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Phylogenetic Relationships of *Escherichia coli* Isolates Associated with Bovine Fecal and Milk samples

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To study the genetic diversity among *Escherichia coli* strains isolated from bovine fecal and milk samples, nineteen isolates of *E. coli* were typed by random amplification of polymorphic DNA (RAPD). Clusters of strains were defined on the basis of the epidemiological data and subsequently phylogenetic tree was created and revealed 4 clusters. The obtained results indicated that the serotypes causing bovine mastitis were similar genetically to that causing diarrhea. This type of information can be used in clinical setting to discriminate ongoing epidemics of *E. coli* from incidentally increased infection rate.

Key words: Phylogenetic tree, *Escherichia coli*, RAPD.

Escherichia coli is ubiquitous in the cow's environment that is contaminated by feces[1,2] Besides, a frequent cause of bovine mastitis is that the *E. coli* serotypes in mastitic milk were similar to fecal isolates. It is known that O157 serotype of *E. coli* isolated from raw milk samples[3, 4] were of fecal origin.[5, 6]

Outbreaks often occur via the fecal-oral route as a result of the low infective dose and the presence of susceptible people[7] Infection may also occur through direct or indirect contact with infected cattle and cattle feces.[8]

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E. coli O157 serotype, the causative agent of colibacillosis, can cause severe disease such as bloody diarrhea, hemolytic uremic syndrome and hemorrhagic colitis in human,[9,10] although other fecal *E. coli* serotypes are known to cause bovine mastitis.[2,11]

The ability to differentiate between these strains is complicated by the clonal nature of this group. Typing methods such as biochemical profiling,[12] plasmid profiling, verocytotoxin typing,[13] phage typing[14], ribotyping[15] and pulsed-field gel electrophoresis (PFGE)[16] have had limited success in intra-sero group differentiation.

As a consequence, there is a need to improve subtyping methods, with particular emphasis on the development of molecular methods, to target the often minimal amount of variation observed between strains.

Current methods are variable in their speed, technical complexity, cost and ability to discriminate reliably between *E. coli* strains.[17] To be able to trace reliably the source and mode of transmission of an outbreak, an epidemiological typing method must be able to discriminate reproducibly between different strains and identify identical strains. Random amplification of polymorphic DNA by PCR (RAPD - PCR) analysis [18, 19] is a molecular typing method which has increasing acceptance due to its simplicity, sensitivity and flexibility to study the genetic diversity among groups of *E. coli* strains.

In the present study, a robust RAPD - PCR protocol was applied to study the inter- and intra serotype specific genotypic variations of the isolated strains of *E. coli* from bovine fecal and milk samples. Bands were then analyzed using a binary system and phylogenetic tree was created to allow robust epidemiological typing of *E. coli* strain.

MATERIALS AND METHODS

Sample collection

Aliquots grams of rectal fecal were collected from 56 diarrheic calves aged from 1 to 4 months. They were from private and governmental farms, located at Giza and EL-Sharkia governorates, Egypt.

Milk samples from the same farms were aseptically collected from 32 quarters with subclinical mastitis and 48 quarters with clinical mastitis. Fecal and milk samples were transferred to the laboratory in an ice box to be cultured immediately.

Isolation and identification of *E. coli*

Milk samples were centrifuged at 3000 rpm for 15 minutes and after centrifugation, the supernatant as well as the sediment were cultured. Both fecal and milk samples were primarily cultured on MacConkey agar medium, incubated aerobically at 37°C. After an overnight incubation, a part of single typical well isolated lactose fermenting colony was tested for sorbitol fermentation by culturing on sorbitol MacConkey agar and sorbitol phenol red agar media, then incubated at 37°C overnight. Morphological, cultural and biochemical examinations were carried out according to Quinn *et al*. [20]

Isolates that were primarily identified by biochemical tests were then subjected to serological identification using diagnostic polyvalent and monovalent *E. coli* antisera (Welcome diagnostic antisera). Diagnostic *E. coli* O157 antisera (Difco) and H7 anti-sera (Difco) were used for serological identification of *E. coli* O157: H7.

