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Genetic Diversity of *Escherichia coli* O157:H7 strains using random amplified polymorphic DNA (RAPD)

I Wayan Suardana[1*], Wayan Tunas Artama[2], Dyah Ayu Widiasih[3] and I Gusti Ngurah Kade Mahardika[4]

[*1]Department of Veterinary Publich Health, Faculty of Veterinary Medicine, Udayana University, Denpasar. Jl. PB. Sudirman Denpasar-Bali, Indonesia
[2]Department of Biochemistry, Faculty of Veterinary Medicine, Gadjah Mada University, Jl. Fauna 2, Karang Malang, Yogyakarta 55281, Indonesia
[3]Department of Veterinary Publich Health Faculty of Veterinary Medicine, Gadjah Mada University, Jl. Fauna 2, Karang Malang, Yogyakarta 55281, Indonesia
[4]Department of Virlology Faculty of Veterinary Medicine, Udayana University, Denpasar. Jl. PB. Sudirman Denpasar-Bali, Indonesia

Abstract

Escherichia coli* O157:H7, an emerging cause of food-borne disease, has now been reported from several countries worlwide. Cattle and sheep are known as a principle reservoir of this agent. It is also prevalent in gastrointestinal tract of other animals like pigs, dogs, cats and birds. This agent can be transmitted to humans by food and water and even person to person contact. The random amplified polymorphic DNA (RAPD) technique has been applied to evaluate zoonotic potency of *Escherichia coli* O157:H7 local isolates. Ten random decamer primers have been evaluated in this study to differentiate isolates originated from humans and animals. The OPA-02, OP-03, OPA-04, OPA-07, OPA-08, OPA-09, OPA-10, OPA-13, OPA-19, and OPA-20 decamer primers show a good differentiation pattern of *E. coli* O157:H7 isolated from human, cattle and chicken feces, as well as from beef. The primers constantly produce 16, 16, 11, 14, 8, 24, 7, 11, 7, and 12 polymorphic bands, respectively. Various human isolates that were isolated from clinically ill and healthy patients share common genetic clusters with animal isolates from beef, as well as cattle and chicken feces with genetic similarity coefficients greater than 70%. According to this result, It has been concluded the transmission of *E. coli* O157:H7 local isolates from animals to humans is potential occur.

Keywords: *E.coli* O157:H7, RAPD, genetic diversity, humans, animals, zoonosis.

INTRODUCTION

Escherichia coli is a bacterium comonly present in both animal and human intestinal tract, and also widespread in the environment. There are numerous strains, some of which are potential pathogens which can cause disease in humans through various infectious and toxinogenic mechanisms (Luciani et al., 2006).

Verotoxin producing *E. coli* O157:H7 strains is the predominant causative agent of hemorrhagic colitis (HC), a bloody diarrhea that can lead to hemolytic uremic syndrome (HUS), a potentially lethal renal failure (Rivas et al., 2006). Cattle and other ruminants appear to be the natural reservoir for *E. coli* O157:H7 (LeJeune et al., 2004). Transmission of agent to humans mainly occurs via comsumption food such as raw or undercooked meat products, which occurs as a result of contact of feces or intestinal content after slaughter and raw milk and even person to person contact (Nataro and Kaper, 1998; Rodolpho and Marin, 2007).

Molecular methods are a potential tool to effectively and efficiently identify genetic relatedness in both animals as a natural reservoir and humans in order to track the pathogenesis of this agent. Traditional methods

of strain typing, such as serotyping and bacteriophage typing have been used. However, newer molecular typing methods have been recently introduced in many laboratories. One of the PCR-based techniques is Random Amplified Polymorphic DNA (RAPD) (Dale and Park, 2004) .

