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Clonal analysis of *Escherichia coli* strains isolated from food by pulsed-field gel electrophoresis

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Abstract: The genetic diversity of 74 isolates of *Escherichia coli* (*E. coli*) recovered from ground beef, sausage, turkey and well water samples was examined by PFGE (Pulsed field gel electrophoresis) of chromosomal DNA digested with *XbaI*. The *E. coli* isolates were classified into different serotypes obtained by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and expressing various *fliC* gene and toxic virulence markers. The restriction pattern profiles showed a high degree of polymorphism among isolates of different serotypes and a close relationship between strains belonging to the same serological group. In addition, some bacterial strains with identical virulence determinants exhibited distinct PFGE types. The results obtained showed that PFGE analysis is a powerful tool to reveal the clonal nature and genetic differences among potentially pathogenic *E. coli* strains.

Keywords: *Escherichia coli*; PFGE; Food

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Introduction

Molecular fingerprinting of bacterial isolates for their clonal definition is currently carried out by means of a broad range of various techniques (Swaminathan, Barrett, Hunter & Tauxe, 2001). Among these molecular typing methods, the analysis of restriction fragment length polymorphism of genomic DNA after digestion with rarecutting restriction enzymes (e. g. *XbaI*) and the separation of the generated large DNA fragments by means PFGE, also PFGE pattern analysis is rather frequently applied. Today PFGE pattern analysis is the most successful fingerprinting technique for epidemiological purposes and it has been applied for a broad range of bacterial species (Swaminathan et al., 2001). However, some bacterial species or particular strains could not be fingerprinted due to a rapid DNA degradation (e. g. (Baggesen, Wegener & Christensen, 1996; Corkill, Graham, Hart & Stubbs, 2000; Ling, Chan & Cheng, 2001; Lyytikainen et al., 2000; Romling & Tummler, 2000)

Among the many potentially pathogenic microorganisms introduced into the environment, coliform bacteria and in particular *E. coli* has been used extensively by authorities as an indicator of water quality. *E. coli* strains were not considered to be major pathogens until 1982 when *E. coli* O157:H7 was recognized as the source of an outbreak in Oregon and Michigan, USA (Riley et al., 1983). Since then, there have been many additional outbreaks reported from various countries (Kaper, O'Brien & pp., 1998.). The Center of Disease Control (CDC) estimates there are 73,000 cases of infection and 61 deaths yearly in the United States (http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.htm). The frequency of reports of this pathogen in various foods and drinking water illustrate the need to

minimize our exposure to this strain by monitoring it using accurate, rapid and cost-effective methods.

Differentiation of chromosomal DNA based on PFGE is a good, sensitive molecular method to analyze the degree of genetic relatedness or variability among different *E. coli* serotypes as well as among the strains of the same serogroup.

The purpose of this study was to determine the genetic relationship between a material of 74 of potentially pathogenic *E. coli* strains isolated from ground beef, turkey, sausage and well water samples in Casablanca, Morocco, belonging to different and to the same serotypes and expressing various *fliC* gene and toxic virulence markers.

Materials and methods

Bacterial strains

Assays were performed on 74 *E. coli* strains from the collection described by (Badri, Filliol, Carle, Hassar, Fassouane & Cohen, 2008). The strains originated from ground beef (n = 14), sausage (n = 21), turkey (n = 36) and well water (n = 3) were classified into different serotypes and expressing various *fliC* gene and toxic virulence markers.

Preparation of genomic DNA

Bacteria were grown on trypton caseine soja (TCS) agar plates at 37℃ for 16–18 h. Cells were removed from the plate to 1.5 ml microcentrifuge tubes containing 1 ml of Cell suspension buffer (CSB) (10 ml Tris-HCl 1M pH 8, 20ml EDTA 0,5 1M pH 8, 70 ml H2O maxima sterile) and the cell density was adjusted at 610 nm. The standardized cell suspension (120 µl) was transferred to 1.5 ml microcentrifuge tubes. Ten microliters of 10 mg/ml lysozyme solution (Sigma) was added and mixed with the cells by pipetting up and down. The mixture was incubated in a waterbath at 37℃ for 10 min. An equal volume of molten 1.6% of Agarose-Incert (BMA) prepared in TE buffer 5X and maintained at 60℃ was added to the cell suspension and mixed by gently pipetting up and down several times. The mixture was transferred into two reusable plugs mold Bio-Rad, Hercules, CA and allowed to cool for 15 min. The agarose plugs were transferred to 2 ml conical tubes containing 1ml of lysis buffer I (50 mM Tris pH 8.0, 50 Mm EDTA, pH 8.0, 0.5% sodium lauryl sarcosine, 1 mg of lysozyme, 10 mg/ml RNAase A), incubated for 2 h at 37℃. After RNA lysis, the lysis buffer solution I was removed and the plugs were washed with 1 ml of TE buffer. One ml of lysis buffer II (50 mM Tris pH 8.0, 50 mM EDTA, pH 8.0, 1% sodium lauryl sarcosine, 50 mg/ml Proteinase K) was added to agarose plugs and incubated 16-20 h at 50℃. After proteolysis, the lysis buffer solution II was removed and the plugs were washed twice with 1 ml of TE buffer 5X followed by tree washes with 1.5 ml of TE buffer 1X for 10 min each in the orbital water bath shaker 50–54℃ at 200 rpm. After the final TE wash, the plugs were sliced 2–2.5 mm slices using a Gel-Cutting Fixture (S&S Service Company, Stockbridge, GA) and prepared for restriction digestion or stored in 1.5 ml TE at 4℃ until ready for restriction.

