# Isolation and identification of Three pathogenic food borne organisms (Staphylococcus aureus ,Salmonella, Escherichia coli) from Dairy and Meats

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**Abstract:**

This study aim toIsolation and identification of four pathogenic food borne organisms (Staphylococcus aureus, Salmonella, Escherichia coli) from Dairy and Meats) by using traditional methods, chromogenic media, API and rapid method. by using traditional methods ,chromogenic media ,API and rapid method Isolation and identification of the isolated bacteria Standard protocols will be followed for isolation of the target organisms . Isolated bacteria will be identified by biochemical tests ,confirmatory test ,API.was used (SPSS) for statistical analysis version 25. Statistical significance was set at $P < 0.05$. results of this studys: Prevalence of Salmonella , E .coli , S .aureus Out of the total 70 Dairy samples examined, 10.0% (7/70) showed the occurrence of Salmonella ,14.3% (10/70) showed the occurrence of E .coli ,24.3% (17/70) showed the occurrence of S .aureus ,Prevalence of Salmonella , E .coli , S .aureus Out of the total 100 Meat samples examined, 29.0% (29/100) showed the occurrence of Salmonella ,20.0% (20/100) showed the occurrence of E .coli ,26.0% (26/100) showed the occurrence of S .aureus .The obvious conclusion to be drawn from the results studyis that:There is contamination in Meats in Khartoum State, and traditional slaughtering processes , Salmonella species and Escherichia Coli and Staphylococcus aureus were isolated from meats and Dairy.we Need Application of the following 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.

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

# عزل وتحديد ثلاثة كائنات حية منقولة بالغذاء من منتجات الألبان واللحوم

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**مستخلص:**

هدف هذه الدراسة إلى عزل وتحديد أربعة كائنات ممرضة محمولة بالغذاء (Escherichia coli ،Salmonella ،Staphylococcus aureus) من منتجات الألبان واللحوم) باستخدام الطرق التقليدية والوسائط الملونة و API والطريقة السريعة. باستخدام الطرق التقليدية والوسائط الكروموجينية وواجهة برمجة التطبيقات والطريقة السريعة. عزل وتحديد البكتيريا المعزولةسيتم اتباع البروتوكولات القياسية لعزل الكائنات الحية المستهدفة. سيتم التعرف على البكتيريا المعزولة عن طريق الاختبارات البيوكيميائية ، والاختبار التأكيدي ، API.was المستخدمة (SPSS) للتحليل الإحصائي الإصدار 25. تم تعيين الدلالة الإحصائية عند P <0.05.نتائج هذه الدراسات: انتشار السالمونيلا ، E.coli ، S. aureus من إجمالي 70 عينة ألبان تم فحصها ، 10.0٪ (70/7) أظهرت وجود السالمونيلا ، 14.3٪ (70/10) أظهرت حدوث E. coli. ، 24.3٪ (70/17) اظهرت حدوث بكتيريا S. aureus ، انتشار السالمونيلا ، E.coli ، S. aureus من اجمالي 100 عينة لحوم تم فحصها ، 29.0٪ (100/29) اظهرت حدوث السالمونيلا. ، 20.0٪ (100/20) اظهرت حدوث بكتريا E.coli ، 26.0٪ (100/26) اظهرت حدوث بكتيريا S. aureus.الاستنتاج الواضح الذي يمكن استخلاصه من نتائج الدراسة هو: وجود تلوث في اللحوم بولاية الخرطوم ، وعمليات الذبح التقليدية ، تم عزل أنواع السالمونيلا و Escherichia Coli و Staphylococcus aureus من اللحوم ومنتجات الألبان.نحتاج إلى تطبيق التنفيذ التالي للنظافة الجيدة في معالجة الألبان ؛ يجب تدريب العاملين في المسلخ ، خاصة أولئك الذين تم تعيينهم في عملية ذبائح اللحوم حول مصادر التلوث والظروف الصحية للحفاظ على جودة اللحوم الذبيحة.