RAPD technique is based on the amplification of DNA segments with a single primer of arbitrary nucleotide sequence. These primers detect polymorphisms that can be used to construct genetic maps (Williams et al., 1990). RAPD has been used successfully in the past for *E. coli* O157:H7 (Al-Darahi et al., 2008; Ji-Yeon Kim et al., 2005). On the order hand, RAPD method has many advantages i.e. time and cost-saving, sensitivity, and no special skills required to perform compare with order methods like Amplified Fragment Length Polymorphism (AFLP), and analysis of chromosomal DNA restriction patterns by Pulsed-Field Gel Electrophoresis (PFGE). Therefore, RAPD fingerprinting analysis was used for this study.

The purpose of this study was to evaluate the possibility of epidemiological typing of *E. coli* O157:H7 isolates based on RAPD analysis, in order to identify and differentiate strains of *E. coli* O157:H7 isolated from either animal or human origin. Futhermore, the data of RAPD analysis also can be used to depict a dendrogram so that the genetic relationship among *E. coli* O157:H7 local isolates can be estimated.

MATERIALS AND METHODS

Bacterial strains and DNA extractions

Escherichia coli ATCC 43894 as a control and nineteen of *E. coli* O157:H7 local isolates were used in this study. The local isolates originated from feces of human with renal failure symtoms i.e. KL-52(7), KL-87(7), KL-30(4), KL-45(1), KL-48(2), KL-85(1), KL-83(5), KL-24(5), KL-68(1), KL-106(3), and KL-55(6), chicken feces i.e. MK-35, and MK-19/8(4), healthy human feces i.e. M-14(4), and M-17(1), feces of cattle i.e. SM-25(1), and SM-7(1), and isolates originated from beef i.e. DS-21(4), and DS-16(2). Genomic DNA was extracted as described by Suardana et al., (2010). The concentration of DNA was determined by spectrophotometer.

Primers

We used ten random decamer primers (OPA-02, OPA-03, OPA-04, OPA-07, OPA-08, OPA-09, OPA-10, OPA-13, OPA-19 and OPA-20) obtained from Operon Incorporation Technologies Inc. (Alameda, California, USA). These were tested on twenty *E. coli* O157:H7 strains as single primers for RAPD-PCR technique.

After an initial screening, on the basis of their ability to detect polymorphisms and to produce reliable and scorable banding patterns, the best RAPD amplification patterns were chosen for data compilation and examination of the genetic diversity and relationship.

RAPD-PCR amplification

RAPD-PCR was carried out in 27 µl reaction volumes containing 12.5 µl FastStart PCR Master (Roche), 100 ng genomic DNA, 20 pmol primer, and 9.5 µl ultrapure distilled water (Invitrogen). Amplification was carried out in a thermo-cycler (Biorad PTC-1148) programmed for 5 minutes at 94^{O}C; for 36 cycles of 1 minute at 94^{O}C, 30 seconds at 35^{O}C and 1 minute at 72^{O}C; and a final extension of 5 minutes at 72^{O}C. The amplified DNA samples were separated on 2% agarose gels and detected by staining with ethidium bromide.

RAPD fingeprinting analysis

The amplified fragments were scored in a descending manner from higher to lower molecular weight product. Presence of products was designated as “1” and its absence as “0” (Al-Darahi et al., 2008).Cluster analysis was generated and analyzed using similarity coefficient and unweighted pair group algorithm with arithmetic averages (UPGMA) with coefficients similarity of each cluster ware showed near the branch of dendrogram. Data were processed using Multivariate Statistical Package (MVSP) 3.1 (Sembiring and Goodfellow, 2010).

RESULTS

RAPD Profile

Ten RAPD primers were applied to differentiate nineteen strains of *E. coli* O157:H7 local isolates and one strain of *E. coli* O157:H7 control (ATCC 43894). Table 1 showed that different primers generated variable numbers of fragments with different lengths of DNA amplified products. Its show that screening of genetic variation by 10 primers generated a various number of bands range from 7 to 16 bands. The highest number of bands (more than 10 bands) were amplified with both OPA-02 and OPA-03 (16 bands), OPA-04 (11 bands), OPA-07 (14 bands), OPA-09 (24 bands), OPA-13 (11 bands) and OPA-20 (12 bands). Total of 126 amplification products, of which 126 (100%) was found to be polymorphic with an average of 12.6 polymorphic bands per primer.

