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Molecular Characterization of Microbial Quality of Ready-to-eat Salads using Multi-locus Sequence Typing

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Abstract

Uncertainty persists concerning the role of ready-to-eat (RTE) salad as a bacterial reservoir. The attention paid to food safety by international agencies and international regulations has not improved food safety despite technological advancements, RTE salad's microbiological quality and safety still seems challenging. The present study's objective was to detect any microorganism in the RTE salads sold in supermarkets of Riyadh, Saudi Arabia, and to interpret the susceptibility pattern of isolated bacteria to a set of antimicrobials. Phenotypic methods and biochemical analysis were used to identify the isolated bacterium from each salad sample. Antibiograms of the isolated bacteria was determined by VITEK system 2. Multi-locus sequence typing (MLST) was performed for 15 *Escherichia coli* isolates for investigating evolutionary relationship and genetic analysis. The culture-based technique showed that the major species identified in samples were *Aeromonas* spp., *Acinetobacter* spp., *E. coli*, *Roultella ornithinolytica*, *Citrobacter koseri*, *Luciferciaadec arboxylata*, *Klebsiella oxytoca*, and *Aerococcus viridians*. Remarkably, *Acinetobacter* spp. showed the highest antibiotic resistance to erythromycin, nitrofurantoin and co-trimoxazole. ST 1887 was the most common one traced in 3 *E. coli* isolates, when total of 12 STs (sequence types) were specified to 15 isolates. A total of three clonal complexes (CC); CC-12, CC-14 & CC-23 were reported in this study. Implementing an accurate, rapid, and easy microbiological analysis method could be valuable for providing higher quality products. Based on the obtained results, dedicated regular RTE salad quality monitoring is recommended, and hand hygiene should be maintained while handling and packaging of RTE.

Keywords: Antibiotic Resistance, VITEK System 2, MLST, Microbial Contamination, RTE Salad, Supermarkets

INTRODUCTION

In the fast-moving world, ready-to-eat (RTE) salads are in escalated demand for getting nutrition without losing time in preparations.[1] RTE items like, pre-packaged fruits and vegetables have become popular among Saudi consumers in recent years. A study by the Centers for Disease Control and Prevention (CDC) indicates that approximately 2.6% of Saudis of working age consume fruits and vegetables on a daily basis.[2]

Ready-to-eat products are not processed and consumed as such by humans without any further washing and decontamination. Therefore, the probability of increased cases of foodborne diseases is very high.[3] During the farming process, vegetables may be exposed to variable sources of contamination, such as irrigation, human handling, harvesting equipment, risk of animal droppings from fresh manure, contamination due to poor hygienic practices, etc. The use of untreated wastewater in irrigation represents an essential route for the transmission of many pathogenic organisms. Heavy rainfall and windy conditions also contribute to the chances of soil being contaminated with microorganisms and hence affecting the salads as well. Sometimes during packaging, unsafe water used for rinsing the vegetables is also a source of contamination. For better production and protection, farmers use chemical pesticides, fungicides, and occasionally antibiotics.[4] Due to the rampant use of antibiotics, escalating antimicrobial drug resistance must be addressed in foodborne pathogens. The latter play an essential role in transmitting genes carrying antibiotic resistance.[5-7] The processing of RTE salad products for eliminating externally wilted or ruined leaves has several selection steps. The chosen items were further managed by cutting, washing, drying, and packaging into plastic boxes. Although strict technical approaches have been implemented, it has not been possible to extend the shelf-life of the product, which resulted in health risk due to microbes remaining in the product after consumption.[8,9]

Over the past several decades, a significant increase has been documented in the number of outbreaks caused by the raw consumption of vegetables that have been associated with many diseases. Epidemiologically-linked outbreaks caused mainly by different pathogens like *E. coli* O157:H7, numerous *Shigella* spp, *Listeria monocytogenes,* many *Salmonella* serotypes and also *Campylobacter*. Other than bacteria, some parasites like *Cryptosporidium parvum*, *Cyclospora cayetanensis* and *Giardia* spp and also some viruses such as Hepatitis A and Norwalk-like viruses related to this occurence.[10-12]

