs, the domestic consumption of chicken per capita drastically increased by 58% in 2018 compared with 2008 (MAFRA, 2019).

The increasing demand for chicken products may have the unintended consequence of the increased risk of chicken-associated food illnesses, so hygiene management should be greatly considered during processing. Salmonella easily forms a biofilm on food contact surfaces (Reij et al., 2004; Rodrigues et al., 2011; Møretrø

et al., 2012). Once the biofilm is formed, it protects the embedded bacteria from external physical and chemical treatment (Milanov et al., 2009; Ashrafudoulla et al.,2021). Consequently, cross-contamination between food and food contact surfaces can occur. Salmonella biofilm capacity has been estimated at a laboratory-scale on diverse surface materials, such as stainless steel (SS), rubber, glass, and synthetic plastics (P) (Brooks and Flint, 2008; Rodrigues et al., 2011).

Conventional methods based on selective media for the detection and identification of Salmonella are timeconsuming, labor-intensive, and require numerous reagents. Advanced molecular biology methods, such as these limitations. This method is cost-effective, fast, accurate, and can easily be conducted in conjunction with other bacteria typing methods, such as pulsed-field gel electrophoresis

(PFGE) (Kim et al., 2006). By producing DNA fingerprints of bacteria, PFGE represents an important tool in identifying the origin of outbreaks (Wattiau et al., 2011). In addition, PFGE analysis is also reliable in determining genetic relationships between bacterial species (Whittam and Bergholz, 2006).

Foodborne Bacteria are the most common cause of foodborne diseases and exist in a variety of shapes, types and properties. Some pathogenic bacteria are capable of spore formation and thus, highly heat-resistant (e.g. Clostridium botulinum, C. perfringens, Bacillus subtilus, Bacillus cereus) .

Some are capable of producing heat-resistant toxins (e.g. Staphylococcus aureus, Clostridium botulinum). Most pathogens are mesophilic with optimal growth temperature range from 20 °C to 45 °C. However, certain foodborne pathogens , such as Listeria monocytogenes, and Yersinia enterocolitica are capable of growth under refrigerated conditions or temperatures less than 10 °C .

Escherichia coli:

Escherichia coli is a Gram-negative, non-spore forming rod. It may or may not be mobile; some rods are flagellated and some are not. The organism is a facultative anaerobe and ferments simple sugars such as glucose to form lactic, acetic, and formic acids; the optimum pH for growth is 6.0 to 8.0; however, growth can occur as low as pH 4.3 and as high as 9 to 10 pH.

Escherichia coli (E. coli) naturally form part of the normal flora in the gut of humans and other animals. In fact, most E. coli are considered harmless to humans (Croxen and Finlay 2010). However, certain pathogenic E. coli strains can infect the gut area and cause severe illness (Croxen et al. 2013). Pathogenic E. coli infection usually causes severe diarrhea. diarrheal disease caused by pathogenic E. coli is preventable by improved environmental sanitation and is treatable by antibiotics. The treatment of diarrheal disease is generally effective with oral rehydration and maintaining electrolyte balance through the diet (Chowdhury et al. 2015).

Multidrug-resistant E. coli: recent treatment and prevention strategies:

Food safety of fresh produce is a matter of increasing concern. Indeed, microbial contamination may occur during any of the steps in the farm-to-table continuum from environmental, animal, or human sources . Therefore, the prevention and treatment of microbial contamination is one of the important food safety issues. In general, E. coli caused diarrheal disease is preventable by improved environmental sanitation and is treatable by oral or intravenous rehydration, antidiarrheal and antibiotics (Croxen et al. 2013). Here, we provide experimental treatment and prevention options that can be applied in food preservation and in the field of infectious diseases.

Salmonella:

Salmonella are a group of bacteria that can cause gastrointestinal illness and fever called salmonellosis. Salmonella can be spread by food handlers who do not wash their hands and/or the surfaces

and tools they use between food preparation steps, and when people eat raw or undercooked foods. Salmonella can also spread from animals to people. People who have direct contact with certain animals, including poultry and reptiles, can spread the bacteria from the animals to food if they do not practice proper hand washing hygiene before handling food.

The genus Salmonella consists of only two species: S. enterica and S. bongori (Grimont & Weill, 2007). Salmonella enterica is divided into six subspecies, which are distinguishable by certain biochemical characteristics and susceptibility to lysis by bacteriophage Felix O1. These subspecies are:

Original subgenera	Current nomenclature
Subspecies I	subspecies enterica
Subspecies II	subspecies salamae
Subspecies IIIa	subspecies arizonae
Subspecies IIIb	subspecies diarizonae
Subspecies IV	subspecies housemate
Subspecies VI	subspecies indica

For the serovars of S. bongori, the symbol V was retained to avoid confusion with the serovar names of S. enterica subsp. enterica.

Staphylococcus:

Staphylococcus aureus is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. S. aureus is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans.

