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Diversity of *Escherichia coli* Outer Membrane Protein

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Abstract: In the present investigation 23 *E. coli* isolates collected from calf diarrhea, mastitis cow, cow meat and chicken meat samples as well as 2 standard strains (O157:H7 ATCC 35150 and O6:H11 NCTC 12241) were investigated to study the diversity of *E. coli* serotypes collected from different sources using SDS-PAGE and Western-blot techniques. Among the used seven serotypes of the *E. coli* strains (2 O27:K1 strains, 4 O78:K80 strains, 3 O86:K61 strains, 3 O111:K58 strains, 2 O119:K69 strains, 2 O127:K63 strains, 5 O157:H7 strains and 2 O157:H$^-$ strains), polymorphism existed in the OMP patterns, yielded by SDS-PAGE with each serotype. The OMP patterns determined by SDS-PAGE revealed the presence of one to six major proteins with apparent molecular masses that varied from 27 to 39 kDa except O27:K1 strain isolated from mastitis cow, some other minor protein bands in the range at 3 to 22.625 kDa were present in all strains except O119:K69 strain isolated from diarrheic calf. Among the Western immunoblot analysis all strains reacted immunologically with the prepared hyperimmune serum against OMP of 3 O157 strains (2 O157:H7 strains isolated from mastitis cow and cow meat and one O157: H$^-$ strain isolated from chicken meat) at protein bands 31.663-36, 21-26 and 11-16 kDa.

Keywords: *E. coli* · Sodium dodecyle sulphate-Polyacrylamide gel Electrophoresis (SDS-PAGE) · Western Blot (WB) · Outer membrane Protein (OMP)

INTRODUCTION

Escherichia coli was first isolated by Theodor Escherich in 1885 as *Bacterium coli commune*, which was isolated from the feces of healthy newborns [1]. It is an important zoonotic pathogen and cattle have been identified as a major source of *E. coli* infection of human [2]. *E. coli* O157:H7 is associated with hemorrhagic colitis (HC) and Hemolytic Uremic Syndrome (HUS) cases among children who drank raw milk [3].

The cell-surface structure of Gram-negative bacteria consists of three essential layers: the cytoplasmic or inner membrane (IM), the outer membrane (OM) and the periplasmic space between the IM and OM. The OM of *E. coli* has a highly specialized structure and is usually associated firmly with the underlying peptidoglycan layer predominantly through lipoprotein/matrix protein and linked with cell-surface lipopolysaccharides (LPS) [4]. The major components of the OM are phospholipids, LPS and proteins which help it to serve as a physical barrier between the bacterial body and its surroundings and make the organism resistant to protects the cell against bile salts, antibiotics, proteolytic enzymes and other hostile factors and also facilities the uptake of nutrients [5, 6]. The aim of the present work was to detect the diversity of *E. coli* collected from different sources estimated by outer membrane protein (OMP).

MATERIALS AND METHODS

Strains: A total of 23 *Escherichia coli* strains collected from different sources as well as 2 standard strains as shown in table 1. All isolates and strains were confirmed to be *E. coli* according to Quinn *et al.* [7] and serotyped using diagnostic *E. coli* antisera (Denka Seiken), O:K monovalent antisera (Wellcome) and *E. coli* H7 antiserum.

Extraction of OMPs of *E. coli* Serotypes: OMPs were extracted by the method described by Deneer and Potter [8] with a minor modification. The bacteria were grown overnight at 37°C in 100 ml of Luria broth (Oxoid) and the cells were recovered by centrifugation (6.000 x g for 10 min at 4°C), suspended in 3 ml of

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Table 1: Serotypes of the examined strains and their sources.

Strain serotype	Source	No. Of strains
O6:H11	Standard strain	
	NCTC No. 12241	1
O26:K60	Mastitis cow	1
O27:K1	Mastitis cow	1
	Diarrheic calf	1
O78:K80	Mastitis cow	1
	Diarrheic calf	1
	Chicken meat	2
O86:K61	Diarrheic calf	2
	Chicken meat	1
O111:K58	Mastitis cow	1
	Diarrheic calf	1
	Cow meat	1
O119:K69	Diarrheic calf	2
O127:K63	Diarrheic calf	2
O157:H7	Standard strain	
	ATCC No. 35150	1
	Mastitis cow	1
	Diarrheic calf	2
	Cow meat	1
O157:H$^-$	Diarrheic calf	1
	Chicken meat	1
Total		25

