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INCIDENCE RATE OF SOME FOOD BORNE PATHOGENS BACTERIA FROM RED MEAT AND CHICKEN MEAT IN DUHOK PREVALENCE

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ABSTRACT

Foods naturally provide nutrients and are easily metabolized, making them good substrates for the growth and metabolism of microbes. Food-borne illness is a widespread issue brought on by consuming tainted food and water. The bacteria that finally cause the spoiling of flesh items are either already present at the time of slaughter, or they are introduced by workers and their cutting equipment, or they are spread by water and air in the dressing, cooling, and cutting rooms.

Duhok local markets, restaurant, abattoirs, contains a significant variety of imported meats and fresh meat from different sources, (minced meat, sheep meat, beef meat, chicken meat) were one of them, the current research aimed to understand an event and prevalence of bacteria in meat of meat and meat product by *using* biochemical in Duhok city. A total of 200 Samples of (minced meat, sheep meat, beef meat, chicken meat), were chosen randomly from different sample of meat and meat product, all the isolates submitted to culture on many of media agar , then the isolates tested by biochemical test to confirm final diagnosis, the study was shown there were many of bacteria reside in (minced meat, sheep meat, beef meat, chicken meat) at a different percentage(*Salmonella SPP* (68%,68%,72%,56%) ,*E.coli* (80%,72%,68%,64%) and *Staphylococcus aureus* (84%,48%,44%,72%), It could consideration meat of (minced meat, sheep meat, beef meat, chicken meat) were a viable nutrient for the growth of numerous bacteria kinds, some of which may be quite hazardous if they were to be transmitted to humans.

KEY WORDS: chicken meat, beef meat, sheep meat, minced meat, *E. coli, salmonella and s. aureus*

1-INTRODUCTION

Meat and meat-based items are the healthiest and best cuisine. with the most nutritional value for humans since they are rich in essential minerals, vitamins, essential amino acids, essential fats, and other nutrients. (Biesalski, 2005). The undamaged tissues of wholesom slain avian and animal life are generally sterile, but during processing, the products may become polluted by worker hands, clothing, knives, the hide, the intestines, or the surroundings, making it subpar or even unsafe for use by humans. Contaminated chicken, beef and meat products may represent a risk to public health (Ahmed and Ismail, 2010 and Datta et al., 2012). The most important bacterial pathogens in meat and meat , that cause food-borne illnesses include, *Salmonellae E. coli* and coagulase positive *S. aureus* (Abdaslam et al., 2014, Ezzat et al., 2014 and Saif ,2015). carcass is the main source of E. coli, which primarily happens during the process of removing the hide or evisceration, can easily result in risks to the public's health. (Phillips et al., 2006).

Salmonella is considered as one of the most important causes of acute gastroenteritis and food-borne

infections worldwide (Ranjbar et al. (2016). Gastroenteritis and diarrheal diseases remain one of the most important health problems worldwide (Cardona-Castro *et al.* (2009). -Lopez et al. (2012). This bacterium can result in a variety of clinical consequences, including self-limited gastroenteritis and life-threatening systemic infections. Stevens et al .(2009). *E. coli* is commonly non-virulent but some strains have acquired toxic or pathogenic virulence factors making them dangerous to humans and animals.)Gi et al., 2009 and Datta et al., 2012). *E. coli* is

one of the principally important bacteria . As a result of poor hygiene standards, this type of bacteria can cause meat and meat products to decay. (Naghoni et al (2010).and Wendlandt et al.(2013) .Escherichia *E. coli* is classified into five pathotypes: enteroaggregative, enterohemorrhagic, Shiga toxin-producing E.coli (STEC), enter invasive, enteropathogenic and enterotoxigenic (Scallan et al.(2011) . In addition, *S. aureus* is a major cause of animal diseases including skeletal infections of poultry, which are a large economic burden on the global broiler chicken industry. *S. aureus* is a significant contributor to food-borne illness, estimated to be responsible for 241,000 infections annually in the United States. (Scallan et al .(2011) However, the true incidence of *S. aureus* food-borne disease. Some outbreaks of food-borne diseases that because illness are caused by bacterial infections. These epidemics are almost never brought on by raw meat, but rather by careless handling or tainted meat being prepared for consumption (Busani et al., 2005). As the level of contamination of meats. The goal of the study was to shed light on the bacterial makeup of common meats products including. (minced beef, sheep meat, chicken meat, and sheep meat) in the Iraqi province of Duhok.