Detection of virulence factors of *E. coli* isolates

Hemolytic activity (hemolysin) was tested using 5% defibrinated sheep blood agar. The ability to produce heat stable enterotoxin was assayed by the infant mouse test as described by Robins - Brown *et al*.[21]

The ability of *E. coli* isolates to invade epithelial cell were tested in rabbit eye model "Sereny test". Detection of cytotoxin activity of *E. coli* strains isolated from the fecal and mastitic milk samples using Vero cells was investigated according to Giugliano *et al*. [22] Congo red binding was adopted as a virulence test among *E. coli* isolates according to Berkhoff and Vinal, [23]

Extraction of DNA

The DNA of 19 *E. coli* isolates representing 5 different serotypes were extracted by hexadecyl trimethyl ammonium bromide (CTAB), according to Sambrook *et al*. [24]

Random amplified polymorphic DNA fingerprinting (RAPD -PCR) according to Pacheco *et al*. 25

The total RAPD reaction volume was 50 µl, containing 1µl (600 ng) of the extracted DNA from *E. coli* isolates, 5 µl 10X PCR buffer, 2 µl dNTPs (40 uM), 1µl (1.5 u) DNA polymerase, 36 µl deionized distilled water and 1 µl (50 pmol) of each of the following five primers of arbitrary sequence: Sequence of primer 1 (D1247) 5\ AACAGCCCGT 3\. Sequence of primer 2 (D1281) 5\ AAC GCG CAA C 3\. Sequence of primer3 (D1283) [1533-3465]: 5\ GCG ATC CCC A 3\. Sequence of primer 4 (D1284) [1533-4165]: 5\ GTG GAT GCG A 3\. Sequence of primer 5 (DI4803) [1533-5165]: 5' AAA CGG TTG GGT GAG 3'

The mixture was overload with 40 µl paraffin oil and the reaction conditions were then placed into thermal cycler programmed as follows: Initial denaturation steps at 94°C for 4 minutes followed by first cycle at 94°C for 45 seconds for denaturation, 37°C for 1 minute for annealing step and 72°C for 2 minutes for extension step repeated for another 30 cycles. While the final extension

were at 72°C for 10 minutes. The PCR products were stored in the thermal cycler at 4°C until they were collected.

The PCR products were visualized by agarose gel electrophoresis according to Sambrook *et al*. [24]

RAPD -PCR data analysis

The banding patterns generated by RAPD - PCR analysis were compared to determine the genetic relatedness of *E. coli* isolates. The amplified fragments were scored either· present (1) or absent (0). Bands of the same mobility were scored as identical.

The similarity coefficient (f) between two isolates was defined using the formula of Nei and Li [26]: $f(xy) = 2N(xy) / N(x) + N(y)$

where = N(xy) is the number of RAPD bands shared by the two isolates.

N(x) and N(y) are the numbers of RAPD bands scored in each sample.

The genetic distance (d) was calculated using the equation:

D = 1-F (xy) given by Hillis and Mortiz. [27]

A dendrogram was derived from the distance by the unweighed paired group method, arithmetic mean (UPGMA) algorithm contained in the computer program MVSP (Multivarite statistical package) version 310 (1988-1999 Kovach Computer Services).

RESULTS AND DISCUSSION

The role of *Escherichia coli* as a pathogen is well known and many *E. coli* isolates have been associated with a wide variety of diseases in animals. It is incriminated in production of severe infection such as gastrointestinal colibacillosis, colisepticaemia hemorrhagic colitis, hemolytic uremic syndrome, bloody diarrhea and also it is a frequent cause of bovine mastitis .Therefore, increasing attention is being given to the role played by livestock in the epidemiology of this organism and to study of genetic diversity among groups of *E. coli* strains rather than biochemical and serological identification.

Bacteriological examination of bovine fecal and milk samples recovered from clinical and subclinical mastitic cases revealed recovery of 22 (39.29%) *E. coli* isolates out of 56 examined fecal samples, 12 (25%) isolates out of 48 examined milk samples obtained from clinical mastitic cases and 8 (25%) out of 32 examined milk samples received from subclinical mastitic cases as shown in Table 1.