Digestion of genomic DNA in agarose plugs

Intact, high-molecular-weight *E. coli* DNA in 2–2.5-mm plug slices was digested with XbaI (Pharmacia) in buffer solution according to the manufacturer's instructions Restriction with *XbaI* was done at an enzyme concentration of 25 units U per plug for 3 h at 37℃.

Electrophoresis, staining and destaining agarose gel

The DNA restriction fragments in plugs were separated by electrophoresis through 1% SeaKem Gold agarose gel in 0.5 X solution of Tris–borate–EDTA (prepared by diluting 10X TBE) buffer in a CHEF-Mapper PFGE apparatus Bio-Rad . The electrophoretic parameters used were as follows: initial switch time, 2.2 s; final switch time, 54.2 s; run time, 20 h; angle, 120°; gradient, 6.0 V/cm; temperature, 14℃; ramping factor, linear. After electrophoresis, the gels were stained for 15–20 min in 250 ml of deionized water containing 25 ml of ethidium bromide 10 mg/ml and destained by three washes of 20–30 min each using 500 ml of deionized water.

Analysis

DNA restriction fragments of *Salmonella Branderup* restricted with *XbaI* was used as the standard/reference strain. A total of 18 bands were found in the standard/reference strain ranging in size from 1135 to 20.5 kb. Analysis of banding patterns was done with the Molecular Analyst Bionumerics software.

Results

PFGE of *XbaI*-digested DNA fragments of 74 potentially pathogenic *E. coli* strains isolated from ground beef, turkey, sausage and well water samples in Casablanca, Morocco, belonging to different serotypes and expressing various *fliC* gene and toxic virulence markers. The *E. coli* isolates tested in this study were classified into 49 (66%) of isolates typeables belonged to 19 serogroups (O5, O6, O15, O16, O17, O18, O21, O25, O42, O74, O76, O83, O110, O115, O119, O121, O147, O157 and O159) and 25 (34%) *E. coli* strains not typeables belonged to 15 different patterns.

Among each serogroup, the strains were different in regard to *fliC* gene patterns and toxic virulence markers. The characteristics of the *E. coli* isolates included in this study are presented in Table 1. Strains of the O119 serogroup (n = 2), which were different in regard to *fliC* gene patterns (H30 and H9), generated two DNA band

types (designated as X21 and X22, respectively). Strains of the O15 serogroup generated three DNA band types (designated as X25, X26, and X27, respectively) which were different in the number and size of the digested fragments. Profile X25 was produced by H9 and *iucD*-positive isolates (n = 2) isolated from turkey and ground beef samples, whereas PFGE types X26, and X27 were obtained with bacteria different by *fliC* gene patterns (H18 and H4) and possessing the gene for the production *iucD*. Digestion with XbaI generated two PFGE types (designated as X33 and X34, respectively) among two *E. coli* isolates of the O21 serogroup: one possessing the gene for the production of *astA* and the second had the genetic marker for *astA*. Strains of the O5 serogroup generated two DNA band types (designated as X37 and X38, respectively). PFGE type X37 (n = 1) was obtained with bacteria of the O5:H11 serotype possessing the genetic marker for *astA* whereas PFGE type X38 (n = 2) was obtained with bacteria of the O5:H4 serotype possessing the genetic markers for *st* and *hlyA.*

On the other hand, when The *E. coli* strains of the R115:F31, R147:F4, R157:F7, R159:F4, R25:F8, R42:F21, R83:F1 serotypes were examined by PFGE, the *E. coli* strains of the same serotype produced the identical PFGE types (Table 1, figure 1). A significant similarity was observed among all strains belonging to the same serogroup.

