Vol. 11. No.4. 2022.

Contents available at:
http://www.crdeepjournal.org

International Journal of Life Sciences (ISSN: 2277-193x) CIF: 5.411; SJIF: 6.431
A Peer Reviewed Journal

Full Length Research Paper

Virulence Factors and Capsule Composition of Antibiotic Resistant Biofilm-producing E*scherichia coli* O157 from Ready-to-eat Foods

[1]Babalola, T. O., *[1]David, O. M., [1]Balogun, A., [1]Ilori, T. O., [1]Owabumoye, J. B., [2]Esan, C. O. and [3]Famurewa, O.

Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria.
[2]University Health Centre, Ekiti State University, Ado-Ekiti, Nigeria.
[3]Department of Biological Sciences, Microbiology Unit, Kings University, Odeomu, Nigeria

ARTICLE INFORMATION

Corresponding Author:
David, O. M

Article history:
Received: 07-10-2022
Revised: 12-10-2022
Accepted: 25-10-2022
Published: 27-10-2022

Key words:
Ready-to-eat foods, Escherichia coli O157, biofilm, virulence factors, biocide, capsule.

ABSTRACT

Ready-to-eat (RTE) food is widely consumed in Nigeria though it has been linked with food-borne illnesses. Biofilm forming pathogens persist in RTE foods. Association of Escherichia coli *O157 with RTE foods has not been reported in the study area hence the aim of this study. Out of 205 food samples screened only 63 (30.73%) were positive for* E. coli *while 24 (38.10%) were O157 strains. Thirteen (57.17%) of O157 serotype produced biofilm out of which only three produced strong biofilm. All the biofilm forming* E. coli *O157 strains were resistant to ampicillin, 11 (84.6%) were resistant to amoxicillin/clavulanate while none of the isolates was susceptible to nitrofurantoin. The percentage biofilm inhibition was highest when the isolates were exposed to Morning Fresh® (a liquid soap) followed by JIK® (a sodium hypochloride brand). Among the O157 strains, only 3 (23.1%) were positive for haemolysis, while 8 (61.5%) showed haemagglutination of group O RBCs and 4 (30.8%) produced mannose resistance haemagglutination. Only five of the O157 strain produced capsule. The amount of amino acid produced by strong-biofilm producing strains was higher than weak biofilm producers. All the 13 strains possessed adhesion, biofilm producing and small hydrophobic genes. Most ready-to-eat-foods screens in this study were contaminated with* E. coli *O157 that borne both virulent and biofilm forming traits. The isolates from RTE were also resistant to common antibiotics and their biofilm were not easily removed by biocides. Proper hygiene is needed to forestall the spread of biofilm producing pathogens.*

Introduction

Ready-to-eat (RTE) food is generally consumed cooked, raw, chilled or hot without further processing (Food and Environment, 2001). Non-homemade foods are cooked and presented in such a way that fascinates the people. It is not always about the fascination it is about the time to prepare food to eat, e.g. lack of time people of all age from student to job holder have to rely on non-homemade foods. Usually non-homemade foods are not hygienic; in order to attract the attention of customers and earn more money with less effort, vendors compromise the food quality (Gu *et al*., 2016; Beyi *et al*., 2017). People suffer a lot due to consumption of food prepared and/or stored under poor hygiene. In some cases the raw materials or ingredients used may be the source of the contamination (Pankaj *et al*., 2016). Absence from home, studying while at work or need for a change of diet are among reasons people resort to buying street vended foods (Musa and Akande, 2002). There has been clear empirical evidence that most bacteria that persist in and/or on food produced biofilm (Sun *et al.*, 2022). These sessile cells form an irreversible interaction with the one another, substrates and interface. The bacteria is entrenched in a environment made up of extracellular polymeric substances (EPS). The EPS produced allowed nutrients and water to permeate the entire structure and allow every cell an access to them at the same time protects the sessile cells from antimicrobial agents (Donlan and Costerton, 2002; Lazazzera, 2005; Shemesh *et al*., 2007; Meredith *et al*., 2017). Cells in

biofilm have higher antimicrobials resistance compared with planktonic cells (Nascimento *et al*., 2014; Oliveira *et al*. 2015). The ability of pathogens to produce biofilm enhances their survivability in and outside the host. It encourages their persistence in food processing environment and lead to hygienic problems and eventual economic losses (Brooks and Flint, 2008; Zottola and Sasahara, 1994; Ganesh Kumar and Anand, 1998; Lindsay and von Holy, 2006; Nascimento *et al*., 2014). Bacterial biofilm is a major concern in food processing plants and industries. They present persistent source of contamination and resistance to treatments. Biofilm producing bacteria in food producing environment/kitchens cause serious health-related effects. Pathogenic bacteria that produce biofilms have two arsenals, they are very difficult to eradicate and they are resistant to antibacterials. The occurrence of biofilm producing *E. coli* O157 in ready-to-eat food in vending sites and eateries in Ekiti State, Nigeria was investigated in this study.

Materials and Methods

Sample Collection

A total of two hundred and five (205) non-repeat ready-to-eat food samples were randomly obtained from selected food vending sites and eateries in Ekiti State, Nigeria. The samples were collected into sterile specimen containers and immediately transferred in ice packs to the laboratory of the Department of Microbiology, Ekiti State University, Ado-Ekiti for analyses within one hour of collection. The samples were serially diluted and plated on Nutrient Agar, Eosin Methylene Blue Agar, MacConkey Agar (Oxoid Ltd, Basingstoke, UK) and incubated for 24 hours at 37^{o}C. After incubation, colonies with greenish metallic sheen appearance on EMB agar were sub-cultured onto Sorbitol MacConkey Agar supplemented with cefixime and tellurite (CT-SMAC) (Oxoid Ltd, Basingstoke, UK) and incubated at 37^{o}C for 24 hours. Whitish colonies on the plates were picked and sub-cultured on Nutrient agar slants. The isolates recovered were from CT-SMAC were subjected to standard identification procedures according to Oje *et al*. (2019).

