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Protein Patterns and Plasmid Profiles of the Bacterial Strains Isolated from a Poultry Slaughterhouse in Ankara, Turkey

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Summary

A total of 65 identified isolates of coliform bacteria *Salmonella*, including *Campylobacter* and *Staphylococcus* isolated from different control points of a poultry slaughterhouse in Ankara, Turkey were characterized by morphological, biochemical and physiological tests including API 10 S system, and by plasmid profiles on agarose gel electrophoresis and whole-cell protein patterns on SDS-PAGE. Plasmids were detected in 53.8 % of the isolates. The molecular mass of the plasmids was within the range from 0.66 to 12.66 mDa. Electrophoretic banding patterns showed that whole cell protein profiles differed in several protein bands in *Salmonella*, *Campylobacter* and *Staphylococcus* species, but the differences were insufficient for reliable differentiation of bacteria species by SDS-PAGE method.

Key words: poultry, bacteria, SDS-PAGE, plasmid

Introduction

Food-borne illnesses (infections) resulting from bacterial contamination of poultry meat continue to be a health concern for the public at large. Most of the bacterial contaminants are non-pathogenic and associated with meat spoilage. However, poultry meat can become contaminated with a variety of food-borne pathogens, including *Salmonella* serotypes, *Campylobacter* spp. (especially *C. jejuni*), *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Yersinia enterocolitica*, *Aeromonas* spp. and *Streptococcus* spp. (*1–3*). Because of the potential importance of poultry as a vehicle for certain pathogens such as *Salmonella* and *Campylobacter*, specific tests for pathogens as well as indicator bacteria such as *E. coli* are likely to be important components of quality assessment (*4*). The identification of pathogens and indicator bacteria isolated from poultry is commonly employed in quality and hygienic control. In microbiological analysis associated with epidemiological investigation of outbreaks, it is often necessary to obtain a more detailed identification and characterization of the organisms involved than can be provided by conventional methods such as plasmid analysis and whole cell protein analysis. SDS-PAGE, usually combined with dendrograms derived from the numerical analysis of the whole-cell protein patterns of the strains, has been used extensively to study the differences among bacterial genera, species and strains (*5*). Plasmid analysis has also proved a useful method for differentiating isolates (*6*). The purpose of this study was to investigate the protein patterns and plasmid profiles to allow characterization and differentiation of the identified bacterial isolates of poultry.

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Materials and Methods

Bacterial isolates

A total of 65 identified isolates of the coliform bacteria including *E. coli*, *Salmonella*, *Staphylococcus* and *Campylobacter* isolated from different control points of a poultry slaughterhouse in Ankara were used in this study. The isolates were examined and identified in a previous project study of the Scientific and Technical Research Council of Turkey in 2001 at two different intervals (*7*). The control points in the project study were: **1.** entering of water chilling (whole carcass), **2.** chilling water, **3.** exit of water chilling (whole carcass), **4.** carcass box in air chilling (swab), **5.** carcass on the ground of air chilling (swab), **6.** exit of air chilling (whole carcass), **7.** wing piece, **8.** breast piece, **9.** back piece, **10.** thigh piece, **11.** carcass box in processing area, **12.** ground between water chilling and air chilling, **13.** personnel hand (swab), **14.** breast piece before packaging, **15.** packed breast piece, **16.** crop piece before packaging, **17.** packed crop piece, **18.** cutting board (swab), **19.** whole breast before packaging, **20.** plant process water, and **21.** whole packed carcass. A study code number was given for each isolate. The first digit of the code number of the isolates indicates the control point (Table 1). Isolation of coliform bacteria was carried out according to the method using EMB agar as isolation medium (*4*). *Salmonella*, *Staphylococcus* and *Campylobacter* were isolated using BGPR (brilliant green phenol red) agar, BPA (Baird-Parker) agar and CCDA (*Campylobacter* blood-free selective) agar base (Modified CCDA-Preston), respectively (*4*,*8*–*10*).

Reference strains

The strains of *E. coli* ATCC 35218**,** *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* ATCC 13883, *Salmonella enteriditis* ATCC 13076, *Campylobacter jejuni* ATCC 33291 and *Staphylococcus aureus* ATCC 29213 were used as references in this study.

Plasmid DNA analysis

Plasmid DNA of Gram-positive isolates (staphylococci) was prepared according to the method by Anderson and McKay (*11*), whereas plasmid DNA of Gram-negative isolates was prepared according to the method by Maniatis *et al*. (*12*) and Anderson and McKay (*11*). Plasmids were electrophoresed for 4 h at 100 V on a 0.8 % agarose gel in TAE buffer and the gel was photographed under UV illumination using Polaroid film Sigma 667. The approximate molecular masses were determined using plasmids of known size as standards (Lambda-pUC mix Marker 4).

