

# Characterization of Foodborne Pathogens and Enterotoxigenic Staphylococcus Aureus Isolates with Detection of Antibiotic Resistance from Beef Meat

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## Abstract

This study aimed to determine the current status of bacterial contamination in the animal products provided and to analyze *Salmonella* isolates, *Staphylococcus aureus*, and *Escherichia coli* from samples. The status of bacterial contamination was investigated in a total of 52 samples of beef meat intended for human consumption purchased from a general meat shop from September 2019 to December 2019 in Iraq. All meat samples that collected from butchers were contaminated by microorganisms. Isolation and characterization of the bacterial specimens from the samples were performed using conventional cultural techniques and biochemical identification. Five species of bacteria were isolated from all samples including *Salmonella*, *E. coli*, *Staphylococcus aureus*, *Shigella*, and *Enterobacter faecalis*. Three species of bacteria showed significant contamination of meat, these are *Salmonella*, *E. coli*, and *Staphylococcus aureus*. *Salmonella* were recovered from beef meat 30(58%), while *E. coli* 15(29%) and *Staphylococcus aureus* 20(38%). Antimicrobial susceptibility against ten antimicrobial agents commonly used in human was tested by used the disc diffusion method, including Ampicillin (A)(10µg), Amoxicillin/clavulanic acid (AM)(10 µg), Chloramphenicol (C)(30 µg), Streptomycin (S)(10 µg), Trimethoprim (Tr)(5 µg), Tetracycline (T)(30 µg), Ciprofloxacin (Cf)(5 µg), Nalidixic acid (Na)(30 µg), Gentamicin (G)(10 µg), and Kanamycin (K)(30 µg). multidrug resistance was detected in most of bacterial isolates, all isolates of *Salmonella* were resistant to streptomycin, amoxicillin, chloramphenicol, and gentamicin but sensitive to other antibiotics. PCR assay for detection of enterotoxin gene (*sea* gene) of *S. aureus* isolates, the result revealed that only 15 isolates from 20 isolated *S. aureus* carried this

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gene and showed bands after electrophoresis examination. We conclude, the existence of foodborne pathogens and indicator organisms such as toxigenic *Staphylococcus aureus* in meat shows bad food handling and processing practices. Consequently, butchers and meat suppliers should be educated about the side effects of lack of adequate personal and environmental hygiene and sanitation. In addition, consumers must avoid consumption raw and undercooked foods. These results suggest that meat can be a source of resistant bacteria, which can spread to society through the food chain. We conclude that resistant strains of bacteria in beef meat are common. These results support the adoption of strategies for the wise use of antibiotics in food animals and for reducing the number of pathogens existing on farms and slaughterhouses.

## INTRODUCTION

Food safety is an important concern that has increased in international trade. Outbreaks of food-borne pathogens are one of the important things that leads to illness and death, about 24-81 million of food borne disease associated with meat every year were recorded [1]. The eating of contamination foods with pathogenic bacteria and their products such as toxins and enzymes leading to serious diseases [2]. Food spoilage mean any change in food such as taste, smell, and appearance, but it remains safe for the consumer until the number of microorganisms present in the food reaches a certain limit that causes food-borne diseases [3]. Meat and meat products are considered a fertile environment for microorganisms and their toxins, including bacteria [4]. One of the most important bacteria isolated from meat is *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp., *Shigella* spp., *Salmonella* spp., and *Clostridium perfringens* [5]. Food-borne pathogens are colonize the gastrointestinal tracts of consumed domestic animals by human [6]. The slaughtered animals are sterile but despite this, meat gets easily contaminated with microorganisms by several processes [7]. Most of these organisms transmitted into meat and meat products through the hands and clothes of workers and contaminated environment, devices, and knives [8]. Food-borne microorganisms are a major source of disease and death, leading to significant spending on health care. Children are more susceptible to food poisoning because of their weak immune system [9]. There are two types of microbial contaminants, microorganisms that capable to produce disease and the second type that spoils the meat products and makes them unfit for human consumption [10]. The increase consumption of meat especially the contaminated food borne bacteria because including proteins, vitamins, lipids, minerals and other nutrients resulted outbreak of food-borne infections [11], possibly due to the presence of large amounts of water in meat, it provides an environment suitable for bacterial growth [12]. Meat contains about 75% of water, 19% of protein, 2.5% of fat, 1.2% of carbohydrates, and exist of vitamins, minerals, and cholesterol [13]. Cattles are the main

reservoir for Enterobacteriaceae especially *E. coli* where contamination occurs during the process of slaughter and sacrifice of the carcass remains unhealthy and the proliferation of large number of bacteria generates a public health hazard [14]. Contaminated meat can able to transmitted of zoonotic infections [15]. *Salmonella* are the main causative agents of foodborne infections in human and resulted salmonellosis outbreaks in USA and Europe [16]. *E. coli* is indicated to the presence of fecal contamination in food directly or indirectly. The conventional methods for detection of pathogenic bacteria in food are perfect by which the selective media were used for identification of morphology [17]. *E. coli*, *Salmonella*, *Shigella*, *Proteus*, and others are genus of the family Enterobacteriaceae and its presence in meat indicates to enteric contamination [6]. *Salmonella enterica* and *Typhimurium* are the main strains associated with human salmonella infection (salmonellosis). The contamination of meat products with these strains are the main source of infection [18]. Therefore, consumption of uncooked beef can pose severe health complications for consumers [19]. Salmonellosis leftovers one of the most foodborne diseases worldwide, especially in developing countries. The appearance of antibacterial resistance in *Salmonella* isolates from food products can influence the treatment of this infection [20].

In developing countries, the food-borne diseases not recognized and not reported. Therefore, the statistical data are continuous increased [21]. In fact, even cleaning and disinfection process of product surfaces to reduce the adherent bacteria, but it does not remove all bacteria, and a small amount of them remains on surfaces and devices, and therefore it can be transmitted directly to meat by knives, slicers, or conveyor belts or indirectly by floors [22]. The emergence of antibiotics and their excessive uses have generated resistant strains of bacteria. In animals, antibiotics have been used to stimulate growth and cure diseases. This in turn has led to the generation of resistant bacterial strains that are transmitted to humans through eating their meat [23].

