Jewel Journal of Scientific Research (JJSR) 3(1): 152- 157, 2015
©Federal University, Kashere-Nigeria (ISSN: 2384 - 6267)
www.fukashere.edu.ng

Genetic relatedness of *Escherichia coli* O157:H7 strains from Lettuce and flies collected from irrigation farms in Kano metropolis

Dahiru, M.
Department of Biological Sciences, Faculty of Sciences, Federal University Kashere, P.M.B. 0182, Gombe.
Email: musahanifa@yahoo.com

Abstract
The genotypic characteristics of 17 E. coli 0157:H7 strains from lettuce and 11 flies around lettuce farms were investigated. Relatedness was determined by plasmid DNA analyses and chromosomal DNA restriction digest with Sma 1. Plasmid analyses revealed 3 different plasmids profiles that contained plasmids ranged from 1 – 4 (0.523 kilobase (kb) to 11.495 kb) in some strain. Most strains (76.5%) had only one plasmid (11.495 kb) mainly strains from flies. While restriction digests analyses revealed 9 clusters, strains in cluster one had up to 70% similarity. Similarly, most strains from flies (FS1, 2, 7, 8, 9, 19, 22 and 26) were closely related (> 50%) than that from lettuce. Nevertheless, a strain from lettuce was observed to be similar at more than 56% with a strain from flies (LS96 and FS 1). Other observed to be similar were FS30/LS72, FS11/LS96 and FS8/LS69 at 39%, 48% and 49% respectively. The result showed a considerable level of relatedness, this indicates they were probably transmitted from same source. The ubiquitous nature of flies made this relationship of public health importance, and therefore further researches are needed to identify the original source of the organism for proper mitigation.

Key Words: Mitigation, plasmid, restriction digest, similarity, strain

Introduction

Escherichia coli strains of the O157:H7 and O157- serogroups emerged as important public health problem was first recognized in 1982, following an outbreak in the USA (Schlundt *et al*., 2004). The pathogenicity of *E. coli* O157 is mainly located either on chromosome or on transmissible plasmid (Jacek, 2002). Humans are infected with EHEC primarily through the consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Faecal contamination of water and other foods (such as vegetables), as well as cross contamination during food preparation (originating from beef and other meat products, contaminated surfaces and kitchen utensils), or at pre/post harvest period of vegetables can occur and cause infection (Schlundt *et al*., 2004). Vegetables and many other leafy greens are important sources of fibers, minerals and vitamins, they plays an important role in lifestyle associated illnesses, these has increase demand and consumption rate especially in urban centers. The World Health Organization has issued reports that correct fresh produce intake alone could save 2.7 million lives a year and that 31% of heart disease cases are due to an insufficient intake of such foods (Johnson *et al*.,

2006), it also recommends an intake of 400g, or five to nine portions, of fresh fruits and vegetables per day (Mathew, 2006). Vegetables production through irrigation is mostly informal in Kano metropolis; it usually takes place on open urban spaces and unutilized plots continuously throughout the year, in areas with water access or close to streams and drains, which allow dry season production of quick market and highly valuable crops with corresponding profits, due to market proximity. A survey in 13 countries of West Africa showed that in 16 of 20 cities, men are mostly involved in open-space urban vegetable farming while in most cases; women dominated the vegetable retail sector (Drechsel *et al*., 2006). This made them one of the most ready to eat food and consumed (minimally processed food) and therefore the rapidly growing agricultural economic sector in the world (Chen, 2007) which peoples heavily relied on in developing countries.

This relationship had therefore made the identification of *E. coli* strains of public health important, for both clinical and epidemiological purposes. Understanding plasmid patterns and molecular characterization of other genetic material are also epidemiologically useful for establishment of adequate surveillances mechanism. A part from the need for periodic screening of emerging bacterial pathogens to determine their plasmid profile from different sources, for epidemiological data base and clinical studies (Farshad *et al*., 2012). The aim of the study was to investigate the genotypic characteristics of *E. coli* 0157:H7 on lettuce irrigated with wastewater and flies that were attracted by the foully smell of dirty in the wastewater canal, determined the relationship of *E. coli* 0157:H7 isolates from the lettuce and the flies, by plasmid profile and restriction digest of the chromosomal gene.

