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Effects of Water Treatment Methods on Protein Profile of *Escherichia coli* O157:H7 and *Escherichia coli* Non-O157 Isolated from Drinking Water Sources in Ado-Ekiti, Ekiti State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author BMO designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors OF, JOO and TOF managed the analyses of the study. Authors AOO and COE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To examine the effects of water treatment methods on the protein profile of *Escherichia coli* O157 and *Escherichia coli* non-O157 isolated from different drinking water sources in Ado-Ekiti.
Study Design: Experimental study design.
Place and Duration of Study: Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria, between December 2015 and June 2016.
Methodology: The test organisms, *E. coli* O157 and *E. coli* non-O157, were charged against some water treatment methods such as silver nitrate, sunlight, low and high temperature and varied pH

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using standard methods. Protein expression profiles of these organisms, before and after exposure, were studied with cell crude proteins extract using standard SDS-PAGE method. Statistical analysis was carried out on the data generated using correlation matrix.

Results: The survivability testing showed that *E. coli* O157 survived better than *E. coli* non-O157. Silver nitrate, among all other agents used, exhibited the most lethal effect on both serotypes. The protein profile of the two serotypes was similar before their exposure (r = 0.9897, P-value = 0.941), while after their exposure, they exhibited different characteristic responses (r = 0.693, P-value = 0.018).

Conclusion: This study showed that the treatment methods had effects on the expression of bacterial protein profile following exposure.

Keywords: Escherichia coli; physico-chemical agents; survivability; protein profile; SDS-PAGE.

1. INTRODUCTION

Pollution of drinking water sources is a reoccurring problem in developing countries including Nigeria [1]. Pollution or contamination of water sources could arise from point sources (such as industrial effluents and discharge from municipal waste water treatment plants) or non-point sources (such as runoff from farm lands, percolation of septic effluents into ground water, indiscriminate dumping of domestic wastes into streams or rivers among others) [2]. Consequently, different water treatment methods have been harnessed in different homes to combat this menace and also to ensure the safety of drinking water available. Among various water treatment methods include: The use of chlorine, sunlight (solar disinfection), silver compounds, Moringa oleifera, boiling, storage, lime, e. t. c. [3]. Unfortunately, some bacterial pathogen especially the enteric bacteria, which are mostly implicated in contaminated drinking water, have devised means of resisting some of these water treatment methods. This poses a serious threat to health of any community.

Enteric bacteria such as *Escherichia coli (E. coli*) which are Gram negative rod shaped bacteria within the family Enterobacteriaceae [4] has been implicated among bacteria which are capable of resisting some of these water treatment plans. This is made possible as a result of their ability to up-regulate a general stress response regulator, δ^S, known as *RpoS. RpoS* is a sigma subunit of RNA polymerase which is up-regulated in *E. coli* during stationary phase and upon exposure to environmental stresses [5]. The δ^s replaces the house keeping sigma factor, δ^{70}, on the RNA polymerase and thus change its regulatory properties [6]. This δ^s polymerase recognizes a different promoter sequence which is present in front of genes that encode proteins which protect the bacteria against different types of cell injuries. Thus, allowing their survival under different water treatment methods.

The effect of environmental stresses (water treatment methods) on *E. coli* can be buttressed, apart from microbiological culturing method, by a molecular technique known as sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE). This method allows for investigation of the effect of water treatment methods on the protein profile of the bacterial cell. Therefore, this study was carried out to investigate the effect of different water treatment methods such as low and high temperature, sunlight and silver nitrate and varied pH on the protein profile of *E. coli* O157:H7 and *E. coli* non-O157 isolated from drinking water samples following exposure by using SDS-PAGE.

2. MATERIALS AND METHODS

2.1 Bacterial Culture

Escherichia coli O157:H7 (S89) and non-O157 (S3b) isolated from drinking water samples and identified using PCR techniques in a previous study (Unpublished) were used in this study.