HEPES (N-2 hydroxy ethyl piperazine-N'-2ethane sulfonic acid), pH 7.4 (Sigma Chemical Co.) and disrupted by sonication for 10 min at 4°C, then centrifuged at 6.000 x g for 10 min at 4°C. The supernatant was added to 0.75 ml of 2% N-lauroylsarcosine (Sarkosyl) from Sigma Chemical Co. and incubated for 10 min at room temperature. The mixture was centrifuged at 100.000 x g for 1 h (Bechman 70.1 Ti 39.000 rpm) in order to recover the detergent-solubilized OMPs. The pelleted protein was suspended into 3 ml of 10 HEPES (pH 7.4), indicated with volume of Sarkosyl at room temperature for 20 min and recovered by ultracentrifugation as described above. The final protein pellet was resuspended in 1 ml of 10 mM HEPES and stored at-20°C. Protein concentration of the prepared OMPs was measured by the method of Lowry *et al.* [9]. SDS-PAGE was carried out according to Laemmli [10] with 4% stacking and 9% separating gel after the OMP preparation were solubilized at 100°C for 7 min in 0.05 M Tris-HC1 buffer (2.5% SDS, 5% 2-mercaptoethand, 25% glycerol and 0.03% bromophenol blue). Protein bands were detected with sliver stain (Sigma Chemical Co.) using mid molecular weight protein markers (Sigma). Protein markers 210-4 kDa and 130-19.5 kDa were used for SDS-PAGE and protein marker 118-6.5 kDa was used for immunoblot assay.

Preparation of Hyperimmune Serum for *E. coli* O157: Three New Zeeland rabbits with an average weight 1700 g were used for preparation of hyperimmune sera against the OMP of *E. coli* O157. These animals were observed for 4 weeks before the starting of experiment for complete accommodation and daily given well nourished rations with daily doses of minerals and vitamins mixture to increase their immunity. All animals were free from *E. coli* antibodies. Pools of purified outer membrane proteins from 3 strains of O157 (O157:H7 isolated from mastitis cow, O157:H7 isolated from cow meat and O157: H- isolated from chicken meat) with the same concentration (7 mg/ml) was mixed [11] with equal volume of complete Freund's adjuvant. 0.5 ml from emulsion was injected S/C in each rabbit. After 9 days, 0.5 ml from emulsion of equal volume of the prepared OMP and incomplete Freud's adjuvant was injected S/C [12]. At day 19, the rabbits were bled and the blood was collected from each rabbit. The serum samples were separated and the antibody titers were estimated by passive hemagglutination (PHA) test according to Laboratory Manual, BgVV. Service laboratory, Berlin, Germany). The reactivity of hyperimmune serum against the prepared outer membrane proteins was tested by Western blot analysis.

Immunoblotting Technique: The protein bands of the prepared outer membrane proteins were electrophoretically transferred from SDS-PAGE to a nitrocellulose sheet using the modified Towbin *et al.* [13] technique. The gel was evenly pressed against the nitrocellulose sheet. The assembly was put to an electrophoretic chamber containing a transfer buffer with the nitrocellulose sheet facing the anode. The electrophoretic chamber was put at 4°C and a voltage gradient of 100 V was applied for one hour. The nitrocellulose sheets were soaked in blocking buffer (5% bovine serum albumin in 0.3% PBS-T) for 2 hours then washed in washing buffer (0.3% PBS-T) 2 times for 5 minutes each. The prepared hyperimmune serum was diluted (1:500 dilution) and the nitrocellulose sheet was exposed to the diluted sera for one hour. The nitrocellulose sheet washed 2-3 times for 5 minutes each in washing buffer. The nitrocellulose sheets were exposed to anti-rabbit IgG peroxidase (1:1000 dilution) (Bio-Rad Chemicals) for one hour. The sheet was washed 2-3 times for 5 minutes each in washing buffer and exposed to the AEC substrate (Sigma) for 30 minutes. The sheet was then rinsed thoroughly with distilled water to stop the reaction and the reaction was estimated by Gel pro-analyzer.

RESULTS

The outer membrane proteins of the examined 25 *E. coli* strains were grouped in 3 groups and investigated by SDS-PAGE as shown in photos (1, 2 and 3). Group I contained OMP profile analysis of nine strains as shown in photo 1. It was clear that, all nine samples exhibited the same major binding protein bands at 39.806, 31.839 and 23.786 kDa. Major OMP band at 27.125 kDa was observed in 7 strains: 3 O157:H7 strains (standard strain, one isolated from diarrheic calf and one isolated from cow meat), O157: H⁻ strain isolated from diarrheic calf, O119:K69 strain isolated from diarrheic calf and 2 O78:K80 strains isolated from diarrheic calf and mastitis cow. Also, It was observed the presence of minor OMP band at mol. wt. 3.7391 kDa with strong peptide band in 5 strains, 4 O157:H7 strains (standard strain, one strain isolated from mastitis cow and 2 strains isolated from diarrheic calves) and O78:K80 strain isolated from mastitis cow.