Because strains of bacteria have different nucleotide sequences, they may exhibit distinguishable patterns. RAPD profiles of each isolate isolated from feces of humans, feces of animals, beef and feces of chickens were distinctly difference from each other generated using 10-base primers. Figure 1a, and 1b show some

Table 1. Number of bands and polymorphism revealed by RAPD

Primers	Sequence	Total bands	Bands quality	Polymorphic bands	discriminatory Efficiency
OPA-02	TGC CGA GCTG	16	Clear	16	1
OPA-03	AGT CAG CCAC	16	Unclear	16	1
OPA-04	AAT CGG GCTG	11	Clear	11	1
OPA-07	GAA ACG GGTG	14	Clear	14	1
OPA-08	GTG ACG TAGG	8	Clear	8	1
OPA-09	GGG TAA CGCC	24	Clear	24	1
OPA-10	GTG ATC GCAG	7	Clear	7	1
OPA-13	CAG CAC CCAC	11	Clear	11	1
OPA-19	CAA ACG TCGG	7	Unclear	7	1
OPA-20	GTT GCG ATCC	12	Clear	12	1
All primers		126			
Average				12,6	

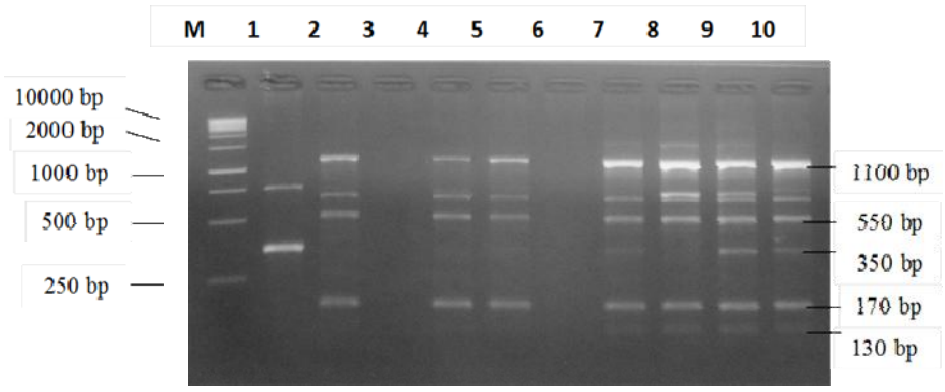

Figure 1a. RAPD patterns of *E. coli* O157:H7 strains produced by OPA-2 primer. Electrophoresis was performed on 2% agarose gel. Line 1 positive control : ATCC 43894; 2: KL52(7); 3: KL87(7); 4: KL30(4); 5: KL45(1); 6: KL(48(2); 7: KL85(1); 8: KL83(5); 9: KL24(5); and 10: KL68(1); M: 1 kb DNA *Ladder* Marker (Microzone Ltd cat. 1DLL-500).

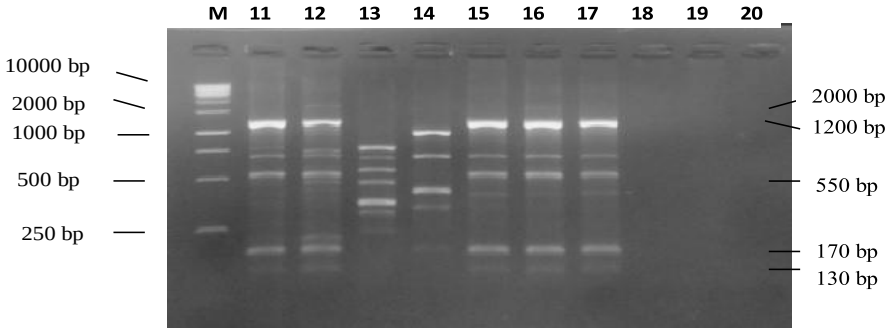

Figure1b. RAPD patterns of *E. coli* O157:H7 strains produced with OPA-2 primer. Electrophoresis was performed on 2% agarose gel. Line 11: KL-106(3); 12: KL-55(6); 13: MK-35; 14: MK-19/8(4); 15: M-14(4); 16: M-17(1); 17: DS-21(4); 18: DS-16(2); 19: SM-25(1); and 20: SM-7(1) M: 1 kb DNA *Ladder* Marker (Microzone Ltd cat. 1DLL-500).

