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ORIGINAL ARTICLE

Characterization of *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Albany Isolated from Chickens and Ducks using Random Amplified Polymorphic DNA (RAPD)-PCR

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Abstract

The objective of this study was to characterize *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Albany strains isolated from chicken and ducks to determine their relatedness using Random Amplified Polymorphic Deoxyribonucleic Acid (RAPD)-PCR. RAPD-PCR analysis of the *Salmonella* serovars produced DNA bands that ranged from 242 to 3189bp for *Salmonella* Typhimurium, 252 to 2756bp for *Salmonella* Enteritidis and 232 to 2612 bp for *Salmonella* Albany. Cluster analysis at a coefficient of 0.85 grouped the *Salmonella* serovars into various clusters and singletons. *Salmonella* Typhimurium were grouped into 4 clusters and 1 singleton at a discriminatory index of 0.85.*Salmonella* Enteritidis were grouped into 2 clusters and 2 singletons at a discriminatory index of 0.64. *Salmonella* Albany were grouped into 3 clusters and 1 singleton at a discriminatory index of 0.71. One *Salmonella* Typhimurium isolated from chicken carcass was not characterized as the RAPD-PCR employed failed to produce any DNA band from that isolate. Characterizing *Salmonella* serovars from different sources is important to determine their genetic relatedness, and source of contamination and spread.

Key words: Chicken, Ducks, *Salmonella* Albany, *Salmonella* Enteritidis, *Salmonella* Typhimurium, RAPD-PCR.

1.0 Introduction

Salmonella species are well recognized as important cause of foodborne infections worldwide. Various animal species including poultry and cattle are important sources of *Salmonella species*, and have been implicated in a number of *Salmonella* infections (Adams and Moss, 2008; Frederick and Huda, 2011; Addis *et al*., 2011; Adzitey *et al*., 2012a; Adzitey *et al*., 2012b; EFSA, 2012; CDC, 2013; Geck *et al*., 2014). Different serovars of *Salmonella* have been isolated from poultry (Paiva *et al*., 2009; Adzitey, 2012a; EFSA, 2012; CDC, 2013). Of all the *Salmonella* serovars, *Salmonella* Typhimurium, and *Salmonella* Enteritidis are considered the most common serovars involved in most outbreaks (Defra, 2010) while *Salmonella* Pullorum and *Salmonella* Gallinarum are noted as poultry host specific serovars (Paiva *et al*., 2009).

Molecular characterization of foodborne pathogens is important in several ways. For instance, it helps in determining the genetic relatedness of foodborne pathogens, tracing the primary source of foodborne infections, understanding the route of spread of foodborne pathogens, elucidating the mechanisms by which they cause infection and many more (Albufera *et al*., 2009; Adzitey *et al*., 2014; Adzitey, 2013). The molecular methods employed to achieve these include enterobacterial repetitive intergenic consensus (ERIC), repetitive extragenic palindromic (REP), random amplified polymorphic deoxyribonucleic acid (RAPD) etc, (Khoodoo *et al*., 2002; Albufera *et al*., 2009; Adzitey *et al*., 2012c; Adzitey *et al*., 2013a; Adzitey *et al*., 2013b; Adzitey *et al*., 2013c; Adzitey *et al*., 2014; Patel *et al*., 2014).

This study was carried out to characterize *Salmonella* serovars isolated from chickens and ducks

in Penang using RAPD-PCR to determine their genetic relatedness.

2.0 Materials and Methods

2.1 Bacterial Strains

A total of 36 *Salmonella* serovars comprising of 12 *Salmonella* Typhimirium, 12 *Salmonella* Enteritidis, and 12 *Salmonella* Albany, isolated from chickens and ducks in Penang, Malaysia between 2010 and 2012 were used for this study. Table 1 shows the various *Salmonella* serovars, their source of isolation and assigned code.