Group II contained OMP profile analysis of eight strains as shown in photo 2. The strains in this group had band at 63.966 kDa except O111:K58 and O86:K61 strains isolated from diarrheic calves with strong peptide band in O119:K69 strain isolated from diarrheic calf.

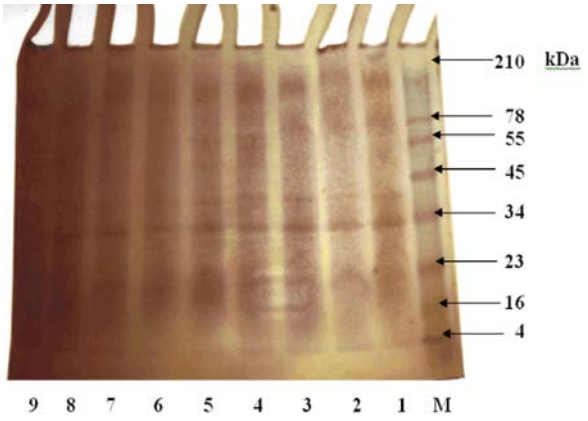

Photo 1: Outer membrane profile analysis of *E. coli* strains of Group I.
Lane M: protein marker (mol. wt. 210-4 kDa). Lane 1: O157:H7 standard strain (ATCC No. 35150). Lane 2: O157:H7 isolated from mastitis cow. Lane 3: O157:H7 isolated from diarrheic calf. Lane 4: O157:H7 isolated from diarrheic calf. Lane 5: O157: HÎ isolated from diarrheic calf. Lane 6: O157: H7 isolated from cow meat. Lane 7: O119: K69 isolated from diarrheic calf. Lane 8: O78: K80 isolated from diarrheic calf. Lane 9: O78:K80 isolated from mastitis cow.

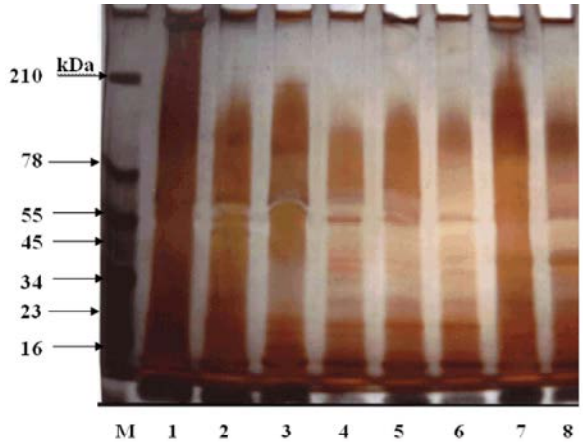

Photo 2: Outer membrane profile analysis of *E. coli* strains of Group II.
Lane M: protein marker (mol. wt. 210-4 kDa). Lane 1: O119:K69 isolated from diarrheic calf. Lane 2: O111:K58 isolated from cow meat. Lane 3: O27:K1 isolated from mastitis cow. Lane 4: O27:K1 isolated from diarrheic calf. Lane 5: O111:K58 isolated from diarrheic calf. Lane 6: O86:K61 isolated from diarrheic calf. Lane 7: O86:K61 isolated from diarrheic calf. Lane 8: O127:K63 isolated from diarrheic calf.

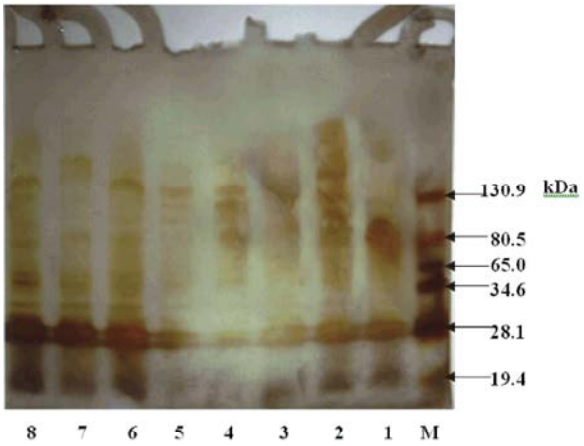

Photo 3: Outer membrane profile analysis of *E. coli* strains of Group III.
Lane M: protein marker (mol. wt. 130-19.5 kDa). Lane 1: O78:K80 isolated from chicken meat. Lane 2: O127:K63 isolated from diarrheic calf. Lane 3: O26:K60 isolated from mastitis cow. Lane 4: O111:K58 isolated from mastitis cow. Lane 5: O157: H⁻ isolated from chicken meat. Lane 6: O6:H11 standard strain (NCTC No. 12241). Lane 7: O86:K61 isolated from chicken meat. Lane 8: O78:K80 isolated from chicken meat.