2.2 Bacterial Growth Condition

The bacterial cultures were sub-cultured in Tryptone Soy Broth (TSB) (Lab M, LAB011 U.K.) at 37℃ for 24 hours and the cultures were used for the following experiments to determine the effect of different physicochemical agents on their survival as described by Albashan [7].

2.3 Control Culture

Each volume of 500 ml Tryptone Soy Broth (TSB) (Lab M, LAB011 U.K.) was inoculated with an overnight culture of each of the test organisms. They were placed in a 37℃ orbital

shaking incubator, with constant shaking (200 rpm) for 30 hrs.

2.4 Effect of pH

A volume of 500ml TSB each was adjusted with IM phosphate buffer to pH 5, 6, 7 and 8 and was sterilized at 121℃ for 15 minutes. Each was then inoculated with an overnight culture of the test organisms. They were placed in a 37℃ orbital shaking incubator, with constant shaking (200 rpm) for 30 hrs.

2.5 Effect of Low and High Temperature

Overnight cultures of the test organisms were used to inoculate 500 ml TSB and incubated at different high temperatures (40℃, 50℃ and 60℃) in an orbital shaking incubator, with constant shaking (200 rpm) for 30 hrs. While the other inoculated broth was incubated at 4℃ and 20℃ using a refrigerator for 30 hrs.

2.6 Effect of Sunlight

An overnight culture of the test organisms was used to inoculate 500 ml TSB and was exposed to sunlight for 6 hours and after which was incubated in a shaking incubator for 30 hrs.

2.7 Effect of Silver Nitrate

Minimum inhibitory concentration of 0.063 mg/ml of 0.01 M of silver nitrate was prepared and added to 500ml TSB. This was incubated in a 37℃ orbital shaking incubator, with constant shaking (200 rpm) for 30 hrs.

2.8 Determination of Survivability of the Test Organisms and Statistical Analysis of Each Test

The bacterial survivability was determined by taking the optical density (O.D. at 600 nm) of each of the test experiments at every 6 hrs interval. The data obtained from each test experiment was analyzed using t-test to determine the correlation and significance difference (set to 5%) between the control and the test experiments.

2.9 Harvesting of Bacterial Protein and SDS–PAGE gel Electrophoresis

Bacterial cells were harvested by using cold ultra centrifuge (4℃) at 10,000 rpm for 20 min. Bacterial intracellular proteins were extracted from the cell pellets by using bacterial protein extraction reagent (Thermo Scientific) and ammonium persulfate (Sigma Aldrich) precipitation. SDS-PAGE gel electrophoresis was carried out using 12% resolving gel (1.5 M Tris-HCl, pH 8.8 (Bio Basic Canada, Inc), 10% SDS (Fisher Scientific), 40% bis-acrylamide and 4% stacking gel (0.5 M Tris-HCl (pH 6.9), 10% SDS, 40% bis-acrylamide). Bacterial proteins were treated with dithiothreitol (DTT) and boiled before it was loaded into the wells. For ease of molecular weight estimation and comparison, protein ladder (Spectra Multicolor Broad-Range Protein Ladder, Thermo Scientific) was loaded onto each gel. The SDS-PAGE gels were run using constant electric current (30 mA) until the bromophenol blue dye front reached the bottom of the gel plate. The protein gels were then put into a container containing staining solution, Coomassie Brilliant Blue (CBR) R-250 (Fisher Scientific). Gels were left in the staining solution overnight and also followed by overnight destaining in distilled water [8].

2.10 Determination of Molecular Weight of Proteins and Statistical Analysis of the Protein Profile

The molecular weight of each of the expressed bands was determined by using a molecular weight standard marker which ranged from 20kDa to 97KDa. The marker comprised of five different proteins which included phosphorylate (97kDa), Bovine serum albumin (66kDa), Chicken ovalbumin (45kDa), Soyabeans trypsin inhibitor (30kDa) and Bovine lactoglobulin (20kDa). A standard graph was plotted for each of the organisms by the mobility (retention factor, R_f) values on the x-axis and logarithm of molecular weight on the y-axis. The molecular weight of each of the expressed bands was extrapolated from the graph. The protein banding pattern for each sample was scored for the presence and absence of bands as 1 and 0 respectively. The statistical analysis included correlation between the protein profile expressions by the test organisms.

