Pakistan Journal of Biological Sciences 16 (20): 1173-1178, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.1173.1178

Genetic Diversity of *Escherichia coli* Isolated from Ducks and the Environment Using Enterobacterial Repetitive Intergenic Consensus

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Abstract: *Escherichia coli* are mostly free living bacteria that harbour the gastrointestinal tract of poultry. Howbeit, pathogenic *Escherichia coli* are very important foodborne pathogens that can cause severe complications, illnesses and deaths in humans. The objective of this study was to determine the genetic diversity or relatedness of 62 *Escherichia coli* strains isolated from ducks and the environment using Enterobacterial Repetitive Intergenic Consensus (ERIC). The analysis of the *Escherichia coli* strains by ERIC produced DNA bands of different sizes for differentiation purposes and cluster analysis at a coefficient of 0.85 grouped the strains into different clusters and singletons. At this coefficient the *Escherichia coli* strains were grouped into thirteen clusters and eleven singletons with discriminatory index (D value) of 0.946. The ERIC PCR adapted in this study showed to be a useful genotyping tool for determining the genetic relatedness of the duck *Escherichia coli* strains. Comparison of the genetic relatedness among foodborne pathogens is important for foodborne diseases outbreak investigations.

Key words: Ducks, genetic diversity, *Escherichia coli*, ERIC

INTRODUCTION

Bacteria of the Enterobacteriaceae family which are Gram-negative, rod-shaped and facultative anaerobic include *Escherichia coli* (Feng *et al*., 2002). They are generally motile bacteria capable of fermenting glucose and/or lactose which lead to the production of acid and/or gas as side products (Feng *et al*., 2002). Pathogenic *Escherichia coli* cause a wide range of human diseases or conditions such as gastroenteritis, diarrhoea, dysentery-like illness, haemorrhagic colitis and haemolytic uraemic syndrome (Feng *et al*., 2002; Pereira *et al*., 2008; Frederick, 2011). Pathogenic *Escherichia coli* have been isolated from or found in poultry, goats, sheep, cattle, etc. (Ghadrdan-Mashhadi *et al*., 2006; Moniri and Dastehgoli, 2007; Mirmomeni *et al*., 2008; Karou *et al*., 2009; Ali *et al*., 2010; Mihdhdir, 2009; Islam *et al*., 2011; Mahalakshmi *et al*., 2011; Lawan *et al*., 2012).

The importance of *Escherichia coli* and other pathogenic foodborne pathogens in the cause of human diseases warrant the need to employ better and accurate isolation, identification and characterization/genotyping methods. Comparing the DNA bands of bacteria isolated from different sources provide a means of tracing their primary source and this contributes to establishing the source of human foodborne infections (Adzitey *et al*., 2012a, 2013a). The DNA bands of various types of bacteria have been compared using several genotyping tools such as Pulsed Field Gel Electrophoresis (PFGE), Random Amplified Polymorphic Deoxyribonucleic acid (RAPD), Enterobacterial Repetitive Intergenic Consensus (ERIC), Repetitive Extragenic Palindromic (REP), ribotyping and many more (Ling *et al*., 2000; Salem *et al*., 2006; Hollmen *et al*., 2011; Adzitey *et al*., 2013b). Effective and appropriate genotyping methods are needed for epidemiological studies or outbreak investigations to ensure accurate and/or precise reporting of foodborne disease outbreaks.

Poultry including ducks have been reported to be important sources of foodborne pathogens responsible for foodborne disease outbreaks in humans. For instance *Salmonella* spp. isolated from ducklings, duck eggs, meats or products have been associated with *Salmonella* infection or death of affected persons (Merritt and Herlihy, 2003; Noble *et al*., 2012). Malaysia makes significant contribution to the total duck meat consumed worldwide, in that, Malaysia is the third world producer of duck meats (FAO, 2009; Adzitey and Adzitey, 2011; Adzitey *et al*., 2012b, 2013b). Ducks in Malaysia have also been showed to be important sources of other foodborne pathogens (Adzitey *et al*., 2012b, c, 2013b). Thus the presence of pathogenic foodborne pathogens in ducks in Malaysia can pose the risk of acquiring foodborne diseases from the consumption of contaminated duck eggs, meats or products. Determining the genetic diversity or relatedness among duck bacteria

isolates and their sources of isolation provide an idea about the extent to which these bacteria can contaminate or cross contaminate other foods.