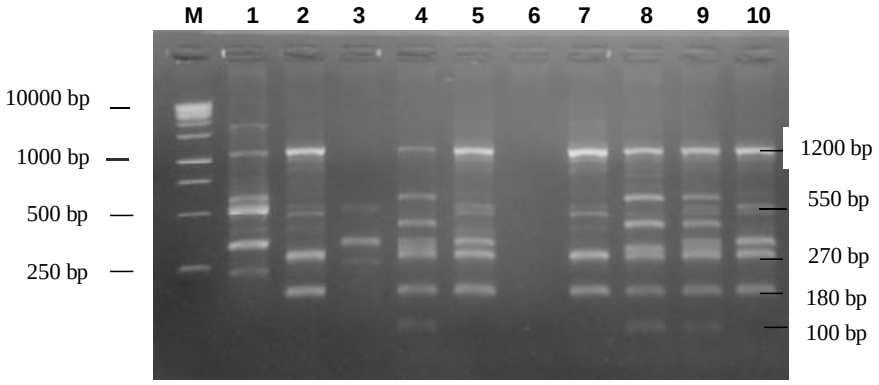

Figure 2a. RAPD patterns of *E. coli* O157:H7 strains produced with OPA-9 primer. Electrophoresis was performed on 2% agarose gel. Line 1 positive control : ATCC 43894; 2: KL52(7); 3: KL87(7); 4: KL30(4); 5: KL45(1); 6: KL(48(2); 7: KL85(1); 8: KL83(5); 9: KL24(5); and 10: KL68(1); M: 1 kb DNA *Ladder* Marker (Microzone Ltd cat. 1DLL-500).

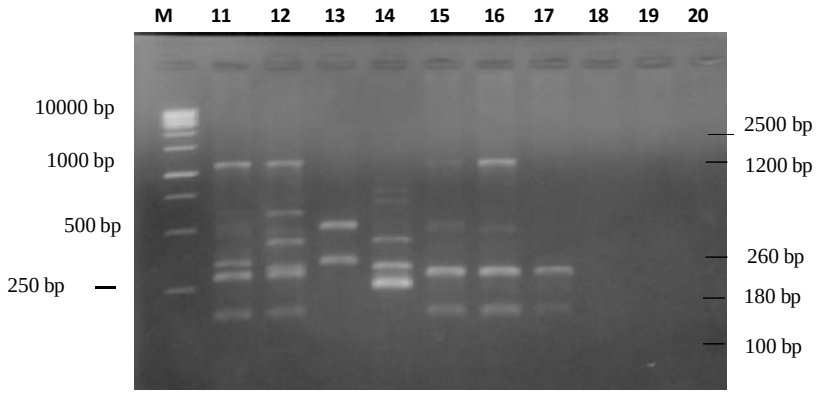

Figure 2b. RAPD patterns of *E. coli* O157:H7 strains produced with OPA-9 primer. Electrophoresis was performed on 2% agarose gel. Line 11: KL-106(3); 12: KL-55(6); 13: MK-35; 14: MK-19/8(4); 15: M-14(4); 16: M-17(1); 17: DS-21(4); 18: DS-16(2); 19: SM-25(1); and 20: SM-7(1) M: 1 kb DNA *Ladder* Marker (Microzone Ltd cat. 1DLL-500).

variable bands amplified by OPA 2 primer i.e: 130, 170, 250, 350, 420, 500, 550, 600, 700, 750, 850, 950, 1100, 1200, 1450 and 2000 bp. Futhermore, Figure 2a, and 2b show the results of amplification by OPA-9 primer i.e: 100, 180, 250, 260, 270, 300, 325, 350, 450, 500, 550, 560, 650, 680, 700, 750, 850, 900, 950, 1000, 1200, 1350, 2000 and 2500 bp.