2-MATERIALS AND METHODS

2.1 -Sample collection:

Meat samples were collected from randomly selected (restaurant, supermarkets, and abattoirs) in Duhok city. Samples were collected and in total 200 samples of beef (n=50), sheep (n=50), chicken meat (n=50), and minced meat (n=50) were collected from June to October.

2.2 -Preparation of the sample and bacterial isolation

To prepare a total about 100 grams of sample under sterile sanitary condition. We cut it into small pieces with sterilized knives in a hood to get rid of contamination and weight by balance (Akiba et al .(2011).

2.3- Processing of samples

Twenty-five grams from each sample Each sample and about 25gm of aseptically triturated meat sample were placed in separate sterile plastic bags to which 225 ml of buffered peptone water was added (BPW) as described by (Akiba et al .(2011). After being transported to the laboratory. The bags were vigorously shaken, and the rinsed material was collected in sterile bottles before being immediately incubated at 37° for 24 hours (pre-enrichment).

2.4- Isolation and Identification of *Salmonella:*

After incubation at 37°C for 18hrs about 0.1ml of the pre-enriched culture was inoculated into 10 ml of Rappaport-Vassiliadis (RV)enrichment medium and incubated at43°C for 18-24 hrs. Thereafter, a loop full of each broth was streaked on Salmonella-Shigella agar(S.S. agar) and Xylose Lysine Deoxycholate agar (XLD-agar) and chromogenic salmonella agar incubated for 24 hrs. at 37°C.Colonies of typical growth were picked up and confirmed morphologically according to (Ranjbar et al . (2014).

2.5-Isolation and Identification: *of E. coli:*

Each sample and about 1gm of aseptically meat sample will pre-enriched in 9 ml of buffered peptone water (PBW) as described by (ISO,2002; Kramarenko et al .2014). 1g of sample mixed with 9mL of BPW (ISO 6887-1). And incubated at 37c for 24hrs.then a loop full streaked on MacConkey agar and TBX (Tryptone bile-Glucuronide)agar and incubate for 24 hrs. at 37c and confirmed by indol test.

2.6-Isolation and Identification of *S. aureus*:

We will add 10 g of food to 90 mL of P.W.(1st dilution, mother solution). Then Mix the mother solution by vortex for 30 sec. I made serial dilution (adding 1 mL of mother suspension to 9 mL of PW) as required by Iraqi Standard Specifications, and Incubate at 37˚C for 24 hrs. Finally Confirmation of suspected colonies by using Firstly, Brain Heart Infusion Broth by using test tubes containing 5 mL of previously prepared and incubates at 37˚C for 24 hrs. Secondly, Coagulase Test by both Tube method and Slide method. In tube method 0.5mL rabbit plasma in test tube and add 0.5 ml of broth and incubate at 37˚C and read the result after 4-6 hrs. or after 24 hrs. Positive coagulase will produce solid (ISO,2002; Kramarenko et al .2014).