The present study aimed to highlight on the prevalence and enumerate of foodborne pathogens from beef meat, it

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was bought from different butcher shops. Isolation, identification, and determine the antimicrobial resistance of the bacterial pathogens from beef meet. Also, verify staphylococcal virulence genes.

### MATERIALS AND METHODS

**Place Selection:** Places for taking meat samples were chosen from butchers that are characterized by failure to observe the health conditions to maintain human health and safety from diseases.

**Sample Collection:** A total of 52 samples of beef meat were collected from different places in Al-Najaf Al-Ashraf Province/Iraq. These places included Al-Krama, Al-Naser, Al-Jazeera, Al-Hindia, Al-Askari, Al-Wafaa, Al-Muhandiseen, Al-Nafut, Al-Ansaar, Al-Jamiaa, Al-Jameia, Al-Nedaa, and Al-Melaad Quarter. Samples collected were purchased from butchers to the consumer. From each area, four samples were collected, and each sample weighs 250 grams. After collection all samples, they are brought transferred in sterile plastic bags directly to the Laboratory of Microbiology at the Faculty of Science, University of Kufa, and placed in clean containers in order to isolate and diagnose the bacteria in it.

**Sample Preparation:** From each sample, about 1 gram of meat is cut with a clean, sterile knife, then the surface layer exposed to air is removed, the samples are weighed and prepared for subsequent processing.

**Sample Processing:** Each sample of meat is subject to two ways to isolate the bacteria, including: The first method, the sample is taken and placed directly on a brain heart infusion agar medium. Bacteria grow around and under the meat sample. The second method is the meat cut into small pieces and placed in a buffer to the second day, then 1-2 drops of solution are taken and cultured on brain heart infusion agar medium by streaking method.

**Bacterial Counting:** All samples were subjected to bacterial counting by taking 25 gram of meat sample, they are added to 225 ml of buffered peptone water (BPW) in 500 ml Erlenmeyer flask, leave for 1-2 min for homogenized and make serial dilutions out of them. Serial dilutions were done with ten test tubes containing 9 ml of normal saline or phosphate buffer solution and labeled  $10^{-1}$ - $10^{-10}$ . Add 1 ml of diluted meat sample into first test tube to formed  $10^{-1}$  dilution, then transferred 1 ml of first tube to the second tube after well mixed to form  $10^{-2}$  dilution and so it continues until reach to  $10^{-10}$  dilution. 1-2 drops of sample from  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  dilutions were inoculated on triplicate nutrient agar plates, incubated at 37°C for 24-48 hours. The bacterial colonies were calculated with Colony-Forming Unit (CFU) [24, 25].

**Detection of Bacterial Species: Isolation of *E. coli*.** The ability of *E. coli* to ferment lactose gives us an option to choose the MacConkey agar for discriminate between lactose fermenting strains from other lactose non-fermenting strains, such as *Salmonella* and *Shigella*. Take 1 ml of  $10^{-1}$  dilution and inoculated onto MacConkey agar medium and cultured by spreading method by using glass spreader. Incubated the Petri dishes at 37°C for 24-48 hours. The colonies which characterized by red/pink color, round, medium sized, and non-mucoid picked as suspected *E. coli* colonies [26]. Expected *E. coli* colonies can be confirmed by gram stain and biochemical tests which including indole test, methyl red test, oxidase test, and voges-proskauer test. **Isolation of *Salmonella* and *Shigella*.** 1 ml of  $10^{-1}$  dilution from meat sample was

inoculated and streaked onto MacConkey agar. The plates were incubated at 37°C for 24-48 hours and then it picks up colorless and transparent colonies supposed to be *Salmonella* and is subject to subsequent processing including motility test, Gram's staining, and biochemical tests. To obtain pure culture the colonies from MacConkey agar can be subcultured on Salmonella Shigella (SS) agar, incubated at 37°C for 24-48 hours. The colorless colonies with black center were regarded as *Salmonella* and subjected to further study, while the colorless colonies that grew on SS agar are considered as *Shigella* [27]. **Isolation of *Staphylococcus aureus*.** 1 ml of  $10^{-1}$  dilution from meat sample was inoculated and streaked onto Mannitol Salt Agar (MSA) as a selective and differential medium, incubated at 37°C for 24-48 hours. Yellow colonies with yellow zones pick up as positive *S. aureus*, while colorless and red colonies with red zones were staphylococci other than *S. aureus* such as *Staphylococcus epidermidis* [28].

**Gram's staining Method:** one drop of water is placed on slide and a small colony of potential colonies cultured on Brain Heart Infusion agar is taken and placed over a drop of water and mixed well until it becomes white and spread on slide with an area of 1cm<sup>2</sup>. The smear is fixed by air or gentle heat. First, crystal violet was applied on the smear for two minutes and washed with running water. Second, some drops of iodine were added for one minute and washed with tap water. Third, add acetone alcohol for few second and washed with tap water. Forth, add safranin for two minutes as a counter stain and washed with water. The slide leave for dried in air and examined under the microscope with power 40x and then 100x by using oil immersion [29].

**Biochemical Tests:** All bacterial isolates were biochemically tested by using IMViC pattern including indole (I), methyl red (M), voges-proskauer (Vi), and citrate utilization test (C). addition to that Kligler's iron agar (KIA), urease test, and oxidase test also used for identification of bacteria. For the diagnosis *Staphylococcus aureus*, coagulase and catalase tests are used.

**Methyl red test:** Inoculate the liquid medium of glucose phosphate peptone water from a young agar culture of the test bacteria and incubate at 37°C for 48 hours. Add 5 drops of the methyl red reagent. Mix and read immediately. Positive tests are red color and negative tests are yellow color [30].

**Voges-Proskauer test:** Inoculate single colony of test bacteria in glucose phosphate peptone water. Incubate at 37°C for 48 hours, then add 1 ml of 40% potassium hydroxide and 3 ml of 5% solution of  $\alpha$ -naphthol in ethanol. After 5 min showed pink color indicated to positive reaction [30].

**Citrate utilization test:** Inoculate a small colony of the test organism onto Simmon's citrate medium. Incubate for 96 hours at 37°C and read the results. Positive result represented blue color with growth, negative result is original green color and no growth [30].

**Kligler's Iron Agar:** Slants of kligler iron agar were inoculated with stock culture of bacteria in the conventional method. Incubated at 37°C for 24 hours and read the result [31].