Serological characterization of E. coli O157

Two drops of normal saline (0.85% sodium chloride) was placed on a grease-free clean glass slide and thoroughly mixed with a loopful of 18 h culture of *Escherichia coli*. One drop of antisera was added to the bacterial suspension and to the control to the control (normal saline). The antiserum was mixed with the bacterial suspension. The slide was gently rocked for one minute and observed for agglutination using a magnifying lens. Clumping in only test slide within one minute was taking to be a positive result.

Antibiotic Sensitivity Test

The disc diffusion method was used for the susceptibility testing of the isolates to different antibiotics as described by Clinical and Laboratory Standard Institute (CLSI, 2012). Each of the isolates was grown at 37ºC in Mueller-Hinton broth (Oxoid) for 18 h and diluted to an optical density of 0.1 (0.5 McFarland Standard) according to CLSI (2012). The isolates were seeded on sterile Mueller-Hinton Agar (Oxoid), the antibiotic discs gently place on the plate at equidistance and incubated at 37 ºC for 18 hours. The zone of clearance around each of the antibiotics was measured and interpreted according to CLSI (2012). The commercial antibiotics tested include: ampicillin (10 µg), amoxicillin-clavulanate (30 µg), ceftazidime (30 µg), gentamicin (10 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), ofloxacin (5 µg) and cefuroxime (30 µg).

Detection of Biofilm Production

Biofilm Formation Assays (Qualitative Assay)

Biofilm producing *E. coli* O157 strains was detected by streaking the isolates on nutrient agar supplemented with 0.08 % Congo Red as described by Mathur *et al*. (2006). The plates were incubated at 37^{o}C in aerobic condition for 24 h and left at room temperature for 48 h. The result was interpreted according to their colony phenotypes. Isolates that developed black colonies with dry and smooth consistency with rough and round edges and shiny surface were considered a positive indication of biofilm production while colonies with no black pigmentation was considered to be non-biofilm producers.

Quantification of Biofilm

All biofilm forming strains of *E. coli* O157 were grown at 37^{o}C for 18 h as a pure cultures on Tryptone Soya Broth and diluted to equal 0.5 MacFarland Standard. This dilution was used as the inoculum in the test tubes. Each of the isolates was inoculated into the test tubes and incubated at 37^{o}C for 24 h according to Christensen *et al*. (1982). The content of each tube was aspirated and gently washed three times with sterile physiological saline. To each of the tubes, 5 mL crystal violet (0.5%) solution was added and allowed to stay for 15 minutes at room temperature. The tubes were gently washed with sterile distilled water three times. Five mililitre of 95% ethanol was added to each if the tubes and the optical density (OD) of the content of the tube was measured spectrophotometer at 520 nm. The result was interpreted according to Siegfried *et al*. (1994).

Detection of Virulence Factors in the Isolates

Test for Haemolysis

Test *E. coli* strains were streaked onto Mueller-Hinton agar supplemented with 5% human blood agar and incubated for 18 h at 37^{o}C. Colonies surrounded with a clear haemolytic zone was interpreted to be due to the production of haemolysin as described by Raksha *et al*. (2003).

Haemagglutination of Human Group O Erythrocytes

A drop of erythrocytes of human (with blood group O) and a drop of phosphate-buffered saline (PBS) with and without 3% mannose were placed on a clean grease free glass slide. The slide was gently rocked for 5 min at room temperature. The formation of haemagglutination was observed when viewed under a light microscope with 40× magnification. Haemagglutination was recorded as mannose-resistant (MRHA). When it occurred in the presence of D-mannose and mannose sensitive (MSHA), when it was inhibited by the presence of D mannose (Siegfried *et al*., 1994).

Cell Surface Hydrophobicity (CSH)

A 40 µL of 0.2 M phosphate-buffered saline PBS (pH 6.8) was dropped on a three sterile grease free slides while another forty microliter of different concentrations (1.0 M, 1.4 M and 2.0 M) of ammonium sulphate were added to the slides. Forty microliter of 18 h old culture of *E. coli* suspension (5 x 10^9 cfu/mL) was added to each of these slides. The agglutination formed in different molar concentrations of ammonium sulphate were observed at low power magnification of light microscope. Strains were considered hydrophobic if aggregation in a concentration of 1.4 M was observed (Siegfried *et al*., 1994).

Determination of Minimum inhibitory Concentration (MICs) and Maximum Tolerance Concentrations (MTCs)

Twofold serial dilutions of biocides were prepared and added to make up to final concentrations in Mueller Hinton broth. The tubes were incubated at 37°C for 24 h. The tube with least concentration and with no sign of growth was taken for MIC while the highest concentration with visible sign of growth was recorded as maximum tolerance concentrations.

Determination of Biofilm Inhibitory Potential of the Biocide

The method of David and Afolayan (2013) was used to determine the percentage biofilm inhibition of selected biocides on the *E. coli* O157. Mueller-Hinton broth (5 mL) was prepared in the test tubes and inoculated with the test organism. The tubes were incubated at 37°C for 6 h and maximum tolerance concentrations (MTCs) of biocides were added before incubation aerobically for 24 h at 35°C. After the incubation, the contents of tubes were discarded and the tubes washed three times with sterile distilled water. The remaining attached bacteria were fixed with methanol after 15 min of which the tubes were emptied; air dried and stained with 10 ml of 1.0% crystal violet for 5 min. Excess stain was rinsed off with water. After the plates were air dried, the dye bound to the adherent cells was extracted with 10 mL of 33% (v/v) glacial acetic acid (Merck, Darmstadt, Germany) per tube. The optical density of each tube was measured at 570 nm using UV-3000 PC spectrophotometer (Optima Scientific). The percentage reduction in biofilm was calculated as

%Biofilm inhibition= [(AC-AT)/AC] x100

Where, AC = absorbance of the control, AT = absorbance of the test

Determination of Biofilm Eradication Potential of the Biocide

Tubes containing Mueller-Hinton broth (5 mL) was inoculated with the biofilm-forming *E. coli* O157 and the tubes were incubated at 37°C for 6 h and concentrations 2 x MIC of biocides. The tubes were further incubated aerobically for 24 h at 35°C. The quantity of biofilm produced after incubation was determined as reported earlier.

