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MOLECULAR TYPING OF *Escherichia coli* STRAINS ISOLATED FROM FOOD IN CASABLANCA (MOROCCO)

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Abstract – Serotyping of O- and H-antigens is regarded as the gold standard in classification of *E. coli* for taxonomic and epidemiological purposes similar to the Kaufmann-White scheme for *Salmonella enterica*. Molecular methods to replace or to support the serotyping were recently applied. Using the molecular polymorphism of the somatic (O-antigen) gene *rfb* cluster and flagella (H-antigen) gene *fliC*, 74 *E. coli* strains carrying the virulent genes isolated from food were characterised by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The results showed that 49 (66%) of the isolates revealed a reproducible and clear cut classification, with a very good correlation to the collection of reference strains, were found.

Key words: *E. coli*, Molecular typing**,** PCR-RFLP, Food, Morocco

INTRODUCTION

E. coli strains are normal inhabitants in the gut of warm-blooded animals, including human beings, that cause intestinal infections such as diarrhea or hemorrhagic colitis, or extra-intestinal infections such as neonatal meningitis, nosocomial septicaemia, haemolytic uremic syndrome, urinary tract and surgical site infections (7). On the basis of their distinct virulent properties and the clinical symptoms of the host, pathogenic *E. coli* strains were divided into numerous categories or pathotypes, designated as enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and extra-intestinal pathogenic *E. coli* (ExPEC) (14).

Serotyping has long served to help distinguish between various *Escherichia coli* by the combination of their O- and H- (and sometimes K-) antigens. In conventional serotyping, an anti-*E. coli* polyclonal antiserum is used to agglutinate somatic and flagellar antigens. Unfortunately, *E. coli* are the most difficult members of the *Enterobacteriaceae* to serotype. The large collection of cross-absorbed sera required for complete *E. coli* serotyping is only available in a few international reference laboratories.

In order to circumvent the manifold problems related to conventional serotyping, rapid molecular methods for identifying different *E. coli* serogroups are developed. Alternatively, (4) and (10) proposed the use of a molecular method based on the analysis of operon O (*rfb*) and flagellin gene (*fli*C) polymorphisms, for O-antigen and H-antigen determination of *E. coli* strains without the use of antisera.

The purpose of the present study was: (*i*) to characterize *E. coli* somatic and flagellar genes by restriction of their amplified sequences, (*ii*) to compare restriction patterns with databases of restriction patterns (R-types) and (F-types) compiled by (10) and (4) respectively in order to deduce O-types and H-types, (*iii*) to classify the strains employed in this study in EPEC, STEC, EAggEC, and ETEC and ExPEC strains, based on the relationship between the results of

molecular typing and virulent properties of *E. coli* strains.

MATERIALS AND METHODS

Bacterial strains

The relevant characteristics of the 74 *E. coli* carrying virulence factors strains used in this study were from the collection described by (1). The strains originated from ground beef (n = 14), sausage (n = 21), turkey (n = 36) and well water (n = 3) in Casablanca (Morocco).

DNA preparation

Strains were streaked on tryptocasein soy agar (Sanofi Diagnostics Pasteur, Marnes-la- Coquette, France) and were subjected to the two DNA extraction methods. Due to the size of O-antigen gene clusters, intact DNA macromolecules were necessary for use as templates for PCR. Thus, bacterial DNA was isolated using Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA). For *fliC* flagellin gene, bacterial DNA was isolated using InstaGene Matrix kit (Biorad), following the instructions given by the manufacturer.

Amplification of the rfb and fliC genes

The O antigen gene cluster was amplified with the following two primers: 482 (5′-CAC TGC CAT ACC GAC GAC GCC GAT CTG TTG CTT GG-3′) and 412 (5′-ATT GGT AGC TGT AAG CCA AGG GCG GTA GCG T-3′) (17).

The *fliC* flagellin gene was amplified with the following two primers: 5′-CAA GTC ATT AAT AC (A/C) AAC AGC C-3′ (primer 1) and 5′-GAC AT(A/G) TT(A/G) GA(G/A/C) ACT TC(G/C) GT-3′ (primer 2). A/C, A/G, G/C and G/A/C in parentheses indicate A or C, A or G, G or C, G or A or C (respectively). The synthesized product had to contain a mixture of oligonucleotides representing all these combinations (5).