Dendrogram Analysis

The genetic relatedness among *E. coli* O157:H7 strains was ilustrated through a tree-like structure that is generally known as dendrogram. Dendrogram generated using 10-base primers are presented in Figure 3.

Figure 3 shows that *E. coli* strains were divided into 19 sub-clusters with 18 nodes. Sub-cluster 1 consists of KL-48(2) human and SM-25(1) cattle strains with similarity coefficient “1” or 100% similarly. Sub-cluster 2 consists of SM-7(1) cattle isolate with similarity coefficient “ 1” or 100% similarly to sub-cluster 1 (node 1). Sub-cluster 3 consists of DS-16(2) beef isolate with similarity coefficient 0,99 or 99% similarly to sub-cluster 2 (node 2). Sub-cluster 4 consists of KL-87(7) human isolate with similarity coefficient 0.98 to node 3. Sub-cluster 5 consists of M-14(4) human with similarity

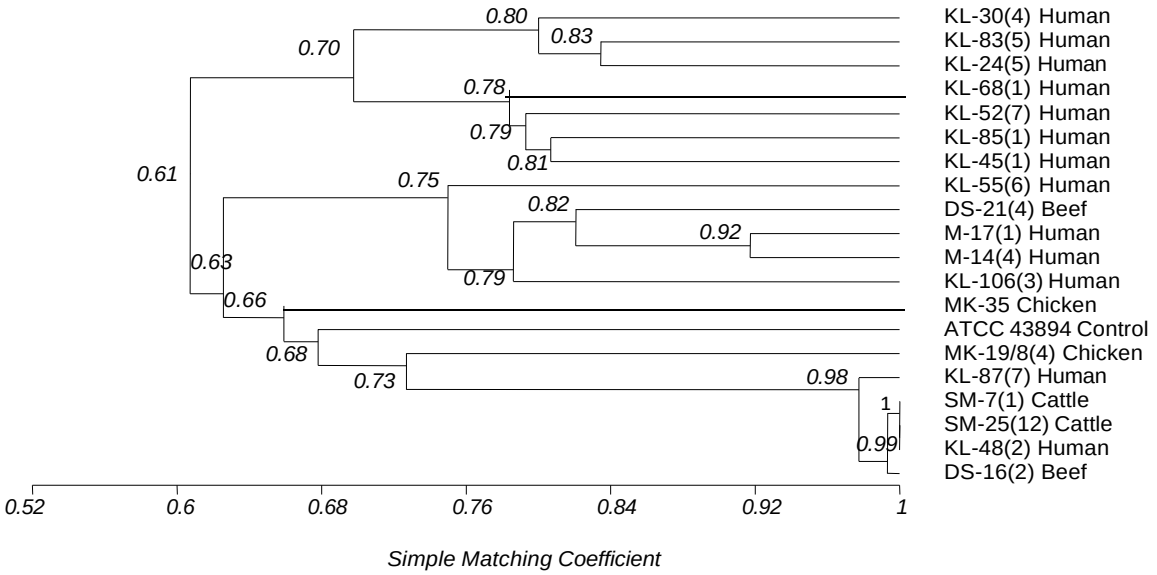

Figure 3. Dendrogram of *E. coli* O157:H7. Dendrogram was generated using Simple Matching Coefficient (Ssm) of similarity and algorithm Unweighted Pair-Group Method using Arithmetic Average (UPGMA) based on 126 RAPD fragments produced by 10 random decamer primers.