S. aureus is a Gram-positive, non-spore forming spherical bacterium that belongs to the Staphylococcus genus. The Staphylococcus genus is subdivided into 32 species and subspecies. S. aureus produces staphylococcal enterotoxin (SE) and is responsible for almost all staphylococcal food poisoning (Montville and Matthews 2008; FDA 2012).

S. intermedius, a Staphylococcus species which is commonly associated with dogs and other animals, can also produce SE and has been rarely associated with staphylococcal food poisoning (Talan et al. 1989; Khambaty et al. 1994; Le Loir et al. 2003).

Food Safety:

Food safety can be defined as the system that keeps food and food products free from substances hazardous to human health. Food safety should be a part of governments' strategies to ensure secure food for the consumers. In this context, a "hazard" refers to any biological, chemical or physical property

that may cause unacceptable risk (FAO, 1998). The emergence and discovery of new food-borne pathogens and other food-related hazards has increased the need for food-safety measures. The intensification of food production has also changed food processing and handling systems and raised new challenges for food- safety institutions. Intensification has led to large amounts of potentially infectious material being concentrated at single sites, such as large industrial production establishments or processing plants, and has therefore contributed to the potential for large-scale outbreaks of infection.

Changing consumption patterns – street vendors and home cooking of primary products are giving way to the purchase of processed food from supermarkets – make food-safety an issue of public concern rather than just a matter for individual consumers. Developing countries face difficulties in achieving food-safety goals in animal production systems. These difficulties result from inter alia unstable administrative and political structures, lack of infrastructure, and lack of investment in food-safety measures and research, as well as from inadequate consumer information. Responsibility for ensuring safe food for the consumer has traditionally been seen as the responsibility of public institutions. However, with the intensification and industrialization, responsibility has been shifted to a wider set of stakeholders including the private producer and the consumer.

3. Methodology

Study design:

This was analytical study.

Study area:

This study was conduct in Khartoum state. Samples collection from most of the poultry companies and farms from Khartoum

Study period:

The study was conduct from the period Sep 2022 - Feb 2023

Study population:

Samples was Chicken meat, eggs, from Worker's Hands, Water, and Alaleaga and grilled Chicken.

Inclusion criteria:

Chicken meat, eggs, from Worker's Hands, Water, Alaleaga and grilled Chicken.

Exclusions criteria:

A foodstuff, water, letters …etc.

Sample size:

The sample size was 700, 220 sample was collected from Chicken meat, 100 sample collected from Eggs, 100 sample collected from Worker's Hands, 100 sample collected from Water, 100 sample collected from Alaleaga and 100 sample collected from grilled Chicken.

Microbiological procedure

Aerobic plate count (APC) and Enterobacteriaceae counts (ENT)

25 g of test sample (meat) was weighed and blended in a stomacher machine for 2 minutes. A gram of the sample was weighed out and homogenised in 9mls buffered peptone water (LabM, UK) to give a dilution of 1:10. A six-fold serial dilution will be prepared. 0.1 ml of dilutions 10-6 and 10-5 for every sample will plated on plate count agar (PCA) (Biomark, India) for aerobic plate count determination and on McConkey agar (MCA) (LabM, UK) for Enterobacteriaceae (enteric bacteria) counts. The PCA and MCA were both incubated overnight (18–24 hours) at 37°C.

Distinct colonies on PCA and MCA were counted using a digital colony counting chamber and recorded in colony forming units per gram (cfu/g) of meat sampled using the formula:

These were further expressed in mean colony forming units per gram (mean cfu/g) and converted to log 10 base values .

Antiprogram of Salmonella

Antiprogram of Salmonella will be determined by using disk diffusion assay following the guidelines of clinical and laboratory standard institute. Pre-incubated 24 h cultures of Salmonella will be diluted to 108 CFU/mL in sterile buffer peptone water and spread over mueller-hinton agar (Merck, Germany). The antibiotic discs will placed over the lawn and incubated at 37 °C for 18–24 h. The clear zone around each antibiotic disc was measure on the following day .

Data analysis:

The statistical package of social sciences (SPSS) was used for statistical analysis version 25. Statistical significance was set at $P < 0.05$.

4. Results

Results of PCR:

All isolated sample was test by polymerase chain reaction and the isolated Salmonella was confirmed.

Table (1) frequency of sample among study population:

Samples	Frequency	Percent
Chicken meat	200	28.6
Eggs	100	14.3
Worker's Hands	100	14.3