Restriction patterns

PCR-generated DNA was purified using GFX PCR DNA and Gel Band Purification Kit (Pharmacia). The purified PCR products *rfb* and *fliC* were cleaved respectively with *Mbo*II and *Hha*I restriction endonucleases (Pharmacia) according to the manufacturer's instructions for 2 h at 37 °C.

Computer identification of isolates

Using the Taxotron software, the restriction patterns of their amplified O-antigen gene cluster and *fliC* gene were identified by comparing databases containing the R and F type reference strains (4, 10) to conclude respectively O and H types.

RESULTS

Using PCR-RFLP for typing of *E. coli* strains that carrying virulence factors, the results showed that 49 (66%) of the isolates revealed a reproducible and clear cut classification, with a very good correlation to the collection of reference strains, were found. Irregular strain typeables belonged to 19 R types and 24 R:F types. Out of 25 (34%) *E. coli* strains non typeables belonged to 15 different patterns (Table 1 and figure 1).

Prevalent types in *E. coli* strains isolated from ground beef samples were: R119:F30 (7%), R157:F7 (14%), R15:F9 (7%), R25:F8 (36%), R5:F11 (7%), R76:F19 (7%), R83:F1 (7%). In 21 *E. coli* strains isolated from sausage samples, 11 types were detected: R119:F9 (5%), R121:F10 (5%), R159:F4 (14%), R15:F18 (5%), R15:F4 (5%), R16:F11 (5%), R21:F10 (5%), R25:F8 (5%), R6:F21 (5%), N11:F10 (10%), N18:M1 (5%), N1:F7 (5%), N2:F25 (5%), N5:F8 (5%), N6:F4 (10%), N7:F10 (5%), N9:F19 (5%) (N: non typeable). Among 36 *E. coli* strains isolated from turkey samples, the prevalent types were: R110:F28 (3%), R115:F31 (17%), R147:F4 (17%), R15:F9 (3%), R17:F5 (3%), R18:F7 (3%), R21:F4 (3%), R25:F8 (6%) R42:F21 (8%), R5:F4 (3%), R74:F29 (3%), R83:F1 (3%). The three *E. coli* strains isolated from well water [ ] corresponded to: R5:F4 (33%), N14:F21, (33%) and N12:F4 (33%).

Each type of sample is characterized by specific types. Also R25 and R15 types found in ground beef and turkey were also found in sausage. Only three types (R147:F4, R115:F31 and R42:F21) were associated with *E. coli* strains isolated from turkey samples, whereas the type R157:F7 colonies were only detected in *E. coli* strains isolated from ground beef.

DISCUSSION

The availability of rapid and specific laboratory tests to identify the foodborne pathogen is essentiel for preventing an otherwise easily treated malaise from developing into a life-threatening disease (6). A specific combination of somatic (O) and flagellar (H) antigens defines the serotype of Enterobacteriaceae members (6). Belonging to a specific serotype or serogroup does not in itself confer virulence on bacteria, but it has been demonstrated that serotyping can be used as an epidemiological probe for pathogenicity, because there is a high positive correlation between certain serotypes and enteropathogenicity. *E. coli* of a specific serotype can be associated reproducibly with certain clinical syndromes, even though the serological antigens do not confer but only correlate with virulence, as in EPEC and EHEC (13).

In this study, PCR-RFLP analysis of the somatic (O-antigen) gene *rfb* cluster and flagella (H-antigen) gene *fliC* of 74 *E. coli* strains carried

the virulence genes isolated from food, showed that molecular types that were found were associated with ETEC, STEC, EPEC, EAggEC and ExPEC phenotypes.