Traditional studies for *E. coli* were always clarified by referring to the serotyping of the incriminated isolates.[28] Serotyping of *E. coli* was carried out according to the available antisera, 22 serotypes of *E. coli* were isolated from fecal samples of diarrheic calves revealed 7 isolates (12.50%) O111: K58, 5 isolates (8.93%) O128: K67, 4 isolates (7.14%) O157: H7, 3 isolates (5.36%) O26: K60, 2 isolates (3.57%) O119: K69 and one isolate (1.79%) were untypable. This result confirms that mentioned by Wells *et al.* [29] and Karmali *et al.* [9] who mentioned that such *E. coli* strains are the most frequently isolated from fecal samples of diarrheic calves.

Serotyping of *E. coli* isolates yielded from bacteriological examination of milk samples received from clinical mastitic cases revealed 6 strains (12.50%) O111: K58, 3 strains (6.25%) O128: K67, 2 strains (4.17%) O157: H7 and one strain (2.08%) untypable.

Table 1. Serological identification of *E. coli* isolates recovered from fecal and mastitic milk samples

Bovine samples	No. of examined samples	*E. coli* Serovars						
		O111:K58	O128:K67	O157:H7	O26:K69	O119:K69	Unty-pable	Total
Fecal samples	56	7 (12.5%)	5 (8.93%)	4 (7.14%)	3 (5.36%)	2 (3.57%)	1 (1.79%)	22 (39.29%)
Clinically mastic milk samples	48	6 (12.5%)	3 (6.25%)	2 (4.17%)	-	-	1 (2.08%)	12 (25%)
Subclinical mastitic milk samples	32	3 (9.38%)	2 (6.25%)	1 (3.12%)	-	2 (6.25%)	-	8 (25%)

Table 2. Virulence determinants of *E. coli* strains isolated from diarrheic calves and milk samples

Virulence factors	Fecal samples		Clinical mastitic samples				Subclinical mastitic samples				Total					
	O111	O128	O157	O26	O119	Unty-pable	O111	O128	O157	Unty-pable	O111	O128	O157	O119	Unty-pable	%
	(7)	(5)	(4)	(3)	(2)	(1)	(6)	(3)	(2)	(1)	(3)	(2)	(1)	(2)	-	(42)
Haemolytic activity	3	2	4	1	1	0	2	3	2	0	2	1	1	1	0	23	54.76
Enterotoxin production	0	2	0	1	1	0	0	3	0	0	0	1	0	0	0	8	18.05
Verotoxin Production	3	0	4		0	0	3	0	2	0	0	0	0	0	0	13	30.95
Invasiveness	3	2	4	2	2	1	3	3	2	0	1	1	1	1	0	26	61.90
Congo red binding	3	2	4	1	1	0	2	3	2	0	0	1	1	0	0	20	47.62

%:was calculated according to the number of the isolated *E. coli* strains. (42)

While serotyping of *E. coli* isolates yielded from the examined milk samples of the sub-clinical mastitic cases revealed 3 isolates (9.38%) O111: K58, 2 isolates (6.25%) O128: K27, 2 isolates (6.25%) O119: K69 and one isolate (3.12%) O157: H7 as shown in Table (l). It was clear that there was no specific serotype involved in mastitis. These results agreed with the results of many authors who cited that *E. coli* recovered from mastitis cases belonged to different sero groups and varied greatly in O-groups.[30, 31]

Furthermore, *E. coli* recovered from mastitis had different O-serotypes and may not be attributed to epizootic strains.[32] Serotyping was not useful as a distinguishable reaction for specifying the pathogenic group of *E. coli* mastitis.[33]Also no obvious association between O-serogroup of *E. coli* causing mastitis. [34]

The virulence factors are thought to play important role in diseases caused by *E. coli.*

Thus, the virulence factors of the isolated *E. coli* serotypes were evaluated in this study.

The virulence attributes of *E. coli* isolates recovered from diarrheic calves and mastitic cows were carried out as shown in Table (2). It is clear that 23 isolates were hemolytic with the percentage of 54.76% by the using of 5% defibrinated sheep blood agar and belonging to serotypes 0111 (7), O128 (6), O157: H7 (7), O119 (2), and O26 (1).