Material and Methods

E.coli O157:H7 isolates

A total of 17 *E.coli* O157:H7 isolates were obtained from previous research (Dahiru and Enabulele, 2014). Six from lettuce samples and 11 from flies scavenging in lettuce farms that were irrigated with wastewater. Of the 11 *E.coli* O157:H7 from flies 7 isolates were collected from farms along Jakara wastewater canal and the remaining from Sharada wastewater canal. Only one lettuce isolate was obtained from Jakara farms, while the remaining 6 strains were from Sharada wastewater canal irrigation farms.

Plasmid Isolation

E. coli 0157:H7 strains were grown on tripticase soy broth (TSB) at 37^0C for 24 hrs and cells were harvested by centrifugation at 5000 g for 5 min at room temperature. Plasmid deoxynucleotide acid (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). Plasmid DNA were treated with ribonuclease (RNase) (Fermentas) thereafter, accordance with manufacturer's instructions and run on 1% agarose (Bio Rad) by horizontal gel electrophoresis.

Restrictions Digest Analysis

Genomic DNA for restriction digest analysis was extracted in accordance with Bio Basic Inc (Canada) DNA extraction Kit instructions. DNA was treated with ribonuclease RNase (Fermentas) thereafter to remove the possible RNA contaminant from the sample, in accordance with manufacturer's guide. Restriction digest protocol was adapted from Murry and Gonzalez (Murrey *et al*., 1990 and Gonzalez *et al*., 2007). 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), for 1:30 minutes.

Electrophoresis of Samples.

Ten micro litter (10 µl) each of sample was properly mixed with 2 µl of loading dye (Bromocrysol purple) loaded into TAE submerged gel, inside electrophoresis machine tank. Ten (10 µl) of sample each was loaded in each well of the gel, except first well, in which 1 kb DNA (Fermentas) marker was loaded . The power was turn on and run at 60 V for 1.30 minutes and the gel was viewed under UV trans-

illuminator Gel Documentation machine, (Sambrook *et al*., 1989 and Desmond, 2008).

Fragment Size Estimation and Computation of Strain Similarities.

Restricted fragment sizes were estimated by Gel Doc XR+ with computer aided program, soft ware 3.0 (BioRad) UV trans- illuminator, by comparison with 1 kb molecular mass markers (Farmentas). Similarities among strains were determined by the Dice coefficient, and cluster analyses was based on the complete linkage.

Results

Plasmid Profile Analysis

Analysis of plasmid DNA of 17 isolates from lettuce and flies were shown in (Table 1). The results demonstrate three (3) different plasmids profiles, harboring plasmids ranged from 1 – 4 in some strain. In general there were only 6 different plasmids sizes, which ranged from 11.495 to 0.523 kb. Majority (76.5%) of the *E. coli* O157:H7 strains had only one plasmid (11.495) was observed in the entire number of isolates from flies, as shown in plasmid profile serial number 1 (Table 2). Others profiles had 3 plasmid and 4 plasmid were observed with 5.9% and 15.7% respectively.

Table 1: Plasmid profile patterns of *E. coli* 0157:H7 Strains from lettuce and flies sampled along wastewater irrigation farms in Kano, Nigeria.

