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# Efficacy of Using Lactic Acid and Beefxide to Reduce Pathogenic *E. coli* and *Salmonella* spp. on Beef Carcasses in Mosul Slaughterhouse, Iraq

**Israa M. Jweer and Omar A. Al-Mahmood***
Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Iraq
***Corresponding Author:** Omar A. Al-Mahmood, **E-Mail:** omar.a.almoula@uomosul.edu.iq

## ABSTRACT

Meat contamination occurs in a variety of ways, the most significant of which include live animals prior to slaughter (infected animals), workers and tools used in the slaughter process, and post-slaughter activities to prepare the carcasses (removing the skin and viscera). Raw meat remains the primary source of many diseases caused by microbes that are transmitted to humans, especially those that cause food poisoning, such as *Escherichia coli* and *Salmonella* spp., which are considered among the most important health problems facing the world. Therefore, we decided to study the efficiency of using some organic acids (lactic acid and beefxide) to reduce the number of pathogenic *Escherichia coli*, *Salmonella* spp., on the surfaces of beef carcasses in the slaughterhouse of Mosul city. Eighty samples (beef carcass sponge swabs) were collected over the course of three months between September 13, 2023, and December 11, 2023, using culture method and polymerase chain reaction. Our findings of pathogenic bacteria including Shiga toxin-producing *E. coli*, showed the presence of 4 isolates out of 40 carcass samples (4/40: 10%); all were isolated from the sample's prior treatment with organic acids, 3 of which possessed the *stx1* gene and one isolate had the *stx2* gene. The study also revealed that there were only two isolates of *Salmonella* spp. (2/40: 5%) in beef carcasses that possessed the *invA* gene and these are samples that have not been treated with organic acids. This study concludes the effectiveness of lactic acid and beefxide solutions in removing pathogenic bacteria such as Shiga toxin-producing *E. coli* and *Salmonella* spp. from beef carcasses.

***Keywords:*** Beefxide, *E. coli* (STEC), Lactic acid, Mosul, *Salmonella* spp., Slaughterhouse.

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## INTRODUCTION

Meat and its products are a large portion of the typical diet in many countries because they are linked to health and cultural reasons. Food contamination can occur during production, transportation, and storage **(Otu-Bassey *et al.*, 2017)**, leading to food poisoning risks. *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* are frequent pathogenic microbes that cause food poisoning **(Elmonir *et al.*, 2021; Kareem and Al-Ezee, 2020)**. From this standpoint, many countries are keen on the safety of food, including meat, which is considered of utmost importance to public health, especially when the environment of the slaughterhouses is highly contaminated **(Soriyi *et al.*, 2008)**.

*Salmonella* spp. and Shiga toxin-producing *E. coli* are the primary causes of foodborne diseases **(Havelaar *et al.*, 2015)**. Ruminants, particularly cattle, are reservoirs and asymptomatic carriers of *Salmonella* spp. and *E. coli* O157 **(Gutema *et al.*, 2021)**. Studies have found these infections in cattle feces and hides on farms and in slaughterhouses in developed nations **(Essendoubi *et al.*, 2019)**. The presence of *Salmonella* spp. and *E. coli* O157 in bovine feces and hides may cause them to be transferred to carcasses during hide removal and evisceration. *E. coli* and *Salmonella* spp. are commonly used to assess the quality and safety of meat. The Food Safety and Inspection Service (FSIS) in the US requires a maximum of 2.7% *Salmonella* in beef carcasses **(USDA-FSIS, 1996)**. *Salmonella* spp. is the primary cause of foodborne outbreaks in several countries **(Majowicz *et al.*, 2010)**.

The standard culture approach is often used to detect pathogenic bacteria. It identifies bacteria that can be cultivated. However, culturing is difficult and time-consuming **(Kawasaki *et al.*, 2003)**. Food samples may contain competitively inhibiting bacteria or dormant or metabolically aberrant bacteria that cannot be grown, leading to missed detections and false-negative results. Molecular biology techniques like polymerase chain reaction (PCR) are commonly

employed to detect harmful bacteria. These are simple, quick, inexpensive, and versatile **(Xie *et al*., 2020)**.