In 1996, Japan witnessed the famous vegetable-borne outbreak in history. Around 6,000 different microorganisms were confirmed, 11,000 people were affected, and hundreds were admitted to hospitals. Many required kidney dialysis because of hemolytic uremic syndrome (HUS), and involved the death of three children caused by *E. coli* O157:H7.[13] The European Union reported 60 outbreaks of foodborne-related infectious intestinal diseases associated with fruits, salads, and vegetables between 1992 and 1999 out of a total of 1408 outbreaks reported during the period.[14] Pathogenic bacteria were traced from 10% of various RTE salad samples collected from retail premises in Sweden causing foodborne illness.[15] Leafy vegetables (lettuce and spinach) were the source of >25% of all reported *E. coli* outbreaks in different states in the USA, causing severe gastroenteritis. [16]

The Ministry of Health (MOH) in Saudi Arabia reported that foodborne illnesses had increased steadily.[17] A total of 264 cases of food poisoning outbreaks were reported in 2010 from households and commercial sources in Saudi Arabia.[18] In the subsequent year, 2066 people, mainly children, fell ill as a result of 255 incidents.[19] Furthermore, Hajj and Umrah seasons in Makkah, Saudi Arabia, are another setting where high numbers of outbreaks have emerged. This is primarily due to sprawl and crowdedness of people with different cultural backgrounds and socioeconomic statuses that may carry poor food hygiene standards around crowded food shops.[17] Salads have become increasingly popular among Saudis due to health concerns, which has led to an increase in their consumption. According to a nationwide study that investigated the dietary habits of the Saudi population, about 50% of those surveyed reported eating green salad. Thus, investigating the microbial quality of minimally-processed salads is essential to mitigate adverse outcomes. A study by Khiyami et al. in Riyadh, showed that hand handling of vegetable salads during processing and packaging posed a potential health risk due to the presence of fecal coliforms and associated pathogens.[20] Recently, a study from Nigeria showed the prevalence of multidrug-resistant bacteria (MRB) in a total of 640 RTE samples. This study showed the presence of MRB in the RTE serving 46.54 to 60.83% of restaurants.[21]

Until recently, diagnostic laboratories for microbial identification primarily focused mainly on traditional phenotypic and gene sequencing identification approaches. Implementing MLST (Multi-locus Sequence Typing) have transformed routine microbe epidemiological identification laboratories by offering simple, quick, high data rate passing and reliable approaches. This method has proved its applicability in various studies and is already being used in many laboratories/hospitals and government surveillance organizations as a routine procedure.[22]

Therefore, the aim of the current study was to investigate the microbiological quality of RTE salads sold in supermarkets of Riyadh, Saudi Arabia. Molecular characterization of the isolated bacteria was performed by MLST methods, and antimicrobial susceptibility was determined by VITEK system2.

MATERIALS AND METHODS

Evaluation of Microbiological Quality in RTE salads

Sample collection

A total of 70 different varieties (Fattoush, Greek, Chicken Caesar, Tabbouleh, and Green Salad) of RTE salads, kept at 4°C, were collected from popular supermarkets in Riyadh to ensure diversity. Ready-to-eat salads were purchased on the packaging date, transported aseptically to the laboratory. Simulating home-refrigeration stored condition was evaluated for microbial quality from RTE salad after 36 h storage at 4°C and the samples were processed within 2 h after removal from the refrigerator.

Sample preparation

Dilutions of 1:10 were prepared for aerobic plate and bacterial counts, 25 g of sample was blended in 225 ml buffered peptone water (0.1%) (pH 7.2). Different types of nutrient agar media were used (TSA, VRGB agar, and VRBL agar; SPML). The diluted suspensions (0.1 ml) were spread over the plate and incubated in the incubator (Memmert Gmbh, Germany) for 24-48 h at 37°C using an already established protocol.[21,23]

Table 1. Isolated microorganisms and Antimicrobial Susceptibility Testing using Disk Diffusion Assay of bacterial isolates of RTE salad