% Biofilm eradication = [(AC-AT)/AC] x100

Where, AC = absorbance of the control, AT = absorbance of the test

Detection and Harvest of Bacterial Capsule

The biofilm-producing *E. coli* strains were examined for the production of capsule according to Fawole and Oso (2004). Capsule (exo-polysaccharide) produced by the isolates was harvested as described by Thangapandian *et al*. (2014) with slight modification. Briefly, strains of capsule producing *E. coli* were inoculated into 100 ml MacConkey broth supplemented with 20 % sucrose and incubated at 37 ^{o}C for 24 h. After the initial inoculation, 8 % inoculum was further inoculated into 250 ml of 20% glucose-MacConkey broth for the production of capsule. The capsule was recovered by using ethanol precipitation. The broth was centrifuged at 8000 rpm for 20 min. The supernatant was collected and cell pellets were discarded. Then, chilled ethanol was added in 2:1 (ethanol: supernatant) ratio. The solution was stirred and stored at room temperature for 24 h. After 24 h the supernatant was carefully separated from the capsules.

Determination of Amino Acid Profile of the crude capsule

The composition of amino acid in the crude capsule produced by the isolates was carried out using High performance liquid chromatography (HPLC) with detecting wavelength of 200 nm. The Kromasil SIL column used for the analyses of the amino acid profile has a 250 mm length, 4.6 mm diamemter and 5 m particle sizes. The mobile phase of the column A and B was 25:75, whereas mobile phase A was 2.5 mM Potassium dihydrogen phosphate with pH 2.85 and mobile phase was acetone nitrile, for the quantification of amino acid. The column oven temperature was maintained 30^{o}C in all experiments.

Detection of Virulence Gene by Polymerase Chain Reaction

The presence of *IcaA, SH* and *Clf A* genes were detected in the isolates using the PCR method as described by Olsvik and Strockbin (1993). The following primers were used in this study: Adhesion (*IcaA*) gene primer with forward sequence of

5'TCTCTTGCAGGAGCAATCAA3' and reverse sequence of 5'TCAGGCACTAACATCCAGCA3' (Paluch-Oles *et al*., 2011), Biofilm producing, ClfA gene primer forward: 5'CCGGATCCGTAGCAGATGACC3' reverse: 5'GCTCTAGATCACTCATCAGGTTGTTCAGG3' (Arciole *et al*., 2001), Small hydrophobic (SH) gene primer, forward: 5'AACTCTGTATGTCTGGACTATATTG3' and reverse of 5'CTTGTTCTAGCGTGACGGAT3' as previously described by Depardieu *et al*. (2004).

A 2 µL of PCR amplification mixture contained deionized sterile water, 12.5l Green Go Taq Master Mix pH 8 (Promega, USA) contained [(50unit/ml) of Go Taq DNA polymerase, (400 Mm) of each dNTPs and (3 mM) of MgCl2], 1pmol for specific primers (Alpha DNA, Canada). The PCR cycles for the genes were as follow initial denaturation at initial temperature-second (94-120), number of cycles (30), denaturation temperature 54 for 60 seconds, extension temperature of 72 for 60 seconds and the final extension of 72 for 600 s using Gradient PCR (TechNet500, USA). PCR products were electrophoresed on 1.5% agarose gels in TAE buffer (40 mM Trisacetate pH 8.0; 2 mM EDTA). Electrophoresis was performed on horizontal gel and the DNA samples were directly loaded into the gels. Samples were electrophoresed alongside a 100-bp DNA ladder (Sigma-Aldrich). Electrophoresis was performed at a constant voltage (100 V) until the loading buffer fronts had moved to nearly the end of the gel. After electrophoresis, gels were stained in 0.5 mg/L ethidium bromide and visualized on a UV transilluminator. Photographs were taken by a digital camera.

Results

In this study, 205 non-repeat ready-to-eat food samples were randomly obtained from the food vending sites and eateries in Ekiti State, Nigeria. *Escherichia coli* was detected in only 63 of the samples while only 24 of the *E. coli* were O157 strains. Thirteen (57.17%) of O157 serotype were biofilm formers. Out of these 13 biofilm-forming isolates, only three were strong biofilm formers, six (6) were moderate biofilm producers while four produced weak biofilm (Table 1). Antibiotic susceptibility testing showed that all the biofilm forming *E. coli* O157 isolates were resistant to ampicillin, 11 (84.6 %) were resistant to amoxicillin/ clavulanate, 8 (61.5%) to ciprofloxacin, cefuroxime and ceftadizime while 7 (53.8%) were resistant to ofloxacin. Only 3 (23.8%) of the isolates were resistant to gentamycin while all the isolates were susceptible to nitrofurantoin (Table 2).

Table 1: Quantification of biofilm produced from *E. coli* isolated from ready-to-eat food

No of isolates	Optical density	Type of biofilm formed
E. coli 28	0.28	Moderate
E. coli 47	0.31	Moderate
E. coli 39	0.31	Moderate
E. coli 24	0.15	Weak
E. coli 45	0.14	Weak
E. coli 42	0.26	Moderate
E. coli 29	0.36	Strong
E. coli 14	0.18	Weak
E. coli 48	0.36	Strong
E. coli 30	0.35	Strong
E. coli 27	0.12	Weak
E. coli 16	0.30	Moderate
E. coli 31	0.30	Moderate

Table 2: Phenotypic Pattern of biofilm forming *E. coli* isolated from ready to eat food samples

Isolates	Resistant Pattern
E. coli 28	CAZ^+, CRX^+, GEN^+, CPR^+, OFL^+, AUG^+, AMP^+
E. coli 47	CAZ^+, CRX^+, AUG^+, AMP^+
E. coli 39	CAZ^+, CRX^+, CPR^+, OFL^+, AUG^+, AMP^+
E. coli 24	CAZ^+, CPR^+, OFL^+, AUG^+, AMP^+
E. coli 45	CAZ^+, AUG^+, AMP
E. coli 42	CAZ^+, GEN^+, CPR^+, AUG^+, AMP^+
E. coli 29	CPR^+, AUG^+, AMP^+
E. coli 14	CRX^+, GEN^+, CPR^+, OFL^+, AUG^+, AMP^+
E. coli 48	OFL^+, AUG^+, AMP^+
E. coli 30	CAZ^+, AUG^+, AMP^+
E. coli 27	CAZ^+, CRX^+,CPR^+,OFL^+, AUG^+, AMP^+
E. coli 16	CAZ^+, CRX^+, OFL^+, AUG^+, AMP^+
E. coli 31	CRX^+, CPR^+, AMP^+

Keys: *CAZ= Ceftazidime, CRX= Cefuroxime, GEN=Gentamicin, CPR=Ciprofloxacin, OFL=Ofloxacin*

AUG= Amoxycilin/Clavulanate, AMP= Ampicillin.