Total protein analysis

Total protein samples were extracted as described by Bushuk *et al*. (*13*). Total protein analysis was carried out by using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) by the method described by Laemli (*14*). Each run included marker proteins of known molecular masses (Bio-Rad). The gels were stained overnight with Coomassie Brillant Blue G-250 according to Bushuk *et al*. (*13*) and Demiralp *et al*. (*15*).

Table 1. Plasmid numbers and sizes of the poultry isolates

Code numbers of the isolates	Numbers of the isolates with plasmid/ total numbers of the isolates	M_r(plasmid profile)/mDa					
E. coli	12/20						
E.3.1.2		12.66	3.66	1.93	1.2	0.9	
E.2.1		12.66	1.2	0.93			
E.14.1		12.66	3.66	1.2	0.73		
E.10.5		12.66	2.8	1.2			
E.17.1		12.66	2.13	1.2			
E.21.1		12.66	6.66	2.8	2.13	1.2	0.6
E.5.2.1		12.66	4	0.93			
E.3.1.1		12.66	3.66	1.93	1.33	0.9	
E.5.2.2		12.66	3.66	0.93			
E.10.2		12.66	5.13	3.33	0.93		
E.3.1.3		12.66					
E.10.4		12.66	1.5				
Campylobacter spp.	12/16						
C.9.2.1		12.66	2.3	0.8			
C.18.1		12.66	1				
C.8.12		12.66	0.8				
C.10.2.1		12.66					
C.15.1.1		12.66					
C.14.1.2		12.66					
C.17.1.1		12.66					
C.1.1		12.66					
C.9.1.2		12.66					
C.21.1.2		12.66					
C.1.1.1		12.66					
C.10.1.2		12.66					
E. cloacae	3/5						
E.11.1		12.66					
E.11.2		12.66					
E.7.1		12.66	0.93				
C. braakii	3/4						
E.7.2		12.66	1.5				
E.7.3		12.66	3.66	2.13	1.5	1.2	0.9
E.16.1		12.66	2.8				
Staphylococcus spp.	2/6						
Se.15.1		12.66					
Se.21.2		12.66	3.66	1.5			
S. aureus	1/9						
Sa.9.2		12.66	4.66	2.8	2.13		
C. farmeri	1/1						
E.10.3		12.66	3.66	0.93			
Salmonella	1/2						
S.21.3		12.66					

Cluster analysis

Different fragments on the gel were numbered sequentially and the presence or absence of fragments in each sample was scored (present 1, absent 0) for comparison with each other. Cluster analysis of whole cell proteins was performed according to the genetic distance method of Nei (*16*).

Results

The isolates were identified as: *Escherichia coli* (20 isolates; 30.76 %), *Enterobacter cloacae* (5 isolates; 7.69 %), *Klebsiella pneumoniae* (2 isolates; 3.07 %), *Citrobacter braakii* (4 isolates; 6.15 %), *Citrobacter farmeri* (1 isolate; 1.53 %), *Salmonella* spp. (2 isolates; 3.07 %), *Campylobacter* spp. (16 isolates; 24.61 %), *Staphylococcus aureus* (9 isolates; 13.84 %) and *Staphylococcus epidermis* (6 isolates; 9.23 %). The isolates of coliform bacteria and *Salmonella* were identified by using the API 10 S system (bioMérieux Sa) on the basis of twelve biochemical tests, which were ONPG, fermentations/oxidations of glucose and arabinose, lysine and ornithine decarboxylase, citrate utilization, H_2S, indole and NO_2 production, urease, tryptophane deaminase and cytochrome oxidase. API 10 S test kit is recommended by the commercial producer firm (API 10 S bioMérieux catalogue) for the identification of Enterobacteriaceae and other non-fastidious Gram-negative rods. It is indicated that some identifications may be extended by the use of API 20 E strip which provides 10 additional tests compared to API 10 S strip. The isolates of *Staphylococcus* and *Campylobacter* were identified by the conventional morphological (Gram reaction and microscopic morphology) and some biochemical tests including catalase, coagulase and thermostable DNase for *Staphylococcus*; and motility test by hanging drop method, H_2S production test in TSI agar, catalase, indole formation and oxidase for *Campylobacter* (*4,9–11*).

Plasmid profiles of the strains isolated from the poultry slaughterhouse are detailed in Table 1. Plasmid profiling demonstrated that 35 of the 65 isolates (over 53 %) contained plasmids. There was variation in the plasmid size observed, with 19 different molecular masses identified. Most of the isolates showed multiple plasmid bands with sizes ranging from 0.66 to 12.66 mDa. The most common plasmid of 12.66 mDa was detected in all strains isolated. The isolates of *K. pneumoniae* were plasmid-free.