**Urease test:** Sterilize basal medium without glucose or urea (which sterilize by filtration). Cool to 50 and add the glucose or urea to reach final concentration of 2%. Dispense into tubes as deep slopes and inoculate with heavy growth of bacteria on the surface of the slants for 4

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days incubation. Positive result represented by development of a purple-pink color [32].

**Oxidase test:** This method described by Kovacs [33], a small piece of filter paper was soak in 1% of Kovacs oxidase reagent and by a loop pick a colony from fresh bacteria and spread on the filter paper. Wait and observed the change in the color, purple color indicates the positive oxidase test. While the color does not change mean the microorganisms are oxidase negative.

**Catalase test:** A colony of bacteria was taking with a toothpick and mixed with a one drop of hydrogen peroxide on a slide. Positive result by indicates the effervescence [9].

**Coagulase test:** 0.2 ml of overnight broth bacterial culture was added to 0.5 ml of diluted rabbit plasma (plasma: saline 1:5) in a tube. Gentle mixing the tube and incubated at 37°C for 2, 4, 24 hours. Positive result by observed the clot in the tube [34].

**Detection of Antibiotic Susceptibility:** An antibiotic sensitivity test (AST) is usually performed to determine which antibiotic is most effective in treating bacterial infection in an organism. Add 1-2 colonies of each bacterial samples to 5 ml of nutrient broth, incubated at 37°C for 24 hours and then mixing and homogenized very well. 1 ml of bacterial suspension was inoculated on plates containing Mueller-Hinton Agar (MHA), spread the suspension over all the agar surface even the edges by sterile glass spreader. Inserted the cultured plates into the fridge for 30 min to absorb the bacterial suspension before putting the antibiotic discs [35]. The antibiotic discs that seeded onto the agar included Ampicillin (A)(10µg), Amoxicillin/clavulanic acid (AM)(10 µg), Chloramphenicol (C)(30 µg), Streptomycin (S)(10 µg), Trimethoprim (Tr)(5 µg), Tetracycline (T)(30 µg), Ciprofloxacin (Cf)(5 µg), Nalidixic acid (Na)(30 µg), Gentamicin (G)(10 µg), and Kanamycin (K)(30 µg) [36].

The results of resistance, intermediate, and sensitive were performed according to criteria of National Committee for Clinical Laboratory Standards (NCCLS, 2004) [37].

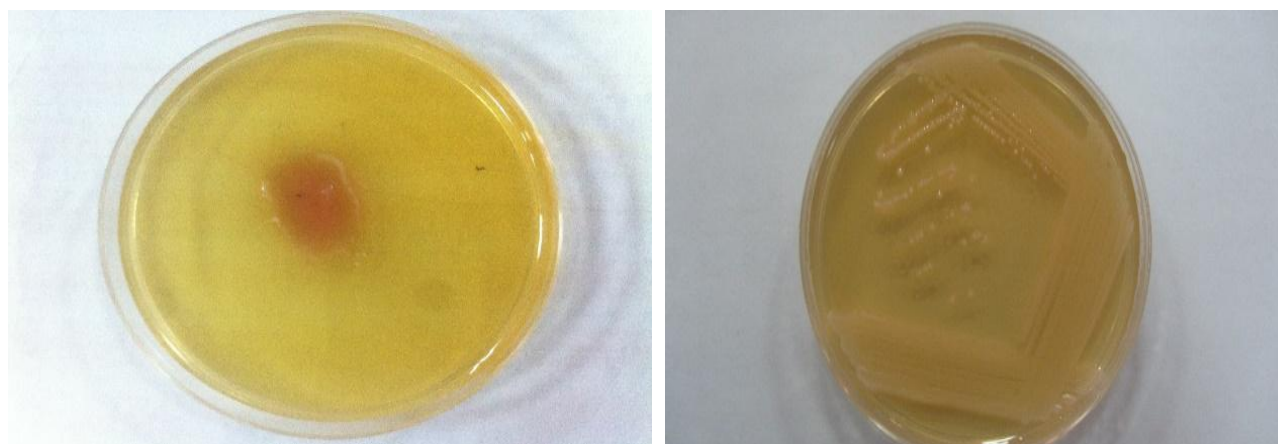
**DNA Extraction:** Phenol/Chloroform method was used for extraction of genomic DNA according to Janet and Adel (2006) [38]. The bacterial isolates were cultured on LB medium at 37°C for 24 hours. Centrifuged the grown isolates at 6000rpm for 2min, discard the supernatant and the pellet was suspended in 400 µL of STE buffer which consist of 2%SDS, 100mM NaCl, 100mM Tris-HCl and 10mM EDTA with pH=8.0, after that the solution was incubated at 55°C for 30min. Add 200 µL of phenol and 200 µL of chloroform to the solution and centrifuged for 10min at 8000rpm, then transferred the upper aqueous phase to a clean tube and adding 100 µL of ice cold isopropanol for precipitated DNA. Centrifuged for 10min at 10000rpm, finally, the pellet was dissolved in 50 µL of TE buffer.

**Polymerase Chain Reaction Assay: Primers Preparation.** The primers were prepared in a lyophilized form by which they dissolved in TE buffer to give final concentration of 100pmol/µL. From the stock solution take 9µL and mixed with the 100 µL of TE buffer, this is the working solution. The primer of enterotoxin gene for *S. aureus* was obtained from Sharma *et al.* (2000) [39], the sequences of this primer (F: TGTATGTATGGAGGTGTAAC, R: ATTAACCGAAGGTTCTGT) at 270 bp size. **PCR Mixture.** The final total volume of PCR mixture in 20 ul included 5 ul of DNA samples, Forward primer 1.5 ul, Reverse primer 1.5 ul, and sterile deionized water 20 ul in Eppendorf tube containing PCR Premix.

PCR Cycling condition: PCR tubes were placed into thermocycler PCR instrument where DNA was amplified as indicating in below (Tab.1).

**Table 1.** Program used for amplifying the enterotoxin gene of *S. aureus*

Stage	Temperature (time)	
Initial denaturation	94°C for 30 s	
Denaturation	92°C for 30 s	52 cycles
Annealing	50°C for 30 s	
Extension	72°C for 30 s	
Final extension	72°C for 2 min	



**Figure 1.** Comparison between two methods for isolation of bacteria from meat samples, to left, 1g of meat sample put directly on culture medium, to right, 1 drop of buffer solution containing meat sample streaked on culture medium.