2.7 Biochemical test

Biochemical characterization of the bacteria was done by performing specific tests were carried out on suspected colonies according to (Markey et al .2013,Benson,et al .2001)

Biochemical test from salmonella ssp such as catalase +ve, citrate, capsule -ve, gram staining -ve ,Gas-ve, H2S +ve ,indol -ve ,oxidase -ve, +veTriple sugar iron TSI alkaoli/Acid ,-ve urea's

Biochemical test from Ecoli such as catalase +ve,Gas+ve, Gram staining -ve, H2S -ve, indol

+ve, oxidase +ve ,Hemolysis -ve,Ureas -ve, citrate -ve ,Tsi Acid/Acid

Biochemical test from staphylococcus aureus such as catalase +ve, coagulase +ve, Gas -ve,H2S -ve, gram staining +ve, urea's +ve, Hemolysis +ve, and +ve indole

3-RESULTS

The result of bacteriological examination on some food born bacteria isolated from meat products. The first were result were show to isolation of bacteria on different culture media.

The 200 meat samples were collected from different places in Duhok city such as (restaurant ,super market, and abattoir). The first fifty samples of (minced meat) were collected from (restaurant and super market). the second fifty samples of (sheep meat) were collected from (abattoir and restaurant). the ,third fifty samples of (beef meat) were collected from abattoir and super market), and the last fifty samples of (chicken meat) were collected from (Ibrahim Khalil customs and super market) from Duhok. The predominant bacterial pathogen isolated(*Salmonella E.coli* and *S.aureus*) in Table (1) were isolated from (142,132,124) positive out of 200 sample .The Salmonella isolation appeared that, total of 50 sample were isolated from 34 Positive samples minced meat (64%), 34sheep meat (64%) and 36 beef meat (72%),28 chicken meat (56%) . *Salmonella* bacteria have the highest rate of beef meat ,and in minced meat and sheep meat are equal, while less in chicken meat . the chicken meat were significant with minced, sheep, beef meat. all used by biochemical test such as catalase, oxidase, gas, gram staining, H2S, indol ,Triple sugar iron ,urea's .in *E.coli* were isolated 58.5%, represented as 40 positive samples (80%) from minced meat, 36 positive samples (72%) from sheep meat ,34 beef meat (68%) ,32chicken meat samples (64%). *E.coli* bacteria in minced meat is high ,while low in chicken meat . All kind of *E.coli* bacteria were non-significant together at the level $p<0.05$ check by biochemical test as indole oxidase ,catalase, gram staining, trippy sugar iron , urea's, Simon citrate .The result Achieved in table that the incidence of *S. aureus* in examined ,50 samples were isolated from 42 samples *from* minced meat (84%),24 samples from sheep meat(48%) and 24samples from beef meat (48%),36 samples from chicken meat (72%) The *S.aureus* in minced meat more than other, but low in sheep meat and beef meat .All type were non-significant together . All isolated strains were coagulase positive *S.aureus* .

Table(1) :.-Number of positive samples for pathogen isolation from samples

Samples	Number of positive samples salmonella	Number of positive samples E.coli	Number of positive samples S.aureus
Minced meat	34	40	42
Sheep meat	34	36	24
Beef meat	36	34	24
Chicken meat	28	32	36
Total	132	142	124

Table(2) : -Number and percentage of positive sample for pathogens bacteria salmonella SSP isolation

Sample	NO. .of sample	Positive Sample+	Percentage %	5%*
Minced meat	50	34	68%	*
sheep meat	50	34	68%	_
Beef meat	50	36	72%	*
Chicken meat	50	28	56%	*

Table(3) :- Number and percentage of positive sample for pathogens bacteria : Escherichia coli isolation

Sample	NO .of sample	Positive Sample+	Percentage %	5%*
Minced meat	50	40	80%	*
sheep meat	50	36	72%	*
Beef meat	50	34	68%	*
Chicken meat	50	32	64%	*

Table (4):- Number and percentage of positive sample for pathogens bacteria *staphylococcus aureus* isolation

Sample	NO.of sample	Positive Sample+	Percentage %	5%*
Minced meat	50	42	84%	*
sheep meat	50	24	48%	*
Beef meat	50	22	44%	*
Chicken meat	50	36	72%	*