SDS-PAGE of whole-cell protein extracts of *E. coli* strains (18 isolates and the reference strain) produced patterns containing 25 to 34 discrete bands with molecular masses of 65–200 kDa. The whole-cell protein patterns of the *E. coli* isolates are fairly homogeneous with

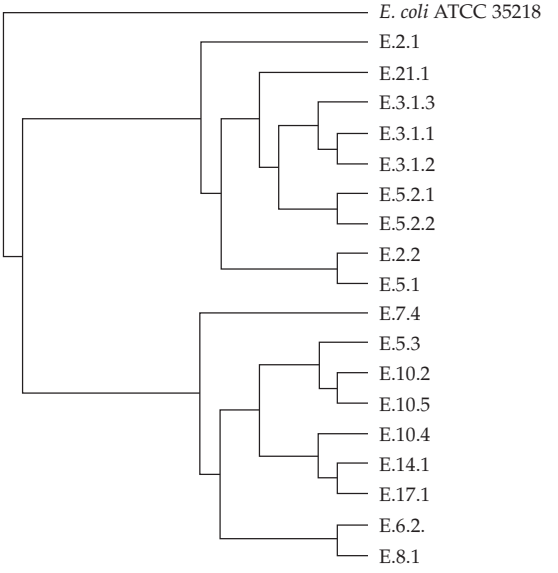

Fig. 1. Dendrogram of the *Escherichia coli* isolates and the reference strain

Table 2. Fraction of genetic distances of *Escherichia coli* isolates in %

Pop ID	E.2.1	E.21.1	E.2.2	E.3.1.1	E.3.1.2	E.3.1.3	E.5.1	E.5.2.1	E.5.2.2	*E. coli* ATCC 35218	E.5.3	E.6.2	E.7.4	E.8.1	E.10.2	E.10.4	E.10.5	E.14.1	E.17.1
	**																		
E.21.1	26	**																	
E.2.2	23	23	**																
E.3.1.1	36	17	20	**															
E.3.1.2	36	17	20	00	**														
E.3.1.3	39	20	23	02	02	**													
E.5.1	26	15	12	17	17	15	**												
E.5.2.1	29	17	26	09	09	12	23	**											
E.5.2.2	29	17	26	09	09	12	23	00	**										
E. coli ATCC 35218	42	50	39	32	32	36	50	32	32	**									
E.5.3	46	46	29	36	36	39	39	42	42	50	**								
E.6.2	54	46	42	23	23	20	32	29	29	42	15	**							
E.7.4	42	36	39	39	39	36	29	32	32	71	23	23	**						
E.8.1	39	32	29	23	23	20	20	29	29	58	20	09	23	**					
E.10.2	32	46	29	36	36	39	39	29	29	50	09	20	29	20	**				
E.10.4	26	32	23	29	29	32	20	29	29	58	20	26	29	15	15	**			
E.10.5	36	42	26	32	32	36	29	32	32	54	12	23	26	23	07	07	**		
E.14.1	39	39	17	23	23	26	26	29	29	50	15	20	23	15	15	09	07	**	
E.17.1	42	36	15	26	26	29	23	32	32	54	12	23	20	17	17	12	09	02	**

some variability, primarily localized in the low molecular mass region (estimated molecular mass, ~31–66 kDa). However, the reference strain of *E. coli* has a slightly different pattern. A dendrogram of the protein profiles revealed two distinct clusters (Fig. 1). The first cluster contained the reference strain (*E. coli* ATTC 35218) only. The second cluster divided into two subclusters, one of which was more heterogeneous with 9 isolates.

Certain *E. coli* isolate pairs isolated from the same control point of the poultry slaughterhouse, such as E.3.1.1 with E.3.1.2 and E.5.1.2 with E.5.2.2, were included in the same cluster with the 0 % genetic distance (Table 2). However, some isolate pairs which were isolated from the same control point, such as E.2.1 with E.2.2, E.5.2.1 with E.21.1, and E.5.2.2 with E.5.3, had slightly different characteristics because there is greater genetic distance between each isolate pair. The results indicate the spread of genetically identical and non-identical strains of a species in the poultry plant.

SDS-PAGE protein profiles of the Enterobacteriaceae isolates were quite similar except of the *E. coli* strains. There are two electrophoretic clusters in the dendrogram related to the protein profiles of the strains (Fig. 2). The *E. coli* strains and the reference strain formed a clearly distinct group in Enterobacteriaceae strains. The strains of *Enterobacter*, *Klebsiella*, *Citrobacter* and *Salmonella* isolates and the reference strains grouped within a separate cluster. This cluster divided into two subclusters. The reference *Enterobacter* strain alone formed the first subcluster. There were two distinct groups in the second subcluster: *Enterobacter/Citrobacter* and *Klebsiella/Salmonella*. The strains of *Enterobacter* were well separated from the strains of *Citrobacter*. The protein profiles of three *Citrobacter braakii* strains were quite different from *C. farmeri* strain. A strain of *C. farmeri* (E.10.3), having genetic distance between 22–53 % from other strains tested (Table 3), was placed in a group of its own due to its slightly different pattern from the other *C. braakii* strains. In the *Klebsiella/Salmonella* group, one isolate of *Salmonella* (*Salmonella* 1.1) grouped in a cluster with three isolates of *Klebsiella*. The other two *Salmonella* strains (one being the reference) were included in a separate subgroup.