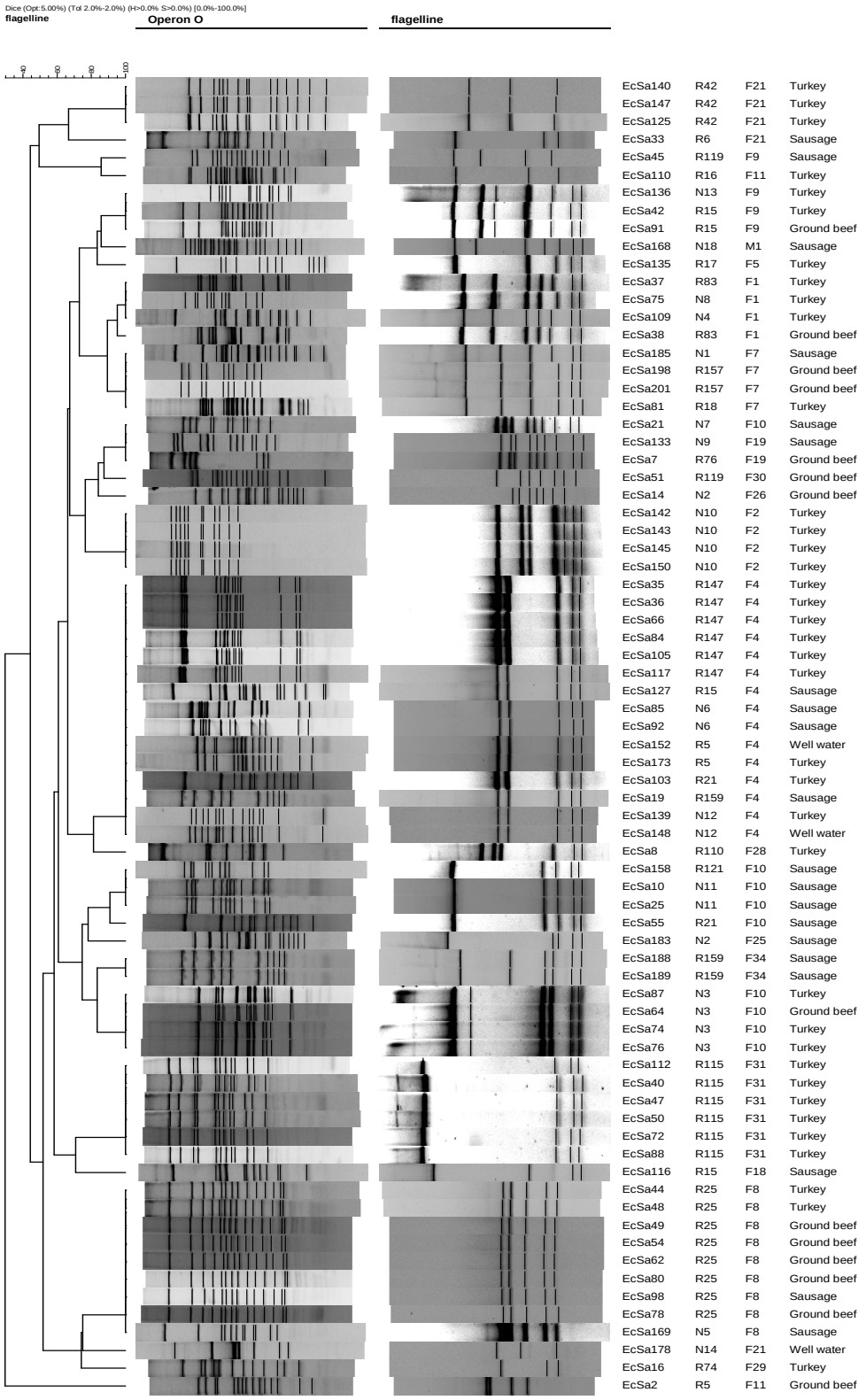

Figure 1. Dendrogram generated by Bionumerics software, showing the relationships between O-antigen (R) and H-antigen (F) generated with MboII and HhaI restriction of *rfb* and *fliC* PCR products respectively. The phenogram was constructed using the Dice coefficient and UPGMA analysis. The degree of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

Table 1. Results of restriction patterns of *rfb* gene (R-type) and *fliC* gene (F-type) obtained by PCR/*Mbo*II and *HhaI* RFLP typing of *E. coli* isolated from ground beef, sausage, turkey and well water samples.

