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Full Length Research Paper

Genetic relatedness of *Escherichia coli* 0157:H7 strains from cabbage irrigated with wastewater in Kano

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Twenty two (22) *Escherichia coli* O157:H7 strains from cabbage collected from Jakara and Sharada Wastewater irrigation farms in Kano city were characterized, to determine possible genetic relatedness. Strains were characterized using plasmid analyses by alkaline lyses method (pH 8) and restriction digest of the chromosomal desoxynucleotide (DNA) by *Sma* 1. The results of plasmid profile analyses revealed 7 plasmids ranged from 1.422 to 12.497 kb distributed into 5 profiles. Most *E. coli* O157:H7 (68.18%) had only one plasmid. The plasmids sizes 11.495 kb were most frequent, and observed among all strains, especially in 63.64% of strains from Jakara cabbage. Strains were similar at 45.45% of Jakara and 22.73% of Sharada strains of the total strains analyzed, respectively. However, strains from same source (farm) were found to exhibit different plasmid profile. The data from restrictions digest analyses showed only little deviation from plasmid profile data, with only 8 clusters of restriction patterns as opposed to 5 cluster of plasmid profile. Strains in cluster one of restriction digest were 65% similar, Five of the clusters had strains from both sources. Interstignly, some strains identified as similar by plasmid profile were equally re-grouped into similar clusters by restriction digest. The interrelationship among strains, clearly demonstrate that, they were probably from same sources, being transmitted through the environment to the cabbages.

Key words: Kano, restriction digest, plasmids, wastewater canals, Nigeria.

INTRODUCTION

Horizontal transmission of pathogenic factors through mobile elements, such as bacteriophages, plasmids, or transposable elements, is important in the emergence of new pathogenic bacteria (García-Aljaro et al., 2009).

The pathogenicity of *Escherichia coli* O157:H7 is mainly mediated by virulent factors located either on chromosome or on transmissible 60 MDa plasmid (Schmid et al., 1995). The main virulence markers responsible for virulence of *E. coli* O157 are Shiga toxins (Stx1, Stx2 or Stx2 variants), and two factors encoded by the pathogenicity island LEE (locus of enterocyte effacement), intimin (a product of the *eaeA* gene) and translocated intimin receptor (*tir*) (Paton and Paton, 1998). Moreover, the plasmid-encoded *E. coli* enterohemolysin (Ehly), which has been found in many O157 strains, has been suspected to has a role in pathogenicity of the infection (Jacek, 2002). Subsequently, *E. coli* O157:H7 was reported to be responsible for the outbreaks linked to the consumption of fresh vegetables and fruits (such as lettuce, spinach, carrots, radish sprouts, alfalfa, unpasteurised apple cider, melon and berries) (Chang et al., 2013). *E. coli* O157:H7 was responsible for 7% of fresh produce outbreaks in US

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(Olaimat and Holley, 2012).

Large amounts of vegetables are produced in Kano and are minimally processed, and served with no further treatment and eaten raw. These minimally processed products may be contaminated with food-borne pathogens, such as *E. coli* O157:H7, *Shigella*, *Listeria monocytogenes, Vibrio* and *Salmonella* (Sela et al., 2009) from a number of sources like waste from slaughter slabs, were goats, sheep and cow are eviscerated and hospitals. The pathogenic potential of *E. coli* strains is thought to be dependent on the presence of virulence factors (VFs), which are located on large plasmids and/or in particular regions, called pathogenicity islands' (PAIs), on the chromosome. Plasmids and phage genomes both can carry antibiotic resistance or toxin genes. Identification of *E. coli* strains is important for both clinical and epidemiological implications. Understanding plasmid patterns and molecular characteristics of microorganisms are useful epidemiological data for tracing of pathogenic bacteria. Therefore, there is a need for periodic screening of common bacterial pathogens to determine their plasmid profile from different sources, especially *E. coli* O157:H7 from vegetables. Epidemiological data have demonstrate 26% contribution to food borne outbreaks in United States, Codex (2003), a place where active surveillance mechanism are in operation. Our previous studies have documented 22% occurrence of *E. coli* 0157:H7 in cabbage from Jakara canal wastewater in Kano Nigeria (Dahiru and Enabulele, 2014). It is against this background, the research find it important to investigate the genetic relatedness by characterizing *E. coli* 0157:H7 from these sources especially their virulent factor as the major determinant of infection, which could show light on the level of risk involved in consumption of cabbages grown from these farms. These could in turn serve as surveillance lead data during possible food borne outbreaks.