3. RESULTS AND DISCUSSION

The survivability of the test organisms after every six hours was determined by taking the O. D (at 600 nm) reading of each sample and this is presented in Table 1 to 3. Table 1 depicts the survival of both *E. coli* O157 and *E. coli* non-O157 following exposure to varied pH. The result showed that the there was a strong positive correlation between the control and test experiments in the response of both organism at pH 5 to pH 8. Statistically, there was a significant

difference between the response of *E. coli* O157 at pH 5, 6 and 8 likewise for *E. coli* non O157 at pH 8. However, there was no statistical significant difference in the response of *E. coli* non-O157 to the effect of pH 5, 6 and 7 when compared with the control experiment.

The result shown in Table 2 explains the response of the two test organisms to varied high and low temperatures. There was a positive correlation between the control and test experiments on both test organisms. Statistically, there was a significant difference in the response of *E. coli* O157 to the effect of temperature 4, 20 and 60℃ as the p-values are less than 0.05 but there was no significant difference at temperature 40 and 50℃. For *E. coli* non-O157, there was a statistical difference between the test and control experiments at temperature 4, 20 and 40℃.

The treatment with sunlight and silver nitrate on both organisms reduced their survivability after exposure. The results showed that that there was a statistical significant difference in the response of both test organisms to the effect of sunlight and silver nitrate (p-value < 0.05) (Table 3).

The extracted crude bacterial proteins were separated on denaturing polyacrylamide electrophoresis gel. Plate1. shows the protein expression profile of *E. coli* O157 and *E. coli* non-O157 respectively following exposure to various treatment conditions (Silver nitrate, sunlight, low and high temperature and pH) for 30 hrs. The result shown on Plate 1 revealed the protein expression pattern of the two strains (*E. coli* non-O157 on the G-plate and *E. coli* O157 on the H-plate). Comparatively, the two strains exhibited different protein expression pattern. Following exposure to different pH, that is pH 5 – 8. It was observed that *E. coli* non-O157 gave a similar pattern of protein expression from GL1 to GL3. Intensity of bands were the same when compared with the control but GL4 gave a different protein expression pattern. Some bands were not expressed when compared with the control while similar protein expression pattern was observed in *E. coli* O157 from Lane HL1-HL4.

Following exposure to varied high temperature, 40℃, 50℃, and 60℃, different pattern of protein expression was observed for both strains. Downstream, the intensity and broadness of bands seen in both strains were completely different from the control. At low temperature, 4℃ and 20℃, protein expression was poorer in *E. coli* non-O157 than O157. Some bands were not expressed in *E. coli* non-O157 when compared with the control while in *E. coli* O157, some protein bands were also not expressed and intensity of bands were more pronounced in comparison with the control. Exposure of *E. coli* O157 and non-O157 to sunlight produced almost similar protein patterns which are different when compared with their control. Some bands were not expressed and also broad and intense protein bands were obtained. Similarly, protein expression following exposure to silver nitrate was very poor in both strains as the expression of protein bands diminished and was completely different from the control.