Table 2. Genetic similarity coefficients among 20 *E. coli* O157:H7 isolates originated from feces of human with renal failure symtoms, feces of chicken, feces of healthy human, feces of cattle and beef

	ATCC 43894 Control	KL-52(7) Human	KL-87(7) Human	KL-30(4) Human	KL-45(1) Human	KL-48(2) Human	KL-85(1) Human	KL-83(5) Human	KL-24(5) Human	KL-68(1) Human	KL-106(3) Human	KL-55(6) Human	MK-35 Chicken	MK-19/8(4) Chicken	M-14(4) Human	M-17(1) Human	DS-21(4) Beef	DS-16(2) Beef	SM-25(12) Cattle	SM-7(1) Cattle
ATCC 43894 Control	1																			
KL-52(7) Human	0.497	1.000																		
KL-87(7) Human	0.690	0.683	1.000																	
KL-30(4) Human	0.517	0.703	0.634	1.000																
KL-45(1) Human	0.490	0.800	0.566	0.669	1.000															
KL-48(2) Human	0.697	0.690	0.979	0.641	0.559	1.000														
KL-85(1) Human	0.517	0.703	0.634	0.697	0.807	0.641	1.000													
KL-83(5) Human	0.497	0.683	0.655	0.786	0.634	0.662	0.731	1.000												
KL-24(5) Human	0.497	0.683	0.614	0.814	0.662	0.607	0.786	0.834	1.000											
KL-68(1) Human	0.524	0.779	0.697	0.717	0.772	0.690	0.800	0.683	0.724	1.000										
KL-106(3) Human	0.607	0.655	0.628	0.607	0.607	0.634	0.607	0.600	0.614	0.669	1.000									
KL-55(6) Human	0.503	0.607	0.552	0.710	0.531	0.559	0.555	0.566	0.555	0.607	0.745	1.000								
MK-35 Chicken	0.655	0.538	0.676	0.490	0.517	0.683	0.517	0.469	0.497	0.524	0.566	0.448	1.000							
MK-19/8(4) Chicken	0.600	0.524	0.745	0.641	0.490	0.724	0.545	0.593	0.566	0.593	0.538	0.600	0.559	1.000						
M-14(4) Human	0.586	0.662	0.690	0.641	0.641	0.683	0.655	0.607	0.607	0.717	0.841	0.779	0.545	0.614	1.000					
M-17(1) Human	0.545	0.621	0.634	0.600	0.614	0.641	0.614	0.566	0.552	0.662	0.786	0.793	0.490	0.586	0.917	1.000				
DS-21(4) Beef	0.600	0.703	0.800	0.655	0.559	0.807	0.641	0.648	0.621	0.703	0.731	0.683	0.600	0.628	0.834	0.807	1.000			
DS-16(2) Beef	0.690	0.683	0.972	0.638	0.552	0.993	0.634	0.655	0.600	0.697	0.628	0.552	0.676	0.717	0.676	0.634	0.800	1.000		
SM-25(12) Cattle	0.697	0.690	0.979	0.641	0.559	1.000	0.641	0.662	0.607	0.690	0.634	0.559	0.683	0.724	0.683	0.641	0.807	0.993	1.000	
SM-7(1) Cattle	0.697	0.690	0.979	0.641	0.559	1.000	0.641	0.662	0.607	0.690	0.634	0.559	0.683	0.724	0.683	0.641	0.807	0.993	1.000	1.000

coefficient 0,92 to M-17(1) human, and continuously up to sub-cluster 19 generated by sub-cluster 18 and sub-cluster 15 with similarity coefficient 0,61. Complete data about the similarity coefficients among each isolate have been showed in Table 2.

DISCUSSION

RAPD, one of the DNA subtyping methods, can be used in studies of epidemiology of foodborne diseases. In RAPD, a number of regions will be amplified that are bounded by nucleotide sequences showing similarity to the primers (Hill and Jinneman, 2000). RAPD is largely used for genetic variability analyses although on the other hand, there are some limitations (Bando et al., 2007).