	N° of isolates (%)			
Molecular type	Ground beef (n =14)	Sausage (n = 21)	Turkey (n = 36)	Well water (n = 3)
N1:F7	-	1(5)	-	-
N10:F2	-	-	4(11)	-
N11:F10	-	2(10)	-	-
N12:F4	-	-	1(3)	1(33)
N13:F9	-	-	1(3)	-
N14:F21	-	-	-	1(33)
N18:M1	-	1(5)	-	-
N2:F25	-	1(5)	-	-
N2:F26	1(7)	-	-	-
N3:F10	1(7)	-	3(8)	-
N4:F1	-	-	1(3)	-
N5:F8	-	1(5)	-	-
N6:F4	-	2(10)	-	-
N7:F10	-	1(5)	-	-
N8:F1	-	-	1(3)	-
N9:F19	-	1(5)	-	-
R110:F28	-	-	1(3)	-
R115:F31	-	-	6(17)	-
R119:F30	1(7)	-	-	-
R119:F9	-	1(5)	-	-
R121:F10	-	1(5)	-	-
R147:F4	-	-	6(17)	-
R15:F18	-	1(5)	-	-
R15:F4	-	1(5)	-	-
R15:F9	1(7)	-	1(3)	-
R157:F7	2(14)	-	-	-
R159:F4	-	3(14)	-	-
R16:F11	-	1(5)	-	-
R17:F5	-	-	1(3)	-
R18:F7	-	-	1(3)	-
R21:F10	-	1(5)	-	-
R21:F4	-	-	1(3)	-
R25:F8	5(36)	1(5)	2(6)	-
R42:F21	-	-	3(8)	-
R5:F11	1(7)	-	-	-
R5:F4	-	-	1(3)	1(33)
R6:F21	-	1(5)	-	-
R74:F29	-	-	1(3)	-
R76:F19	1(7)	-	-	-
R83:F1	1(7)	-	1(3)	-
Total	12(86)	11(52)	25(69)	1(33)

N: R-type unknown, M: F-type unknown

Whereas, ETEC associated with neonatal diarrhoea belong to a limited number of serogroups with O8, O9, O20, O64, O101, O138, O141, O147, O149, and O157 being the most commonly found in several countries ((20); (15); (9); (3); (12); (8)). The present study showed that LT-producing ETEC strains [ ] belonged to type R159:F4 and STa-producing ETEC strains belonged to type R5:F4.

Interestingly, we found 50 % of STEC isolates corresponded to *E. coli* R157:F7. The results are in accordance with earlier observations that the majority of STEC strains that cause human illnesses belong to a large number of serotypes, most outbreaks and sporadic cases of hemorrhagic colitis and hemolytic-uremic syndrome (HUS) have been attributed to strains belonging to *E. coli* O157:H7 (2, 3, 19). In contrast, our study showed that the non-O157 STEC strains [ ] belonged to *E. coli* R6:F21, R76:F19. These findings are not in agreement with those of (16) which reported that infections with non-O157 STEC were associated with serotypes such as O26:H- (nonmotile), O26:H11, O91:H-, O103:H2, O111:H-, O113:H21, O118:H16, O128:H2, O145:H-, O145:H28, and O146:H21.

Furthermore, in the EAggEC isolates employed in this study, that carried *iucD* and/ or *IpaH* genes in addition to *astA* genes, we identified R115:F31, R119:F30, R119:F9, R42:F21, R110:F28, R121:F10, R159:F4, R18:F7, R21:F10, R21:F4, R5:F11 and R74:F29. EPEC are defined as *stx*-negative *E. coli* strains able to produce A/E lesions on intestinal cells, detectable in vitro by positive *eae* and FAS tests (11). In our study, eight strains were *eae* positive (5 from ground beef, 2 from turkey and 1 from sausage) and [ ] belonged to type R25:F8. This serotype was : O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158 (18). The ExPEC, belonged to R147:F4, R15:F9, R15:F18, R15:F4, R15:F9, R16:F11, R17:F5 and R83:F1, R42:F21, R21:F4.

This molecular method to replace or to support the conventional serotyping demonstrated that ETEC, non-O157 STEC, EPEC, EAggEC and ExPEC isolates have certain O groups, that do not belong to the classical serogroups. Furthermore, this molecular method is a rapid and convenient alternative for typing [ ] *E. coli* types, without the need for serological testing and [ ] has proven to be very helpful as an alternative method for typing strains causing outbreaks that affect a large segment of the population and for supplementing conventional serotyping, for example when a strain becomes motionless or rough. However, it is necessary to find a high-performance platform that would enable us to add more antigens and to analyze more samples in a shorter time.

Additional research about the frequency of resistance to common antimicrobial agents in ETEC, non-O157 STEC, EPEC, EAggEC and ExPEC isolates used in this study is certainly needed to identify any associations between virulence and resistance genes in *E. coli* isolates.

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