As well as 8 isolates (18.05%) belonging to serotypes O128 (6), O26 (1), and O119 (2) were positive for enterotoxin production, while 13 isolates (30.95%) were positive for verotoxin production belonging to O157: H7 (7) and O111 (6). The same table represents the invasiveness and Congo red binding activities of the examined serotypes.

An overall looking for the present study (Tables 1 and 2) serotyping of *E. coli* isolated from diarrheic and mastitic cases (clinical and subclinical) as well as trial to detect the virulence factors aiming to find some characters that could be depended upon in identifying pathogenic *E. coli* strains that incorporated in calves diarrhea and bovine mastitis.

Generally, there is no conclusive information of genetic relatedness of the mastitic and diarrheic fecal isolates of *E. coli.* There is an ongoing need for integration of molecular methods of typing of *E. coli* isolates.

Unlike methods that rely on DNA sequence information for unique primer design, RAPD-PCR uses short primers of arbitrary sequence and annealing conditions that favor non-specific template binding. RAPD primer amplifies sequences that are repetitive or unique and amplification products occur wherever the primers bind in a converging orientation. The resulting amplicons can be used as genetic fingerprint of target genomes for which previous molecular genetic analysis has not been performed[35]

The purpose of this RAPD assay is to investigate the genetic relatedness of 19 *E. coli* strains of pathogenic serotypes isolated from diarrheic and mastitic cases.

The results of RAPD - PCR on 19 *E. coli* strains recovered from fecal and milk samples are shown in Photo. (1 and 2). These 19 *E. coli* strains were representative to 5 different typable serotypes (O111: K58 6 strains, O128: K67 3 strains, O157: H7 7 strains,

O119: K69 2 strains and O26: K60 one strain). The distribution of the serotypes in the different lanes and their sources are illustrated in Table (3).

Examination of the chosen 19 strains of *E. coli* serovars by RAPD - PCR assay with the five previously mentioned primers revealed 19 characteristic RAPD fingerprinting patterns based on the presence, size and the intensity of their RAPD products. Moreover, it is noticed that the amplification reactions generated number of bands ranging from 2 to 10 bands with a molecular weight ranging from 78 to 1117.35bpas shown in Table (4). It is important to point out here that the majority of *E. coli* sero types had shared bands at molecular weights 78.45, 220, 307 and 570.62 as shown in Table (4). However, these sharing bands differed in their intensity as shown in Table (5). While the remaining bands at different molecular weight are considered unique for *E. coli* serovars. The strains of the same serotypes showed different banding pattern and also among different serotypes which indicated inter- and intraserotype variation as shown in the similarity index illustrated in Table (6) which ranged from 33.3 to 94.7.

The genetic relatedness of the 19 *E. coli* strains using a dendrogram constructed with Scan

Table 3. The serotypes constitution in the different lanes and their sources

Lanes	Serotypes	Origin
Lane (1)	O111: K58	Fecal sample
Lane (2)	O111: K58	Clinical mastitic sample
Lane (3)	O157: H7	Clinical mastitic sample
Lane (4)	O157: H7	Subclinical mastitic sample
Lane (5)	O111: K58	Fecal sample
Lane (6)	O128: K67	Fecal sample
Lane (7)	O128: K67	Clinical mastitic sample
Lane (8)	O111: K58	Clinical mastitic sample
Lane (9)	O128: K67	Subclinical mastitic sample
Lane (10)	O26: K60	Fecal sample
Lane (11)	O111: K58	Clinical mastitic sample
Lane (12)	O119: K69	Subclinical mastitic sample
Lane (13)	O157: H7	Fecal sample
Lane (14)	O157: H7	Clinical mastitic sample
Lane (15)	O157: H7	Fecal sample
Lane (16)	O119: K69	Fecal sample
Lane (17)	O157: H7	Fecal sample
Lane (18)	O111:K58	Subclinical mastitic sample
Lane (19)	O157: H7	Fecal sample

Table 4. Scoring sheet of 19 serotypes of *E.coli* recovered from fecal and milk samples showing the molecular weight and the number of the DNA banding patterns as well as the presence or absence of shared and unique bands