Despite individual PFGE patterns were obtained with the strains of the serotypes R110:F28, R121:F10, R16:F11, R17:F5, R18:F7, R6:F21, R74:F29 and R76:F19, which presented by one strain each.

On the other hand, the *E. coli* strains not typeables belonged of the same *rfb*-RFLP profiles, the same *fliC*-RFLP profiles and the same toxic virulence markers, generated the same PFGE type (Table 1, figure 1).

Discussion

E. coli strains tested in this study were all potentially pathogenic. Furthermore, these isolates were serotyped using the molecular polymorphism of the somatic (O-antigen) gene *rfb* cluster and flagella (H-antigen) gene *fliC*, by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

In order to produce higher discriminatory potential than molecular typing with PCR-RFLP, PFGE was used to allow the comparison between individual isolates, the determination of a possible clonal relationship and the genetic relatedness between different *E. coli* serotypes as well as between strains of the same serogroups.

The analysis revealed the close relationship between *E. coli* strains of the same serotypes as well as some genetic similarities between the isolates of different serogroups. Despite the presence of polymorphic DNA bands and diverse virulence properties, a significant similarity was observed among all strains belonging to the same serogroup. Isolates with identical, or almost identical PFGE patterns are considered to be of the same clonal origin (Maslow, 1993.; Tenover et al., 1995). There was no visible correlation between PFGE types and sources of isolates (ground beef, turkey, sausage and well water). These data showed that PFGE is a powerful tool to reveal inter- and intra-serotype specific genetic differences among *E. coli* isolated from food.

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FIG. 1: Dendrogram generated by Bionumerics software, showing the relationships between O-antigen (R) and H-antigen (F) generated with MboII and HhaI restriction of *rfb* and *fliC* PCR products respectively. The phenogram was constructed using the Dice coefficient and UPGMA analysis. The degree of similarity (%) is shown on the scale.
N: R-type unknown
M: F-type unknown