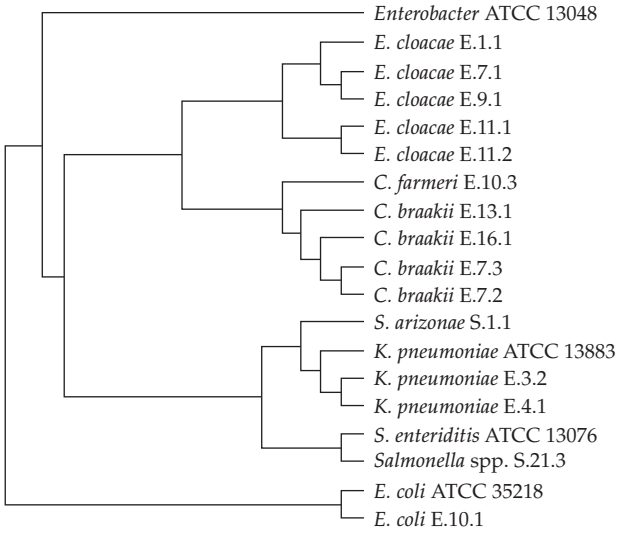

Fig. 2. Dendrogram of the Enterobacteriaceae and the reference strains

Table 3. Fraction of genetic distances of Enterobacteriaceae and the reference strains in %

Pop ID	*Enterobacter* ATCC 13048	E.1.1	E.7.1	E.9.1	E.11.1	E.11.2	*Klebsiella* ATCC 13883	E.3.2	E.4.1	*C. braakii* E.7.2	*C. braakii* E.7.3	*C. braakii* E.13.1	*C. braakii* E.16.1	*C. farmeri* E.10.3	*S. enteriditis* ATCC 13076	*Salmonella* spp. 21.3	*S. arizonae* 1.1	*E. coli* ATCC 35218	E.10.1
Enterobacter ATCC 13048	**																		
E.1.1	38	**																	
E.7.1	38	00	**																
E.9.1	45	05	05	**															
E.11.1	49	19	19	13	**														
E.11.2	49	13	13	08	05	**													
Klebsiella ATCC 13883	38	42	42	35	45	38	**												
E.3.2	28	25	25	19	28	22	19	**											
E.4.1	38	35	35	28	38	31	16	08	**										
C. braakii E.7.2	38	35	35	28	31	25	42	25	35	**									
C. braakii E.7.3	53	42	42	35	38	31	42	31	35	10	**								
C. braakii E.13.1	38	42	42	35	38	31	28	25	28	22	10	**							
C. braakii E.16.1	38	35	35	28	31	25	28	25	28	10	10	10	**						
C. farmeri E.10.3	38	42	42	49	53	45	49	38	42	35	22	22	22	**					
S. enteriditis ATCC 13076	77	38	38	31	42	35	38	35	31	38	38	45	38	53	**				
Salmonella spp. 21.3	77	45	45	38	49	42	38	35	31	45	45	53	45	53	05	**			
S. arizonae 1.1	45	49	49	49	53	53	28	31	28	49	49	42	42	58	38	45	**		
E. coli ATCC 35218	58	62	62	72	67	67	62	77	82	94	94	82	82	53	77	67	82	**	
E.10.1	58	38	38	45	49	42	45	42	45	45	45	45	45	45	42	49	45	35	**