MATERIALS AND METHODS

Plasmid analyses of *E. coli* O157:H7

Twenty one (21) *E. coli* O157:H7 strains were obtained from Cabbage, irrigated with wastewater of Jakara and Sharada canal irrigation farms. Strains were grown on TSB at 37°C for 24 h and cells were harvested by centrifugation at 5000 *g* for 5 min at room temperature. Plasmid DNA was extracted using rapid protocol for plasmid DNA extraction based on the alkaline lyses method of plasmid preparation (extraction at pH 8.0) developed by Simeon et al. (2003). The pellet was treated with ribonuclease RNase (Fermentas|Thermo Scientific) thereafter, accordance with manufacturer's guide.

Isolation of genomic DNA and restrictions digest analysis

Genomic DNA for restriction digest analysis was carried in accordance with Bio Basic Inc (Canada) extraction Kit instructions. DNA was treated with ribonuclease RNase (Fermentas|Thermo Scientific) thereafter to remove the possible RNA contaminant from the sample, in accordance with manufacturer's guide.

Restriction digest protocol was adapted from Murrey et al. (1990) with few modifications such as use of horizontal Gel electrophoreses machine. The genomic DNA (*E. coli* 0157:H7) were digested with *Sma* 1 restriction enzyme (Fermentas), in accordance with manufacturers' instructions. The digest were size-separated by electrophoresis at 60 V in 1% agarose gels (Bio Rad).

Preparing agarose gel and electrophoresis of samples

One gram [1g] agarose powder (Bio Rad) was dissolved in 100 ml of Tris acetate-EDTA (TAE) (40 mM Tris acetate, 2 mM EDTA, pH 8). The solution was boiled and allows to cooled to 55°C and five micro litter (5 µl) of ethidium bromide added. Combs were inserted into plastic tray and the molten agarose gel was poured into the plastic casting tray, allowed to cool and solidify. Ten micro litter (10 µl) each plasmid/DNA was properly mixed with 2 µl of loading dye (Bromocrysol purple) and load in to the agarose gel wells, already submerges in TAE inside the electrophoresis tank. One kb DNA (Fermentas|Thermo Scientific) ruler loaded in to the first. The Gel was runned at 60 V for 1.5 h and viewed under UV trans-illuminator Gel Documentation machine, (Sambrook, 1989; Desmond, 2008)

Fragment size estimation and computation of strain similarities

Restricted fragment sizes were estimated by Gel Doc XR+ with computer aided program, software 3.0 (BioRad) UV trans- illuminator, by comparison with 1 kb molecular mass markers (Fermentas|Thermo Scientific). Similarities among strains were determined by the Dice coefficient (Czekanowski or Sorenson), and cluster analyses was based on the complete linkage by using SPSS statistical package version 16.

RESULTS

Plasmid profile analysis

Analysis of plasmid DNA (Figure 1) revealed that, the 22

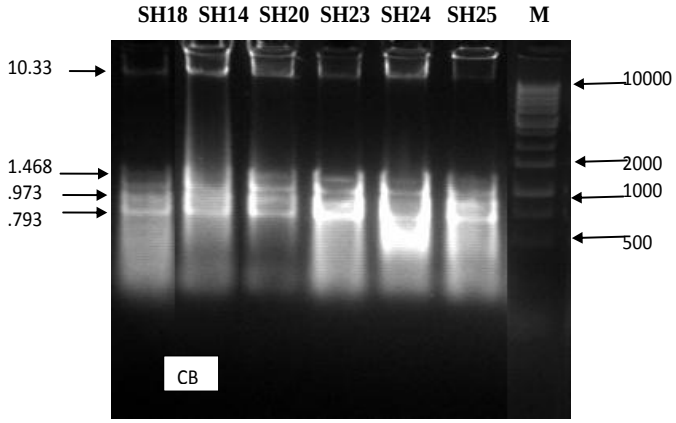


Figure 1. Plasmid profile of *Escherichia coli* 0157:H7 Strains from Sharada cabbage farms (SH). Electrophoresed on 1% agrose gel. Lane-6 samples, 7 (M) 1kb DNA ladder.

Table 1. Plasmid patterns from *E. coli* 0157:H7 from cabbage collected from Jakara and Sharada canal irrigation farms.

Plasmid pattern	Number of strains	Source of strains	
		Jakara cabbages	Sharada cabbages
12.497	1	1	0
11.495	11	7	4
10.573	4	3	1
11.495, 2.776, 2.16, 1.422	1	0	1
11.495, 2.776, 2.16, 1.838, 1.422	5	0	5

isolates from cabbage had 7 different plasmids that ranged from 1.422 to 12.497 kb distributed into 5 profiles (Table 1). Most *E. coli* O157:H7 (68.18%) had only one plasmid, however, few others had 4 to 5 (27.28%) plasmids (Figure 1). Plasmid size 11.495 kb was most frequent and predominantly among strains from Jakara (63.64%) source and 12.497 kb was the least observed also found only among strains from Jakara cabbage. Strains with 4 and 5 number of plasmids were only observed in strains sourced from Sharada cabbage. The strains were genetically similar at 45.45% of Jakara and 22.73% of Sharada strains of the total strains analyzed, as shown in plasmid profile serial number 2 and 3, respectively.