S/N	Plasmid Pattern	Number of Strains	Source of Strains	
			Flies	Lettuce
1	11.495	13	11	2
3	11.495, 1.422, 0.523	1	0	1
4	11.495, 2.16, 1.838, 1.422	3	0	3
	Total	17	11	6

Restrictions Digest Analyses

The results of the DNA restriction digest of the 17 *E. coli* 0157:H7 strains isolated from lettuce and flies by *Sma* 1 enzyme revealed 9 different clusters, with each contained not more than 3 strains per cluster. Strains in cluster one were most closely related than all other strains, for example strains number FS8 and FS 22 (figure 1) were related to about 70% level of relatedness according to Dice similarity coefficient (Table 2). Similarly, most strains isolated from flies (FS1, 2, 7, 8, 9, 19, 22 and 26) were closely related (> 50%) than that isolated from lettuce. However, a strain from lettuce was also observed to show relatedness of more than 56% with a strain from flies (LS96 and FS 1). Significant similarities were also observed between FS30/LS72, FS11/LS96 and FS8/LS69 in figure 2, and had 39%, 48% and 49% (Table 2) respectively in the proximity matrix.

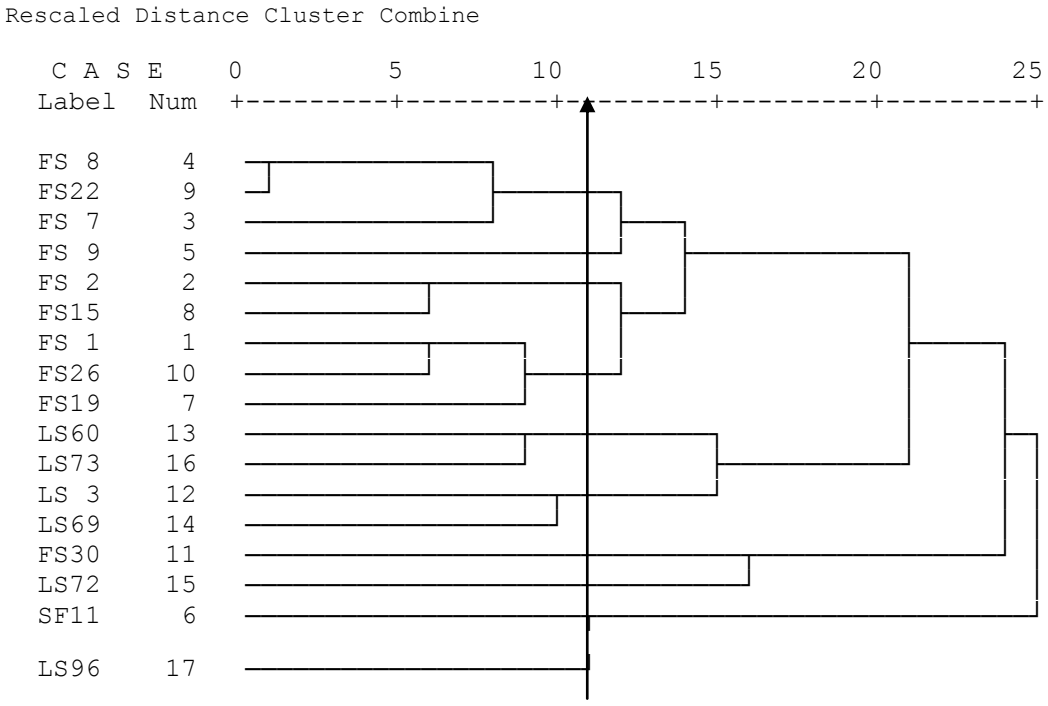

Figure 1: Dendrogram of *Sma* 1 Restriction digests of *E. coli* 1057:H7 isolated from flies and Lettuce by Complete Linkage Method using Dice similarity coefficient

Table 2: A proximity Matrix of the genomic DNA digest of *E. coli* 0157:H7 from lettuce and flies from wastewater irrigation farms in Kano, Dice Similarity Coefficient