S. No	Isolate ID	Results	AST - Disk Diffusion Assay (Zone diameter)						
			NIT	CTX	TET	ERY	AMK	GEN	TOB
1.	S-1	*Acinetobacter pitti*	R	R	S (25)	R	S (35)	S (34)	S (35)
2.	S-2	*Aeromonas caviae*	R	S (34)	S (35)	S (20)	S (40)	S (40)	S (40)
3.	S-3	*Escherichia coli*	S (26)	S (30)	S (20)	R	S (25)	S (27)	S (26)
4.	S-4	*Roultella ornithinolytica*	S (15)	S (24)	S (16)	R	S (26)	S (26)	S (27)
5.	S-5	*Aeromonas Jandaei*	R	R	S (30)	R	S (19)	S (25)	S (25)
6.	S-7	*Citrobacter koseri*	S (17)	S (26)	S (26)	S (18)	S (28)	S (29)	S (29)
7.	S-8	*Aeromona scaviae*	S (27)	S (22)	S (24)	S (14)	S (26)	S (18)	S (26)
8.	S-9	*Lucifercia adecarboxylata*	S (20)	S (26)	S (24)	S (20)	S (30)	S (28)	S (28)
9.	S-10	*Klebsiella oxytoca*	S (27)	R	S (21)	S (23)	S (32)	S (30)	S (29)
10.	S-11	*E. coli*	S (28)	S (30)	S (21)	R	S (26)	S (28)	S (26)
11.	S-12	*Acinetobacter baumannii*	R	R	S (25)	R	S (35)	S (34)	S (35)
12.	S-13	*Aerococcus viridans*	S (19)	S (25)	S (20)	S (19)	S (25)	S (24)	S (28)
13.	S-14	*E. coli*	S (28)	S (30)	S (21)	R	S (26)	S (28)	S (26)
14.	S-15	*E. coli*	S (26)	S (31)	S (20)	R	S (25)	S (27)	S (25)
15.	S-18	*E. coli*	S (28)	S (28)	S (20)	R	S (25)	S (27)	S (24)
16.	S-20	*Aeromona scaviae*	R	S (32)	S (30)	S (21)	S (38)	S (35)	S (31)
17.	S-23	*E. coli*	S (27)	S (28)	S (21)	R	S (26)	S (27)	S (26)
18.	S-24	*E. coli*	S (25)	S (29)	S (22)	R	S (27)	S (28)	S (26)
19.	S-25	*E. coli*	S (28)	S (30)	S (21)	R	S (25)	S (26)	S (25)
20.	S-26	*E. coli*	S (27)	S (31)	S (22)	R	S (25)	S (26)	S (27)
21.	S-29	*Aeromonas caviae*	R	S (30)	S (31)	S (22)	S (34)	S (36)	S (33)
22.	S-31	*E. coli*	S (27)	S (31)	S (22)	R	S (27)	S (26)	S (24)
23.	S-35	*Citrobacter koseri*	S (18)	S (24)	S (25)	S (19)	S (25)	S (28)	S (30)
24.	S-38	*E. coli*	S (28)	S (29)	S (23)	R	S (27)	S (28)	S (24)
25.	S-44	*E. coli*	S (28)	S (28)	S (23)	R	S (26)	S (25)	S (25)
26.	S-46	*E. coli*	S (28)	S (27)	S (22)	R	S (26)	S (25)	S (24)
27.	S-47	*Aeromonas caviae*	R	S (31)	S (33)	S (24)	S (31)	S (31)	S (35)
28.	S-49	*E. coli*	S (26)	S (30)	S (20)	R	S (25)	S (25)	S (25)
29.	S-56	*Citrobacter koseri*	S (20)	S (25)	S (25)	S (20)	S (24)	S (25)	S (28)
30.	S-60	*E. coli*	S (25)	S (27)	S (20)	R	S (25)	S (25)	S (25)

Abbreviations: Nitrofurantoine = NIT, Cotrimoxazole = CTX, Tetracycline = TET, Erythromycin = ERY, Amikacin = AMK, Gentamicin = GEN, Tobramycin = TOB

Enumeration and identification of presumptive colonies

Colony forming units per milliliter (CFU ml^{-1}) of a sample was calculated for cultivable aerobic bacteria. Microorganisms were isolated on the basis of phenotypic characteristic differences, and it stored in the 15% glycerol stock solution at -20°C for further analysis.

DNA isolation

DNA was isolated from overnight growth of 15 *E. coli* isolates using the Qiaquick DNA Isolation Kit (Qiagen) as per user manual. Purity and integrity were checked by running the 5 µl of isolated DNA in the agarose gel electrophoresis and 'Nanodrop' spectrophotometer was used to quantify the concentration of isolated DNA samples. Fixed dilutions of 100 ng/µl were prepared for each isolated DNA samples.