Therefore this study was conducted to determine the genetic diversity of *Escherichia coli* strains isolated from ducks and their environmental sources in Penang, Malaysia.

MATERIALS AND METHODS

***Escherichia coli* strains:** Sixty two *Escherichia coli* strains previous isolated from ducks and the environment were used for this study (Adzitey *et al*., 2012d). The strains used were isolated from duck intestines (n = 17), wash water (n = 9), faeces (n = 22) and soil (n = 14).

DNA extraction: DNA extraction was done using the boiling method according to Adzitey *et al*. (2012c). A loopful of freshly grown *Escherichia coli* strain was added to 500 μL sterile distilled water and boiled in a heater block at 100°C for 10 min.

ERIC analysis of *Escherichia coli* strains: The extracted DNA was genotyped using modified ERIC PCR reaction described by Versalovic *et al.* (1991) and Weigel *et al.* (2004). The (22-mer) primer ERIC-1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') was used. ERIC PCR was carried out in a 25 μL volume containing 12.5 μL GoTaq mastermix (Promega, USA), 7 μL nuclease free water, 2 μL 25 mM $MgCl_2$, 2.5 μL template DNA and 0.5 μL of each primer (2μM concentration). Amplification was done with the following temperature cycle: 1 cycle at 95°C for 2 min; followed by 35 cycles at 90°C for 30 s, 52°C for 1 min and 65°C for 8 min and 1 cycle at 65°C for 16 min.

All amplifications were performed using Biometra® Tprofesssional thermocycler, Germany. Amplicons (10 μL) were stained with EZ-Vision® One DNA Dye (2 μL), loaded on a 1.5% agarose gel and electrophoresed at 90 V and 400 mA for 1 h 30 min. VC 1 kb and VC 100 bp DNA ladders (Vivantis) were used as the molecular weight markers and the amplicons were visualized under UV transilluminator gel imaging system (Bio-Rad Gel Imaging System).

Cluster analysis and calculation of discriminatory index: Cluster analysis was done as described by Adzitey *et al*. (2013a). Briefly, DNA band sizes were determined using the detect band button of the NTSYSpc Version 2.2. Band sizes were then scored as presence of DNA band (a score '1') and absence of DNA band (a score '0'). These scores were entered in NTedit to obtain a data matrix and then inserted in NTSYSpc Version 2.2 computer software for the construction of dendrogram based on simple matching coefficient and UPGMA (Unweighted Pair-Group Arithmetic Average Clustering) cluster analysis to determine the genetic relatedness of the *Escherichia coli* strains. Clustering was defined at a coefficient of 0.85 and *Escherichia coli* strains not belonging to any particular cluster were referred to as singletons (single isolates). The discriminatory index (D value) was calculated according to Hunter and Gaston (1988).

RESULTS AND DISCUSSION

In this study, ERIC was used to analyse 62 *Escherichia coli* strains isolated from ducks and their environment in Penang, Malaysia. ERIC analysis of the *Escherichia coli* strains produced DNA bands of different sizes for differentiation purposes. Figure 1 is a

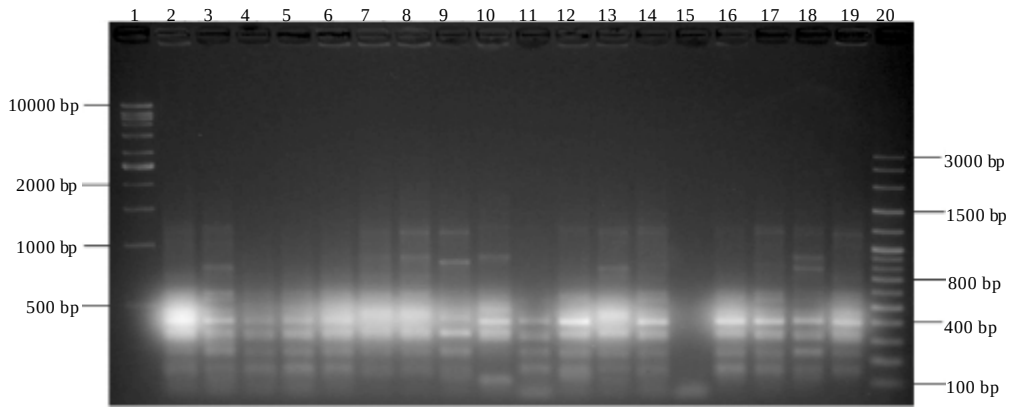

Fig. 1: Representative eRIC agarose gel electrophoresis showing DNA bands of *Escherishia coli* strains. Lane 1: 1 kb DNA ladder, Vivantis, lanes 2-19, *Escherischia coli* strains isolated from ducks and the environment, lane 20: 100 bp DNA ladder, Vivantis