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### RESULTS

In this study, two methods were used to isolate bacteria from meat samples (Fig.1), which is the method of placing 1g of meat sample directly on brain heart infusion agar and the method of placing the meat sample in a buffer solution, then taking drops of buffer and streaking on brain heart infusion agar. The second method was the best because of the isolation of many types of bacteria compared to the first method.

The results showed all meat samples that collected from butchers were contaminated by microorganisms. The samples which collected from most butchers especially from north quarters gave a high level of contamination.

A total of 52 isolates, five species of bacteria were isolated from all samples including *Salmonella* 30, *E. coli* 15, *Staphylococcus aureus* 20, *Shigella* 6, and *Enterobacter faecalis* 3 (Fig.2). Three species of bacteria showed significant contamination of meat, these are *Salmonella*, *Staphylococcus aureus*, and *E. coli* (Tab. 2).

**Table 2.** Clarify the distribution of bacterial isolates according to the places taken from them, where we note that *Salmonella* is the most frequently observed in Al-Jazeera and Al-Anaasr quarter followed by *S. aureus* and then *E. coli*.

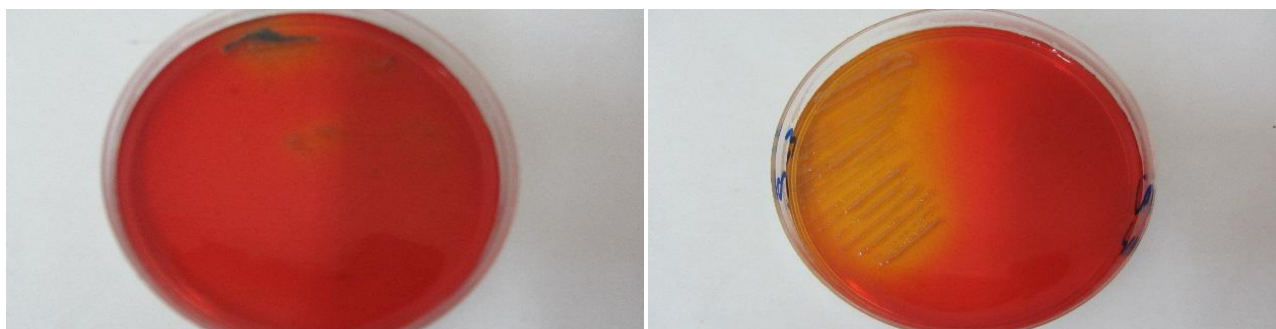
Isolated Bacteria Region	<i>Salmonella</i>	<i>E. coli</i>	<i>Staph. aureus</i>
Al-Krama	1	1	2
Al-Naser	2	1	1
Al-Jazeera	4	2	3
Al-Hindia	3	1	1
Al-Askari	3	1	1
Al-Wafaa	1	1	1
Al-Muhandiseen	1	1	2
Al-Nafut	2	1	2
Al-Ansaar	4	1	1
Al-Jamiaa	1	1	1
Al-Jameia	3	2	1
Al-Nedaa	3	1	3
Al-Melaad	2	1	1
Total	30(58%)	15(29%)	20(38%)

The predominant species was *Salmonella* (58%), followed by *Staphylococcus aureus* (38%), then *E. coli* (29%). Out of fifty-two meat samples collected from thirteen butchers exist in different places in al-Najaf only 30 samples were contaminated with *Salmonella* by culturing

**Figure 2.** Light microscopic view of *Enterobacter faecalis*



on SS agar (Fig. 3), examination with gram stain, and biochemical tests. *Salmonella* colonies will appear colorless with black centers because these bacteria do not ferment lactose, they are a product of hydrogen sulfide gas.

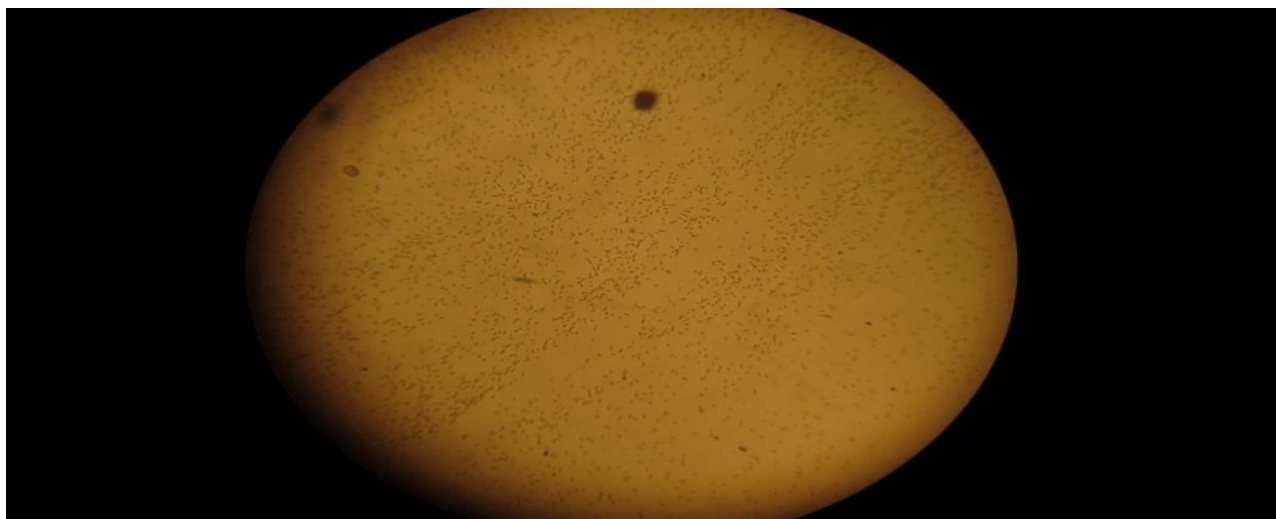


**Figure 3.** Two species of bacteria streaking on Salmonella-Shigella agar. Colorless colonies with black center of *Salmonella* on the left, yellow colonies of *Shigella* on the right.