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

## Introduction:

The World Health Organization defines food- borne diseases (FBD) as diseases of infectious or toxic nature caused by, or thought to be caused by consumption of food or water. The pathogenesis of bacteria causing food –borne poisoning depends on their capacity to produce toxins after ingestion (in the digestive tract ) or intoxication (ingestion of preformed toxins in foodstuff) .Infection caused by microbes that contaminate the food supply are a frequent reminder of the complex food web that links us with animal ,plant and microbial populations around the world .Some

pathogens persist in the environment , or in multiple hosts ,and can contaminate the foods via pathways that reflect the variety, of ecosystems that make up our food supply .Food safety depends on understanding these pathways well enough to prevent them .Substantial progress during the 20th century in animal disease control efforts has greatly reduced the food –borne infections related to zoonotic diseases such as brucellosis and bovine tuberculosis (Tauxe and Esteban,2006). At the same time ,an increasing number of microbes have been recognized that can cause serious illness in humans ,but rarely cause illness in the animal that carry them .The presence of these microbes is thus not apparent to the rancher of farmer ,and the animal appears entirely healthy on inspection at slaughter ,addressing these microbes requires a different prevention based on reducing levels of microbial contamination throughout the food chain .Recent outbreaks show that plants can also be contaminated with human pathogens on the farm , through manures ,water ,and wild animal incursion (Lynch et al .,2009 ). The need to reduce and prevent contamination continues through harvest and slaughter processing, and the food preparation steps in the final kitchen.

New Food-borne pathogens emerge when previously unrecognized pathogens are identified and are linked to food borne transmission from the beginning , or when food –borne transmission is documented for pathogens that are already well known.

The identification of new food safety problem has been accelerated by important improvements in surveillance and response.

These new surveillance tools capture information about infections in humans, as well as in animals and contamination of foods, providing important information that is integrated across sectors.

**Objectives:**

**General objective:**

Isolation and identification of four pathogenic food borne organisms (Staphylococcus aureus, Salmonella, Escherichia coli) from

Dairy and Meats) by using traditional methods, chromogenic media, API and rapid method.

**Specific objective:**

1. Molecular characterization of the species ,virulence gene ,toxin production ,antibiotic resistance plasmid
2. Assess the antimicrobial susceptibility of isolated organisms
3. Determine the activity of some commonly used preservative (Nacl,benzoic acid ,sorbic acid) towards isolated organisms
4. Detection of Antibiotics Residues in collected samples

**Previous studies:**

1.**A previous study conducted by M. Ann S. McMahon , Environmental Stress and Antibiotic Resistance in Food-Related Pathogens**

This study investigated the possibility that sublethal food preservation stresses (high or low temperature and osmotic and pH stress) can lead to changes in the nature and scale of antibiotic resistance (ABR) expressed by three food-related pathogens (Escherichia coli, Salmonella enterica serovar Typhimurium, and Staphylococcus aureus). The study found that some sublethal stresses significantly altered antibiotic resistance. Incubation at sublethal high temperature (45°C) decreased ABR. Incubation under increased salt (>4.5%) or reduced pH (<5.0) conditions increased ABR. Some of the pathogens continued to express higher levels of ABR after removal of stress, suggesting that in some cases the applied sublethal stress had induced stable increases in ABR. These results indicate that increased use of bacteriostatic (sublethal), rather than bactericidal (lethal), food preservation systems may be contributing to the development and dissemination of ABR among important food-borne pathogens.