(Also, this group showed a band at molecular weight 59.483-57.534 kDa except O119:K69 strain isolated from diarrheic calf and O27:K1 strain isolated from mastitis cow. The strains of group II had OMP band at 28 kDa except O119:K69 strain isolated from diarrheic calf and O27:K1 strain isolated from mastitis cow. There was major banding at 22.625 kDa in all strains except O119:K69 strain isolated from diarrheic calf.

Group III contained OMP profile analysis of eight strains as shown in Photo 3. (In this group it was noticed the presence of OMP band at molecular weight 19.405-16 kDa in all strains. A 36 kDa band was found in all strains except O111:K58 isolated from mastitis cow, O157: H$^-$and O86:K61 isolated from chicken meat. A 35 kDa band was detected in all strains except O157: H$^-$ isolated from chicken meat, O86:K61 isolated from chicken meat, O6:H11 standard strain and one of two strains O78:K80 isolated from chicken meat. 34.645 kDa in all strains except O157: H$^-$ isolated from chicken meat, standard strain O6:H11 standard strain, O86:K61 isolated from chicken meat and O78:K80 isolated from chicken meat. 31-30 kDa was found in all strains except one of two strains O78:K80 isolated from chicken meat and O111:K58 isolated from mastitis cow. All strains in this group except O78:K80 strain isolated from chicken meat harbored 28.108 kDa.

Comparative analysis of the groups reveals the presence of protein bands at:

- 28.975-27.125 kDa among 20 strains: O6:H11 standard strain, O26:K60 strain isolated from mastitis cow, O27:K1 strain isolated from diarrheic calf, 3 O78:K80 strains isolated from diarrheic calf, mastitis cow and meat chicken, 3 O86:K61 strains two isolated from diarrheic calves and one isolated from meat chicken, 3 O111:K58 strains isolated from diarrheic calf, mastitis cow and cow meat, O119:K69 strain isolated from diarrheic calf, 2 O127:K63 strains isolated from diarrheic calves, 3 O157:H7 strains (the standard strain, one strain isolated from diarrheic calf and one strain isolated from cow meat) and 2 O157:H$^-$ strains isolated from diarrheic calf and chicken meat.
- 32.875-30 kDa among 17 strains: O6:H11 standard strain, O26:K60 strain isolated from mastitis cow, 3 O78:K80 strains isolated from diarrheic calf, mastitis cow and chicken meat, 2 O86:K61 strains isolated from diarrheic calf and chicken meat, O111:K58 strain isolated from mastitis cow, O119:K69 strain isolated from diarrheic calf, O127:K63 strain isolated from diarrheic calf, all the examined O157:H7 strains (standard strain, strain isolated from mastitis cow, 2 strains isolated from diarrheic calves and strain isolated from cow meat) and 2 O157:H$^-$ strains isolated from diarrheic calf and chicken meat.
- 42.25-39.806 kDa among 14 strains: O27:K1 strain isolated from diarrheic calf, 2 O78:K80 strains isolated from diarrheic calf and mastitis cow, O86:K61 strain isolated from diarrheic calf, O111:K58 strain isolated from diarrheic calf, 2 O119:K69 strains isolated from diarrheic calves, O127:K63 strain isolated from diarrheic calf, 5 O157:H7 strains (standard strain, one strain isolated from mastitis cow, 2 strains isolated from diarrheic calves and one strain isolated from cow meat) and O157:H$^-$ strain isolated from diarrheic calf.
- 23.786-22.625 kDa among 15 strains: 2 O27:K1 strains isolated from diarrheic calf and mastitis cow, 2 O78:K80 strains isolated from diarrheic calf and mastitis cow, 2 O86:K61 strains isolated from diarrheic calves, 2 O111:K58 strain isolated from diarrheic calf and cow meat, O119:K69 strain isolated from diarrheic calf, 5 O157:H7 strains (standard strain, 2 strains isolated from diarrheic calves, strain isolated from cow meat and strain isolated from mastitis cow) and O157:H$^-$ strain isolated from diarrheic calf.
- 68.644-63.966 kDa among 13 strains: O6:H11 standard strain, O26:K60 standard strain isolated from mastitis cow, 2 O27:K1 strains isolated from diarrheic calf and mastitis cow, O78:K80 strain isolated from chicken meat, 2 O86:K61 strains isolated from diarrheic calf and chicken meat, 2 O111:K58 strains isolated from diarrheic calf and cow meat, O119:K69 strain isolated from diarrheic calf, 2 O127:K63 strains isolated from diarrheic calves and O157:H$^-$ strain isolated from chicken meat.
- 59.483-57.534 kDa among 12 strains: O6:H11 standard strain, O26:K60 strain isolated from mastitis cow, O27:K1 strain isolated from diarrheic calf, O78:K80 strain isolated from chicken meat, 3 O86:K61 strains (2 isolated from diarrheic calves and one from chicken meat), 3 O111:K58 strains isolated from diarrheic calf, mastitis cow and cow meat and 2 O127:K63 strains isolated from diarrheic calves.

