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Study of Microbiological Contamination in Babylonian Ruminant and Poultry Slaughter

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ABSTRACT: Unsuitable sanitary conditions and mishandling are the main causes of environmental pollution in slaughter, Slaughter are a major source of bacterial contamination in poultry meat and its products, which is a major health and economic concern for many researchers. 18 samples were taken from six slaughterhouses in Babil Governorate (three samples from poultry slaughterhouses and three samples from ruminant slaughter, where sample numbers 1, 2 and 3 were divided into poultry slaughter and sample numbers 4, 5 and 6 were divided into ruminant slaughterhouses from the period from January 2024 to April 2024. Analyzing the microbial contamination in the slaughter of Babylonian poultry and ruminants. Media Transporter tubes containing peptone water were used to transport, collect and store samples from the slaughterhouses and transport them to the laboratory. The validity of the sample transporter is 24 hours. The results of the experiment showed the emergence of bacterial contamination of pathogenic bacteria Escherichia coli, E.coli bacteria, and bacteria Pseudomonas, Salmonella, and Shigela bacteria were found in all study samples from the above-mentioned sites, some of which were found in high numbers, indicating a lack of interest in cleanliness and sterilization of animal slaughter.

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INTRODUCTION

Many governmental authorities are concerned about the health and financial implications of bacterial contamination of chicken meat, large animals, and their products, which is primarily caused by slaughterhouses. Some research seek to determine risk variables linked to contamination and quantify non-compliance with bacterial contamination on animal meat samples from slaughterhouses (Klaharn et al., 2022). Salmonella and Escherichia coli are the two main harmful bacteria that contaminate meat and equipment. It has been demonstrated that these bacteria spread vertically (Swelum et al., 2021). Poultry meat has a significant global influence on human health and is a major public health problem due to its ability to spread foodborne diseases (Antunes et al., 2016; Goncalves et al., 2018). According to the Department of Disease Control (2021), spoilage bacteria can shorten shelf life and result in product losses in the chicken meat production industry, which can have a substantial negative impact on the environment in addition to foodborne illnesses (Rouger et al., 2017). It has been demonstrated that bacterial contamination happens at every stage of the production process, from primary production at the farm level to slaughtering operations, the environment of the slaughterhouse, and storage until it reaches the consumer (Ananchaipattana et al., 2012). Slaughtering operations in slaughterhouses are crucial to the spread of bacteria and microbes in food and foodborne illnesses. It has been discovered that contamination typically happens during the slaughtering process (Shang et al., 2019). A program is being implemented by the Department of Livestock Development (DLD) to track the levels of Salmonella spp., Staphylococcus aureus, Enterococcus spp., Coliforms, Escherichia coli, and aerobic platelet count (APC) in meat from slaughterhouses (The Department of Livestock Development, 2088). Zoonoses are infectious diseases that humans can contract from animals in a natural way. An estimated 1415 infections harm humans, accounting for roughly 61% of all zoonotic pathogens (Anon, 2011). According to Uzoigwe et al. (2021), standard slaughterhouses with sufficient facilities are necessary for meat production and hygiene. As an essential component of the livestock sector, which supplies meat to millions of people globally, The slaughter and processing of animals on bare floors tainted with blood and feces, which endangers the public, is one of many issues of health and safety protocols at slaughterhouses that must be addressed (Ovuru et al., 2023). The best methods for minimizing and managing