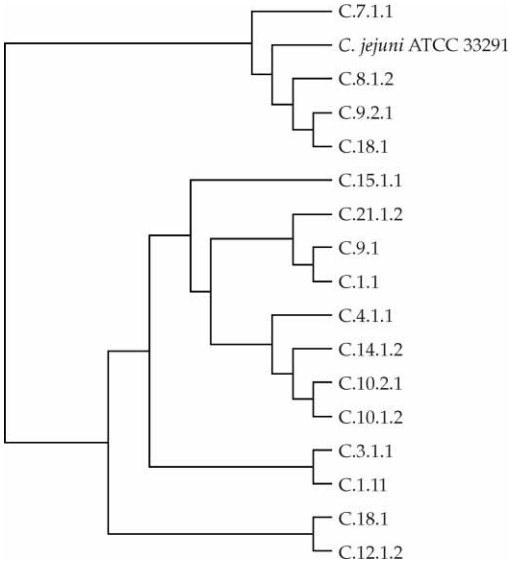

Fig 3. Dendrogram of the *Campylobacter* isolates and the reference strain

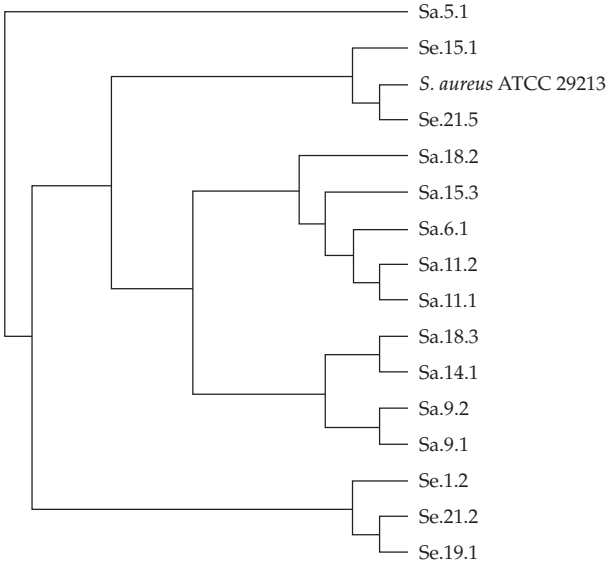

Fig 4. Dendrogram of the *Staphylococcus* isolates and the reference strain

The *Campylobacter* isolates divided into two main clusters according to whole-cell protein patterns (Fig. 3). The first group contained four *Campylobacter* isolates and the reference *Campylobacter jejuni* strain. The whole-cell protein patterns of the isolates in this cluster were fairly similar, except for isolates C.7.1.1, C.8.1.2, C.9.2.1, C.18.1 and the reference strain having the genetic distance between 30–91 % from the rest of the isolates (Table 4). The majority of the *Campylobacter* isolates are in the second cluster. The protein profiles of this group were heterogeneous with two subclusters apparent.

The whole-cell protein patterns of the *Staphylococcus* isolates are fairly homogeneous with some variability, primarily localized in the high molecular mass region. The staphylococci isolates divided into two clusters based on their whole-cell protein patterns (Fig. 4). The first cluster contained only *Staphylococcus aureus* isolate Sa.5.1. The second cluster, including the remaining staphylococci isolates and the reference *S. aureus* strain, divided into two subclusters. The isolates identified as *S. aureus*, as well as the reference *S. aureus* strain, and two isolates of *Staphylococcus* spp. were in the first subcluster. Two

Table. 4. Fraction of genetic distances of *Campylobacter* isolates in %

Pop ID	C. *jejuni* ATCC 33291	C.9.1	C.1.1	C.21.2	C.10.2.1	C.14.1.2	C.10.1.2	C.4.1.1	C.9.2.1	C.18.1	C.17.1.1	C.15.1.1	C.12.1.2	C.3.1.1	C.7.1.1	C.1.1.1	C.8.1.2
C. *jejuni* ATCC 33291	**																
C.9.1	38	**															
C.1.1	44	04	**														
C.21.2	54	10	06	**													
C.10.2.1	47	15	10	12	**												
C.14.1.2	51	17	12	10	02	**											
C.10.1.2	47	18	10	12	00	02	**										
C.4.1.1	54	15	10	12	04	06	04	**									
C.9.2.1	44	51	51	61	54	57	54	47	**								
C.18.1	41	47	47	57	51	54	51	44	06	**							
C.17.1.1	54	30	30	32	38	35	38	32	41	51	**						
C.15.1.1	57	22	17	19	15	17	15	10	51	54	19	**					
C.12.1.2	69	41	41	38	51	47	51	44	41	44	32	47	**				
C.3.1.1	57	27	32	41	47	51	47	41	57	61	30	27	54	**			
C.7.1.1	61	54	47	51	65	69	65	57	47	44	65	54	51	41	**		
C.1.1.1	61	24	24	32	22	24	22	17	47	38	44	30	57	24	51	**	
C.8.1.2	65	73	65	77	61	65	61	69	32	30	77	91	54	82	61	54	**