	FS 1	FS 2	FS 7	FS 8	FS 9	SF11	FS19	FS15	FS22	FS26	FS30	LS 3	LS60	LS69	LS72	LS73	LS96
FS 1																	
FS 2	.516																
FS 7	.576	.438															
FS 8	.571	.557	.554														
FS 9	.515	.562	.471	.492													
SF11	.351	.400	.475	.250	.475												
FS19	.526	.473	.508	.536	.441	.360											
FS15	.508	.590	.554	.613	.431	.321	.571										
FS22	.594	.452	.576	.698	.515	.351	.456	.571									
FS26	.585	.508	.507	.500	.478	.310	.552	.562	.646								
FS30	.421	.436	.305	.464	.373	.200	.360	.286	.456	.414							
LS 3	.333	.462	.393	.453	.393	.255	.340	.415	.444	.400	.340						
LS60	.453	.392	.364	.423	.291	.304	.348	.385	.302	.444	.304	.419					
LS69	.483	.464	.400	.491	.400	.314	.392	.421	.414	.373	.431	.500	.426				
LS72	.340	.431	.364	.346	.436	.348	.478	.269	.377	.407	.391	.372	.238	.383			
LS73	.423	.360	.444	.431	.370	.222	.356	.353	.423	.377	.444	.429	.537	.478	.244		
LS96	.561	.436	.441	.429	.441	.480	.440	.429	.456	.483	.280	.340	.391	.431	.391	.222	

Discussion

The characterization of *E. coli* O157:H7 isolates from lettuce and flies, by plasmid profile and restriction digest analyses, showed some strains the same plasmid profile but had different restriction patterns. It was noted that 9 out of 11 strains from flies analyzed belonged to one large clonal group, although most of these strains were recovered from same sample type but from different location (farms), and possessed the same (plasmid profiles) however had diverse restriction digest patterns (figure 1). Nevertheless, strains with identical plasmid profiles (LS60, LS3 and LS69) were classified into different, although closely related, clonal group. Similarly, farm flies strains differentiated into same restriction patterns cluster (I), recovered from different farms, were identified as one clonal group with a very high degree of molecular similarity. As demonstrated in a previous study, such genetic differences and relationships were observed among strains of O157 serogroups, for example, (Gaddad *et al*., 2011), detected plasmids in toxigenic *E. coli* (STEC) most of which had only one plasmid, only 3 isolates had 4 plasmids in common and belongs to same profile. Previously, Smith and his colleagues (Smith *et al*., 2003), detected plasmids of various sizes in 47% *E. coli* 0157:H7 strains from cows, goats, pigs and rams. Samadpour and colleagues (1993) reported lambda (λ) restriction fragment length polymorphism (RFLP) of *E. coli* 0157:H7 strains from geographically or temporally unrelated sporadic cases, and none of strains had the same (λ) RFLP pattern. In this study the differences in restriction patterns could probably be due to spatial, temporal, and genotypic differences as previously observed in similar study by Oh and his colleagues (2009). In spite of the limited number of *E. coli* 0157:H7 strains, the results revealed some differences and similarities within and between the groups, which implies the interrelationship in the sources of strains which could provide a lead in contact tracing or other epidemiological studies.

Conclusion

All *E. coli* 0157:H7 characterized by plasmid profile and restriction digest have demonstrated a considerable level of relatedness within or between strains. This had therefore demonstrate the role of flies in the transmission of the bacteria (as bacterial carrier), from another source to lettuce or from the lettuce to other sources. The finding is of great economic and public health importance owing to the ubiquitous nature of flies and its relationship with environment. However a more specific research is therefore needed to determine the original source of *E. coli* 0157:H7 along the chain of transmission.

References

Chen, M.F. (2007). Consumer attitudes and purchase intentions in relation to organic foods in Taiwan: Moderating effects of food-related personality traits. *Food Quality and Preference*. 18: 1008-1021.