Bands	MolWt	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12	Lane13	Lane14	Lane15	Lane16	Lane17	Lane18	Lane19
1	1117..34	0	0	1	1	1	1	0	0	0	1	0	1	0	0	0	0	1	1	1
2	1000..00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	912..65	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
4	900.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	800.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	727.95	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
7	700.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	600.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	570..00	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	1	1	1
10	500.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	441..17	1	1	0	0	1	1	0	1	0	0	0	1	1	1	1	0	1	0	1
12	400.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	355.00	1	1	0	1	1	1	0	1	1	0	1	0	1	1	1	0	1	1	1
14	307.00	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
15	300.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	251.19	0	0	1	1	1	1	0	0	0	1	1	0	1	1	0	0	1	1	0
17	220..00	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1
18	200.00	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
19	113..37	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1
20	100.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	93.20	0	1	1	1	1	0	1	1	1	1	0	1	0	0	0	0	0	0	0
22	78..45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
No. of Bands		9	10	8	9	9	9	3	8	6	7	6	6	9	8	7	2	9	7	9

Note: The amplified fragments were scored either present (1) or absent (0). Bands of the same mobility were scored identical

Table 5. The molecular weight, the intensity and the number of DNA banding pattern of 19 *E. coli* serotypes following amplification by RAPD-PCR assay using 5 primers

Bands	Mol.Wt	Lane 1 %	Lane2	Lane3	Lane4	Lane5	Lane6	Marker	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12	Lane13	Lane14	Lane15	Marker	Lane16	Lane17	Lane18	Lane19
1	1117..34			14.19	6.86	4.63	5.09					14.23		8.86						7.02	15.59	7.48
2	1000..00							7.89										7.71				
3	912..65	6.53	5.42	9.71	7.86		8.03															
4	900.00							10.05										8.8				
5	800.00																	8.84				
6	727.95	7.54	6.97					9.82							7.97							6.79
7	700.00							9.76										8.91				
8	600.00							9.46										8.83				
9	570..00	8.46	7.88	8.18	9.72	9.81	7..22			10.43	14	8.78	12.33		8.77	8.45	10.39			9.5	9.12	9.2
10	500.00							9.27										11.51				
11	441..17	17.48	17.39			9.28	10			9.31				23.72	7..9	10.17	10.77			8.31		17.98
12	400.00							10.23										12.4				
13	355.00	7.73	9.35		8.02	9.69	9..33			10.19	10.49		16.64		11.76	10.89	13.64			9..2	12.55	10.9
14	307.00	21.28	18.94	15.56	14.31	12.46	14.84		48.95	14.83	19.61	17.18	19.48	24.12	16.63	18.3	17.45			16.25	16.58	15.18
15	300.00							9.9										11.35				
16	251.19			11.4	12.93	11.22	11.35			11.79		14.96	15.58		11.39	14.9				12.24	12.15	
17	220..00	7.14	12.59	14.31	10.94	17.61	17.94			13.56	9. 81	13.61	18.39	14.03	10.84	11.39	15.32		55.43	10.77	10.9	12.59
18	200.00							10.51										9.89				
19	113..37	5.93	7.11												13.57	10.67	12.58			10.9		8.39
20	100.00							13.13										10.64				
21	93.20		5.33	12.37	11.62	10.44			22.13	13.8	17.24	13.46		8.4								
22	78..45	17.91	9.02	14.29	17.23	14.44	16.2		28.88	16.09	28.85	17.79	17.38	20.88	12.18	15.23	19.85		44.57	15.81	24.11	11.49
No. of Bands		9	10	8	9	9	9	10	3	8	6	7	6	6	9	8	7	10	2	9	7	9

Table 6. Similarity index of RAPD profiles of *E. coli* recovered from milk samples using Nei and Li's coefficient.