On the other hand, lactic acid and other organic acids are safe solutions that have been approved by the Food and Drug Administration (FDA) as antimicrobials for use in beef products at concentrations of 1-5% under the guidance of the Food Safety and Inspection Administration **(USDA-FSIS, 2016)**. Additionally, beefxide is a mixture of lactic acid and citric acid that can be used at a concentration of up to 2.5% to reduce microbes in beef **(Hendricks *et al*., 2014)**. Lactic acid and beefxide are similar in the efficiency of reducing microbes in beef production, and it has been proven by Laury and his colleagues, who conducted a study using a spray method that led to a decrease in the numbers of *Escherichia coli* and *Salmonella* spp. on beef carcasses **(Laury *et al*., 2009a).**

Therefore, the study aimed to explore the presence of pathogenic *E. coli* and *Salmonella* spp. in beef carcasses prior to treatment, as well as the efficacy of utilizing lactic acid and beefxide to reduce these pathogens following treatment in a Mosul slaughterhouse.

## MATERIALS AND METHODS

The study included one slaughterhouse where 80 sponge swabs (40 carcass samples for *E. coli* and 40 carcass samples for *Salmonella* spp.) were collected from the Mosul slaughterhouse (Nineveh Governorate/Iraq), for a period extending from 13/9/2023 to 11/12/2023 through regular weekly visits (12 visits) and over a period of three months (20 samples treated with organic acids and 20 samples not treated with organic acids for each pathogen (the carcasses have not been washed by anything except regular wash (water) in the slaughterhouse).

Organic acids (2.5% lactic acid and 2.5% beefxide solution consisting of lactic acid 60% + citric acid 35% + potassium hydroxide 5%) were used for sample treatment. Sterile sponge swabs (a special kit: Whirl-Pak® bag, gloves, and Butterfield' Phosphate Buffer (World Bioproducts LLC, USA)) were used in this study. Swabs (100 $cm^2$ per swab) were collected from each carcass (either the round or chuck area) after wearing sterile gloves and preparing sponge swabs. A special sterile 10 x 10 cm (100 $cm^2$) template (World Bioproducts LLC, USA) was used to mark the swabbing areas on the carcass, which were taken before treatment with organic acids. As for the method of taking the swab after treatment with organic acids, the carcass was sprayed with organic acid inside the slaughterhouse, and then an hour later the swab was taken from the same carcass. The spraying method was directly on the carcass for 10 seconds and at a distance not exceeding 50 cm with low pressure using a manual sprayer **(Reynolds, 2005).**

The swabbing process included 10 horizontal movements followed by 10 vertical movements for each site, after which the swab was placed in a sample bag (Whirl-Pak® bag, USA) for sponge swabs **(USDA-FSIS, 2014)**, which contains 15 ml of phosphate buffer solution with a unique identification code (Sample ID) for each sample. All samples were placed in a cooler container within five minutes of being collected. Then they were transferred directly to the Veterinary Public Health Research Laboratory at the College of Veterinary Medicine/University of Mosul to perform the tests within a period not exceeding 4 hours. Treated and untreated samples were examined for detection of pathogenic *E. coli* and *Salmonella* spp. using culture and molecular methods.