The light microscopic examination of presumptive *Salmonella* isolates, the results showed Gram-negative

cells quite large about 0.7-1.5  $\mu\text{m}$  in diameters, bacillus to rod shaped (Fig. 4).

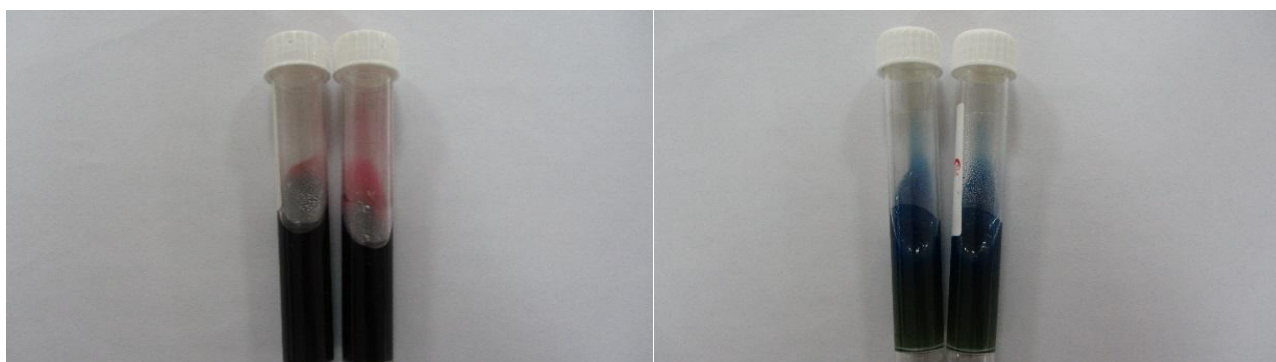
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**Figure 4.** *Salmonella* species under the light microscope, showed gram negative cells bacillus to rod shape.

While, the results of biochemical tests of *Salmonella* showed indole (-), methyl red (+), voges proskauer (-),

simmon's citrate (+), urease (-), kligler's iron agar (Alkaline/Acid with H<sub>2</sub>S), and oxidase (-) (Fig. 5) (Tab. 3).



**Figure 5.** Biochemical tests for *Salmonella* spp. show positive results in a test simmon's citrate (right) and kligler's iron agar, alkaline/acid with H<sub>2</sub>S.

**Table 3.** Biochemical tests of three species of bacteria isolated from meat

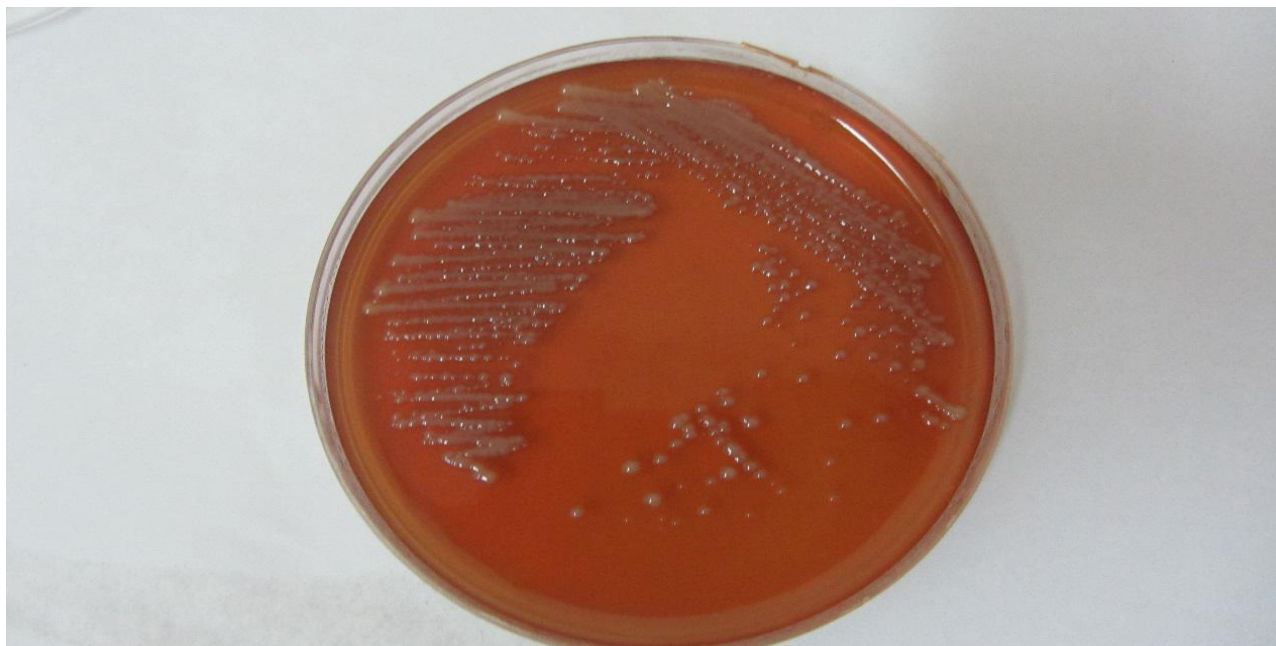
Bacterial species	Biochemical Tests								
	Indole	Methyl red	Voges-proskauer	Simmon citrate	urease	KIA	Oxidase	Coagulase	Catalase
<i>Salmonella</i>	-	+	-	+	-	K/A	-	nil	nil
<i>E. coli</i>	+	+	-	-	-	A/A	-	-	+
<i>S. aureus</i>	nil	nil	nil	nil	nil	nil	nil	+	+

A total of 52 meat samples were used in this study, only 20 (38%) samples were positive to *Staphylococcus aureus* by culturing on mannitol salt agar, the golden yellow colonies with smooth round represented presumptive *S. aureus* bacteria. On the other hand, the microscopic examination showed gram positive bacteria with grape

like cluster.

All strains of *S. aureus* were cultivated on 5% of sheep blood agar, incubated at 37°C for 24 h, then identified by biochemical tests (Fig. 6). The result of biochemical tests for *S. aureus* revealed positive for both coagulase and catalase.

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**Figure 6.** The figure referred to the ability of *S. aureus* to hemolysis of blood agar, where it was found that the strains non hemolysin for blood.