According to Table 1, all of decamer primers, resulted reproducible binding patterns and the efficiency of each primer that was determined by calculating the number of polymorphic bands obtained to the total number of bands produced by each primer, showed the highest calculated efficiency i.e. “1”. Results indicated that all of the primers used in the research had a good discriminatory and reliable efficiency to be used as a DNA fingerprinting method. Grandmann et al., (1995) reported that determining the efficiency and discriminatory power of any primer is important, since it can be used to reduce the extent of blind screening of the primer. This will eliminate the possibility of producing monomorphic patterns between genetically different individuals.

Figure 1a, 1b, 2a, and 2b demonstrate that short primers of arbitrary nucleotide sequence may be used to reproduce amplify segments of genomic DNA from a different source of isolates. Each isolate has a various bands that are specifically depending on nucleotide sequence of each isolate. Results of this research are equivalent with the study of the researcher before. RAPD as one of DNA fingerprinting methods has become a highly valuable molecular tool for intraspecies characterization of *E. coli* (Cave et al., 1994). This technique has been successful to characterize *E. coli* O157:H7 isolated from patients with HC and HUS (Al Darahi et al., 2008), and has been successfully used to determination of DNA polymorphism in *E. coli* strains isolated from vaginal infections too (Lobos and Padila, 2009).

William et al., (1990) reveal that the genetic mapping using RAPD markers has several advantages over other methods: (i) a universal set of primers can be used for genomic analysis in a wide variety of species, (ii) no preliminary work, such as isolation of cloned DNA probes, preparation of filters for hybridizations, or nucleotide sequencing, is required (iii) each RAPD marker is the equivalent of a sequence tagged site.

RAPD profiles exhibited variation within the serotypes, because different bands were obtained depending on the primer used (Koh et al., 1998). As the sequence of the primer changes, different locations on the DNA will be amplified and resulting in different band profiles on the gel (Williams et al., 1990).

Hill and Jinneman (2000) reported that Random Amplified Polymorphic DNA (RAPD) as one of DNA subtyping methods can be used to generate a pattern of fragment sizes or nucleotide sequences. DNA subtyping can be used in studies of epidemiology of foodborne disease. Similarity values between nucleotide sequences or gel patterns from isolates can be used to generate clusters. Dendrograms (i.e. tree diagram) can be used to visualize and sometimes to quantify the relationships between strains of a given bacterial species.

Dendrogram in Figure 3. helps in grouping of *E. coli* O157:H7 originated from feces of human with renal failure symtoms, feces of chicken, feces of healthy human, feces of cattle and beef. Figure 3 show most of isolates have similarity coefficients greater than 0,70 or most of isolates have similarity greater than 70%. This result indicated most of isolates can be grouped in the same species according to the Committee on Reconciliation of Approaches to Bacterial Systematic. The committee wrote that generally would include strains with approximately 70% or greater DNA-DNA relatedness (Rosello-Mora and Amann, 2001; Doolittle and Zhaxybayeva, 2009).

The highest of similarity coefficients among isolates are showed by KL-48(2) human isolate originated from human with renal failure compare with SM-25(1) cattle and SM-7(1) cattle isolates with similarity coefficient “ 1 “ or 100 % similarly. Higher similarity are showed by DS-16(2) beef with the other isolates also. These results showed the opportunity to find *E. coli* O157:H7 local isolates as a zoonotic agent originated from cattle in human are potential occur. Rodolpho and Marin (2007) explained, cattle as a natural reservoir for *E. coli* O157:H7 can be transmitted of agent to humans mainly occurs via comsumption food such as raw or undercooked meat products, which occurs as a result of contact of feces or intestinal content after slaughter and raw milk and even person to person contact.

The success of RAPD method to help understand the epidemiology and ecology, tracking outbreaks and spread of the organisms has been evaluated before by Maiti et al., (2009), Al-Darahi et al., (2008) and Ji-Yeon Kim et al., (2005).

In conclusion, genetic diversity especially the relatedness among *E. coli* O157:H7 local isolates originated from various source can be proved using Random Amplified Polymorphic DNA (RAPD) technique and this result can be implemented to help consideration in order to control of *E. coli* O157:H7 transmission.

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