Multiple antibiotic resistance was exhibited by 4 (30.8%) of *E. coli* O157 biofilm formers in this study showing multiple resistance to three different antibiotics and three resistance patterns (resistitypes). Another 3 (23.1%) exhibited multiple resistance to four antibiotics showing three resistance patterns. Two strains (15.1%) of *E. coli* O157 biofilm formers showed multiple resistance to five antibiotics with two different patterns. Another set of 2 (23.1%) showed multiple resistance to six antibiotics with two different patterns. Only one organism (7.8%) showed multiple resistance to seven antibiotics and it occurs only in one pattern (Table 3). The percentage biofilm inhibition was highest when the isolates were exposed to Morning Fresh (a liquid soap) followed by JIK. The least biofilm inhibitory ability was observed in when the test organisms were treated with maximum tolerance concentration of Dettol. Compared to the control, four isolates were able to produce higher amount of biofilm when exposed to 2 x MIC of JIK while 9 and 8 produced more biofilm when exposed to Dettol and morning fresh respectively. The biofilm forming ability of the *E. coli* 29 was mainly affected when exposed to the biocides at different concentrations (Table 4).

Table 3: Phenotypic resistance pattern of *Escherichia coli* O157 biofilm formers organisms to antibiotics

No of antibiotics	Phenotypic pattern	No of organisms
3	AMP/AUG/CAZ	2
3	AMP/AUG/OFL	1
3	AMP/CPR/CRX	1
Total		4 (30.8)
4	AMP/AUG/CRX/CAZ	1
4	AMP/AUG/OFL/CPR	1
4	AMP/AUG/CPR/CRX	1
Total		3 (23.1)
5	AMP/AUG/CPR/GEN/CAZ	1
5	AMP/AUG/OFL/CRX/CAZ	1
Total		2 (15.1)
6	AMP/AUG/OFL/CPR/CRX/CAZ	2
6	AMP/AUG/OFL/CPR/GEN/CRX	1
Total		3 (23.1)
7	AMP/AUG/OFL/CPR/GEN/CRX/CAZ	1
Total		1 (7.8)

Values in parenthesis are percentage values

Table 4: Percentage inhibition and eradication of *E. coli* O157 biofilm by biocides

Test Organisms	JIK® (a brand of sodium hypochloride)		Dettol® (A disinfectant)		Morning Fresh® (A liquid soap)	
	BI	BE	BI	BE	BI	BE
E. coli 28	47.92	12.52	44.26	-20.40	50.18	5.84
E. coli 47	39.78	-0.40	24.36	-1.60	48.72	-6.08
E. coli 39	48.40	14.00	30.77	25.00	25.90	29.87
E. coli 24	47.92	12.52	48.72	4.00	55.82	-13.80
E. coli 45	35.91	-12.48	24.87	-18.00	73.44	-62.40
`*E. coli* 42	55.49	13.88	38.49	4.00	50.06	-25.40
E. coli 29	66.15	18.52	46.10	20.00	48.71	-3.52
E. coli 14	44.82	12.52	28.20	-13.60	34.26	-11.11
E. coli 48	48.90	-14.04	40.97	-5.60	48.72	1.76
E. coli 30	48.59	18.36	33.33	-17.20	48.76	-28.00
E. coli 27	22.64	18.76	48.72	-6.00	24.62	2.80
E. coli 16	35.64	-0.12	23.00	-18.22	40.46	20.00
E. coli 31	35.10	17.76	51.95	-8.00	69.23	-23.80

BI= biofilm inhibition, BE= biofilm eradication

As shown in Table 5, only 3 (23.1%) were haemolystic, 8 (61.5%) isolates showed haemagglutination (HA) of group O RBCs while 4 (30.8%) were mannose resistance haemagglutination (MRHA) and other 4 (30.8%) were mannose sensitive haemagglutination (MSHA) while 11 (84.6%) isolates were positive for cell surface hydrophobicity (CSH). Out of the 13 biofilm-forming *E. coli* O157 strains only five produced capsule and one each was selected among strong biofilm and weak biofilm formers. *Escherichia coli* (isolate 29), a strong biofilm former had higher amount of amino acid (valine, proline, phenylalanine, tyrosine, methionine and histidine) than the weak biofilm former (isolate 16). However, the total amounts of amino acid in isolate 29 was higher than that of isolate 16 as shown in Table 6.

Table 5: Virulence factors detected in *Escherichia coli* O157 biofilm formers (n=13)

Test	Positive	Negative
Haemolysin	3 (23.1)	10 (76.9)
Haemagglutinin	8 (61.5)	5 (38.5)
MRHA	4 (30.8)	0
MSHA	4 (30.8)	0
CSH	11(84.6)	2 (15.4)

Figures in parenthesis are percentage values. MRHA = D-mannose resistant Haemagglutination, MSHA = D-mannose susceptible Haemagglutination, CSH = Cell surface Hydrophobicity

Table 6: Comparison between amino acid profile of biofilm of *E. coli* O157 isolates 16 and 29 (g/16g of N)

Amino Acids	Biofilm former strains	
	***E. coli* 16 (WBF)**	***E. coli* 29 (SBF)**
Glutamate	12.18357	12.61618
Aspartate	9.37424	9.82523
Alanine	8.91660	9.21538
Leucine	7.89898	8.18171
Glycine	7.06107	7.21654
Lysine	5.94986	6.16367
Valine	5.44894	5.17169
Threonine	4.88502	4.95560
Isoleucine	4.50056	5.91486
Serine	4.39161	4.76441
Proline	4.28994	4.27348
Phenylalanine	4.04772	3.78831
Arginine	3.17606	4.00954
Tyrosine	2.64458	2.32230
Methionine	2.59246	2.01426
Histidine	2.50978	2.03619
Cystine	1.31996	1.39970

Keys: WBF Weak Biofilm Formers, SBF Strong Biofilm Formers

The polymerase chain reaction revealed that all the 13 isolates showed necessary amplification using adhesion (*ICA*) gene and biofilm-producing (*Clf A*) gene primers with band of 1351 bp and 1235 bp, 11 showed amplification with 970 bp while only two isolates did not show amplification using small hydrophobic (*SH*) gene (Fig. 1-3).