representative ERIC PCR agarose gel showing DNA bands of *Escherichia coli* strains. The reproducibility of the ERIC PCR was verified and confirmed by running the same experiment twice and the results of both experiments were consistent. The DNA band sizes of the *Escherichia coli* strains ranged from 120-2300 bp. DNA bands were analysed and a dendrogram constructed from it using NTSYSpc Version 2.2. Clustering was defined at a coefficient of 0.85 and the number of clusters and singletons produced were used to calculate the discriminatory index (D value) as described by Hunter and Gaston, 1988. Figure 2 is a dendrogram showing the

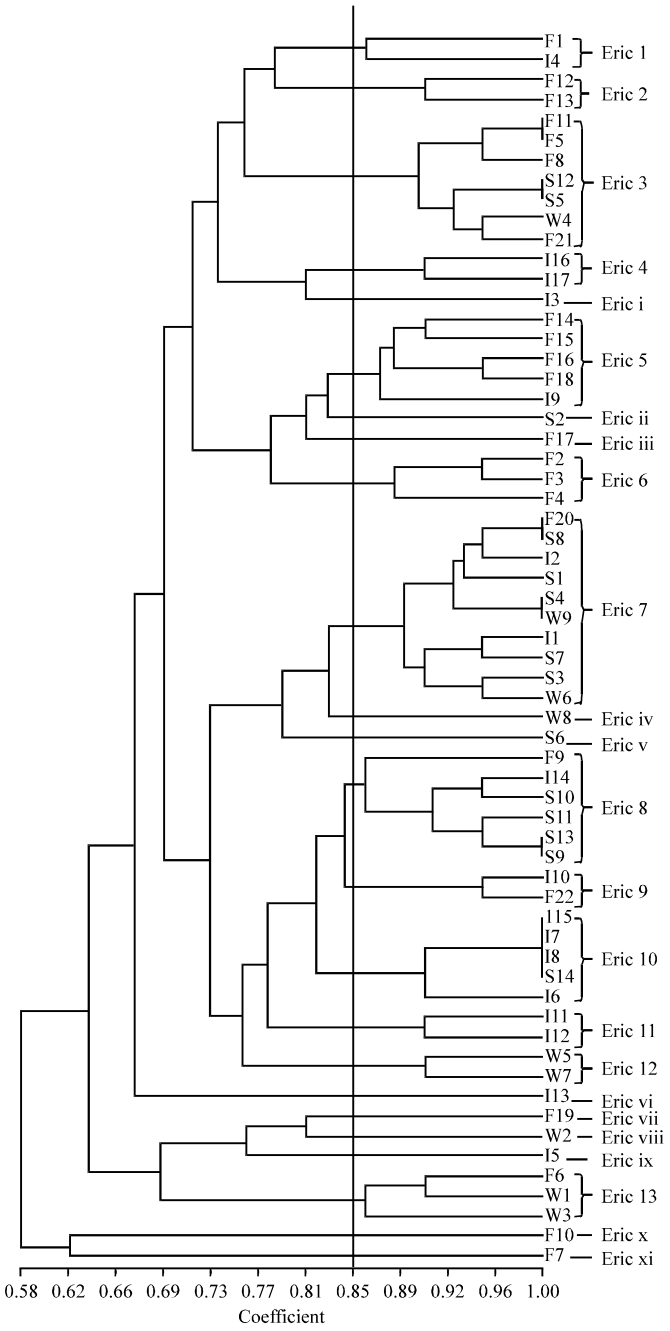

Fig. 2: Dendrogram showing the genetic relatedness of *Escherichia coli* strains isolated from ducks and the environment performed by ERIC-PCR. Eric1-ERIC13 = *Escherichia coli* cluster 1-13; Erici-Ericxi = *Escherichia coli* singleton i-xi; I = intestines; F = faeces; W = wash water and S = soil

genetic diversity/relatedness of the 62 *Escherichia coli* strains. This is indicated by the number of clusters and singletons produced and their location on the dendrogram. ERIC analysis and clustering of the *Escherichia coli* strains at a coefficient of 0.85 produced 13 clusters and 11 singletons at a D value of 0.946.