PCR & MLST Analysis

PCR thermal cycler was used to amplify 7 housekeeping genes (*gyrB, adk, icd, fumC, recA, purA* and *mdh*) for each of 15 *E. coli* isolates. Already available oligonucleotides for above mentioned seven genes were sent for synthesis to

Sigma Aldrich (pub-mlst.org/bigsdb?db=pubmlst_ecoli_achtman_seqdef). Amplified PCR products were run on agarose gel electrophoresis for each of seven genes per isolate. Amplified PCR products were verified by comparing their respective band lengths with DNA ladder for each gene using gel documentation system. Qiagen gel purification kit was used to purify the PCR product from the agarose gel by cutting the gel portion containing the amplicon on UV transilluminator system. After purification, DNA concentration was measured for the purified product followed by DNA sequencing. To check the quality of the sequencing, dendrograms of each gene were checked using 'FINCH TV' software after execution of sequencing. Allele types were confirmed for each gene by submitting the sequence on PubMLST database (website details: pubmlst.org/bigsdb?db=pubmlst_ecoli_achtman_seqdef). Allelic profiles were prepared for each *E. coli* isolates using PubMLST database. These allelic profiles were submitted to PubMLST database, which further give a unique ST (Sequence Type).

Phylogenetic Tree Preparation

In accordance with PubMLST's standard pattern, a concatenated sequence file was prepared by joining sequences of seven housekeeping genes in the FASTA format. Concatenated sequences of all the 15 *E. coli* isolates were placed in a single notepad file. This notepad file carrying all the gene sequences of all isolates was submitted to ClustalX2 software to execute multiple sequence alignment. All the aligned sequences were saved in the form of '.aln' file. This '.aln' file was further opened in the software named 'MEGA' 6th version and further converted and saved as '.meg' file. This '.meg' file was opened in the MEGA software, which analyzed the sequences and Neighbor-joining and 'Maximum Likelihood' & phylogenetic trees were constructed.

Antibiotic-resistant profiles

Antibiotic susceptibility was tested using 'Kirby Bauer Disk Diffusion Assay'. We followed the CLSI guidelines for result interpretation of zone diameter and susceptibility pattern of bacterial isolates. We selected seven commonly used antibiotics: nitrofurantoin (30 µg), gentamicin (10 µg), amikacin (30 µg), erythromycin (30 µg), tobramycin (10 µg), tigecycline (30 µg) and cotrimoxazole (1.25/23.75 µg). We confirmed the results by using VITEK system 2 (BioMɪrieux). Antibiotic susceptibility pattern were given by the VITEK machine in the form of interpretation as resistant or susceptible.

Table 2. MLST profiles of 15 *E. coli* isolates of salad samples

S. No.	Sample ID	ST	MLST Profiles of 7 housekeeping genes							CC
			adk	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*	
1.	S-3	1690	6	11	12	1	20	12	7	ST23
2.	S-11	1615	6	263	12	1	20	13	7	ST23
3.	S-14	1644	6	11	61	50	13	6	3	-
4.	S-15	2089	6	153	261	91	7	78	6	-
5.	S-18	1887	6	17	15	18	53	8	7	-
6.	S-23	1887	6	17	15	18	53	8	7	-
7.	S-24	1612	6	4	15	18	24	26	14	-
8.	S-25	1389	9	6	162	18	7	8	7	-
9.	S-26	1309	8	241	4	8	8	9	2	-
10.	S-31	1309	8	241	4	8	8	9	2	-
11.	S-38	1416	14	14	10	14	8	7	10	ST14
12.	S-44	1798	6	4	3	18	9	7	7	-
13.	S-46	1887	6	17	15	18	53	8	7	-
14.	S-49	1439	13	13	9	13	16	10	2	ST12
15.	S-60	1803	6	6	5	9	9	8	172	-

RESULTS

This study selected Fattoush, Greek, Chicken Caesar, Tabbouleh, and Green Salad, among the products commercially available in Riyadh supermarkets. The presence of microorganisms in RTE salad samples marketed in Riyadh, Saudi Arabia, which phenotypically