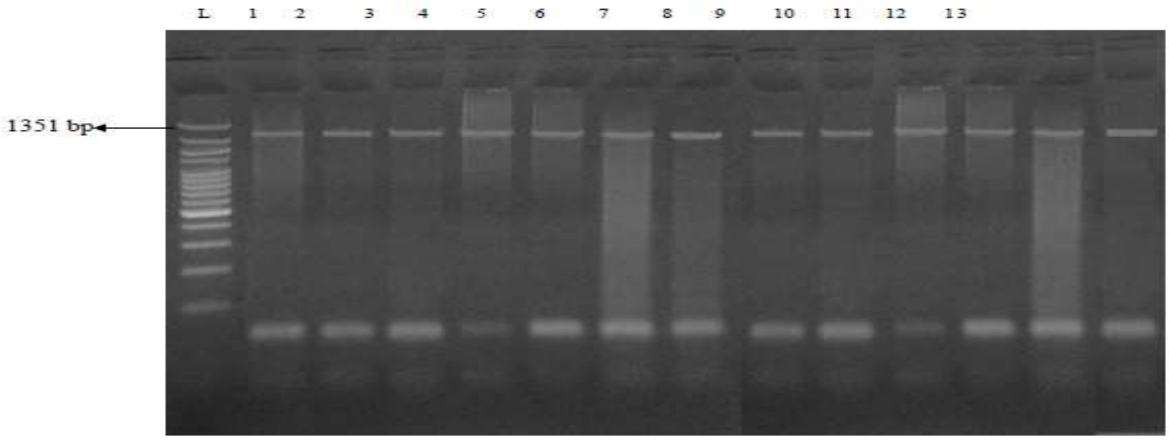

Fig 1: PCR amplification result of *Ica* gene, in *Escherichia coli* isolates Lane M= lane 1500 pb marker, Lane 1= *E. coli* 28, Lane 2= *E. coli* 47, Lane 3= *E. coli* 39, Lane 4= *E. coli* 24, Lane 5= *E. coli* 45, Lane 6= *E. coli* 42, Lane 7= *E. coli* 29, Lane 8= *E. coli* 14, Lane 9= *E. coli* 48, Lane 10= *E. coli* 30, Lane 11= *E. coli* 27, Lane 13= *E. coli* 16 and Lane 13= *E. coli* 31

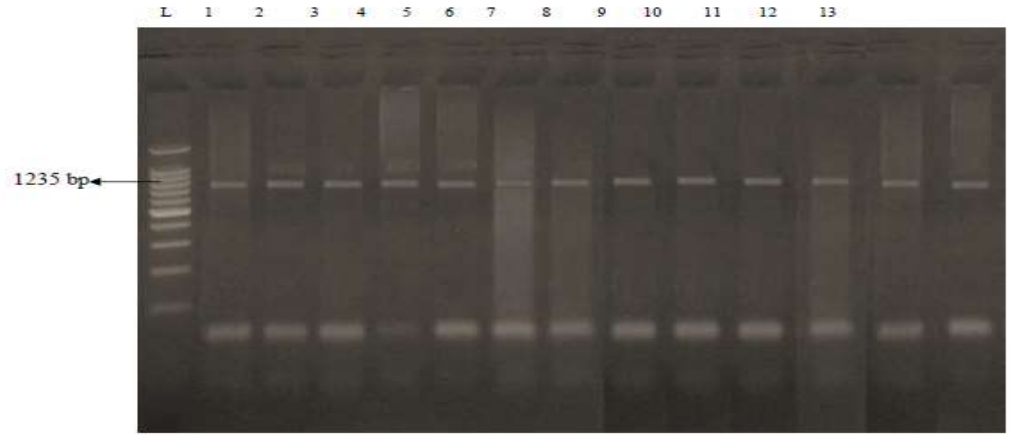

Fig. 2: PCR amplification result of *Clf A* gene, in *Escherichia coli* isolates Lane M= lane 1500 pb marker, Lane 1= *E. coli* 28, Lane 2= *E. coli* 47, Lane 3= *E. coli* 39, Lane 4= *E. coli* 24, Lane 5= *E. coli* 45, Lane 6= *E. coli* 42, Lane 7= *E. coli* 29, Lane 8= *E. coli* 14, Lane 9= *E. coli* 48, Lane 10= *E. coli* 30, Lane 11= *E. coli* 27, Lane 13= *E. coli* 16 and Lane 13= *E. coli* 31