Table 1. Effect of pH on Survival of *E. coli* O157 and *E. coli* non-O157

Time (Hr)	Control (Optical density (nm)		pH 5 (Optical density (nm)		pH 6 (Optical density (nm)		PH7 (Optical density (nm)		pH8 (Optical density (nm)	
	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**
0	0	0	0	0	0	0	0	0	0	0
6	0.362	0.247	0.248	0.306	0.311	0.118	0.125	0.304	0.316	0.211
12	0.579	0.336	0.366	0.411	0.408	0.366	0.396	0.388	0.482	0.266
18	0.683	0.469	0.489	0.532	0.563	0.502	0.775	0.469	0.597	0.403
24	0.772	0.544	0.534	0.488	0.661	0.518	0.725	0.606	0.657	0.388
30	0.846	0.603	0.577	0.483	0.603	0.577	0.756	0.621	0.608	0.393
Pearson Correlation			0.99	0.93	0.98	0.96	0.93	0.99	0.98	0.97
t-test			4.24	0.11	3.30	0.81	1.57	2.67	2.95	2.80
p-value (at 5%)			0.00	0.45	0.01	0.22	0.08	0.02	0.01	0.01

Table 2. Effect of High and low Temperature on Survival of *E. coli* O157 and *E. coli* non-O157

Time (Hr)	Control		4°C		20°C		40°C		50°C		60°C	
	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**
0	0	0	0	0	0	0	0	0	0	0	0	0
6	0.362	0.247	0.117	0.212	0.156	0.198	0.313	0.302	0.223	0.271	0.301	0.191
12	0.579	0.336	0.267	0.239	0.311	0.275	0.676	0.446	0.56	0.339	0.501	0.302
18	0.683	0.469	0.457	0.4	0.356	0.43	0.828	0.538	0.76	0.432	0.622	0.568
24	0.772	0.544	0.552	0.461	0.482	0.441	0.852	0.604	0.771	0.555	0.681	0.483
30	0.846	0.603	0.586	0.489	0.603	0.407	0.88	0.637	0.711	0.524	0.702	0.471
Pearson Correlation			0.95	0.99	0.96	0.97	0.98	0.98	0.96	0.98	0.99	0.93
t-test			4.76	3.85	4.67	2.68	1.78	3.65	1.04	0.83	3.78	0.97
p-value (at 5%)			0.00	0.01	0.00	0.02	0.06	0.01	0.17	0.22	0.01	0.18

The characteristic protein profile of *E. coli* non-O157 and O157 was seen in lane GLC and HLC respectively (Plate 1). Results shown in Fig. 1 and 2 showed the standard curve of the logarithm of molecular weight versus retention factor. The strong linear relationship (r^2 = 0.984 (Fig.1) and r^2 = 0.981(Fig. 2) between the proteins' molecular weight and migration distance (R_f) demonstrated exceptional reliability in predicting their molecular weight. Therefore, from Fig. 1 and 2, the molecular weight of each of the expressed protein bands in both organisms was extrapolated and shown in Table 4. The protein profile of *E. coli* O157 demonstrated 14 proteins which ranged from 81.66 kDa to 16.96 kDa likewise *E. coli* non-O157 demonstrated 14 proteins which ranged from 81.66 kDa to 19.41 kDa. Statistically, a strong positive correlation exist between the molecular weights of the proteins of the two serotypes and that there was no statistical difference in their protein profile before exposure (r = 0.9897, P-value = 0.941. Comparatively, statistical analysis showed that there was a slight positive correlation in the frequency of protein bands shown in both serotypes after exposure as r = 0.693 and that there was a statistical difference between the two serotypes (P-values = 0.018) in their responses to the environmental stress.

Table 3. Effect of Sunlight of Survival of *E. coli* O157 and *E. coli* non-O157

Time (Hr)	control		Sunlight		Silver nitrate	
	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**	***E. coli* O157**	***E. coli* non-O157**
0	0	0	0	0	0	0
6	0.362	0.247	0.104	0.167	0.238	0.209
12	0.579	0.336	0.116	0.171	0.311	0.284
18	0.683	0.469	0.218	0.396	0.466	0.411
24	0.772	0.544	0.453	0.419	0.533	0.463
30	0.846	0.603	0.502	0.448	0.578	0.485
Pearson Correlation			0.86	0.97	0.98	0.99
t-test			4.39	3.96	4.32	3.55
p-value (at 5%)			0.00	0.01	0.03	0.01