Table 5. Fraction of genetic distances of *Staphylococcus* isolates in %

Pop ID	*S. aureus* ATCC 29213	Se.21.5	Se.21.2	Se.19.1	Se.15.1	Se.1.2	Se.15.3	Se.18.3	Se.18.2	Sa.14.1	Sa.11.2	Sa.11.1	Sa.9.2	Sa.9.1	Sa.6.1	Sa.5.1
S. aureus ATCC 29213	**															
Se.21.5	00	**														
Se.21.2	34	34	**													
Se.19.1	26	26	19	**												
Se.15.1	19	19	43	34	**											
Se.1.2	53	53	43	19	43	**										
Se.15.3	19	19	43	19	43	26	**									
Se.18.3	19	19	43	19	43	26	26	**								
Se.18.2	34	34	43	19	26	26	26	43	**							
Sa.14.1	19	19	43	19	43	26	26	00	43	**						
Sa.11.2	19	19	43	19	43	43	12	26	12	26	**					
Sa.11.1	19	19	43	19	43	43	12	26	12	26	00	**				
Sa.9.2	34	34	43	19	63	26	26	12	26	12	12	12	**			
Sa.9.1	34	34	43	19	63	26	26	12	26	12	12	12	00	**		
Sa.6.1	19	19	43	19	43	43	12	26	12	26	00	00	12	12	**	
Sa.5.1	43	43	04	88	53	04	53	75	04	75	75	75	04	04	75	**

Table 6. Fraction of genetic distances of Enterobacteriaceae, *Campylobacter*, *Staphylococus* isolates and reference strains in %

Pop ID	*E. coli* ATCC 35218	E.2.1	E.10.5	*Enterobacter* ATCC 13048	E.9.1	E.11.1	*Klebsiella* ATCC 13883	E.3.2	E.4.1	E.16.1	E.10.3	*S. enteriditis* ATCC 13076	Sa.21.3	*S. arizonae* 1.1	*C. jejuni* ATCC 33291	C.3.1.1	C.1.1	Se.21.5	Sa.15.3
E. coli ATCC 35218	**																		
E.2.1	08	**																	
E.10.5	05	08	**																
Enterobacter ATCC 13048	21	18	14	**															
E.9.1	36	32	36	18	**														
E.11.1	32	28	32	14	02	**													
Klebsiella ATCC 13883	32	28	32	21	14	18	**												
E.3.2	36	32	36	25	11	14	02	**											
E.4.1	44	40	44	32	18	21	14	11	**										
E.16.1	44	40	44	32	18	21	14	11	00	**									
E.10.3	40	36	40	28	21	25	11	08	14	14	**								
S. enteriditis ATCC 13076	53	49	53	40	32	36	21	18	25	25	08	**							
Sa.21.3	53	49	53	40	25	28	21	18	18	18	14	05	**						
S. arizonae 1.1	58	53	58	44	36	40	25	21	28	28	18	21	21	**					
C. jejuni ATCC 33291	49	53	58	44	36	40	32	28	36	36	32	44	44	40	**				
C.3.1.1	53	58	63	49	40	44	36	32	40	40	36	40	40	36	14	**			
C.1.1	53	58	63	49	40	44	36	32	40	40	36	40	40	36	14	00	**		
Se.21.5	44	49	53	40	32	36	28	25	32	32	28	32	32	28	08	05	05	**	
Sa.15.3	53	58	63	49	40	44	36	32	40	40	36	40	40	36	14	11	11	05	**