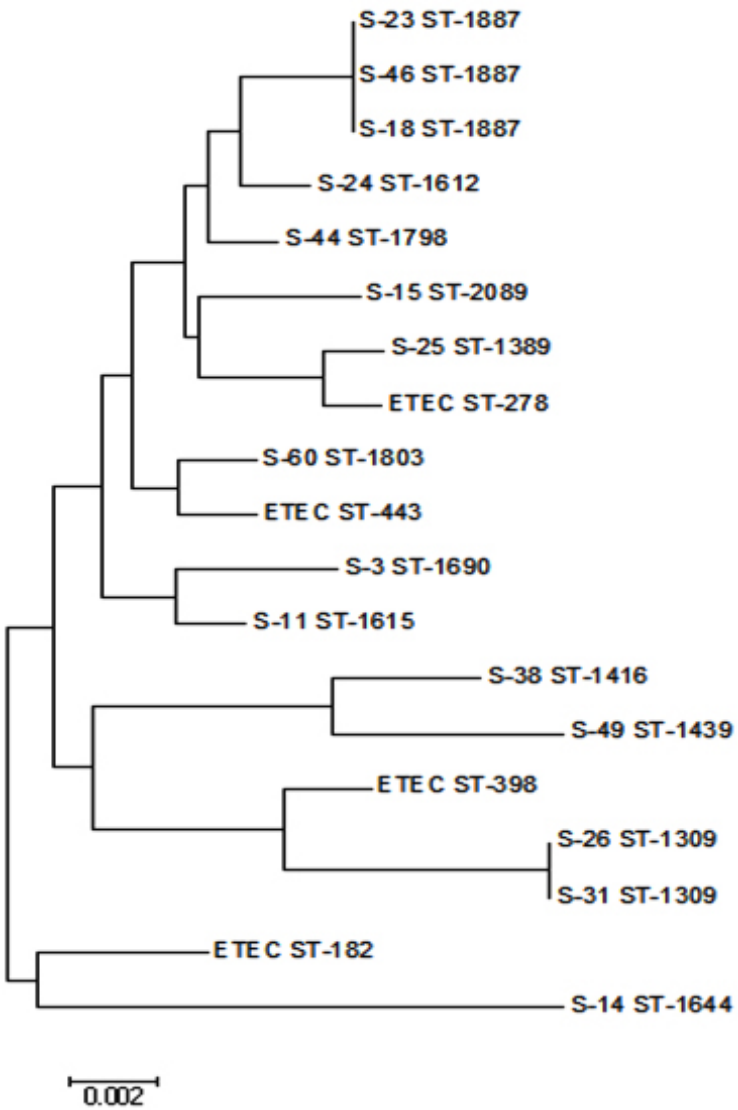

Figure 1. Evolutionary relationships of taxa
The evolutionary history was inferred using the Neighbor-Joining method.[24] The optimal tree with the sum of branch length = 0.07549033 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method.[25] and are in the units of the number of base substitutions per site. The analysis involved 19 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 3414 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.[26] Four ETEC isolates with ST-182, ST-278, ST-398 & ST-443 were taken as standard strains to compare the genetic relatedness

identified a total of 30 microorganisms from 70 RTEs samples as presented in Table 1. Out of 30 identified microorganisms, 15 were *E. coli*, 6 were *Aeromonas* spp., 3 were *Citrobacter koseri*,