Water	100	14.3
Alaleaga	100	14.3
grilled Chicken	100	14.3
Total	700	100.0

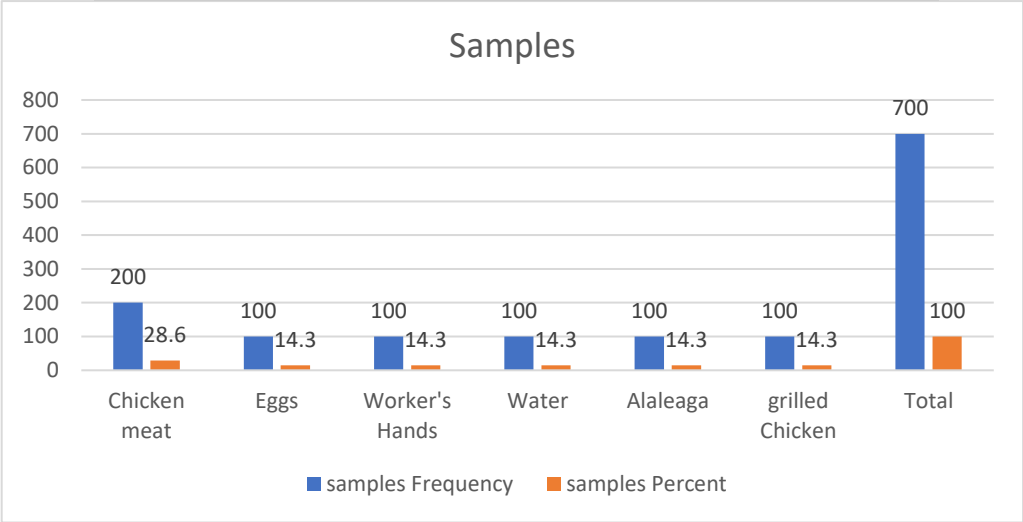

Figure (1) frequency of sample among study population

Results of isolated bacteria:

Table (2) Frequency of isolated bacteria among study population:

	Frequency	Percent
Isolated	24	3.4
Non-isolated	676	96.6
Total	700	100.0

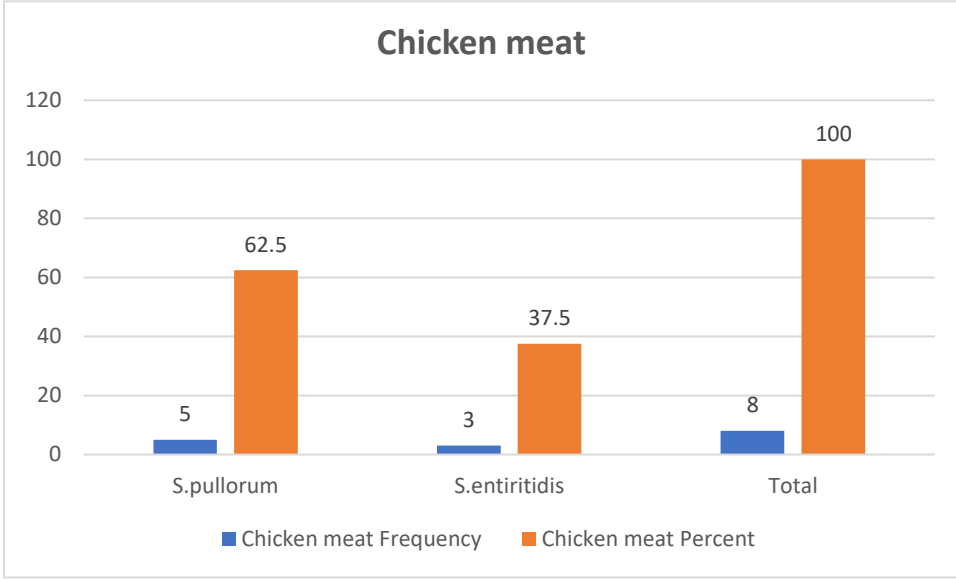

Figure (2) Frequency of isolated bacteria among study population

Table (3) Frequency of isolated Salmonella among Chicken meat:

Chicken meat	Frequency	Percent
S.pullorm	5	62.5
S.entiritis	3	37.5
Total	8	100.0

Figure (3) Frequency of isolated Salmonella among Chicken meat:

5. Conclusion

-The study concluded that out of 700 samples, 24 (3.4%) Salmonella spp was isolated while 676 (96.6%) were non-isolated.

-The study observed that the most isolated species of Salmonella was isolated from Chicken meat.

-The study concluded that the most isolated species was S.pullorum and S.entiritidis.

-The study concluded that isolated bacteria were froze and then placed at room temperature and then cultured in xylose lysine agar, and the results showed the growth of bacteria at the frozen temperature.

Recommendations:

Need Application of the following:

1/ Current standards for microbiological control to ensure quality assurance and safety of meats slaughter process.

2/ Risk management and processing of meats getting

3/ Implementation of good hygiene in Dairy processing; Training should be given to workers in the abattoir, especially for those who are assigned in meats carcass process about the contamination sources and hygienic conditions to maintain the quality of the meats carcasses .

4/ Sanitation in the breeding farms .

5/ Application of HACCP in Dairy and Meats, based on the use of multi - functional strategies (sanitizers &modern disinfections techniques).to reduce bacterial contamination.

6. Consider personnel hygiene, handsgloves, masks, head cover, contaminated equipment cross-contamination from raw material .

7/ Cooking at high temperatures of 100c° will help to eliminate pathogens before consumption.

8/ Application of hygienic measurements appears to be important to reduce the contamination of bacteria .

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