pollutants throughout the slaughtering process can be found by studying the microbiomes of meat. Furthermore, meat's microbiological properties can be a reliable predictor of its nutritional value (Ovuru et al., 2023). High amounts of harmful germs in meat could be caused by improper slaughterhouse cleanliness, corpse handling, and facilities. Most of the time, the state of the animals before they are killed and processed. The quantity of germs discovered on corpses is greatly influenced by the cleanliness of the slaughterhouses, prep rooms, and marketing facilities. To ensure meat safety, only healthy and generally clean animals should be killed, and clean meat must be obtained. Finding pathogen-free meat from sick and dirty animals is one of the most difficult tasks (Vidyarthi et al., 2021). Because bacterial contamination can occur through contact with meat surfaces, such as the hands and clothing of meat handlers outside slaughterhouses, hardwood tables, cutting blades, weighing scales, and water-holding equipment like metal and plastic containers, carts, and meat elevators, the quantity of bacteria in a sample is commonly used as a gauge of potential bacterial contamination (García-Díez et al., 2023; Hauge et al., 2023). According to reports, foodborne illnesses affect over 30% of the population in developed nations each year (Devleesschauwer et al., 2018). An estimated 76 million foodborne illness cases occur worldwide each year, leading to about 325,000 hospital admissions and 5,000 fatalities (Rasool et al., 2020). Whether the microorganism originates from plants or animals also affects the type of bacterium and the degree of contamination. The current study's objectives were to identify bacterial contamination in beef and poultry slaughter in Babylon Governorate, demonstrate the health effects of this contamination on people, assess the variations among slaughterhouses and their definitions, and describe the

MARTIALS AND METHOD

Sample Collection

Assessing the degree of bacterial contamination in slaughterhouses that can lead to meat spoilage was the aim of this investigation. 18 samples were collected over the course of three months, six for each month, three for the ruminant slaughterhouses and three for the chicken slaughterhouses of the Babil Governorate. From January 2024 to April 2024, samples 1, 2, and 3 were separated into slaughterhouses for poultry, and samples 4, 5, and 6 were separated into slaughterhouses for ruminants. Six slaughterhouses spread around the Babil Governorate in various directions provided the samples. Three ruminant and three poultry slaughterhouses in various parts of the Babil Governorate provided samples for the study. Media Transporter tubes filled with peptone water were utilized to transport, collect, and store samples from the slaughterhouses and bring them to the laboratory. The samples were gathered early in the morning, precisely between 7:00 and 9:00 a.m. The sample transporter has a 24-hour validity period. Before and after the slaughter, samples were taken from several points within the slaughterhouses.

Sample Examination

To find out if there were any bacteria present, 60 Petri dishes filled with different nutrient agars were poured, where 10 Petri dishes were distributed for each medium specific to bacteria and placed for 24 hours or more in the refrigerator (Rai, 2016). The morphology of bacterial colonies is examined on a solid nutrient medium as part of the diagnostic procedure for Escherichia coli, E.coli, Pseudomonas, Salmonella, Shigela bacteria and the bacteria were counted and diagnosed to confirm the diagnosis of bacterial infection, which included:

Ten Petri dishes were used to cultivate the resultant bacteria on Mackonkey agar, a selective culture medium for Gram-negative bacteria. Ten Petri dishes were used to cultivate the resultant bacteria on nutrient agar, which was also utilized as a selective culture medium for bacteria. Ten Petri dishes were used to cultivate the resultant bacteria on EMB agar, which is also a selective culture medium for bacteria. Additionally, ten Petri dishes were used to cultivate the resultant bacteria on pseudomonas agar, a selective growth medium for bacteria. Ten Petri dishes were used to cultivate the resultant bacteria on S-S agar, which is also a selective growth medium for bacteria. Each sample from the carrier was taken in 1 ml and put in a Petri dish. Then, the designated medium was added, stirring clockwise and counterclockwise to ensure homogeneity. The samples were then incubated for 48 hours at 37°C in an upside-down position in the incubator. The samples were then identified based on the culture media.

RESULTS AND DISCUSSION

As seen in Table No. (1), the total number of bacteria and dangerous bacteria in the samples increased. The total number of bacteria in sample No. (1) was 232*103, the total number of bacteria in the colon was 211×101, there were 47 E. coli bacteria, 38 Pseudomonas bacteria, 16 Salmonella bacteria, and 2 Shigela bacteria. The total quantity of bacteria in sample No. (2) was reported as follows: 34 Pseudomonas bacteria, 18 Salmonella bacteria, 17 Shigela bacteria, 124*103 Colon bacteria, and 127*101 E. coli bacteria. The total amount of bacteria (324*103), colon bacteria (120*101), E. coli bacteria (55), Pseudomonas bacteria (39), Salmonella bacteria (19), and Shigela bacteria (22) are all recorded in sample No. (4). The overall number of bacteria (368*103), the total number of colon bacteria (178*101), the number of E. coli bacteria (77), the number of Pseudomonas bacteria (43) salmonella bacteria (16), and the number of Shigela bacteria (18) are all recorded in sample No. (5). Noted In the sixth

sample, Five hundred and ninety-nine bacteria, 108 and ten thousand colon bacteria, 91 E. coli bacteria, 53 Pseudomonas bacteria, 17 Salmonella bacteria, and 19 Shigela bacteria were found overall.