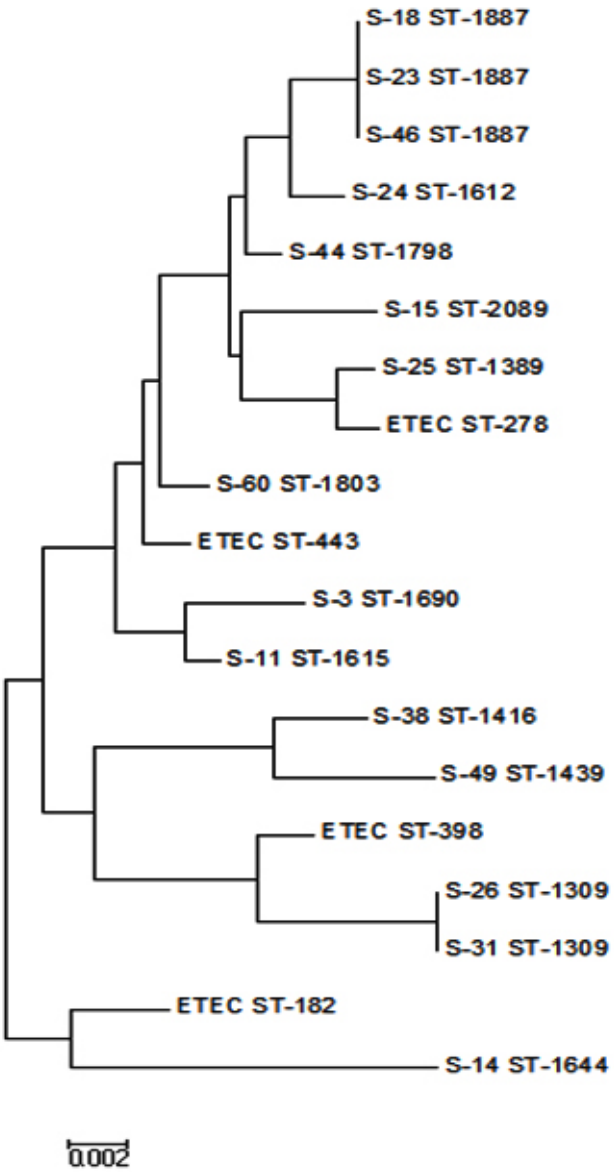

Figure 2. Molecular Phylogenetic analysis by Maximum Likelihood method
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model.[27] The tree with the highest log likelihood (-6779.5754) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 19 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 3414 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.[26] Four ETEC isolates with ST-182, ST-278, ST-398 & ST-443 were taken as standard strains to compare the genetic relatedness

2 were *Acinetobacter* spp. and each of *Roultella ornithinolytica*, *Luciferci adecarboxylata*, *Klebsiella oxytoca*, and *Aerococcus viridians* (Table 1). After 36 h of storage at 4°C, all the analyzed samples displayed an aerobic mesophilic count (TAMC) concentration $\geq 10^6$ CFU/g All the eight types of bacterial isolates showed different colony morphologies on nutrient agar media plates.

Antibiotic sensitivity test was done for all the recovered isolates. Table 1 showed the antibiotic susceptibility/resistance pattern for RTE isolated bacteria. Among the isolated microorganisms, both *Acinetobacter* spp., and *Aeromonas caviae* showed antibiotic resistance to erythromycin, nitrofurantoin, and cotrimoxazole (100%). All 15 isolates of *E. coli* were resistant to erythromycin. Four of 5 (80%) *Aeromonas caviae* isolates were resistant to nitrofurantoin. *E. coli* was sensitive to amikacin, gentamycin, tobramycin, tetracycline.

MLST was performed for 15 *E. coli* isolates for finding out evolutionary relationship and genetic relatedness. ST 1887 was the most prevalent one, which was traced in 3 *E. coli* isolates in this study. A total of 12 STs (Sequence Types) were assigned to 15 isolates. ST-1309 was represented by two isolates. A total of three clonal complexes (CC-12, CC-14 & CC-23) were reported in this study (Table 2). High genetic diversity among all the *E. coli* isolates was shown in Neighbor-Joining and Maximum-Likelihood phylogenetic trees (Figure 1 & 2). Three different clads were presented by *E. coli* isolates in the Maximum Likelihood tree (Figure 2).

DISCUSSION

Foods, microorganisms, and humans have had an interesting association that developed long before history. Humans have learned to harness the metabolism of microbes to achieve various purposes. Sometimes a fine line exists between the microbial enhancement and degradation of foods. The detection and control of pathogens and spoilage microorganisms are essential for human health.

This study investigated the presence of microorganisms in the commercially available Fattoush, Greek, Chicken Caesar, Tabbouleh, and Green Salad, which are routinely sold in Riyadh supermarkets. The result showed eight different types of bacteria, which suggested the probability of microbial contamination by improper handling and negligence for sanitary precautions by supermarket employees. The breach in the cycle of preparation, processing, packaging, and storage may allow the entry of pathogens to the food items. Our study identified *A. baumannii* and *A. pitti* from RTE salads samples, which are frightening nosocomial human pathogens all over the globe. Till date, only a very few studies have witnessed the recovery of *Acinetobacter* in food items, particularly on lettuces and fruits. In salad vegetables and carrots two *Acinetobacter* spp has been identified by Hamilton-Miller and Shah.[28] The current study showed that *Acinetobacter* spp. were resistant to nitrofurantoin, co-trimoxazole and erythromycin. *A. baumannii* has been listed on the top of global priority list of multidrug resistant bacteria, recently published by World Health Organization (WHO).[29] Transmission of *Acinetobacter* species from hospital to the environment or vegetation suggested the alarming need of more surveillance investigations.