The count number of *S. aureus* colonies are  $4.98 \times 10^3$  CFU/g in Al-Naser Quarter, this result show lowest count, while  $6.45 \times 10^3$  CFU/g in Al-Nedaa Quarter represented the highest count of *Staphylococcus*.

*E. coli* was confirmed in 15 isolates from 52 meat samples through morphological characteristics on MacConkey

agar as selective and differential medium that used for isolation and differentiation of Enterobacteriaceae (Fig. 7). Colony of *E. coli* on MacConkey agar showed red or pink color (because the production of acid from lactose) and non-mucoid.



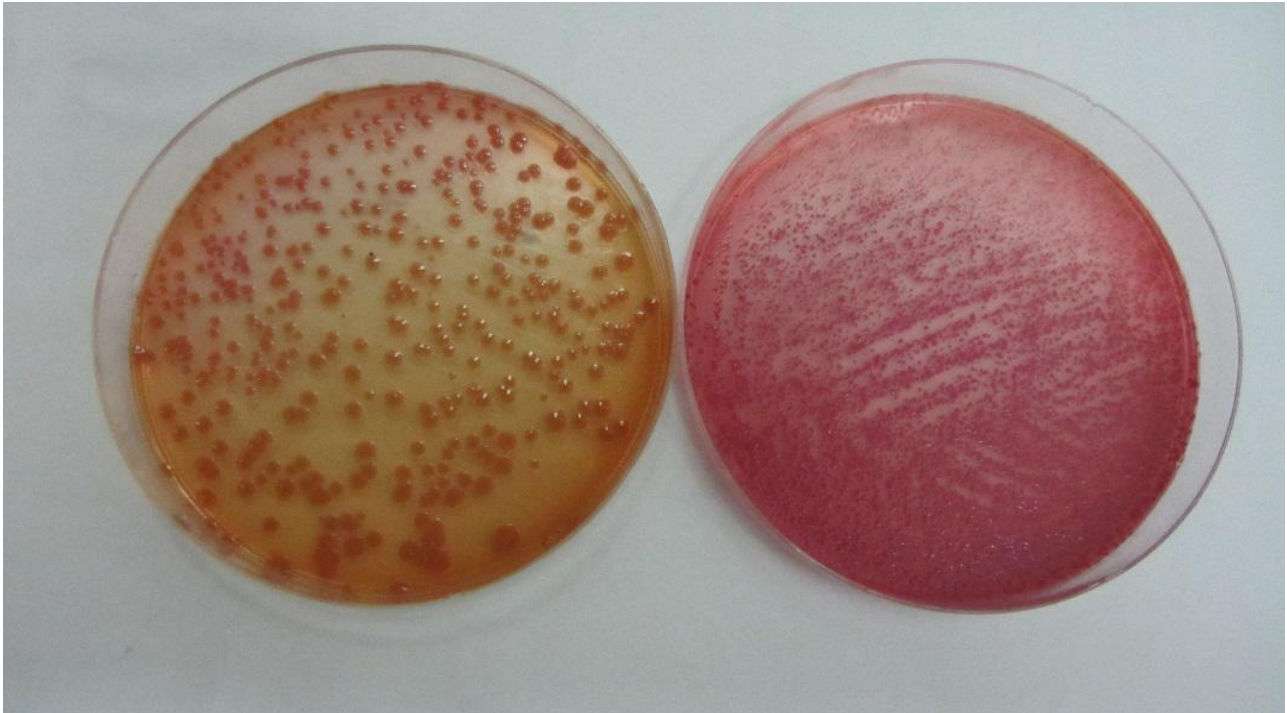
**Figure 7.** Pink color and non-mucoid colonies of *E. coli* because the production of acid from lactose, cultured on MacConkey agar plate.

Mean counts of *E. coli* were  $2.26 \times 10^3$  CFU/g for local butcher's shops in Al-Naser Quarter and  $4.35 \times 10^3$  CFU/g

for small butcher's shops in Al-Nedaa Quarter (Fig. 8).



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**Figure 8.** Counting of *E. coli* isolates isolated from meat samples, two Petri dishes cultured by one drop of *E. coli* serial dilutions.

The isolated pathogens were evaluated for susceptibility to 10 antimicrobial agents used in humans. Resistance of bacterial isolates to antimicrobials was commonly observed. The majority of these bacteria revealed high resistance to several antimicrobials, especially ampicillin and gentamicin (Tab. 4). bacterial isolates were resistant to ampicillin, amoxicillin/clavulanic acid,

chloramphenicol, streptomycin, trimethoprim, tetracycline, gentamicin, and kanamycin. Multidrug resistance was observed in three isolates. All isolates of *Salmonella* were resistant to streptomycin, amoxicillin, chloramphenicol, and gentamicin but sensitive to other antibiotics.

**Table 4.** Number, percentage of isolates resistant to antibiotics (%)

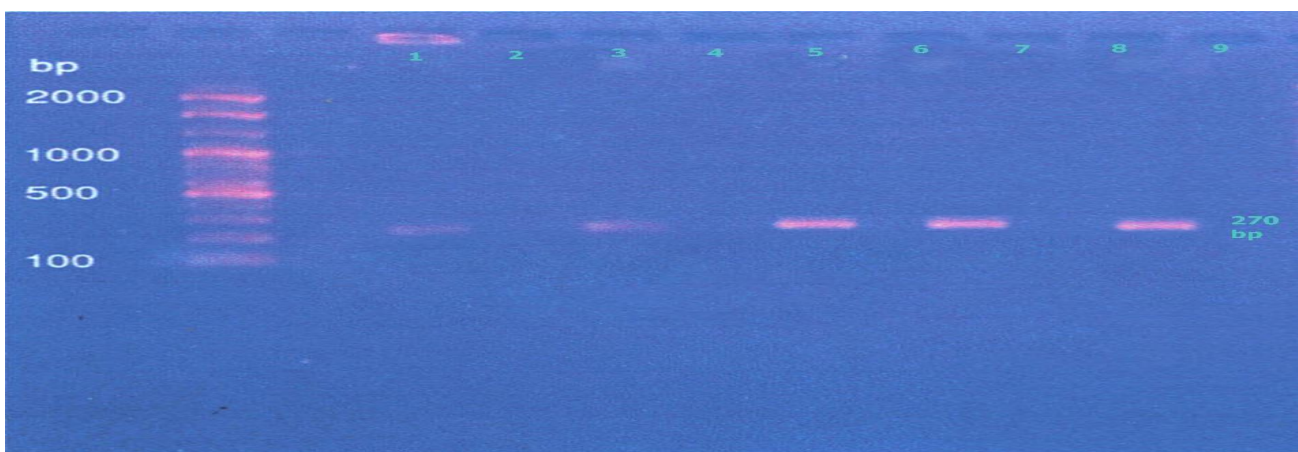
Bacterial Isolates	Antibiotics									
	A	AM	C	S	Tr	T	Cf	Na	G	K
<i>Salmonella</i> n=30	30(100)	30(100)	25(83.33)	10(33.33)	0	0	0	0	30(100)	0
<i>S. aureus</i> n=20	10(50)	10(50)	0	0	20(100)	16(80)	0	0	19(95)	0
<i>E. coli</i> n=15	15(100)	15(100)	0	0	0	1(6.66)	0	0	0	5(33.33)