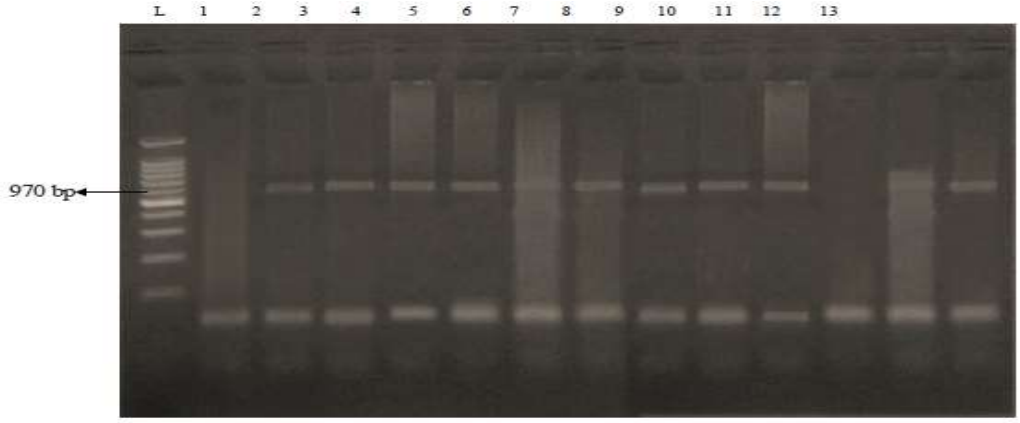

Fig. 3: PCR amplification result of *SH* gene, in *Escherichia coli* isolates Lane M= lane 1500 pb marker, Lane 1= *E. coli* 28, Lane 2= *E. coli* 47, Lane 3= *E. coli* 39, Lane 4= *E. coli* 24, Lane 5= *E. coli* 45, Lane 6= *E. coli* 42, Lane 7= *E. coli* 29, Lane 8= *E. coli* 14, Lane 9= *E. coli* 48, Lane 10= *E. coli* 30, Lane 11= *E. coli* 27, Lane 13= *E. coli* 16 and Lane 13= *E. coli* 31

Discussion

In this study, two hundred and five (205) RTE food samples were screened for the detection of *E. coli*. Out of 63 strains of *E. coli* isolated from the food samples only 24 were detected to be O157 strains. This is in line with the works of Hojjat *et al*. (2016) that isolated *E. coli* O157 from RTE foods in Nigeria. Human infections with *E. coli* O157 have been reportedto be transmitted through consumption of contaminated foods (Hojjat *et al*., 2016; Beyi *et al*., 2017).

There was high antibiotic resistance among biofilm producing *E. coli* O157 in this study. This may be because of the possession of biofilm and could be attributed to the extra-cellular polymeric substances (EPS) acts as obstruction, thus preventing infiltration antibiotics (Thien and O'toole, 2001; Donlan and Costerton, 2002; Mahmoud, 2016). Biofilm production makes producing organisms resistant and increases the chances of contamination of the final products; ready-to-eat foods (Olawale *et al.*, 2015a,b). Raw materials provide an excellent growth environment for the bacteria. Morris *et al*. (1997) reported that basil, celery, Chinese cabbage, leeks, lettuce, parsley and spinach encouraged the growth of and EPS formation by *E. coli* O157:H7. Apart from the floors, waste water pipes, rubber seals and stainless steel surfaces, the major factor that responsible for the spreading and persistence of biofilm-forming pathogens in ready-to-eat foods are improperly cleaned and sanitized equipment (Kumar and Anand, 1998). Disinfectants and dish washing soaps are well used for the removal of germs and for cleaning purpose in most of our canteen and eateries investigated in Ekiti State. The ability of the three biocides commonly used in the eateries investigated was evaluated. Prior to the formation of the biofilms the biocides were very effective in removing the organism and considerably inhibited the biofilm forming ability. On the other hand, 2xMIC of the biocides were unable to eradicate the preform biofilm. An effective cleaning procedure should be able to break up or dissolve EPS giving way to sanitizers to get in contact to the viable sessile cells (Olawale *et al.*, 2015a,b; Stiefel *et al*., 2016). As reported by Chmielewski and Frank (2003) alkaline cleaners with metal chelators agents have low biofilm eradication concentrations compared to the acidic cleaners. Disruption of bacterial EPS makes them more susceptible to

sanitizers (Bridier *et al*., 2015; Wang *et al*., 2016). The increase of carbon/nitrogen ratio in the substrate correspondingly resulted in the increase in the polysaccharide/protein ratio (Dickson and Koohmaraie, 1989; Huang *et al*., 1994; Boyer *et al*., 2007). In most cases the tested concentrations of the biocides in this study encouraged the formation of biofilm. Kumar and Anand (1998) reported that oxidizing disinfectants depolymerize and detached biofilm. The organisms in the biofilm were resistant to the concentration of the biocides. Planktonic cells, of the same species, are about 10 - 1000 times more susceptible to antibiotics compared to the cells in biofilm (Nickel *et al*., 1985; Mah and O'toole 2001).

Haemolytic activity of *E. coli* means the ability of this organism to lyse red blood cells (in vitro). Out of the 13 *E. coli* biofilm formers isolated in this study only three has haemolytic effect on red blood cells. Stimulation of bacterial growth and acquisition of iron from the host appears tobe the main way by which haemolysin contributes to the virulence of *E. coli* (Fatima *et al*., 2012). The ability of the *E. coli* strain to produce agglutination of human erythrocytes by shows an indirect possession of fimbriae on that particular *E. coli* strains (Kallenius *et al*., 1980). Only four of eight that were positive for agglutination of human erythrocytes were mannose resistant (MRHA) which means that the strains possess P fimbriae which is the adhesin that has been most closely associated with uropathogenic *E. coli*. P fimbriae bind specifically to the P blood group antigen which contain D-galatose-D-galatose residue. The change of environmental conditions forces an adaptive modification in the microorganism which enhances its ability to survive. One of the many mechanisms involved in this process is the release of outer membrane vesicles (MV) in Gram- negative bacteria causing a significant increase in cell surface hydrophobicity and an enhance tendency to form biofilms (Baungarten *et al*., 2012). The hydrophobic of microbial surface are conducive to adhesion to abiotic and biotic surface and to penetration of host tissues (Rodrigues and Elimelech, 2009). Eleven out of the biofilm producing *E. coli* isolated in this study were positive for CSH while other two were negative. The general trend is that the amount of *E. coli* biofilm formed increased with increasing metabolizable substrate concentrations, as previously demonstrated by Buhler *et al*. (1998). This present study also conforms to this study, when the isolates were exposed to 2 x MIC some of the isolates produced more biofilm. As reported by Holubar *et al*. (1999) the amount of the biofilm produced by the bacterial isolates is largely dictated by the amount of available substrate available. Polymerase chain reaction (PCR) amplification was used to test for adhesion (*Ica*) gene, biofilm producing (*Clf* A) and small hydrophobic (*SH*) gene as described previously. Deighton and Balkau (1990) reported a high correlation between biofilm forming and the virulence of the producers. There is a dearth of information on polysaccharide intercellular adhesin (*ica*) gene in the *E. coli* O157 strains from ready-to-eat foods. Heilmann *et al*. (1996a) reported that the polysaccharide intercellular adhesin genes in bacteria coded for the biosynthesis of the polysaccharide intercellular adhesion which initiates the accumulative stage of biofilm formation. The cell aggregation and biofilm accumulation offer protection for the sessile cells in the matrix of the polysaccharide against biocides (Heilmann *et al*., 1996; Ziebuhr *et al*., 1997).