Table 4. Molecular Weight of the Different Expressed Protein Bands of *E. coli* O157 and *E. coli* non-O157

	***E. coli* O157**		***E. coli* non-O157**		**Statistical Value**	
	Retention Factor (R_f)	**Molecular Weights (KDA)**	**Retention Factor (R_f)**	**Molecular Weights (KDA)**	**Correlation (*r*)**	**Significance at 5% (*P*-value)**
1	0.11	81.65072	0.11	81.65824	0.9897	0.941
2	0.19	69.63699	0.18	71.77943		
3	0.28	58.22105	0.22	66.68068		
4	0.32	53.76752	0.34	53.45644		
5	0.38	47.71775	0.41	46.98941		
6	0.42	44.06766	0.45	43.65158		
7	0.46	40.69678	0.51	39.08409		
8	0.52	36.11769	0.55	36.30781		
9	0.58	32.05384	0.6	33.11311		
10	0.64	28.44723	0.67	29.10717		
11	0.71	24.74913	0.88	19.76970		
12	0.77	21.96443	0.78	23.76840		
13	0.83	19.49306	0.86	20.51162		
14	0.9	16.95899	0.89	19.40886		

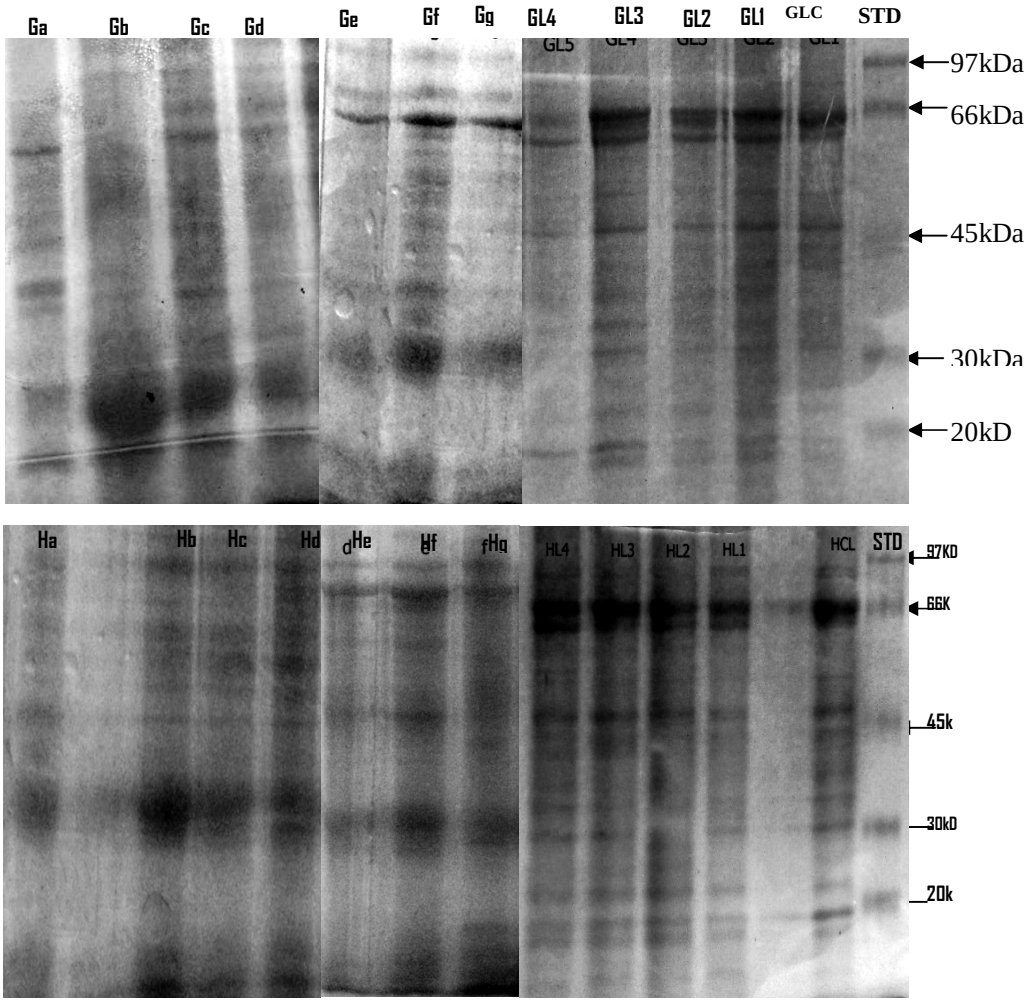

Plate 1. SDS-Poly Acrylamide Gel Electrophoresis Analysis of Bacterial Proins G-Plate consists of *E. coli* non-O157 (S89) protein bands while H-Plate consists of *E. coli* O157 (S3B) protein bands

Key: Lane STD-Standard bands: Phosphorylate (97KDa), Bovine serum albumin (66 KDa), Chicken ovalbumin (45KDa), Soyabeans trypsin inhibitor (30KDa), Bovinelactoglobulin (20KDa). Lane Ga and Ha (silver nitrate), Lane Gb and Hb (sunlight), Lane Gc and Hc (4℃), La ne Gd and Hd (20°C), Lane Ge and He (60°C), Lane Gf and Hf (50°C), Lane Gg and Hg (40℃), Lane GL4 and HL4 (pH 8), Lane GL3 and HL3 (pH 7), Lane GL2 and HL2 (pH 6), GL1 and HL1 (pH 5) and Lane GLC and HLC (Control). (R = 0.693002, P- value = 0.018)

The effect of different treatments carried out on the two serotypes in this study revealed their ability to survive under various treatment methods. Silver nitrate is toxic to microorganisms and this was confirmed in this study as it inhibited the growth of the two serotypes after an exposure. Studies have revealed that when *Escherichia coli* is treated or exposed to silver compound, there is a morphological change in its cell membrane which led to increase in its membrane