2.**A previous study conducted by Federica Giacometti& Hesamaddin Shirzad-AskiAntimicrobials and Food-Related Stresses as Selective Factors for Antibiotic Resistance along the Farm to Fork Continuum**

Antimicrobial resistance (AMR) is a global problem and there has been growing concern associated with its widespread

along the animal–human–environment interface. The farm-to-fork continuum was highlighted as a possible reservoir of AMR, and a hotspot for the emergence and spread of AMR. However, the extent of the role of non-antibiotic antimicrobials and other food-related stresses as selective factors is still in need of clarification. This review addresses the use of non-antibiotic stressors, such as antimicrobials, food-processing treatments, or even novel approaches to ensure food safety, as potential drivers for resistance to clinically relevant antibiotics. The co-selection and cross-adaptation events are covered, which may induce a decreased susceptibility of foodborne bacteria to antibiotics. Although the available studies address the complexity involved in these phenomena, further studies are needed to help better understand the real risk of using food-chain-related stressors, and possibly to allow the establishment of early warnings of potential resistance mechanisms.

**Literature review:**

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.

**methods**

**Study design:**

This was analytical study.

**Study area:**

This study was conducted in Khartoum state.

**Study period:**

The study was conducted from the period 2022

**Study population:**

Samples was Dairy and Meats.

**Materials and Methods:**

**1. Isolation and identification of the isolated bacteria:**

i. Standard protocols will be followed for isolation of the target organisms (enrichment medium if necessary medium ,chromogenic medium )

ii. Isolated bacteria will be identified by biochemical tests ,confirmatory test ,API.

**2. Immunological examinations:**

i. Immunological examinations will be carried out to determine species of the isolated bacteria using polyvalent O Antisera.
ii. Antimicrobial susceptibility of the isolated organisms
iii. Antimicrobial susceptibility will be determined by using disk diffusion assay following the guidelines of clinical and laboratory standard institute
iv. Effective concentration will be determine by MIC & MBC testes
v. Low temperature storage effects on isolated organisms
vi. The survival of isolated organisms under low temperature condition will be determine at freezing point.
vii. Thermal death point will be determine using 100˚C (Boiling temperature) D100˚C.
viii. Detection of Antibiotics Residues.

**Data analysis:**

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

**Results:**

Prevalence of Salmonella , E .coli , S .aureus Out of the total 70 Dairy samples examined, 10.0% (7/70) showed the occurrence of Salmonella ,14.3% (10/70) showed the occurrence of E .coli ,24.3% (17/70) showed the occurrence of S .aureus ,

prevalence of Salmonella , E .coli , S .aureusin Dairy samples is summarized in Table(1)

**Table (1) analysis of Salmonella, E .coli, S .aureusin prevalence in Dairy samples**

| Dairy samples | No.of tested | positive | Percentage | P- value |
|---|---|---|---|---|
| Salmonella | 70 | 7 | 10.0% | 0.021 |
| E .coli | 70 | 10 | 14.3% | 0.03 |
| S .aureus | 70 | 17 | 24.3 | 0.001 |

Prevalence of Salmonella , E .coli , S .aureus Out of the total 100 Meat samples examined, 29.0% (29/100) showed the occurrence of Salmonella ,20.0% (20/100) showed the occurrence of E .coli ,26.0% (26/100) showed the occurrence of S .aureus ,

prevalence of Salmonella , E .coli , S .aureusin Meats samples

is summarized in Table(2)

**Table (2) analysis of Salmonella, E .coli, S .aureusin prevalence in Meats samples**

| Meats samples | No.of tested | positive | Percentage | P- value |
|---|---|---|---|---|
| Salmonella | 100 | 29 | 29.0% | 0.007 |
| E .coli | 100 | 20 | 20.0% | 0.02 |
| S .aureus | 100 | 26 | 26.0% | 0.015 |

**Conclusion:**

The obvious conclusion to be drawn from the results is that:

1/ There is contamination in Meats in Khartoum State, and traditional slaughtering processes

2/ Salmonella species and Escherichia Coli and Staphylococcus aureus were isolated from meats .

3/ contamination were at all stages of Dairy.

4/ Most of Meats Slaughter house and Traditional slaughtering processes are not Applying HACCP System.

**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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