For detection of Shiga toxin-producing *E. coli* (culture method), swab samples were cultured in Tryptone Soy Broth for 24 hours at a temperature of 41 °C in the incubator to enrich the bacteria **(ISO 16654, 2001)**. Then, the bacterial isolates were grown on MacConkey agar to observe their phenotypic characteristics and select *Escherichia coli* colonies in selective medium, which were incubated at a temperature of 37°C for 24 hours **(El-Mongy *et al*., 2017)**. After that, the differential medium (chromogenic agar) was used to distinguish the pathogenic Shiga toxin-producing *E. coli* isolates with a purple or turquoise color from the other serotypes. Later, the Vitek2 Compact System was used to identify *Escherichia coli* isolates. Moreover, other swabs were cultured for the detection *Salmonella* spp. in beef carcasses. Under sterile conditions, swabs were grown on Buffer Peptone Water (BPW) medium and incubated for 24 hours at 37°C for the pre-enrichment stage for *Salmonella* spp. **(ISO 6579, 2002)**. Then, 1 ml of cultured peptone water broth was taken and added to 9 ml of selenite broth (enrichment medium) and incubated for 24 hours at a temperature of 37 °C. Then, a loop of selenite medium was taken and cultured on the Xylose Lysine Deoxycholate agar (XLD agar) and *Salmonella shigella* agar (SS agar) (selective and differential media), and after 24 hours, the media was examined to clearly see the growth of colonies. *Salmonella* spp. appeared in black colonies. Later, the tubes containing Triple Sugar Iron (TSI) medium were inoculated by presumptive *Salmonella* and incubated at 37 °C for 24 hours.

To confirm the isolation, we used conventional PCR to detect some virulent genes in both *Salmonella* spp. and Shiga toxin-producing *E. coli*. The DNA extraction kit manufactured by Addbio was used to extract DNA from bacterial samples. Different primers were used in molecular detection; all primers were manufactured by Macrogen (Korea) as follows **(Table 1)** and different protocols were used for DNA amplifications **(Table 2).**

Table 1: Primers used for detection *E. coli* and *Salmonella* spp.

| Gene | primer | Sequence (5'-3') | Product size (bp) | Reference |
|---|---|---|---|---|
| *uidA* | F | CCAAAAGCCAGACAGAGT | 623 | Moyo *et al.*, 2007 |
| | R | GCACAGCACZTCAAAGAG | | |
| *stx1* | F | ACACTGGATGATCTCAGTGG | 614 | Fratamico *et al.*, 1995 |
| | R | CTGAATCCCCCTCCATTATG | | |
| *stx2* | F | CCATGACAACGGACAGCAGTT | 779 | Gannon *et al.*, 1992 |
| | R | CCTGTCCAACTGAGCAGCACTTTG | | |
| *invA* | F | TCATCGCACCGTCAAAGGAACC | 284 | Rahn *et al.*, 1992 |
| | R | GTGAAATTATCGCCACGTTCGGGCAA | | |
| *sdiA* | F | AATATCGCTTCGTACCAC | 274 | Halatsi *et al.*, 2006 |
| | R | GTAGGTAAACGAGGAGCAG | | |

Table 2: PCR amplification protocols for different genes

| Steps | *uidA* | *stx1* | *stx2* | *invA* | *sidA* | |
|---|---|---|---|---|---|---|
| | Temp/ Time | Temp/ Time | Temp/ Time | Temp/ Time | Temp/ Time | |
| Initial denaturation | 95° C/ 3 min | 95° C/ 5 min | 94° C/ 5 min | 95° C/ 2 min | 95° C/ 5 min | 1 cycle |
| Denaturation | 95° C/ 1 min | 95° C/ 40 sec | 94° C/ 1 min | 95° C/ 30 sec | 95° C/ 1 min | 35 cycles |
| Annealing | 57° C/ 40 sec | 59° C/ 40 sec | 62° C/ 1 min | 65° C/ 30 sec | 54° C/ 1 min | |
| Extension | 72° C/ 1 min | 72° C/ 40 sec | 72° C/ 1 min | 72° C/ 45 sec | 72° C/1 min | |
| Final extension | 72° C/ 5 min | 72° C/ 5 min | 72° C/ 5 min | 72° C/ 7 min | 72° C/ 5 min | 1 cycle |

## RESULTS

The results of *Escherichia coli* isolated using the traditional culture method showed the detection of six isolates out of 40 carcass samples, at a rate of 15%, using culture media (MacConky Agar and Chromogenic Agar), as shown in **Fig. 1.**