Table (1) Investigation of the numbers of pathogenic bacteria and total	d (CFU) in some slaughters in Babylon Governorate
during the first month	

Slaughters NO.	Total bacteria	Total E.coli	E.coli	Pseudomonas	Salmonella	Shigela
1	10 ³ *232	101*211	67	38	16	21
2	10 ³ *124	101*127	71	34	18	17
3	10 ³ *272	101*224	60	41	21	26
4	103*324	101*120	55	39	19	22
5	10 ³ *368	10^{1} *178	77	43	16	18
6	10 ³ *579	101*108	91	53	17	19

As shown in Table (2), there are now more pathogenic and total bacteria in the samples. There are 511*103 bacteria in sample (1), 727*101 bacteria in the colon, 80 E. coli bacteria, 55 pseudomonas bacteria, 11 Salmonella bacteria, and 14 Shigela bacteria among the total number of bacteria. A total of 502*103 bacteria were found in sample (2), 731*101 bacteria were found in the colon, 76 E. coli bacteria, 61 pseudomonas bacteria, 16 Salmonella bacteria, and so on. Sample (3) shows that there are 400*103 bacteria overall. As can be seen in sample No. (4), there are 521*103 bacteria overall, 981*101 coli bacteria overall, 82 E. coli bacteria, 58 Pseudomonas bacteria, 17 Salmonella bacteria, and 20 Shigela bacteria. 20 Shigela bacteria in sample No. (5) is 418*103, the total number of coli bacteria is 970*101, the proportion of E. coli bacteria is 79, the proportion of Pseudomonas bacteria is 50, the proportion of Shigela bacteria is 20, and the proportion of Salmonella bacteria is 17. E. coli bacteria (87), Pseudomonas bacteria (60), Shigela bacteria (23), total (594*103), and total colon bacteria (822*101) are the number of bacteria found in sample No. (6).

Table (2) Investigation of the numbers of pathogenic bacteria and total (CFU) in some slaughterhouses in Babylon Governorate during the second month

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Slaughters NO.	Total bacteria	Total E.coli	E.coli	Pseudomonas	Salmonella	Shigela
1	10 ³ *511	101*727	80	55	11	14
2	10 ³ *502	101*731	76	61	16	18
3	10 ³ *400	101*836	70	59	20	20
4	10 ³ *521	10 ¹ *981	82	58	17	21
5	10 ³ *418	10 ¹ *970	79	50	17	20
6	10 ³ *594	101*822	87	60	22	23

The number of pathogenic and total bacteria in the samples has increased, as indicated in Table (3). The total number of bacteria in sample No. (1) is 324*103, the total number of bacteria in the colon is 201*101, the number of E. coli bacteria is 59, the number of Pseudomonas bacteria is 41, the number of Salmonella bacteria is 19, and the number of Shigela bacteria is 23. The total number of bacteria in sample No. (2) is 104*103, the total number of colon bacteria is 140*101, the number of E. coli bacteria is 68, the number of Pseudomonas bacteria is 38, the number of Salmonella bacteria is 21, and the number of Shigela bacteria (50), the number of bacteria (358*103), the total number of coli bacteria (173*101), the number of E. coli bacteria (50), the number of Pseudomonas bacteria (44), the number of Salmonella bacteria (20), the number of Shigela bacteria (26), the number of Pseudomonas bacteria (41), the number of Shigela bacteria (25), and the number of E. coli bacteria (62) are all recorded in sample No. (4). The overall number of bacteria (320*103), the total number of Salmonella bacteria (19), and the number of E. coli bacteria (26), are all recorded in sample No. (5). Total bacteria (472*103), total colon bacteria (111*101), E. coli bacteria (92), Pseudomonas bacteria (55), Salmonella bacteria (17), and Shigela bacteria (21) are all present in sample No. (6).