permeability [9]. Hence, leading to improper regulation of transport through the membrane and resulting into cell death. In addition, it is noticed that silver penetrates the bacterial membrane and causes damage by interacting with phosphorus and sulfur containing compounds such as DNA [10]. The DNA loses its replication ability and cellular protein become inactive. Besides, silver compound may react with proteins and bind protein molecules thereby leading to inhibition of cellular metabolism causing death of the microorganism [9]. This explained the protein profile seen after exposure to silver nitrate.

Studies have shown that there was a significant reduction in coliform forming unit of *E. coli* after exposure to sunlight [3,11]. This corroborates the results of this study. The ultraviolet radiation from sunlight creates highly reactive oxygen species such as superoxide (O^{2-}), hydrogen peroxides (H_2O_2), and hydroxyl radicals (OH^-) which in turn oxidizes microbial cellular components such as nucleic acids, enzymes, and membrane lipids, which kills the microorganisms [3].

Effect of low and high temperature on the two serotypes showed that they were able to survive at low temperature even though, their O. D. readings were reduced. It was noticed that the ability of *E. coli* O157 to survive when exposed to high temperature was much better than the survival of *E. coli* non-O157. This may probably be as a result of the induction of the stress sigma factor, *rpoS,* which allowed *E. coli* O157 to overcome environmental stresses. In a similar study, Ansay et al. [12] reported that *E. coli* O157:H7 survived freezing with some decline in concentration. Equally, Clavero and Beuchat [13], recovered 5 strains of *E. coli* O157:H7 at holding temperature of 60℃ for 0, 15 and 30 minutes by using a non-selective medium (Trypticase Soy Broth), with decrease in the microbial load in the samples, as the heating time increased. This signifies that, *E. coli* O157:H7 could survive at temperatures above 45℃.