Also, the presence of high molecular weight protein bands at: 192.63, 183.13 and 146.92 kDa was observed among standard strain O157:H7, O27:K1 isolated from mastitis cow and O127:K63 isolated from diarrheic calf, respectively. Protein band in the range at 153.35-150.08

kDa was observed among 2 O78:K80 strains isolated from diarrheic calf and mastitis cow, O86:K61 strain isolated from diarrheic calf, O119:K69 strain isolated from diarrheic calf, 3 O157:H7 strains (2 isolated from diarrheic calves and one from cow meat) and O157: H^{-} strain isolated from diarrheic calf. *E. coli* serotypes O26:K60 isolated from mastitis cow, O78:K80 isolated from chicken meat, O86:K61 isolated from chicken meat and O127:K63 isolated from diarrheic calf had a protein band at 134.19-127.61 kDa. While, *E. coli* serotypes O6:H11 standard strain, O78:K80 (one isolated from diarrheic calf and other from chicken meat), 2 O86:K61 strains isolated from diarrheic calves, 2 O111:K58 strains isolated from cow meat and diarrheic calf, O119:K69 strain isolated from diarrheic calf, 2 O127:K63 strains isolated from diarrheic calves and O157:H7 standard strain had a protein band 118.3-100.78 kDa.

Antibody Titration of the Prepared Hyperimmune Serum: Passive hemagglutination antibody titer of the prepared hyperimmune serum was > 4896 agglutination unit/ml.

Identification of Immunoreactive OMP Bands in Western Immunoblot: It was clear that all strains in the 3 groups reacted immunologically with the prepared hyperimmune serum as recorded in photos 4, 5 and 6.

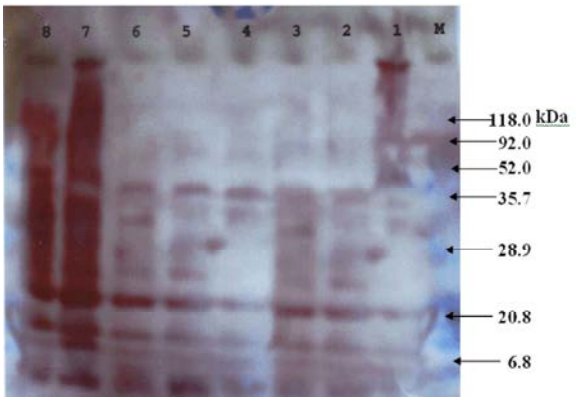

Photo 4: Immunoreactive OMP bands in Western immunoblot (Group I).
Lane M: protein marker (mol. wt. 118-6.8 kDa). Lane 1: O157:H7 standard strain ATCC No. 35150. Lane 2: O157:H7 isolated from mastitis cow. Lane 3: O157:H7 isolated from diarrheic calf. Lane 4: O157:H7 isolated from diarrheic calf. Lane 5: O157: H^{-} isolated from diarrheic calf. Lane 6: O157:H7 isolated from cow meat. Lane 7: O119:K69 isolated from diarrheic calf. Lane 8: O78:K80 isolated from diarrheic calf.

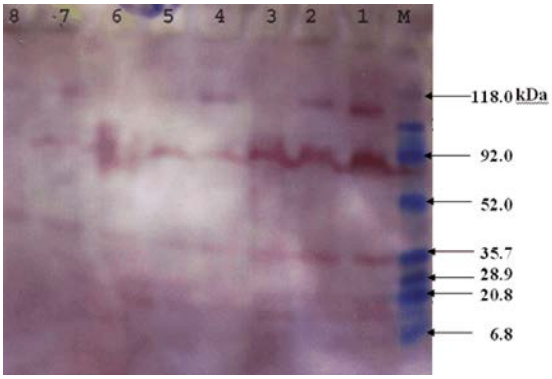

Photo 5: Immunoreactive OMP bands in Western immunoblot (Group II).
Lane M: protein marker (mol. wt. 118-6.8 kDa). Lane 1: O78:K80 isolated from mastitis cow. Lane 2: O119:K69 isolated from diarrheic calf. Lane 3: O111:K58 isolated from cow meat. Lane 4: O27:K1 isolated from mastitis cow. Lane 5: O27:K1 isolated from diarrheic calf. Lane 6: O111:K58 isolated from diarrheic calf. Lane 7: O86:K61 isolated from diarrheic calf. Lane 8: O86:K61 isolated from diarrheic calf.