The apparent presence of virulence and biofilm enhancing in the thirteen *E. coli* O157 biofilm formers strongly suggests that the isolate could poses a threat to the health of the consumers. Deighton and Balkau (1990) reported a high correlation between biofilm forming and the ability of the organism to cause disease in a clinical setting. There is a dearth of information on polysaccharide intercellular adhesin (*ica*) gene in the *E. coli* O157 strains from ready-to-eat foods. Heilmann *et al*. (1996a) reported that the polysaccharide intercellular adhesin genes in bacteria coded for the biosynthesis of the polysaccharide intercellular adhesion which starts the acquisitive phase of biofilm development. The cell aggregation and biofilm accumulation offer protection for the sessile cells in the matrix of the polysaccharide against biocides (Heilmann *et al*., 1996b; Ziebuhr *et al*., 1997).

Conclusion

From this study, it is evident that some ready-to-eat-foods are contaminated with *E. coli* O157. Aside from biofilm production, most of the isolates possessed virulence factors. The isolates from RTE were also resistant to common antibiotics and their biofilm were not easily eradicated by biocides. Not all the isolates produced capsule. There were differences in the composition of the amino acids of the capsule of both weak and strong biofilm forming *E. coli* O157 from RTE. Proper hygiene should be a priority in food industry.

Acknowledgements

The authors acknowledged the technical assistance of Mr. S. S. Ajayi, the chief technologist in the Departments of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria for his technical assistance.

References

Arciola, C. R. Collamati, S. Donate, E. and Montanoro, L. (2001). A rapid PCR method for detection of slime –producing strain of S. epidermidis and S. aureus in peripros thesis infections. *Diagnostic Molecular Pathology*, 10:130-137.

Baungarten, T., Sperling, S., Seifert, J., von Bergen, M., Steiniger, F. and Wick, L. (2012). Membrane vesicle formation as a multiple-stress response mechanism enhances *Pseudomonas putida* DOT-T1E cell surface hydrophobicity and biofilm formatiom. *Applied Journal of Microbiology and Biotechnology*, 93,837-845.

Beshiru, A., Okoh, A. I. and Igbinosa, E. O. (2022). Processed ready-to-eat (RTE) foods sold in Yenagoa Nigeria were colonized by diarrheagenic *Escherichia coli* which constitute a probable hazard to human health. *PLoS ONE,* 17(4): e0266059.

Beyi, A. F., Fite, A. T., Tora, E., Tafese, A., Genu, T., Kaba, T., Beyen, T. J., Beyene, T., Korsa, M. G., Tadesse, F., De Zutter L., Goddeeris, B. M. and Cox, E. (2017). Prevalence and antimicrobial susceptibility of Escherichia coli O157 in beef at butcher shops and restaurants in central Ethiopia. *BMC Microbiology*, 17:49.

Boyer, R. R., Sumner, S. S., Williams, R. C., Pierson, M. D., Popham, D. L. and Kniel, K. E. (2007). Influence of curli expression by *Escherichia coli* O157:H7 on the cell's overall hydrophobicity, charge, and ability to attach to lettuce. *Journal of Food Protection*. 70: 1339-1345.

Buhler, T. S. and Ballestero, A. M. (1998). Generation of a reproducible nutrient depleted biofilm of *Escherichia coli* and *Burkholderia cepacia*. *Journal of Applied Microbiology.* 85(3): 457-462.

Clinical and Laboratory Standards Institute, CLSI. (2012). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second informational supplement. CLSI document M100-S22. CLSI, Wayne, PA

David, O. M. and Afolayan, A. J. (2013). Activity of *Sansevieria aethiopica* (Thunberg) leaf extracts on the planktonic and the biofilm of *Candida albicans* ATCC 10231. *Journal of Medicinal Plants Research.* 7(36): 2662-2668.

Deighton, M. A. and Balkau, B. (1990). Adherence measured by microtiter assay as a virulence marker for *Staphylococcus epidermidis* infections. *Journal of Clinical Microbiology*. 28(11): 2442–47.

Depardieu, F., Podglajen, I., Leclercq, R., Collatz, E. **and** Courvalin, P. ***(*2007). *Modes** and modulations of antibiotic resistance gene expression. Clinical Microbiology Reviews.* 20**:** 79-*114.*

Dickson, J. S. and Koohmaraie, M. (1989). Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. *Applied Environmental Microbiology.* 55: 832-836.

Fatima, N., Agrawal, M., Shukla, I. and Khan PA (2012) Characterization of uropathogenic *Escherichia coli* in relation to virulence Factors. 1:342. *doi:10.4172/scientificreports*.342.

Fawole, M. O. and Oso, B. A. (2004). *Laboratory Manual of Microbiology*. Spectrum Books Limited, Nigeria. pp 22-33.

Heilmann, C., Gerke, C., Perdreau-Remington, F. and Gotz, F. (1996a). Characterization of Tn*917* insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. *Infection and Immunity*. 64:277–282.

Heilmann, C., Schweitzer, O., Gerke, C., Vanittanakom, N., Mack, D. and Gotz, F. (1996b). Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. *Molecular Microbiology*. 20: 1083–1091.

Holubar, P. and Andorfer, C. (1999). Effects of nitrogen limitation on biofilm formation in a hydrocarbon-degrading trickle-bed filter. *Applied Microbiology and Biotechnology.* 51(4): 536-540.