Clusters consisted of 2 or more *Escherichia coli* strains and include Cluster 1 (Eric1), Cluster 2 (Eric 2), Cluster 3 (Eric 3), Cluster 4 (Eric 4), Cluster 5 (Eric 5), Cluster 6 (Eric6) and many more (Fig. 2). *Escherichia coli* strains in the same cluster are more homogenous and genetically closely related. Furthermore, *Escherichia coli* strains in Clusters 1 and 2 are more related to each other than those in Cluster 3. Similarly, *Escherichia coli* strains in Clusters 8 and 9 are more related to strains in Cluster 10 than those in Cluster 11. Singletons (single isolates) were also observed e.g., Singleton i (Erici), Singleton ii (Ericii), Singleton iii (Ericiii), Singleton iv (Ericiv), Singleton v (Ericv) and so on. *Escherichia coli* strains belonging to these groups are heterogenous and show more distant relation to other *Escherichia coli* strains.

Escherichia coli strains in the same cluster but isolated from different sources suggest possible cross contamination. An example of such a cluster is Cluster 3 (Eric 3) which consists of four isolates of faecal origin, two isolates of soil origin and one isolate of wash water origin (Fig. 2). Wash water and soil *Escherichia coli* isolates could have been contaminated by faecal isolates. This is because *Escherichia coli* normally harbours the intestines of farm animals and are shed through faeces during defecation (Feng *et al*., 2002; Frederick, 2011). Contaminated faeces can contaminate the soil when faeces come into contact with the soil. During carcass dressing and washing, faecal samples can contaminate wash water samples following the rupture of the intestines or the occurrence of any fault during processing.

In this study, faecal and soil samples were obtained from duck farms while intestinal and wash water samples were obtained from the wet market. *Escherichia coli* strains isolated from these sources were distributed across the dendrogram rather than grouping only strains isolated from the farm or wet market together. This suggests that *Escherichia coli* strains of similar genotypes were circulating within ducks and their environmental samples in Penang, Malaysia. This is not surprising since the strains were isolated from the same animal species, similar environment and the same geographical area.

ERIC depends on repetitive DNA elements within *Escherichia coli* strains which are amplified during PCR process to produce DNA fingerprints of different sizes (Shi *et al*., 2010). This technique has been used to successfully determine the genetic diversity/relatedness and for epidemiological studies of *Escherichia coli* (Ling *et al*., 2000; Hollmen *et al*., 2011). Ling *et al*. (2000) characterized 30 strains of *Escherichia coli* O157:H7 isolated from beef and chicken burger by ERIC and suggested that there was considerable genetic heterogeneity among the *Escherichia coli* O157:H7 strains and ERIC can be used to trace its dissemination. Hollmen *et al*. (2011) used ERIC and PFGE to characterize *Escherichia coli* strains and found evidence of avian pathogenic *Escherichia coli* (APEC) strains associated with Steller's eiders and harlequin ducks. They also reported that the genetic profile of two *Escherichia coli* strains from water matched an isolate from a Steller's eider providing evidence of transmission between near-shore habitats and birds.

Hunter and Gaston (1988) reported that a D value >0.900 is desirable and the typing results can be interpreted with confidence. This study showed that the discriminatory index at a coefficient of 0.85 was 0.946. Thus though only one primer was used in this study, the results can be interpreted with confidence and the ERIC PCR was a valuable genotyping tool for determining the genetic diversity of the duck *Escherichia coli* strains. ERIC can be used together with PFGE and MLST to study the genetic diversity or outbreak investigations involving *Escherichia coli*. This is because PFGE and MLST are generally known to have better discriminatory power and/or reproducibility which can sometimes be lacking in ERIC (Wassenaar and Newell, 2000; Adzitey *et al*., 2012a). ERIC has advantage over PFGE and MLST in that, it is easy to perform, rapid and cheaper (Wassenaar and Newell, 2000; Adzitey *et al*., 2012a).

CONCLUSION

This is the first report on the use of ERIC PCR to determine the genetic diversity of *Escherichia coli* strains isolated from ducks and their environment in Penang, Malaysia. ERIC analysis at a coefficient of 0.85 grouped the 62 *Escherichia coli* strains into thirteen clusters and eleven singletons. The ERIC PCR adapted was a useful tool for determining the genetic diversity of the *Escherichia coli* strains isolated from ducks and their environmental samples.

ACKNOWLEDGMENT

This project was supported by grants from the Postgraduate Research Grant Scheme (1001/PTEK1ND/843007) of the Universiti Sains Malaysia.

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