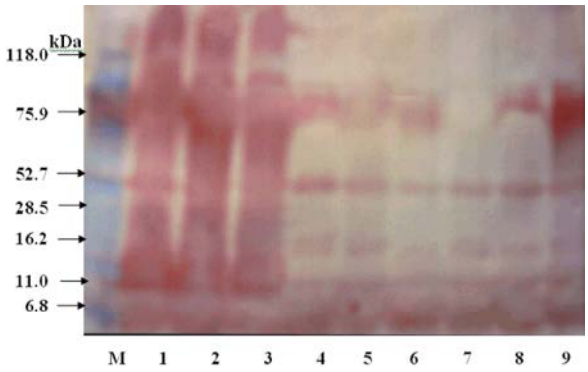

Photo 6: Immunoreactive OMP bands in Western immunoblot (Group III).
Lane M: protein marker (mol. wt. 118-6.8 kDa). Lane 1: O127:K63 isolated from diarrheic calf. Lane 2: O78:K80 isolated from chicken meat. Lane 3: O127:K63 isolated from diarrheic calf. Lane 4: O26:K60 isolated from mastitis cow. Lane 5: O111:K58 isolated from mastitis cow. Lane 6: O157: H-isolated from chicken meat. Lane 7: O6:H11standar strain NCTC No. 12241. Lane 8: O86:K61 isolated from chicken meat. Lane 9: O78:K80 isolated from chicken meat.

At the first group, all strains exhibited strong response to molecular weight protein bands at 34, 22 and 12-13 kDa as shown in photo (4). All strains in the second group

exhibited strong antibody response to molecular weight protein band at 36-31.663 kDa. There also response molecular weight protein bands at 16-11 kDa among the examined OMP except for strains O86:K61 isolated from diarrheic calves (25.063 and 22.932) and O111:K58 isolated from diarrheic calf (18.8kDa) as shown in photo (5). The last group revealed that all isolates had strong response to molecular weight protein band between 26 kDa and 21 kDa, as well as to molecular weight protein band at 14-11 kDa (Photo 6).

DISCUSSION

Meanwhile human and most worm blooded animals carry *E. coli* in the intestinal tract, as harmless commensal, diarrheagenic *E. coli* are the most common bacterial pathogens implicated in diarrhea worldwide. The cell surfaces of Gram-negative bacteria are composed of lipopolysaccharide which found exclusively in the outer leaflet of the asymmetric outer membrane (OM), where it forms a barrier to the entry of toxic hydrophobic molecules into the cell [14]. In the present investigation 23 *Escherichia coli* isolates collected from calf diarrhea, mastitis cows, cow meat and chicken meat samples as well as 2 standard strains (O157:H7 ATCC 35150 and O6:H11 NCTC 12241) were investigated to study the diversity of *E. coli* serotypes collected from different sources using SDS and Western-blot techniques. Firstly the collected strains were examined bacteriologically and serologically to confirm *E. coli* serotypes. The OMP was prepared from *E. coli* strains under investigation by the method described by [8] and SDS-PAGE was carried out by the method described by [10].

It is clear that, 17 strains (All strains of O157 which were O157:H7 standard strain, 4 O157:H7 strains isolated from mastitis cow, cow meat and 2 strains isolated from diarrheic calf and 2 O157:H-strains isolated from diarrheic calf and chicken meat), O119:K69 strain isolated from diarrheic calf, 3 O78:K80 strains isolated from diarrheic calf, mastitis cow and chicken meat, 2 O86:K61 strains isolated from diarrheic calf and chicken meat, O127:K63 isolated from diarrheic calf, O26:K60 isolated from mastitis cow, O111:K58 isolated from mastitis cow and O6:H11 standard strain) had band at 30-32.875 kDa, as well as 4 strains (O78:K80 isolated from chicken meat, O127:K63 isolated from diarrheic calf, O26:K60 isolated from mastitis cow and O111:K58 isolated from mastitis cow) had peptide band located at 35 kDa (Photos 1, 2, 3). OmpT is a 33.5 kDa protease that cleaves preferentially between two basic amino acids [15]. The enzyme has been suggested to be involved in urinary tract disease [16], in DNA excision repair [17] and in the breakdown of antimicrobial peptides [18], but the exact biological function remains unclear. [19] examined enteropathogenic *Escherichia coli* strains (EPEC) and described an outer membrane protein (OMP) of 32 kDa and reported to be involved in the adhesion of EPEC to HeLa cells, a comparable OMP of 35 kDa was detected in strains of EPEC and found to be heat-modifiable and peptidoglycan associated and considered to be the porin protein OmpF. *E. coli* K-12 has two major porins: OmpF (38 kDa) and OmpC (37 kDa). Sugar transport such as OmpF and the maltose regulon are down regulated at low pH, as sugar fermentation generates short-chain acids [20]. OmpA has been implicated as an important virulence factor in several Gram-negative bacterial infections such as *Escherichia coli* K1, a leading cause of neonatal meningitis associated with significant mortality and morbidity [21]. In *E. coli* O157:H7, OmpA plays a role in adherence to intestinal epithelial cells [22] and is also believed to mediate stimulation of dendritic cells to produce cytokines [23].