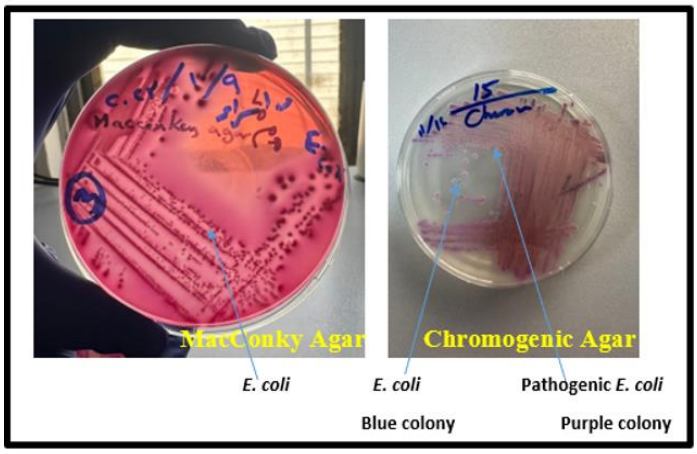


Fig. 1: Growth of *Escherichia coli* on MacConkey and chromogenic agar.

Furthermore, the results of the Vitek2 Compact System test showed that the isolates that were examined gave a 99% probability of being *E. coli*, as shown in **Fig. 2.**

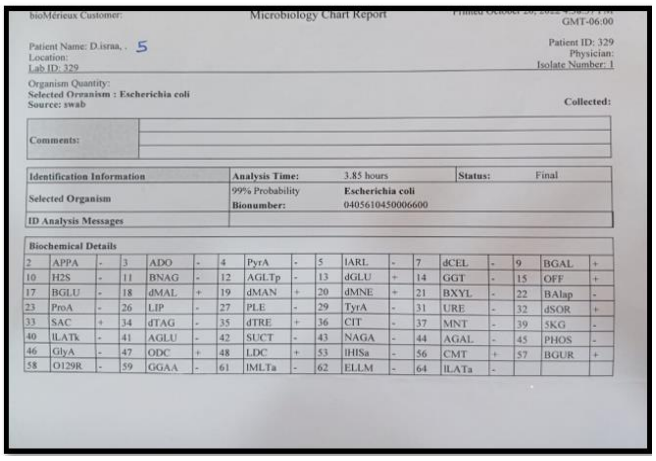


Fig. 2: Shows the results of the Vitek2 system test for *Escherichia coli.*

While the results of the molecular analysis for *Escherichia coli* isolates confirmed the presence of 6 isolates out of 40 samples, which possessed the *uidA*

gene with a 623bp after the DNA amplification using the PCR technique (4 isolates in pre-treatment samples and 2 isolates after treatment with organic acids), as shown in **Fig. 3 and Table 3.**

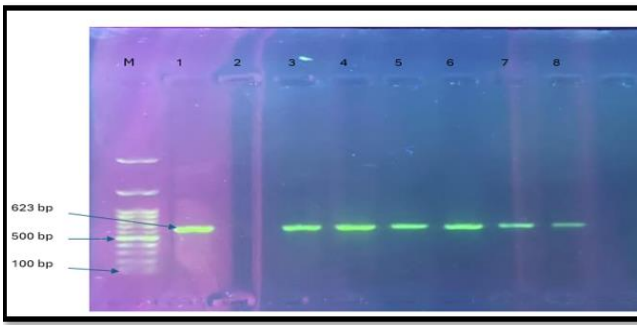


Fig. 3: The result of PCR (*uidA* gene) on an agarose gel with a molecular weight of 623 bp. Well No. 1 was positive control; the negative control was in well No. 2; Wells 3-8 were positive for *Escherichia coli*; Well M was for the Ladder 100bp.