An antibiotic susceptibility test was performed on all *S. aureus* isolates for 10 antibiotics; resistance recorded in 100% for ampicillin, amoxicillin, trimethoprim, tetracycline, and gentamicin.

Among *E. coli* isolates, maximum resistance was observed against ampicillin (100%) and amoxicillin/clavulanic acid (100%), followed by kanamycin (33.33%) and then tetracycline (6.66%). A high degree of susceptibility was observed against chloramphenicol, streptomycin,

trimethoprim, Ciprofloxacin, nalidixic acid.

The detection of enterotoxin gene (*sea* gene) of *S. aureus* isolates by using conventional PCR assay (Fig. 9), the result revealed that only 15 isolates from 20 isolated *S. aureus* carried this gene and showed bands after electrophoresis examination. Certain strains that secrete large amounts of SEA, regardless of the environment, are likely to contribute to increased food safety risks.





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**Figure 9.** PCR amplification of enterotoxin genes (*see*=270bp) of *Staphylococcus aureus*: L-DNA ladder 100bp, lanes (1,3,5,6,8)) positive isolates. While lanes (2,4,7,9) negative isolates for *see* gene.

### DISCUSSION

Meat microbial spoilage is a complex happening that many different bacterial groups may contribute to, depending on storage temperature and packing conditions. Damage can result from microbial development and consumption of meat feeders by bacteria, resulting in the release of unwanted metabolites [40]. Food-borne pathogenic microbes are considered the leading cause of disease and death in developing countries, causing heavy losses in health care. In addition, more and more attention has recently been paid to food safety, especially after the outbreak of diseases due to *E. coli* and *Salmonella*, among other causes, as well as attention to eating raw and unpasteurized foods [41]. The World Health Organization has estimated that 1 in 10 people are exposed to the disease and about 4 million and 20 thousand people die each year as a result of eating contaminated food. The bacteria that cause disease in food, such as *Salmonella*, *Escherichia*, *Staphylococcus*, and *Listeria* are considered a real danger to human health [42]. During the logarithmic phase of bacterial growth, bacteria are existing on the surface of the meat. When the protein-degenerating bacteria (proteolytic bacteria) approach the maximum cell density, the protease enzyme secreted by the bacteria appears to break the connective tissue between the muscle fibers, allowing the bacteria to penetrate the meat. While, Non-proteolytic bacteria do not penetrate meat, even when grown with proteolytic proteins [43].

In this study, the most prevalent isolates were isolated from meat samples are *Salmonella* spp. at a percentage of 58%. This result was corresponding to the result of Bodhidatta *et al.* (2013), they isolated 84% of *Salmonella* from food samples [44].

The presence of *Staphylococcus* and *Salmonella* in meat samples has an indication that the samples were contaminated from air, water, and hands while being slaughtered and cut in addition to that the toxins produced by these bacteria are thermostable and are not affected by temperature up to 100°C and for several hours [45]. This study agreed with the study of Bodhidatta *et al.*, they showed the most common species isolated from children with diarrhea were *Salmonella* spp. (84%), *Arcobacter* (74%), and *Campylobacter* (51%) [44]. *Salmonella* are one of foodborne pathogenic bacteria that causing severe diseases in human and animals, therefore, the meat regarded as a main source of *Salmonella*. Ye Y. and his coworkers showed 13% of meat samples were positive for *Salmonella* [46]. The transmitted of *Salmonella* among humans and animals occurred during consumption of contaminated food, therefore the isolation and identification of *Salmonella* form samples outbreak food-borne detection and prevent the entering the contaminated food into the food supply. *Salmonella* can cause several different syndromes including gastroenteritis, bacteremia, and typhoid fever, the most common of which is gastroenteritis, which is symptoms like abdominal pain, diarrhea nausea, vomiting, and headache [47]. Other study by Bodhidatta *et al.*, revealed the most commonly isolated pathogens from meat were *Salmonella* (84%), in addition to *Arcobacter butzleri* (74%) and *Campylobacter* (51%) [44].

In this study, *Staphylococcus aureus* was found in 38% from meat samples. This prevalence is lower than

previous studies by Bantawa *et al.* (2018), they showed *S. aureus* in 68% samples [48]. While, the prevalence of *S. aureus* in meat was higher than the study of Rong *et al.* (2017), the results of them showed 37.2% of samples were positive for *S. aureus* [49]. The increased prevalence of *S. aureus* refers to insufficient cleaning, unsatisfactory handling, and post-pollution contamination from contaminated air around stores. A high percentage of *S. aureus* in uncooked meat and processors has health risks such as toxin-mediated virulence and consumer invasion [50].