4-DISCUSSION

Foodborne illnesses caused by *Salmonellae* species *E. Coli*, and *Staphylococcus aureus* represent a great public health problem worldwide. These infections are mostly spread by eating tainted food, and the presence of these organisms in meat and other raw meat products has important public health ramifications. (Normanno et al., 2007 and Sousa, 2008). It is a well-known fact that contaminated food is the primary means by which pathogenic bacteria spread. In underdeveloped nations, gastrointestinal infections caused by contaminated food are the leading cause of mortality and morbidity. (Gunasegaran et al 2011).According to our study, The results of Food- borne pathogens that isolated from contamination of meat , such *as Salmonella spp* ,*Ecoli*, and *Staphylococcus aureus* .(Table, 1) revealed that, (116,94 ,94,96) out of 200 samples (58.5%,47%,46%,48) minced meat ,sheep meat ,beef meat ,chicken meat for *Salmonella*, *E coli S. aureus*, represented as 34 ,40,42 positive samples (68%,,80%,84%) from minced meat samples followed by 36,34,24 sheep meat (72%,68%,48%),34,36,22 beef meat (68%),32,28,36 chicken meat (64%,56%,72%) samples and most samples showed mixed isolates. This could be as a result of a number of factors, including the use of low-quality beef carcasses, the spread of bacteria in meat through grinding, subpar manufacturing procedures, insufficient cleaning and disinfection of both equipment and surfaces, poor personal hygiene, and the employment of untrained personnel.

. These results came in accordance with that obtained by Maarouf and Nassif 2008, Lamada et al., 2012 and Abd El-Salam (2014). A total of were recovered from 200 samples, includes *Salmonellae* (132=66%),*E. coli* (142=71%) and *S. aureus* (124=62%). They were isolated mostly from minced meat samples (116=58%), sheep meat (94=66%) , beef meat (94=46%) and finally chicken samples (96 =48%) as shown in Table (2). Nearly similar results were recorded by Maarouf and Nassif (2008), Lamada et al., (2012) and Abd El-Salam (2014). These bacterial pathogens in meat and its products are of public health importance for consumers (Leloir et al., 2003 and Sousa, 2008). The results of E. coli isolation (Table, 3) showed that (142) strains were isolated mostly from minced meat samples (40=80%) followed by sheep samples (36=72%), beef meat (34=68%), chicken meat (32= 64%) samples . Nearly similar results were obtained by Maarouf and

Nassif (2008), Ramadan et al . (2015) and Saif (2015). The biochemical profile of the isolated *E. coli* was identical to those previously reported, including the colonial appearance and the fermentation of specific sugars or enzymatic activity. (Quinn et al., 2002, and Ezzat et al., 2014).

Additionally, the contamination rate of meat sold in supermarkets is higher than that in open markets. Our findings concur with a prior study conducted in most countries. (Minami et al., 2010). Our findings are unexpected given that supermarkets are thought to be more hygienic and as a result, Salmonella prevalence should be lower than in outdoor markets .One of the primary causes of the supermarket's increased contamination level may be the lack of complete hygiene in the meat section, especially the lack of cover-free surfaces ,cutting boards, knives, and a refrigerator. There could be cross-contamination across various types and batches of meat if there is any Salmonella present. The findings of the genotyping revealed diverse genotypes among the *Salmonella* isolated from the same location, demonstrating the diversity and breadth of the sources of *Salmonella* infection. *Salmonella i*solated from several types of meat at various times at the same location, however, shared the same genotype, indicating that the market may have been cross-contaminated as a result of insufficient cleaning. This could be one of the primary causes of the recent rise in *Salmonella* positivity rates. It has been demonstrated that using this technique can yield data that conventional serotype analysis cannot.

methods (Zhao et al., 2012). In our work, we show that seasonal variations affect the rate of *Salmonella*, *E.coli* , and *Staphylococcus auras* contamination in meat and meat product. The detection rates were higher in the summer and fall than they were in the spring and winter, and this was likely due to the weather. In the summer and fall, especially in Iraq/Duhok, warmer temperatures and increased humidity encourage the growth of germs in food.

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