In addition, the results revealed the presence of 4 pathogenic isolates of Shiga toxin-producing *Escherichia coli* (STEC) out of 40 samples (10%), 3 of which possessed the *stx1* gene, and all of them belonged to the prior treatment samples, as shown in **Fig. 4 and Table 3.**

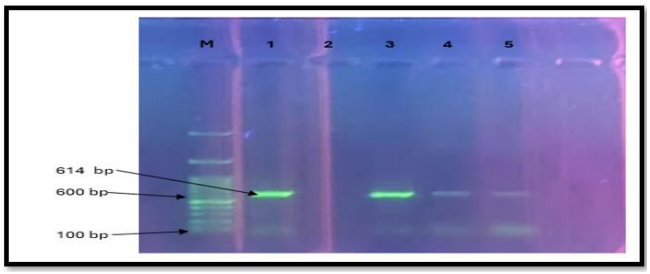


Fig. 4: The result of PCR (*stx1* gene) on an agarose gel with a molecular weight of 614 bp. Well No. 1 was positive control; the negative control was in well No. 2; Wells 3-5 were positive for STEC; Well M was for the Ladder 100bp.

Table 3: The number and percentage of presence of specific genes in *Escherichia coli*

| Genes | Total number of isolates | Number of isolates possessed genes prior treatment (%) | Number of isolates possessed genes post treatment (%) |
|---|---|---|---|
| *uidA* | 6 | 4 (66.6%) | 2(33.4%)* |
| *stx1* | 3/4 | 3 (75%) | 0 |
| *stx2* | 1/4 | 1 (25%) | 0 |

*: The two *E. coli* isolates that had *uidA* gene did not have the Shiga toxin genes and it might be pathogenic.

Whereas the findings of the molecular analysis also confirmed possession of STEC to the *stx2* gene (1 out of 4 isolates), and it belonged to the prior treatment samples, as shown in **Fig. 5 and Table 3.**

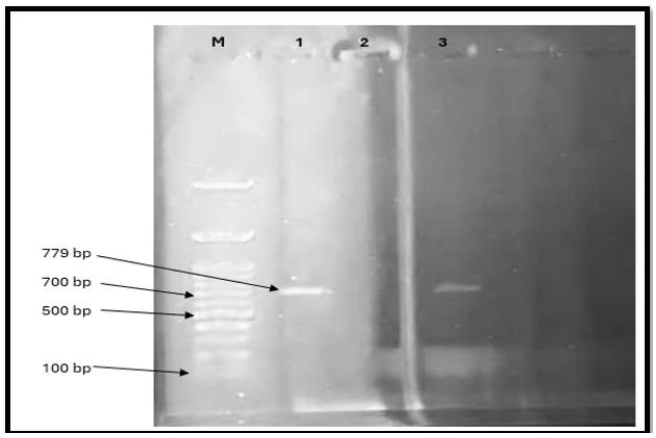


Fig. 5: The result of PCR (*stx2* gene) on an agarose gel with a molecular weight of 779 bp. Well No. 1 was positive control; the negative control was in well No. 2; Well 3 was positive for STEC; Well M was for the Ladder 100bp.

On the other hand, the findings regarding *Salmonella* spp. isolates using the traditional culture method showed that the percentage of contamination was lower compared to *Escherichia coli*, as it was confirmed that there were only two isolates of *Salmonella* spp. in beef carcass samples, which belonged to the untreated organic acid samples, and we did not have any positive samples post-treatment. The percentage of contamination with *Salmonella* spp. was 5% (2 out of 40 samples) of the total number of samples tested using culture media (XLD and SS agar), as shown in **Fig. 6.**


Fig. 6: Growth of *Salmonella* spp. on XLD and SS agar.

*Salmonella* did not ferment lactose or sucrose, so the *Salmonella* spp. produced alkaline slant (pink), and the bottom of the tube was yellow (Acidic), in addition to producing $H_2S$ gas and a black color during biochemical test (TSI test). Moreover, the molecular

detection method using PCR confirmed that the two isolates possessed the *invA* gene in *Salmonella* spp as in **Fig. 7,** while the isolates themselves did not possess the *sdiA* gene.