Dahiru, M. and O. I. Enabulele (2014). Prevalence and antibiogram of pre-harvest Escherichia coli 0157:H7 on cabbage irrigated with Jakara wastewater in Kano metropolis. *Jewel Journal of Scientific Research*. 2(1&2): 147 - 151

Desmond, S. T. N. (2008). An Introduction to Genetic Engineering**.** Third Edition, Published in the United States of America by Cambridge University Press, New York, 564 pp.

Drechsel, P. S., Graefe, M. S. and Cofie, O. O. (2006). Informal Irrigation in Urban West Africa: An Overview. IWMI Research Report 102.

Farshad, S., Ranjbar, R., Japoni, A., Hosseini, M., Anvarinejad, M., Mohammadzadegan, R. (2012). Microbial Susceptibility, Virulence Factors, and Plasmid Profiles of Uropathogenic Escherichia coli Strains Isolated from Children in Jahrom, Iran. *Arch Iran Med*. 15(5): 312 – 316.

Gaddad, S. M., Kesava, Naidu, G., Rajendra, G. N. and Shivannavar, C.T. (2011). Detection of Shiga toxin genes (*stx*1 & *stx*2) and Molecular Characterization of

toxigenic *Escherichia coli* Isolated from diverse sources in Gulbarga region, India; *Pharmacophore.* 2 (5): 253 -265

González, A., María, A. F., Rosa, G. and Javier, H. (2007). Molecular fingerprinting of *Campylobacter* and *Arcobacter* isolated from chicken and water; *J. Int. Micrbiol*. 10: 85-90

Jacek, O. (2002). Genetic relatedness of *Escherichia coli* o157:H7- strains isolated from pigs determined by random amplification of Box DNA sequences. *Bull. Vet. Inst. Pulawy* 46: 3-10

Johnson, L. M., Moe, C. L., Moll, D. and Jaykus, L. (2006).The epidemiology of produce-associated outbreaks of foodborne disease. In: Microbial Hazard Identification in Fresh Fruits and Vegetables (edited by J. James). New Jersey: John Wiley and Sons. Pp. 38-52.

Mathew, K. R. (2006). Microorganisms associated with fruits and vegetables. In: Microbiology of Fresh Produce (edited by K. R. Matthews). Washington DC: ASM Press. Pp. 1-21.

Murray, B. E., Singh, K.V., Heath, J. D., Sharma, B. R. and Weinstock, G. M. (1990). Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition *J. Clin. Microbiol.* 28(9):2059-206

Oh, D., Pan, Y., Berry, E., Cooley, M., Mandrell, R., and Breidt, F. JR. (2009) *Escherichia coli* O157:H7 Strains Isolated from Environmental Sources Differ Significantly in Acetic Acid Resistance Compared with Human Outbreak Strains. *Journal of Food Protection*, 72 (3): 503–509

Samadpour, M., Grimm, L.M., Desai, B., Alfi, D., Ongerth, J.E. and Tarr, P.I. (1993). Molecular epidemiology of *Escherichia coli* O157:H7 strains by bacteriophag lambda restriction fragment length polymorphism analysis. Application to a multistate food-borne outbreak and a day-care center cluster. *J. Clin. Microbiol.* 31(12): 3179-.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, Salem, MA.

Schlundt, J., Toyofuku, H., Jansen, J. and Herbst, S.A. (2004). Emerging food-borne zoonoses. *Rev. Sci.Tech. Off. Int. Epiz*. 23 (2): 513-533

Simeon, O. K., Emma, W. G., Eriola, B. and Olusola, O. S. (2003). A homemade kit for plasmid DNA mini-preparation. *Afri. J. Biotechnology*. 2 (4): 88–90

Smith, S.I., Aboaba, O.O., Odeigha, P., Shodipo, K., Adeyeye, J.A., Ibrahim, A., Adebiyi, T., Onibokun, H. and Odunukwe, N.N. (2003). Plasmid profile of *Escherichia coli* 0157:H7 from apparently healthy animals. *Afri. J. Biotech*. 2 (9): 322-324