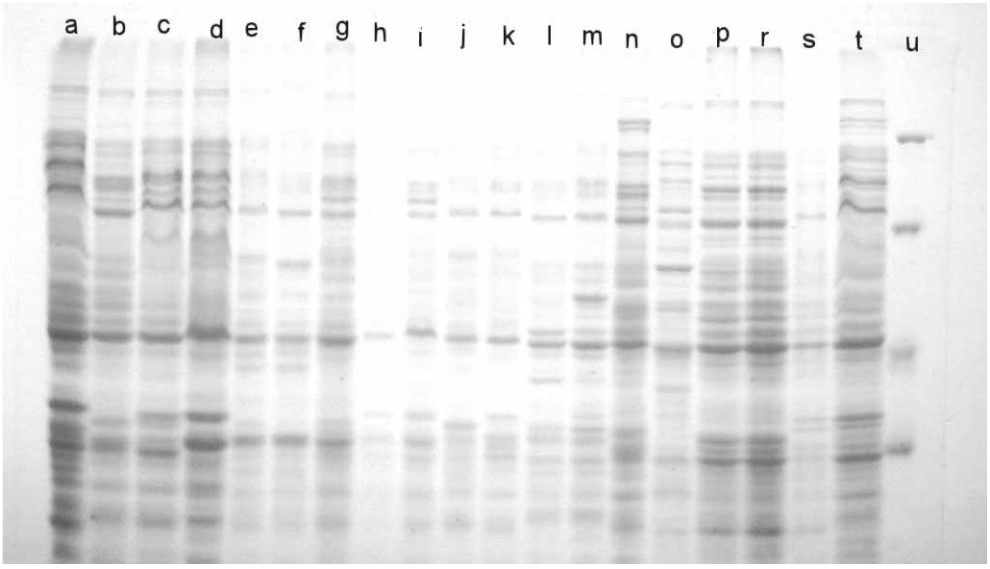

Fig. 5. SDS-PAGE protein profiles of Enterobacteriaceae, *Campylobacter*, *Staphylococcus* isolates and the reference strains. Lanes: **a.** *E. coli* ATCC 35218, **b.** *E. coli* E.2.1, **c.** *E. coli* E.10.5, **d.** *Enterobacter* ATCC 13048, **e.** *E. cloacae* E.9.1, **f.** *E. cloacae* E.11.1, **g.** *Klebsiella* ATCC 13883, **h.** *K. pneumoniae* E.3.2, **i.** *K. pneumoniae* E.4.1, **j.** *Citrobacter braakii* E.16.1, **k.** *C. farmeri* E.10.3, **l.** *Salmonella enteriditis* ATCC 13076, **m.** *Salmonella* spp. S.21.3, **n.** *S. arizonae* S.1.1, **o.** *Campylobacter jejuni* ATCC 33291, **p.** *C. jejuni* C.3.1.1, **r.** *C. jejuni* C.1.1, **s.** *Staphylococcus* spp. Se.21.5, **t.** *Staphylococcus* spp. Sa.15.3, **u.** molecular mass standards (kDa), 116 (β-galactosidase), 66 (bovine serum albumin), 45 (ovalbumin), 35 (lactate dehydrogenase), 25 (restriction endonuclease *Bsp*981), 18 (β-lactoglobulin), 14 (lysozyme)

Staphylococcus spp. isolates were closer to the reference *S. aureus* strain than other isolates of *S. aureus* in the same subcluster, and especially isolate number Se.21.5 shows no differences with the reference strain (genetic distance 0 %) (Table 5). The second subcluster contained three isolates of *Staphylococcus* spp. only.

Whole cell proteins of different genera were electrophoresed in the same gel (Fig. 5). The results indicated that although most of the isolates and reference strains grouped separately in the dendrogram (Fig. 6), the genetic differences (Table 6) of the isolates were not distinctly different from those of the other members of other bacterial groups studied.

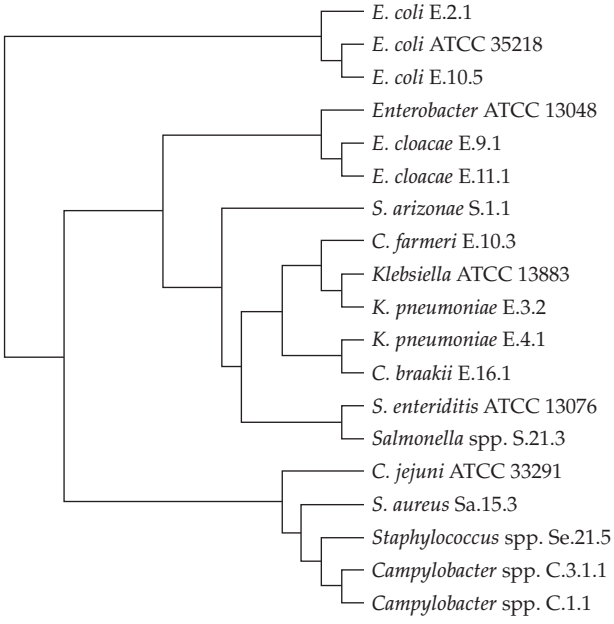

Fig. 6. Dendrogram of the Enterobacteriaceae, *Campylobacter* and *Staphylococcus*

Discussion

The isolates were characterized by their plasmid profiles. However, plasmid profiles do not reveal stable genetic differences among the strains. In fact, plasmid profile tests of the isolates are generally a useful tool for obtaining knowledge about resistance of the isolates to an antimicrobial substance and transfer of a plasmid among closely related isolates from different sources. Plasmid profiling is one of several useful methods for determining the relatedness or unrelatedness of bacterial strains that contain plasmid DNA. Thus, it should be used in combination with, but not in isolation from, other epidemiological methods.

This result underlines the importance of the protein pattern of the bacterial strains in determining their clustering. The protein pattern revealed a significant heterogeneity among strains of the same species such as *E. coli*. All the results of this study clearly show high discriminatory potential of the protein profile analysis in differentiating the bacterial strains isolated from a poultry plant on the isolate level. Strains of the same species with similar or different protein patterns, or plasmid profiles, could be isolated from the same control points in the poultry slaughterhouse. This situation indicates the importance of preventing the spread of pathogens in poultry processing and controlling microbial contamination in poultry plants. Methods of controlling pathogens continue to gain recognition as a serious research priority for many regulatory agencies (2). Two overall strategies are used by poultry processors to control pathogens in plants: good manufacturing practices (GMPs) and hazard analysis critical control points (HACCP). Differ-

entiating among different strains of the same species is a critical part of any epidemiological study, allowing us to detect outbreaks at all levels from local to global. Food-borne pathogens cause significant health problems in the population, as well as economic problems, loss in human productivity, medical expenses, and increased animal production costs. Conventional identification methods still have an important role to play in routine microbiological testing. However, it is believed that the use of molecular tests for differentiation of bacteria is a useful approach for establishing control strategies in plants and controlling bacterial outbreaks.