	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12	Lane13	Lane14	Lane15	Lane16	Lane17	Lane18
Lane2	0.947																	
Lane3	0.588	0.667																
Lane4	0.667	0.737	0.941															
Lane5	0.667	0.737	0.824	0.889														
Lane6	0.778	0.737	0.824	0.889	0.889													
Lane7	0.333	0.462	0.545	0.500	0.500	0.333												
Lane8	0.706	0.778	0.750	0.824	0.941	0.824	0.545											
Lane9	0.667	0.750	0.714	0.800	0.800	0.667	0.667	0.857										
Lane10	0.500	0.588	0.933	0.875	0.875	0.750	0.600	0.800	0.769									
Lane11	0.667	0.625	0.714	0.800	0.800	0.800	0.444	0.857	0.833	0.769								
Lane12	0.553	0.625	0.714	0.667	0.800	0.667	0.667	0.714	0.667	0.769	0.500							
Lane13	0.889	0.842	0.588	0.667	0.778	0.778	0.333	0.824	0.667	0.625	0.800	0.533						
Lane14	0.824	0.778	0.625	0.706	0.824	0.824	0.364	0.875	0.741	0.667	0.857	0.571	0.941					
Lane15	0.875	0.333	0.533	0.625	0.750	0.750	0.400	0.800	0.769	0.541	0.769	0.615	0.875	0.933				
Lane16	0.364	0.737	0.400	0.364	0.364	0.364	0400	0.400	0.500	0.444	0.500	0.500	0.364	0.400	0.444			
Lane17	0.778	0.588	0.706	0.778	0.778	0.889	0.333	0.824	0.667	0.750	0.800	0.667	0.889	0.941	0.857	0.364		
Lane18	0.625	0.588	0.800	0.875	0.875	0.875	0.400	0.800	0.769	0.857	0.923	0.615	0.750	0.800	0.714	0.444	0.875	
Lane19	0.889	0.842	0.588	0.667	0.778	0.778	0.333	0.706	0.667	0.625	0.667	0.667	0.889	0.824	0.875	0.364	0.889	0.750

Pack-3.0 software. The resulting matrix of pair wise distance was used to generate a phenogram based on the outweighed pair-group method with arithmetic mean (UPG- MA) method.

A phylogenetic tree based on the proportion of identical bands in RAPD profiles was constructed in fig. (1). Four clonal clusters were identified; the first cluster included 7 characteristic RAPD profiles for 7 strains. (2 *E. coli* serovars O111 recovered from fecal and clinical mastitis samples were present in lane 1 and lane 2, respectively and 5 *E. coli* serovars O157: H7 recovered from fecal samples were present in lanes 19, 15, 13 and that recovered from mastitic samples were placed in lanes 14 and 17). The second cluster included 10 characteristic RAPD profiles for 10 strains (4 *E. coli* serovars O111, 2 strains of serotype O157: H7 and one strain of serotype O26, 2 strains of serotype O128 and one strain of serotype O119), while cluster three and four included only 2 characteristic RAPD profile for serotypes O128 and O119, respectively.

It is obvious from fig. (1) that different strains of the same serotype genotyped into the same cluster or genotyped into different clusters with similarity index ranged from 33.3-94.7 as shown in Table (6).

Concerning, O119 serovars, they were genotyped in different clusters with a low similarity index (50). While O111: K58 in lane (5) and that in lane (8) were genotyped in the same cluster with a high similarity index (94.1).

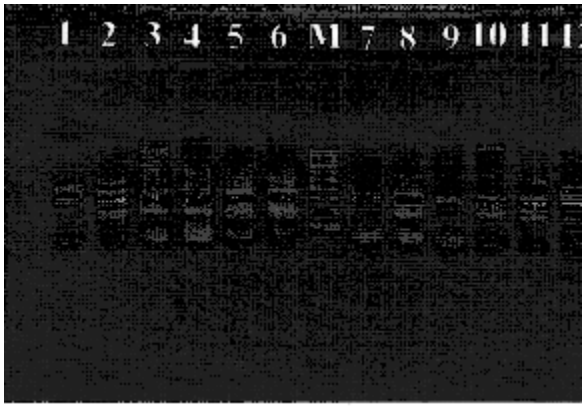

Plate 1. Agarose gel electrophoresis showing RAPD-PCR profiles using 5 RAPD primers. Fragment sizes are illustrated as bps and read from left to right where lane M showing 100 base pair ladder. The serotype distributions are illustrated in table 3 from lane 1 to 12