Table (3) Investigation of the numbers of pathogenic bacteria and total (CFU) in some slaughterhouses in Babylon Governorate during the third month

ſ	Slaughters NO.	Total bacteria	Total E.coli	E.coli	Pseudomonas	Salmonella	Shigela
Ī	1	10 ³ *324	101*201	59	41	19	23
Ī	2	10 ³ *104	10 ¹ *140	68	38	21	20

3	10 ³ *245	10 ¹ *198	62	41	25	26
4	10 ³ *358	101*173	50	44	20	24
5	10 ³ *320	101*210	81	49	19	26
6	10 ³ *472	$10^{1*}111$	92	55	17	21

Around the world, poultry meat is the source of numerous foodborne zoonoses that not only seriously affect public health but also have a significant financial impact (Álvarez-Astorga et al., 2002). Salmonella, Shigela, Pseudomonas, Escherichia coli, and E. coli are some of the significant bacteria linked to these illnesses. This study offered information on bacterial contamination in animal slaughterhouses at the Babylon Governorate level. High levels of E. coli and Salmonella contamination in the samples were the primary conclusions. It is believed that infections are mostly caused by harmful bacteria in humans. Staphylococcal food poisoning, a kind of gastroenteritis brought on by eating meat that contains one or more ready-to-eat meats carrying staphylococcal enterotoxins, has been linked to this pathogen (Seok and Bohach, 2007). Consuming or handling tainted foodborne items can occasionally spread staphylococci across the community, and illnesses brought on by these bacteria have been clearly detected (Capita and Alonso, 2013; Buzon-Duran et al., 2017). Poor hygiene during slaughtering and handling, as well as the quality of the water used during the entire meat production, slaughtering, handling, and consumer marketing processes, can lead to meat contamination (Endale and Hailay, 2013). According to earlier studies, slaughterhouses without an automated chain and those with deteriorating walls and floors are more vulnerable to microbial contamination. Salmonella, Shigela, Pseudomonas, Escherichia coli, and E. coli are among the common bacteria detected in these slaughterhouses. According to a study by Collobert et al. (2002), a higher bacterial presence in these slaughterhouses is a sign of poor hygiene standards and insufficient health regulations. Hazardous germs can spread throughout the slaughterhouse as a result of contaminants building up on the floors and then getting onto workers' shoes. Floors and drains may provide ideal circumstances for the growth and reproduction of germs, especially when cleaned with high-pressure water (Eisel et al., 2018). One of the main causes of the spread of bacteria like E. coli species is poor hygiene managementBensid (2018) states that the primary sources of salmonella are the hair and excrement of dead animals. If the instruments and materials used by the practitioners are not hygienic, bacterial infections may begin with the initial skin cut made to extract blood. This can be brought on by inadequate evisceration and poor cleanliness, which can hasten the growth of bacteria on the carcasses (Sulieman et al., 2023; Korkmaz et al., 2022). Cutting instruments, blades, and other equipment may thereby become polluted, allowing dangerous microbes to infect them. In order to prevent cross-contamination, several writers have emphasized the significance of cleaning the knife between carcasses (Nastasijevic et al., 2023). Additionally, meat may become contaminated during slaughterhouse rinse because of the usage of water (Antic et al. 2021). Regular water analysis is necessary to ensure the quality of the drinking water used for cleaning and operations in slaughterhouses, which makes having adequate potable water essential (Adebowale et al., 2010). The occurrence of antimicrobial-resistant E. coli in animalbased foods is an increasing public health concern, and aside from sexually transmitted E. coli, sometimes known as "non-STEC E. coli," these bacteria obviously pose a risk to food safety. Therefore, the presence of drug-resistant, non-pathogenic E. coli on meat or poultry products may cause resistance genes to be transferred to other bacteria, including possible pathogens, that are present in these foods or in the human gut after ingestion (Zhao et al., 2001). The potential to acquire, carry, and transmit resistance genes to pathogens that may be present in animals, the environment, or the human intestinal system has been proven for non-pathogenic E. coli, a normal and stable part of the gut microbiota. It's crucial to keep in mind that, despite the fact that bacterial infections are acquired through sexual contact, exposure to food sources—especially when selling poultry—is thought to be the primary cause of the rise in drug-resistant ExPEC infections, which include recurrent UTIs. Genetic material, such as genes for virulence or antibiotic resistance, can be readily switched between E. Coli strains. Another finding is that, although several studies have found that the levels of E. coli and Enterobacteriaceae on the surfaces of poultry carcasses are fairly similar (Althaus et al., Buess 2017 et al., 2019), the levels of E. coli in beef carcasses are typically low and lower than the levels of Enterobacteriaceae that were examined from the same samples (Barco et al., 2017). Before being de-feathered in the plucker, the feathers are loosened from the skin by scalding in warm water along the slaughter line. In the USA, a "soft scald" at 51-54°C for 120–210 seconds is typically used, whereas a "hard scald" at temperatures exceeding 60°C for 45–90 seconds is commonly used. Depending on the operation, the process cleanliness varies. Particularly in water that is warmer than 55°C, scaling has been demonstrated to significantly reduce bacterial burdens by 1.0 to 1.5 log per gram (ICMS, 1998). The risk of contamination during feather removal is increased by the intense pressure of plucking, which might result in fecal leaks (Allen et al., 2003). Other studies have shown that cleaning corpses with cold spray for 5-6 seconds both inside and outside can slightly reduce infection (Abu-Ruwaida et al., 1994; Berrang and Dickens, 2000) (Oyarzabal et al., 2004). But, according to Loretz et al. (2010), it can also spread contamination from a small area to a bigger one. Carcasses can be chilled in a variety of ways, such as air cooling with or without extra water spray and different air speeds, or cooling in water with or without ice and chemicals. Immersion cooling in water has been shown to prevent contamination more effectively than air cooling (Corry et al., 2007). Due to single-day sampling