Many studies showed contamination of foods by resistant bacteria[30-32] that suggested the presence of fecal enterobacteria. Because *E. coli* is typically found in the digestive tracts of humans and animals, it is frequently used to indicate fecal contamination. The presence of *E. coli* in RTE salads could be attributed to the farming process, such as using animal dropping, untreated manure, or contaminated irrigation water, as *E. coli* often emanate from the intestinal tract of humans and animals. Similar results have been shown by another study where 25.7% of samples from 16 University were found harboring *E. coli*. [33]

MLST analysis is very significant in finding out genetic relatedness in amongst bacterial isolates. In a recent study, authors observed a considerable diversity amongst various isolates of *E. coli*.[34] *E. coli* isolates in this study were found scattered, which showed an evolutionary divergence. Two islolates, S-26 and S-31 with ST 1309 were found related with the standard ETEC (Enterotoxigenic *E. coli*) strains (Figure 1 & 2). In the Pubmed search, there is no report till date on detection of food borne pathogens in the vegetable salad. However, in 2011, first report of contamination of food borne reported *E. coli*

along with *Salmonella* spp., *Shigella* spp. and *Enterobacter aerogens* from minimally processed salad was observed from Riyadh city.[20] In 2017, another study from Hail city of Kingdom of Saudi Arabia detected the presence of microbes and parasites on morphological basis only.[35] They could not identify the pathogens up to genus level using any of the molecular technique. However, our study for the first-time highlighted global clonal complexes like; CC 12, CC 14 and CC 23 in the vegetable salad samples in the Saudi Arabia, the results indicated more numbers of *E. coli* clones in the region. Although, we took quite a few numbers of isolates in the present study, yet this study suggested the dire need for a large-scale study. Till date there is no MLST based characterization study in the KSA, which could detect the prevalence of global clonal complex in KSA.

The present investigation aimed to trace the food spoiling and disease-causing microorganisms.[36] The current study showed high microbial counts of *Enterobacteriaceae* and coliform, which suggests that local markets of Saudi Arabia should apply effective control measures to limit the growth of microbiological quality of RTE salads. Current investigations suggested that supermarket workers should follow controlled measures for proper food safety practices like proper handwashing. Also, customers' hands and personal belongings like mobile phones could act as a transmission vehicle for both pathogenic and nonpathogenic microorganisms.[37] Hence, providing gloves and hand sanitizers around open vegetable display refrigerators for customers may aid in restricting the growth of microorganisms. The most frequently used method to control microbial contamination of vegetables, fruits, etc., is to apply chemical-based preservation regulated by the Food and Drug Administration (FDA). Moreover, other treatment approaches like; low acidic electrolyzed oxidizing water, sodium nitrite, organic acids, peroxyacetic acid, sulfite and hydrogen peroxide have shown considerable antimicrobial effect in fresh vegetables and sprout.[38, 39]

CONCLUSION

The current study is the first attempt to investigate the microbial diversity in the RTE salad samples from different supermarkets in Riyadh, Saudi Arabia. We also described the molecular characterization and epidemiological investigation of 15 different *E. coli* isolates using MLST. We found out interesting global clonal complex in this study, which are evolutionary closer to standard ETEC strains. Antimicrobial susceptibility of the all the recovered bacterial isolates was determined by disk diffusion assay and VITEK system 2 (BioMʹrieux). We could identify 30 bacterial strains where some of them were nosocomial human pathogens such as *E. coli*, *K. oxytoca*, *A. baumannii* and *A. pitti* from RTE salads samples. These findings will be of interest to control the food poisoning outbreaks in Saudi Arabia. Presence of pathogenic bacteria in RTE salads could be a source of transmission of the latter from hospital to environment. Therefore, the findings from the present investigation suggested that vendors and supermarket owners should monitor the maintenance of hand hygiene and follow good practices while packaging of RTE salads in Saudi Arabia; thereby limiting the growth of the microorganisms.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

RA and JA conceptualized the study. FKA, SM, AA, SH and RA performed investigation. RA and SK applied methodology. RA, HA and JA supervised the study. RA and FKA wrote original draft. HA, AA, SK and SH wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Institutional

Review Board (IRB) of Imam Mohammed Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia, (HAPO-01-R-0011, Project No. 70-2021).

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