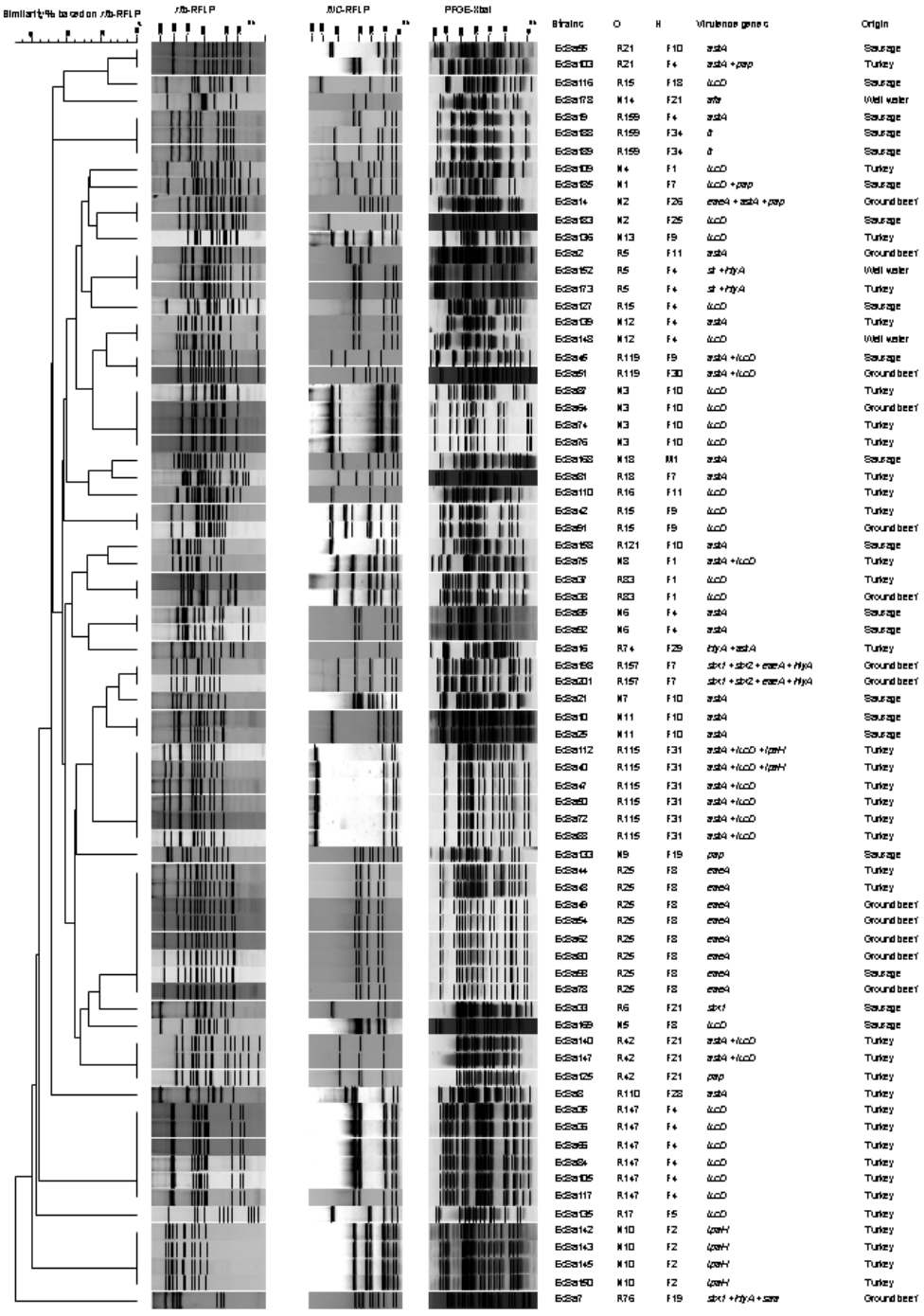

Table 1: Genotypic characteristics of *E. coli* isolates obtained from food in Casablanca, Morocco

Origin	*rfb*	*fliC*	Virulence genes				PFGE type	Number of strains
Sausage	N1	F7	*iucD*	*pap*			X1	1
Turkey	N10	F2	*IpaH*				X2	4
Sausage	N11	F10	*astA*				X3	2
Turkey	N12	F4	*iucD*	*pap*			X4	1
Well water	N12	F4	*iucD*				X5	1
Turkey	N13	F9	*iucD*				X6	1
Well water	N14	F21	*afa*				X7	1
Sausage	N18	M1	*astA*				X8	1
Ground beef	N2	F26	*eae*	*astA*	*pap*		X9	1
Sausage	N2	F25	*iucD*				X10	1
Ground beef	N3	F10	*iucD*				X11	1
Turkey	N3	F10	*iucD*				X12	1
Turkey	N3	F10	*iucD*				X12	2
Turkey	N4	F1	*iucD*				X13	1
Sausage	N5	F8	*iucD*				X14	1
Sausage	N6	F4	*astA*				X15	2
Sausage	N7	F10	*astA*				X16	1
Turkey	N8	F1	*astA*	*iucD*			X17	1
Sausage	N9	F19	*pap*				X18	1
Turkey	R110	F28	*astA*				X19	1
Turkey	R115	F31	*astA*	*IpaH*	*iucD*		X20	2
Turkey	R115	F31	*astA*	*iucD*			X20	4
Ground beef	R119	F30	*astA*	*iucD*			X21	1
Sausage	R119	F9	*astA*	*iucD*			X22	1
Sausage	R121	F10	*astA*				X23	1
Turkey	R147	F4	*iucD*				X24	6
Ground beef	R15	F9	*iucD*				X25	1
Sausage	R15	F18	*iucD*				X26	1
Sausage	R15	F4	*iucD*				X27	1
Turkey	R15	F9	*iucD*				X25	1
Ground beef	R157	F7	*stx1*	*stx2*	*eae*	*hlyA*	X28	2
Sausage	R159	F4	*LT*				X29	2
Sausage	R159	F4	*astA*				X29	1
Sausage	R16	F11	*iucD*				X30	1
Turkey	R17	F5	*iucD*				X31	1
Turkey	R18	F7	*astA*				X32	1
Sausage	R21	F10	*astA*				X33	1
Turkey	R21	F4	*astA*	*pap*			X34	1
Ground beef	R25	F8	*eae*				X35	5
Sausage	R25	F8	*eae*				X35	1
Turkey	R25	F8	*eae*				X35	2
Turkey	R42	F21	*pap*				X36	1
Turkey	R42	F21	*astA*	*iucD*			X36	2
Ground beef	R5	F11	*astA*				X37	1
Turkey	R5	F4	*ST*	*hlyA*			X38	1
Well water	R5	F4	*ST*	*hlyA*			X38	1
Sausage	R6	F21	*stx1*				X39	1
Turkey	R74	F29	*astA*	*hlyA*			X40	1
Ground beef	R76	F19	*stx1*	*hlyA*	*saa*		X41	1
Ground beef	R83	F1	*iucD*				X42	1
Turkey	R83	F1	*iucD*				X42	1