As shown in the same Photos, 15 strains (2 O27:K1 strains isolated from diarrheic calf and mastitis cow, 2 O78:K80 strains isolated from diarrheic calf and mastitis cow, 2 O86:K61 strains isolated from diarrheic calves, 2 O111:K58 strain isolated from diarrheic calf and cow meat, O119:K69 strain isolated from diarrheic calf, 5 O157:H7 strains (standard strain, 2 strains isolated from diarrheic calves, strain isolated from cow meat and strain isolated from mastitis cow) and O157:H$^-$ strain isolated from diarrheic calf) had band at mol.wt. 23.786-22.625 kDa. Also, 20 strains (O6:H11 standard strain, O26:K60 strain isolated from mastitis cow, O27:K1 strain isolated from diarrheic calf, 3 O78:K80 strains isolated from diarrheic calf, mastitis cow and meat chicken, 3 O86:K61 strains two isolated from diarrheic calves and one isolated from meat chicken, 3 O111:K58 strains isolated from diarrheic calf, mastitis cow and cow meat, O119:K69 strain isolated from diarrheic calf, 2 O127:K63 strains isolated from diarrheic calves, 3 O157:H7 strains (the standard strain, one strain isolated from diarrheic calf and one strain isolated from cow meat) and 2 O157:H$^-$ strains isolated from diarrheic calf and chicken meat) had band at mol.wt. 28.875-27.125 kDa.[24] showed that a 25 kDa protein contributes to the complement resistance of some *E. coli*. Some minor protein bands in the range of 18 to 23 kDa were present in all strains [25]. The production of biofilm is also regulated by *AggR* and requires several genes, including *Fis*, which codes for a DNA-binding protein involved in growth regulation and *yafK*, which codes for a 28-kDa protein[26].

Eleven strains (2 O111:K58 strains isolated from cow meat and mastitis cow, 2 O27:K1 strains isolated from mastitis cow and diarrheic calf, 2 O78:H80 strains isolated from chicken meat, O127:K63 isolated from diarrheic calf, O26:K60 isolated from mastitis cow, O157:H$^-$isolated from chicken meat, O6:H11 standard strain and O86:K61 isolated from chicken meat) had band located at 19.75-14.875 kDa, as shown in photos (2 and 3). An 18 kDa fimbrial adhesion from an Indian strain of EAEC (T7) inhibited HEp-2 cell adherence and agglutinated human blood group A erythrocytes in presence of 5 mM Ca^{2+} at 25°C and pH 6.5 [27]. Virulence factors that are believed to be associated with EAEC are 18 and 30 kDa outer membrane adhesions [28] and [27]. [29] excised 16 kDa band and determined through mass spectrometry that this protein is OmpX. OmpX is a member of a protein family that may be important to virulence by neutralizing host defenses. Kuehn and co-workers noted in their pioneering studies of LT secretion via vesicles that OmpX is present in ETEC outer membrane and vesicle preparations, that the abundance of OmpX differs as a function of the growth medium and that this protein is not detected in preparations from non-pathogenic strains [30].

The results reveal that, 12 strains (O6:H11 standard strain, O26:K60 strain isolated from mastitis cow, O27:K1 strain isolated from diarrheic calf, O78:K80 strain isolated from chicken meat, 3 O86:K61 strains (2 isolated from diarrheic calves and one from chicken meat), 3 O111:K58 strains isolated from diarrheic calf, mastitis cow and cow meat and 2 O127:K63 strains isolated from diarrheic calves) had band at 59.483-57.534 kDa, 13 strains (O6:H11 standard strain, O26:K60 standard strain isolated from mastitis cow, 2 O27:K1 strains isolated from diarrheic calf and mastitis cow, O78:K80 strain isolated from chicken meat, 2 O86:K61 strains isolated from diarrheic calf and chicken meat, 2 O111:K58 strains isolated from diarrheic calf and cow meat, O119:K69 strain isolated from diarrheic calf, 2 O127:K63 strains isolated from diarrheic calves and O157:H$^-$ strain isolated from chicken meat) had band at 68.644-63.966 kDa. Colicins are proteins produced by strains of *Escherichia coli* carrying a colicinogenic plasmid and lethal for related *E. coli* strains. They are classified into two groups (A and B), according to various characters. Colicin A is a pore-forming protein of 63 kDa organized into three domains, it has been shown to accumulate in the cytoplasm before being released in the medium with the help of Cal at the end of induction [31]. Colicin A rapidly becomes the major cell protein and is detected as a thick band with an apparent molecular mass of 60 kDa on SDS gels stained with Coomassie blue [32].