Restrictions digest analyses

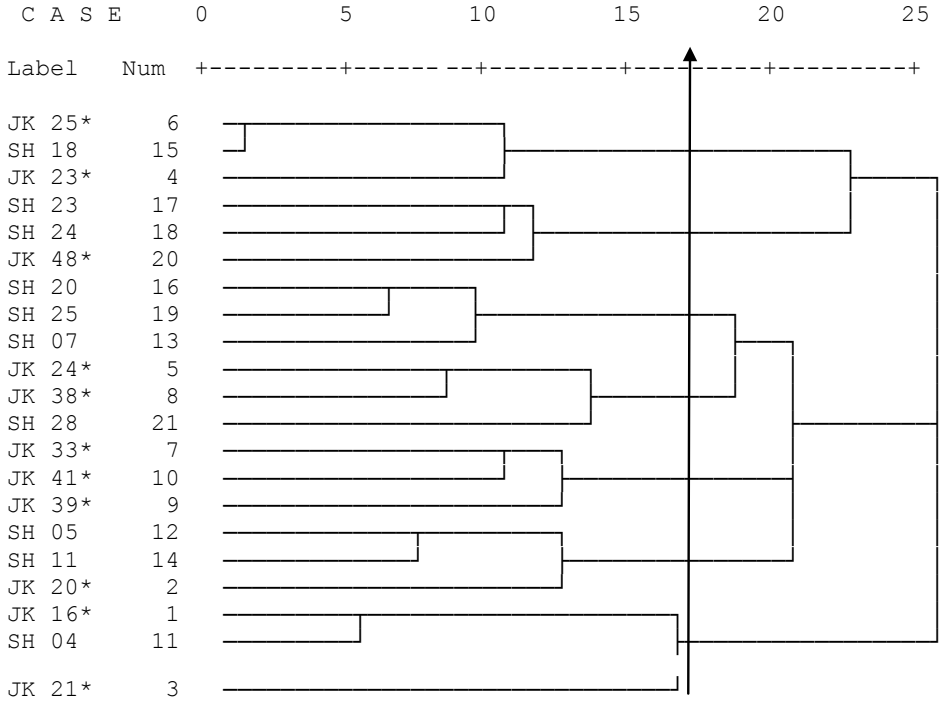
Restriction digests analysis of DNA extracted from the 21 *E. coli* 0157:H7 isolates from cabbage sources was carried out by digestion with *Sma* 1 restriction enzyme, SH14 was lost before restriction digest analyses. All isolates with similar restriction pattern were grouped in accordance with Dice similarity coefficient for determination of relatedness. Eight clusters of restriction enzyme digestion patterns were identified. Each cluster had 3 individual members, except the last 2 which had 2 and 1 strain respectively. Cluster number 1 had isolates from both sources (JK25 and SH18) closely related at 65% similarity (Table 2). Others found in the same clusters (Figure 2) from different sources had similarity of 57%. Moreover, 5 of the 8 cluster contained isolates from both Jakara and Sharada strains, while cluster 3 contained only strains from Sharada cabbages and 5 and 8 clusters contained only strains from Jakara Cabbages.

DISCUSSION

The genetic analyses by plasmid profiles of *E. coli* 0157:H7 has revealed different strains isolated from different locations but closely related in the same profile.

Table 2. Proximity Matrix of the genomic DNA digest of *E. coli* 0157:H7 on cabbage from Jakara and Sharada canals wastewater irrigation farms in Kano, Dice (Czekanowski or Sorenson) Similarity Measure.