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References

1. D.E. Conner, M.A. Davis, L. Zhang: Poultry-Borne Pathogens: Plant Considerations. In: *Poultry Meat Processing*, A.R. Sams (Ed.), CRC Press (2001) pp. 137–158.
2. B.M. Hargis, D.J. Caldwell, J.A. Byrd: Microbiological Pathogens: Live Poultry Considerations. In: *Poultry Meat Processing*, A.R. Sams (Ed.), CRC Press (2001) pp. 121–135.
3. G.C. Mead: HACCP in Primary Processing: Poultry. In: *HACCP in the Meat Industry*, M. Brown (Ed.), CRC Press, Cambridge (2000) pp. 121–153.
4. W.F. Harrigan: *Laboratory Methods in Food Microbiology*, Academic Press (1998) p. 532.
5. T.M. Walia, T. Williamson, A. Kaiser, R. Tewari, Usefulness of protein patterns, serotyping and plasmid DNA profiles in the epidemiologic finger printing of *P. aeruginosa*, *Eur. J. Clin. Microbiol. Infect. Dis. 7* (1988) 248–255.
6. C. Neuwirth, E. Siebor, J. Lopez, A. Pechinot, A. Kazmierczak, Outbreak of TEM-24-producing *E. aerogenes* in an intensive care unit and dissemination of the extended-spectrum beta-lactamase to other members of the family Enterobacteriaceae, *J. Clin. Microbiol. 34* (1996) 76–79.
7. A. Temiz, R. Yılmaz: HACCP Application and Its Control in Poultry Slaugtherhouse and Processing Plants, *A Project Study of the Scientific and Technical Research Council of Turkey*, *Project No: TARP*, *2404* (2003).
8. K.H. Schleifer: Gram-Positive Cocci. In: *Bergey's Manual of Systematic Bacteriology*, *Vol. 2*, P.H.A. Sneath, N.S. Mair, J.G. Holt (Eds.), Baltimore (1986) pp. 999–1100.
9. The Oxoid Manual, Unipath Limited, England (1992).
10. J.F. MacFaddin: *Biochemical Tests for Identification of Medical Bacteria*, Williams and Wilkins, Baltimore (1980).
11. D.G. Anderson, L.L. McKay, Simple and rapid method for isolating large plasmid DNA from lactic streptococci, *Appl. Environ. Microbiol. 46* (1983) 549–52.
12. R. Maniatis, E.F. Fritsh, J. Sambrook: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York (1982).
13. W. Bushuk, R.L. Hay, N.G. Larsen, R.G. Sara, L.D. Simmons, K.H. Sutton, Effect of mechanical dough development on the extractability of wheat storage proteins from bread dough, *Cereal Chem. 74* (1997) 389–395.
14. U.K. Laemli, Cleavege of structural proteins during the assembly of the head of bacteriophage T4, *Nature*, *227* (1970) 680–684.
15. H. Demiralp, S. Çelik, H. Köksel, Effects of oxidizing agents and defatting on the electrophoretic patterns of flour proteins during dough mixing, *Eur. Food Res. Technol. 211* (2000) 322–325.
16. M. Nei, Genetic distance between populations, *The American Naturalist, 106* (1972) 283–292.

Sastav proteina i profil plazmida u bakterijskih sojeva izoliranih u klaonici peradi u Ankari (Turska)

Sažetak

S raznih kontrolnih mjesta u klaonici peradi u Ankari izolirano je i identificirano 65 izolata koliformnih bakterija, *Salmonella*, *Campylobacter* i *Staphylococcus*. Izolati su okarakterizirani morfološkim, biokemijskim i fiziološkim testovima, uključujući API 10 S sustav, te profilom plazmida dobivenim elektroforezom na agaroznom gelu i određivanjem ukupnih staničnih proteina na SDS-PAGE. Plazmidi su otkriveni u 53,8 % izolata. Relativna molekularna masa plazmida iznosila je od 0,66 do 12,66 mDa. Prema rasporedu elektroforetskih vrpca vidi se da se proteini cijelih stanica razlikuju u rodovima *Salmonella*, *Campylobacter* i *Staphylococcus*, ali su razlike nedovoljne da bi se pouzdano utvrdile vrste bakterija primjenom samo SDS-PAGE metode.