Also, it is clear that the SDS-PAGE profile analysis reveals the presence of high molecular weight protein bands among the examined isolates. *E. coli* serotypes O78:K80 (one isolated from diarrheic calf and other from chicken meat), 2 O86:K61 strains isolated from diarrheic calves, 2 O111:K58 strains (isolated from cow meat and diarrheic calf), O119:K69 isolated from diarrheic calf, 2 O127:K63 strains isolated from diarrheic calves and O157:H7 standard strain had a protein band 118.3-100.78 kDa. A correlation between diffuse adherence (DA) and the presence of the 100 kDa protein was observed by [33]. As a prerequisite for its function as an adhesin, the 100-kDa protein was localized to the surface of the bacteria by electron microscopy using colloidal gold-labeled protein A. The distribution of the gold particles indicated an even distribution on the bacterial cell surface and further showed that the 100-kDa adhesin is not part of a filamentous pilus like structure. Direct evidence for the adhesive properties of the 100-kDa protein stemmed from saturable specific binding of the isolated protein to HeLa cells. Thus, the adhesion involved in diffuse adherence (AIDA-l) is the 100-kDa protein [33]. The EPEC adherence factor (EAF) was suggested to correspond to a 94-kDa protein [34]. However, not all EAF^+ strains express a 94-kDa protein and the expressed proteins of this size were further shown to be serologically different [35].

Our data reveals that the presence of bands which have not been observed by others. It may be due to the used media, pH, temperature, method of OMP extraction etc. OMP of sizes 41 kDa and 48 kDa were observed at pH 4.0 only and were not detectable at control pH 7.4, besides OMP of size 17 kDa was 3-fold high at pH 4.0 than at pH 5.5 and control pH 7.4 [20].

In the present investigation hyperimmune serum for *E. coli* O157 (from 3 strains: 2 O157:H7 strains isolated from mastitis cow, cow meat and one isolate O157: H$^-$ isolated from chicken meat) was prepared in rabbits according to [11]. Immunoblot analysis of the extracted OMP of different strains was investigated among the prepared hyperimmune serum according to [13] as shown in Photos (4, 5 and 6). Photo (4) reveals that *E. coli*: O157:H7 standard strain, 4 O157:H7 strains isolated from mastitis cow, 2 from diarrheic calves and cow meat, O157: H$^-$ isolated from diarrheic calf, O119:K69 isolated from diarrheic calf and O78:K80 isolated from diarrheic calf had bands at 34, 22 and 12-13 kDa. Photo (5) shows that investigated *E. coli* strains: O78:K80 isolated from mastitis cow, O119:K69 isolated from diarrheic calf, 2 O111:K58 strains isolated from cow meat and diarrheic calf, 2 O27:K1 strains isolated from mastitis cow and diarrheic calf and 2

O86:K61 strains isolated from diarrheic calves had bands at 31.663-36.03 kDa. Photo (6) illustrates that *E. coli* strains: 2 O127:K63 strains isolated from diarrheic calf, 2 O78:K80 strains isolated from chicken meat, O26:K60 isolated from mastitis cow, O111:K58 isolated from mastitis cow, O157: H^{-} isolated from chicken meat, O6:H11 standard strain and O86:K61 isolated from chicken meat had bands at 26, 21 and 11-14 kDa. Immunoblot analysis of transblot of SDS-PAGE separated bacteria showed that both of the anti-35.5 kDa and 7.5 kDa OMP antibodies were bound by intact *E. coli* O55 cells [36]. Immunoblot analysis of the supernatant from *E. coli* C600 cells shows a single band of 12 kDa that corresponds to thioredoxin. With *E. coli* HMS 262 (*trxA*) cells harboring pTrx-TAP, two bands of 32 and ˜13 kDa are visible. The 32-kDa protein corresponds to TAP-tagged thioredox [37].

It could be concluded that there is a high diversity among *Escherichia coli* strains estimated by analysis of OMPs by using SDS-PAGE. Immunoblotting analysis revealed that all strains reacted immunologically with prepared anti O157 strain to bands at 31.663-36, 21-26 and 11-16 kDa. The results of this study had further development of OMP as a vaccine to protect against *E. coli*. Further studies on the possible relationship between this heterogeneity and the virulence properties of these groups of strains are needed.

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