	CB 16	CB 20	CB 21	CB 23	CB 24	CB 25	CB 33	CB 38	CB 39	CB 41	CB 54	CB 55	CB 57	CB 61	CB 68	CB 70	CB 74	CB 75	CB 76	CB 48	CB 79
1:CB 16																					
2:CB 20	.304																				
3:CB 21	.364	.381																			
4:CB 23	.316	.222	.176																		
5:CB 24	.356	.372	.195	.457																	
6:CB 25	.340	.356	.233	.486	.364																
7:CB 33	.286	.340	.267	.410	.391	.333															
8:CB 38	.391	.364	.190	.444	.512	.444	.298														
9:CB 39	.408	.426	.356	.256	.391	.333	.440	.426													
10:CB 41	.280	.292	.304	.400	.383	.367	.471	.333	.431												
11:CB 54	.571	.370	.423	.391	.377	.436	.246	.481	.421	.379											
12:CB 55	.375	.435	.182	.263	.444	.426	.408	.435	.367	.400	.429										
13:CB 57	.292	.304	.364	.316	.444	.340	.367	.435	.367	.280	.357	.500									
14:CB 61	.531	.426	.311	.308	.435	.375	.400	.383	.280	.392	.526	.531	.408								
15:CB 68	.377	.314	.327	.465	.480	.654	.481	.471	.370	.400	.393	.377	.453	.407							
16:CB 70	.311	.372	.244	.286	.429	.409	.522	.326	.478	.511	.340	.533	.489	.435	.440						
17:CB 74	.372	.195	.256	.303	.350	.238	.409	.341	.227	.311	.353	.279	.372	.409	.417	.400					
18:CB 75	.465	.341	.256	.303	.350	.381	.364	.488	.409	.222	.471	.372	.419	.455	.375	.350	.474				
19:CB 76	.233	.293	.205	.242	.350	.381	.364	.390	.364	.444	.235	.419	.512	.364	.375	.550	.421	.316			
20:CB 48	.333	.423	.200	.273	.353	.340	.327	.462	.364	.321	.452	.407	.407	.400	.441	.392	.449	.449	.408		



Key: CBX* = strain isolated in Jakara cabbage, CBX = strain isolated in Sharada cabbage.

Figure 2. *Sma*1 Restrictions Digest *Escherichia coli* 0157:H7 DNA isolated in cabbage from Jakara and Sharada Canals wastewater irrigation farms.

It was also noted, most of the strains grouped in the second profile were not from cabbage of the same source, but had identical profile. However, strains from same sources (cabbage from a particular farm) were found to exhibit different plasmid profile (Table 1). The results of this work was in harmony with Gaddad et al. (2011), who detected plasmids in toxigenic *E. coli* (STEC) most of which had only one plasmid, only 3 isolates had 4 plasmids in common and belonging to same profile. Similarly Smith et al. (2003), detected plasmids of various sizes in 47% *E. coli* 0157:H7 isolates from cows, goats, pigs and rams. Although, *E. coli* 0157:H7 analyzed in this work were from vegetable (cabbage) irrigation with contaminated wastewater, but result had shown consistency with strains isolated from animal sources. This probably point to the sources of contamination, as waste from both Kano city Abattoirs and Infectious Disease Hospital are channel into these canals for final disposal. Therefore cultivars grown or irrigated with wastewater from these canals stands a high risk of possible contamination by zoonotics and other infectious pathogenes. The interrelationship among strains, clearly demonstrate that, they were from same sources, being transmitted through the environment and subsequently contaminate the cabbages.

Restriction fragment length analyses of strains used in this work revealed little variation in grouping the strains into clusters compared with plasmid profile, thus some strains that were grouped into same profile by the plasmid profile data were equally grouped into another same cluster by restriction digest analyses. For example, SH20 and SH25 (both from same source) and SH07 and JK23 grouped into same profiles (data not shown) by plasmid profile, were equally grouped as similar, in cluster 3 of the restriction digests pattern. Similarly, strains not from different sources were grouped as similar, into profile 2 and 3 of the plasmid analyses and cluster 1, 2, 4 and 6 of restriction digest pattern respectively. Moreover, strains grouped by restriction digest analyses in cluster 6 and 7 were also grouped by plasmid analyses into another genetically similar group. However, some strains identified as dissimilar according to plasmid profiles analyses, were classified as similar by the restriction digest analyses.

The variance of the restriction patterns could probably be due to spatial, temporal, and genotypic differences as reported by Oh et al. (2009). Samadpour et al. (1993), working on *E. coli* 0157:H7 strains from geographically or temporally unrelated sporadic cases, used lambda (λ) restriction fragment length polymorphism (RFLP) and report, none of the strains had the same (λ) RFLP pattern. Also the λ RFLP patterns of four *E. coli* 0157:H7 strains isolated from two patients differ considerably from the pattern of another outbreak strain. Elsewhere, Gaddad and his colleagues (2011) analyzed *E. coli* 0157:H7 isolated from diverse sources and reported a great diversity by SDS-PAGE analysis.

Conclusion

All *E. coli* 0157:H7 characterized by plasmid profile and restriction digest have demonstrated a considerable level of relatedness. This had therefore demonstrated the likelihood of the bacteria, being release from same source to the cabbages and therefore prompt identification and treatment of the source will help drastically in reducing the risk of produce contamination by *E. coli* 0157:H7 on cabbages and public health risk of consumption of contaminated produce and of disease outbreak in the community.

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