on different days, the study design has limitations. Additionally, the comparison between these two groups is impacted by daily fluctuations in the cleanliness of the operations because samples were collected from various locations in both positive and negative slaughterhouses on different days (Nagel Gravning et al., 2021). According to the European Food Safety Authority (EFSA), this strategy is "from farm to company," and infections that are harmful to human health must be controlled. According to Pessoa et al. (2021), this strategy entails early interventions in chicken farms, the implementation of efficient control measures for consumers and slaughterhouses, and the identification of factors that can lower harmful bacteria in slaughterhouses.

CONCLUSION AND RECOMMENDATION

Through bacterial studies, it is possible to predict early the suitability of any food product for human consumption. It was found that the results above showed that the numbers of pathogenic bacteria were high in some samples, and that this bacterial contamination may not be caused by poor sterilization in slaughterhouses, but rather that these infections with these studied bacterial species are present in abundance in animal soil fields (poultry and large animals), as there are currently vaccines against salmonella and E. coli, which allows for an increase in the numbers of these bacteria when conducting and performing swabs for the purpose of bacterial studies.

It is recommended to apply all biosecurity measures in animal husbandry fields, as most fields lack the application of the minimum levels of biosecurity in animal fields. This requires concerted efforts by the Ministry of Agriculture and the Veterinary Department to implement and follow up on biosecurity programs, take blood samples from birds and animals raised in the fields and ensure that they are free of bacterial infections, specifically Salmonella, before marketing those animals to slaughterhouses, apply the highest levels of biosecurity in animal slaughterhouses to reduce bacterial contamination, use modern sterilizers, especially nano ones, due to their great effect in reducing bacterial contamination (sterilization of devices and equipment), and implement projects to manufacture animal protein concentrates accompanying slaughterhouses to benefit from slaughterhouse waste, as this waste is considered a carrier of pathogens and a real problem that prevents the implementation of biosecurity programs.

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