Huang, C. T., Hassett, D. J., Ma, J. F., Elkins, J. G., McDermott, T. R., Ochsner, U. A., West, S. E., Fredericks, J., Burnett, S., Stewart, P. S., McFeters, G., Passador, L. and Iglewski, B. H. (1994). Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. *Molecular Microbiology.* 34: 1082–1093.

Igbinosa, E. O., Beshiru, A., Igbinosa, I. H., Ogofure, A. G., Uwhuba, K. E. (2021). Prevalence and characterisation of food-borne *Vibrio parahaemolyticus* from African salad in Southern Nigeria. *Frontials in Microbiology.* 12: 632266.

Kallenius, G., Mollby R. and Winberg, J. (1980) *In vitro* adhesion of uropathogenic *Escherichia coli* to human periurethral cells. *Infection and Immunity.* 28: 972-980.

Kumar, C. G. and Anand, S. K. (1998). Significance of microbial biofilms in food industry: a review. *International Journal of Food Microbiology.* 42: 9-27.

Mathur, T., Singhal, S., Khan, S., Upadhyay, D. J., Fatma, T. and Rattan, A. (2006). Detection of biofilm formation among the clinical isolates of *Staphylococci*: An evaluation of three different screening methods. *Indian Journal of Medicine and Microbiology.* 24: 25-29.

Morris, C. E., Monier, J. M. and Jacques, M. A. (1997). Methods for observing microbial biofilms directly on leaf surfaces and recovering them for isolation of culturable microorganisms. *Applied Environmental Microbiology.* 63: 1570-1576.

Nickel, J. C., Ruseska, I., Wright, J. B. and Costerton, J. W. (1985). Tobramycin resistance of *Pseudomonas aeruginosa* cells growing as a biofilm on urinary catheter material. *Antimicrobial Agents and Chemotherapy* .27:619-624.

Oje, O. J., Adelabu, O. A., Adebayo, A. A., Adeosun, O. M., David, O. M., Moro, D. D. and Famurewa, O. (2019). Antibiotic resistance profile of β-lactamase-producing *Escherichia coli* O157:H7 isolated from ready-to-eat foods in Ekiti State, Nigeria. *Global Journal of Current Research.* 7(1): 20-27.

Olawale, A. K., David, O. M., Oluyege, A. O., Osuntoyinbo, R. T., Laleye, S. A. and Famurewa, O. (2015a). Histopathological changes induced in an animal model by potentially pathogenic *Enterococcus faecalis* strains recovered from ready-to-eat food outlets in Osun State, Nigeria. *Infection and Drug Resistance.* 8: 1–7.

Olawale A. K., Onasanya A., Oyelakin O. O., Olawale A. O., Olaolu A. O., Oje O. J., David O. M., and Famurewa O. (2015b). Molecular characterization and distribution of virulence determinants among *Enterococcus faecalis* from ready-to-eat food outlets. *International Journal of Microbiology and Application*. 2(6): 88-92.

Oliveira, N.M, Martinez-Garcia, E., Xavier, J., Durham, W.M., Kolter, R., Kim, W. and Foster, K.R. (2015). Biofilm formation as a response to ecological competition. *PLoS Bioologyl.* 13(7): e1002191. doi:10.1371/journal.pbio.1002191

Olsvik, O. and Strockbin, N. A. (1993). PCR detection of heat-stable, heat-label and shiga-like toxin genes in Escherichia coli. In: Persing DH, Smith TF, Tenover FC, White TJ. *Diagnostic Molecular Microbilogy.* 9th ed. American Society for Microbiology. Washington, DC.

Paluch-Oles, J., Magrys, A., Kozioł-Montewka, M., Niedzielski, A., Wiadek J. N., Niedzielska G. Y. and Kotowski, M. (2011). The phenotypic and genetic biofilm formation characteristics of coagulase negative staphylococci isolates in children with otitis media. *International Journal of Pediatric Otorhinolaryngology.* 75: 126-130.

Raksha, R., Shrinivasa, H. and Mawcaden, R. S. (2003) Occurrence and characterization of uropathogenic *Escherichia coli* in urinary tract infection. *Indian Journal of Medical Microbiology.* 21: 102-107.

Rodrigues, D. F. and Elimelech, M. (2009). Role of type 1 fimbriae and mannose in the development of *Escherichia coli* K12 biofilm: from initial cell adhesion to biofilm formation. Biofouling. *The Journal of Bioadhesion and Biofilm Research.* 25(5): 401 - 411.

Sanchez, F., Fuenzalida, V., Ramos, R., Escobar, B., Neira, V., Borie, C. (2021). Genomic features and antimicrobial resistance patterns of Shiga toxin-producing *Escherichia coli* strains isolated from food in Chile. *Zoonosis and Public Health.* 68: 226–238.

Siegfried, L., Kmetová, M., Puzová, H., Molokácová, M. and Filka, J. (1994) Virulence associated factors in *Escherichia coli* strains isolated from children with urinary tract infections. *Journal of Medical Microbiology.* 41: 127-132.

Sivakumar, M., Abass, G., Vivekanandhan, R., Singh, D. K., Bhilegaonkar, K., Kumar, S. (2021). Extended-spectrum beta-lactamase (ESBL) producing and multidrug-resistant *Escherichia coli* in street foods: a public health concern. *Journal of Food Science and Technology.* 58(4): 1247–1261.

Sun, Y., Ma, Y., Guan, H., Liang, H., Zhao, X. and Wang, D. (2022). Adhesion mechanism and biofilm formation of *Escherichia coli* O157:H7 in infected cucumber (*Cucumis sativus* L.). *Food Microbiology.* 105: 103.

Thangapandian, V., Ponmurugan, K., Shine, K., Nalin, K., Sivakumar, T. and Meenatchisundaram, S. (2014). Production, purification and characterization of chicken egg yolk immunoglobulin against *Cryptococcus neoformans* capsular antigen. *Journal of Advancement in Medical and Life Sciences.* 1(3): 1-4. doi: 10.15297/JALS.V1I3.07.

Ziebuhr, W., Heilmann, C., Gotz, F., Meyer, P., Wilms, K., Straube, E. and Hacker, J. (1997). Detection of the intercellular adhesion gene cluster (*ica*) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. *Infection and Immunity.* 65(3): 890–896.