*Staphylococci* are colonized on the skin and nose and easily transferred to the food causing food contamination. *S. aureus* could be able to produce toxins called Staphylococcal enterotoxins, these toxins are characterized by resistant to several conditions such as proteolytic enzymes and heat, therefore they resistant the cooking and to the gastrointestinal tract of the human [51]. The existence of these pathogens is a major concern because some strains are able to produce stable heat enterotoxins that cause food poisoning in people, and therefore must be taken into account in risk valuation [52]. *Staphylococcus aureus* is one of the leading causes of foodborne diseases. The animal products such as milk and meat are often contaminated with enterotoxigenic strains of *Staphylococcus*. The contamination of food may occur directly from infected food-producing animals or may consequence from poor hygiene throughout meat processes, or the storage of retail and food items, because humans may carry microorganisms [53]. Staphylococcal enterotoxins (SEs) are a group of exotoxins, identified in 1959 as extracellular proteins produced by some strains of *Staphylococcus aureus*. These toxins are known as pyrogenic toxins characterized by thermo-stable proteins that are resistant to many protein-degrading enzymes such as pepsin, trypsin, chymotrypsin, and renin. virulence factors of staphylococci responsible for food poisoning in humans [54]. Although *S. aureus* are eliminated, toxins may remain and cause food poisoning [55]. Foodborne staphylococcal diseases acquired from eating food contaminated with intestinal toxins are the second most common kind of food-borne disease. The high level of food poisoning with *Staphylococcus* is due to the inadequate pasteurization / disinfection of the source of the contaminated product [56].

Foodborne diseases are the causes of illnesses and deaths in developing countries and lead to the loss of billions of dollars in medical care, the most important of which is *Salmonella*, which causes foodborne diseases and food poisoning [57]. Meat is a major source of *Salmonella*, detecting *Salmonella* infection in meat samples is very important to control and prevent foodborne diseases [58]. Diarrheal diseases caused by *Salmonella* are more spreading between children in developing countries comes after malaria then respiratory infections [59]. Several studies revealed the contamination of meat by microorganisms which can be supported this study, from which, the study of Sharma and Chattopadhyay they isolated *E. coli* (98%), *Enterococcus faecalis* (90%), *Staphylococcus aureus* (20%), *Salmonella* spp. (2%) and other organisms. They revealed high prevalence of *E. coli* in meat products [60]. These results are almost identical to the results of our study but differ in the high prevalence of bacteria in this study is *Salmonella*.

## Characterization of Foodborne Pathogens and Enterotoxigenic *Staphylococcus Aureus* Isolates with Detection of Antibiotic Resistance from Beef Meat

*E. coli* is the main bacterium that causes diseases that are transmitted through food. Therefore, foods are preserved in many ways, including chemical factors, for example the use of antibiotics, but they also generate bacteria that are resistant to antibiotics [61]. The isolated of *E. coli* from meat sample is a good indicator to intestinal contaminated during slaughtering process because *E. coli* regarded as normal flora of intestine [62].

The differences in bacterial count among meat samples due to the differences in raw materials and sanitary measures [63]. The result count of *Staphylococci* among Quarters was agreement with the study of Goja *et al.* (2013), they reported highest count of *Staphylococcus* in Omdurman ( $7.07 \times 10^4$  CFU/g) and lowest count in Khartoum ( $4.46 \times 10^3$  CFU/g) [64]. The presence of *Staphylococcus aureus* on meat samples may be contaminated from skin, nose, and nose of butchers.

Only three *Shigella* was isolated from beef meat sample, similar to another result from Ethiopia which showed only 3 *Shigella* from 150 meat samples [65].

There are several reasons explain the increased of bacteria in food production environments. Often times, circumstances in the food production environment are similar to those required for the product, such as temperature, nutrients, and stress factors; Therefore, treatment surfaces may act as reservoirs for damaged bacteria. Additional, numerous studies have indicated that the fate of pathogens introduced into the treatment environment may be affected by non-pathogenic bacteria [22].

Resistant bacteria is a known public health problem, the results revealed that all isolates of *Salmonella* were multidrug-resistant which mean resistance to four antibiotics at least (streptomycin, amoxicillin, chloramphenicol, and gentamicin) [66]. When consumption of contaminated food with multi drug resistant food-borne pathogens will causes more serious diseases comparison with susceptible bacteria [62]. Excessive use of antimicrobials and lack of hygiene during meat processing leading to undesirable risks to human health, for example the presence of leftovers of antimicrobial drugs, non-typhoidal *Salmonella*, and antimicrobial resistance *Salmonella* [67]. Multidrug resistant bacteria, including methicillin-resistant *S. aureus*, extended spectrum beta-lactamase Enterobacteriaceae, and vancomycin-resistant Enterococci have a challenge to the human health care. These bacteria were detected in environment outside the hospital, also found in meat and meat products [68]. Animals are usually administered with antibiotics in their feed, but there is present attention in raising animals that are only administered with antibiotics throughout active infection. *Staphylococcus aureus* is a common pathogen for both humans and cattle raised for human ingesting. *S. aureus* has attained high levels of antibiotic resistance, nevertheless the origins and position of choice of resistance are poorly understood [69].

In the present study, the frequency of sea positive isolates of *S. aureus* showed in meat samples was 75%. Therefore, extra study for the expression of *sea* gene remains proposed. Our study measured the high frequency of *sea* gene in meat samples and the reason explain this state is unknown. May be due to *S. aureus* carrying this gene are transferred from hands and nose of persons to meat during meat processing and can cause infection when eat uncooked meat. Our study disagreed with the study of Lampugnani *et al.* (2020) [70], they revealed that 95.2%

of the isolates were confirmed to be *S. aureus*. From them 42.4% were carrying enterotoxin genes.

### CONCLUSION

The results indicate the importance of continuous monitoring of foodborne pathogens in butcher meat to reduce the risk of contamination. The availability of data on pathogenic microorganisms and their transmission in food from different countries would offer common ground for reaching an international agreement on regulations of food safety. The high load of microorganisms and presence of *E. coli* and *Salmonella* indicates that the meat may be contaminated with instictual content and that consumers are at risk of developing a transmitted disease when ingested raw. The detection of *Salmonella* in meat, even in low-incidence cases, has important implications. A strict public health and food safety system is urgently needed to reduce the human health risks associated with *Salmonella* disease. we conclude that our results showed the presence of multidrug-resistant bacteria in fresh meat and possibility of presence of enterotoxin genes in some isolates of *S. aureus*, suggesting that fresh beef meat products may act as reservoirs of drug-resistant bacteria and facilitate the spread of resistance genes. Also, the existence of enterotoxigenic and antimicrobial strains of *S. aureus* bacteria has become noticeable in foods. This requires better control over the sources of food contamination and the proliferation of antibiotic-resistant organisms.

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

### AUTHORS CONTRIBUTION

SA drafted the manuscript, compiled information from the literature, and designed the figures and tables. WA reviewed and edited the manuscript.

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### DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

### ETHICS STATEMENT

None.

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