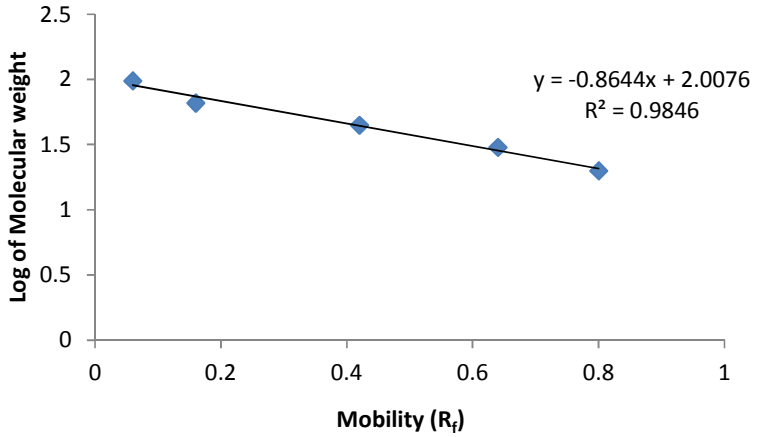

Fig. 1. *E. coli* O157 Standard Curve for Determination of Molecular Weight of an Unknown Protein by SDS- PAGE

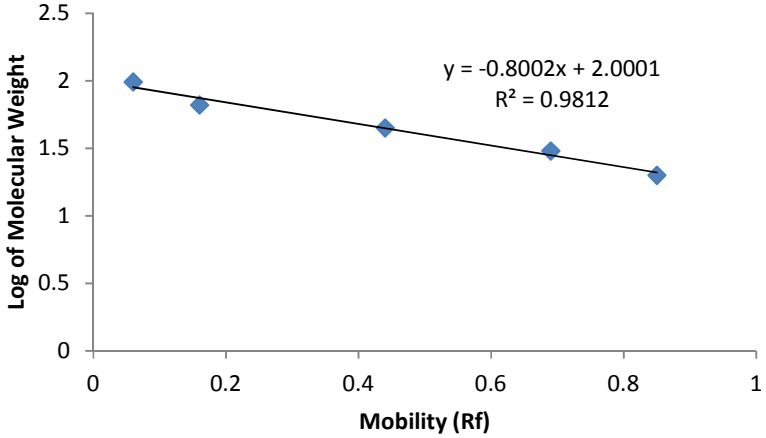

Fig. 2. *E. coli* non-O157Standard Curve for Determination of Molecular Weight of an Unknown Protein by SDS-PAGE

The observation that of *E. coli* O157 survived better than *E. coli* non-O157 at low pH in this study confirmed the findings of other researchers who also reported the survival of *E. coli* O157 in a slightly acidic medium [7,14,15]. Although, *E. coli* O157 strains still showed different capacities to survive in acidic environment, they are superior in their survival over *E. coli* non-O157 [16]. Variation in *rpoS* induction levels might explain the variability in acid resistance of different *E. coli* O157:H7 serotypes [6].

In this study, analysis of protein profile pattern of the two serotypes has been used to distinguish between their characteristic responses towards treatment conditions [17]. This was clarified by Berber [18] who stated that chemical characteristics based on comparative analysis of protein profile can be used for rapid microbial identification. Other researchers have also stated that SDS-PAGE protein profiling is a unique molecular tool which can be trusted and can be repeated with good separation power which has been widely used to identify various microorganisms for taxonomic and epidemiological purposes and in animals for phylogenetic relationship [17,19,20,21,22]. The similarity in molecular weights of their protein bands corroborates the study by Durrani et al. (8) who also recorded similar molecular weights of protein bands of *E. coli* isolated from stool and urine samples. In a similar study, Chong *et al.* [23] and Elshayeb et al. [24] also recorded similar protein profile pattern of *E. coli* in their studies.

4. CONCLUSION

The treatment methods adopted in this study on the test organisms exerted an inhibitory effect on the organisms. This was also buttressed by the protein profile pattern expressed after treatments. In conclusion, the study showed that *E. coli* O157 survived better than *E. coli* non-O157 following exposure to the treatment methods.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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