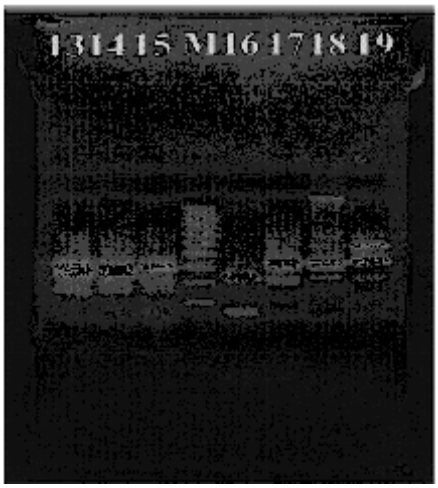

Plate 2. Agarose gel electrophoresis showing RAPD-PCR profiles using 5 RAPD primers. Fragment sizes are illustrated as bps and read from left to right where lane M showing 100 base pair ladder. The serotype distributions are illustrated in table 3 from lane 13 to 19

Examination of 19 strains of *E. coli* representing 5 different serotypes revealed 19 RAPD profiles. Similarity among *E. coli* isolates ranged between 33.3 and 94.7 and the amplification reactions generated a number of bands ranging from 2 to 10 bands with molecular weights ranges from 78.45 to 1117.34 bp. It is noticed that different strains of the same serotypes may constitute the same cluster or different clusters. *E. coli* serotypes O157: H7 and O111 strains of fecal and mastitic cases constituted the same clusters with similarity index 94.7 and 94.1. The obtained results indicated that the serotypes causing bovine mastitis were similar to that causing diarrhea and *E. coli* isolated from cows with mastitis probably are endogenous in origin and that infection results from environmental exposure to *E .coli* strains. Moreover, the same serotypes constituted different clusters which highlighted the importance of RAPD fingerprinting in studying inter- and intra-serotype variation and epidemiology of different *E. coli* serotypes, rather than biochemical and serological identification. This agrees with Pacheco *et al*. [25], William *et al*. [19] and Hopkins and Hilton [36].

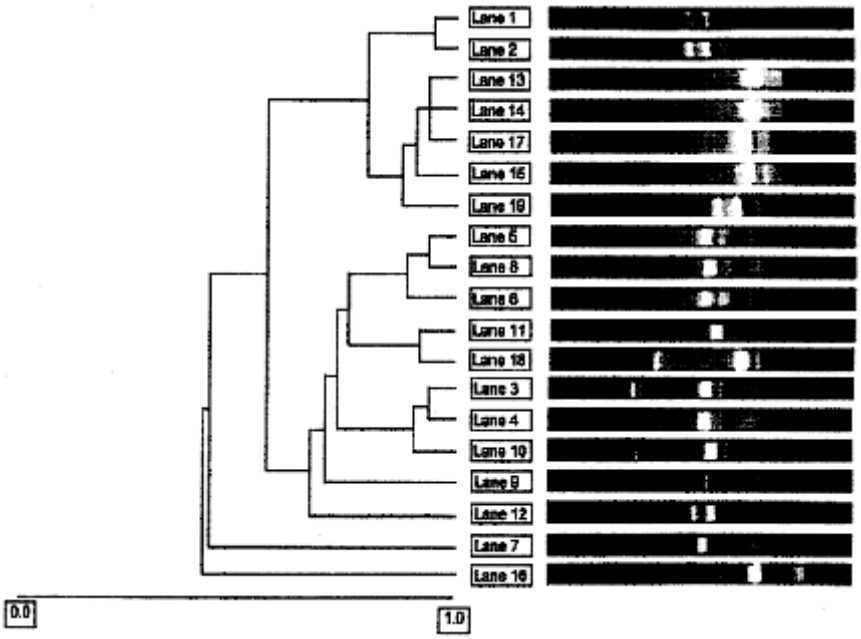

Fig. 1. Phylogenetic tree showing the genetic relatedness among 19 *E. coli* serotypes recovered from fecal and milk samples with 19 RAPD profiles generated by 5 RAPD primers

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