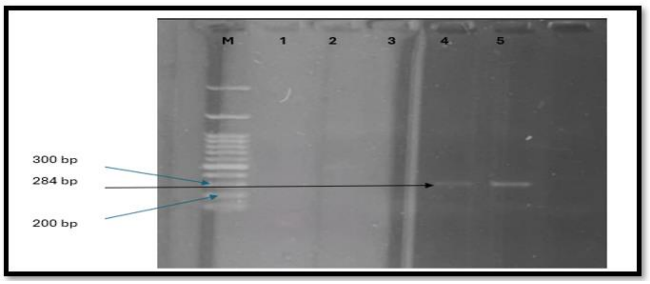


Fig. 7: Positive result for the *invA* gene with molecular weight (284bp), the negative control was in well No. 1, while wells No. 4 and 5 were the positive samples. Wells No. 2 and 3 were negative for salmonella spp., and well No. M was for Ladder—100bp.

## DISCUSSION

In 1994, the US Department of Agriculture/Food Safety Inspection Service (USDA-FSIS) established a zero-tolerance principle for the presence of *E. coli* O157:H7 in meat **(USDA-FSIS, 2004)**. Therefore, organic acids such as lactic acid and citric acid were used to treat beef carcasses to reduce the numbers of pathogenic *E. coli* and *Salmonella* on meat **(Laury *et al*., 2009b).**

The results of the study showed the effectiveness of organic acids in reducing the number of pathogenic bacteria, as no pathogenic bacteria, whether Shiga toxin-producing *E. coli* or *Salmonella* spp., were detected in the post-treatment samples of the carcasses. This is what many researchers agreed with about the ability of lactic acid to reduce the pathogenic *Escherichia coli* present in beef carcasses **(Harris *et al*., 2006; Kalchayanand *et al*., 2008; Kalchayanand *et al*., 2016)**.

In general, lactic acid reduced levels of pathogenic *E. coli* much better than acetic acid, while beefxide solution performed well in its ability to reduce *Salmonella* spp. **(Hardin *et al*., 1995)**, which was consistent with the results of our study. In addition, a study indicated that lactic acid at a concentration of 2.0% reduced *Salmonella* spp. by 1.0 log on carcass surfaces and by 2.0 log for pathogenic *Escherichia coli*. This was different from our study, as a slightly lower concentration was used compared to our study despite its efficiency in reducing the number of germs **(Harris *et al*., 2006)**.

There is much variation in research undertaken worldwide in the reductions of pathogenic bacteria that can be achieved, mainly due to differences in the concentrations and types of acids that are used in their studies, the method of application of the acids, the types of samples tested, and the initial microbial load of the samples **(Acuff, 2005).**

Finally, samples containing pathogenic bacteria had a higher rate of contamination with indicator microorganisms compared to negative samples. This confirms the benefit of using indicators for microorganisms using the petrifilm method and the ease of working with them to indicate pathogenic bacteria. This agrees with a study that encouraged the use of these indicators to predict the presence of pathogens in meat samples, especially *E. coli* O157 and *Salmonella* spp. **(Arthur *et al*., 2004; Al-Mahmood, O. A. 2020; Al-Mahmood and Fraser, 2023).**

## CONCLUSION

The rate of bacterial contamination with pathogenic bacteria (Shiga toxin-producing *E. coli* and *Salmonella* spp.) in beef carcasses was relatively high prior to treatment, as it did exceed the maximum permissible limit internationally. However, the study demonstrated the efficiency of using organic acids (lactic acid and beefxide solution) in reducing the pathogenic microorganisms to a level that does not cause diseases. This study encourages the use of organic acids, such as lactic acid or beefxide solution in Iraqi slaughterhouses to reduce the microbial load, prolong the shelf life of meat, and delay its spoilage. Further research is recommended to study other acids, such as acetic acid, ascorbic acid, and propionic acid, and study their effect on the reduction of pathogenic bacteria in beef carcasses.

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### Declaration of Conflicting Interests

The author of this manuscript stated there is no conflict of interest regarding the writing process or data analysis.

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