2.2 DNA Extraction

A single colony of pure *Salmonella* was inoculated into 10ml Trypticase-Soy Broth and incubated at a temperature of 37^{o}C overnight. 1ml of the overnight culture was centrifuged for 2min at 14,000 x g. Pelleted bacterial cells were subjected to DNA extraction using Wizard® Genomic DNA Purification Kit by following the manufacturer's instructions available at http://www.promega.com/~/media/Files/Resources/Protocols/Technical%20Manuals/0/Wizard%20Genomic%20DNA%20Purification%20Kit%20Protocol.pdf.

2.3 RAPD Analysis of *Salmonella* Isolates

The C-05 (10-mer) primer ′5-GATGACCGCC-′3 was selected for RAPD-PCR after a panel of 8 random primers (designed and manufactured by 1st BASE, Singapore) had been screened. The PCR was performed in a 25µl volume containing 12.5µl Go Taqmastermix (M5132, Promega, USA), 6.25µl nuclease free water, 2.5µl 25mM $MgCl_2$, 2.5µl template DNA (10µM concentration) and 1.25µl primer (5µM concentration). Amplification was performed with the following PCR conditions: initial denaturation at 95°C for 2min, followed by 35 cycles at 95°C for 30s, 45°C for 30s, and 72°C for 1min; terminating at 72°C for 7min (Adzitey *et al*., 2013a). Amplifications were performed using Biometra® Tprofesssional thermocycler. Amplicons (10µl) were stained with EZ-Vision® One DNA Dye (2µl), loaded on a 1.5% agarose gel and electrophoresed at 90V for 1h 30min. VC 1kb and VC 100bp DNA ladders (Vivantis) were used as the molecular weight marker and the amplicons were visualized under UV transilluminator gel imaging system (Bio-Rad Gel Imaging System).

2.4 Cluster Analysis and Calculation of Discriminatory Index

DNA fingerprint positions were determined as described by Adzitey *et al*. (2012b). Clustering was defined at a coefficient of 0.85. *Salmonella* serovars not belonging to any particular cluster were referred to as singletons (single isolates). Discriminatory index was calculated according to Hunter and Gaston (1988) based on the number of clusters and singletons identified.

3.0 Results and Discussion

Random amplified polymorphic deoxyribonucleic acid (RAPD)-PCR was employed to characterize thirty six serovars of *Salmonella* isolated from chickens and ducks in Penang, Malaysia. RAPD-PCR analysis of the *Salmonella* serovars produced DNA bands of different sizes to aid in the differentiation of the various *Salmonella* serovars. The reproducibility of the RAPD-PCR was checked and confirmed by repeating the same experiment twice, and the results of both experiments were consistent with each other. DNA bands were scored as presence (a score of 1) or absence (a score of 0) and dendograms (Fig 1 to 3) were constructed from these scores using NTSYSpc Version 2.2 computer software. Separate dendrograms were constructed for *Salmonella* Typhimirium, *Salmonella* Enteritidis and *Salmonella* Albany which helped in the clustering of the *Salmonella* strains. Clustering was defined at a coefficient of 0.85, and discriminatory index calculated according to Hunter and Gaston (1988) based on the number of clusters (2 or more isolates) and singletons (single isolates).

RAPD-PCR analysis and clustering of the *Salmonella* strains at a coefficient of 0.85 produced 4 clusters and 1 singleton for *Salmonella* Typhimirium with a discriminatory index of 0.84, 2 clusters and 2 singletons for *Salmonella* Enteritidis with a discriminatory index of 0.66, and 3 clusters and 1 singleton for *Salmonella* Albany with a discriminatory index of 0.71. Clusters consisted of 2 or more *Salmonella* strains and are *Salmonella* Typhimurium cluster 1 (TRapd1), *Salmonella* Typhimurium cluster 2 (TRapd2), *Salmonella* Typhimurium cluster 3 (TRapd3), *Salmonella* Typhimurium cluster 4 (TRapd4), *Salmonella* Enteritidis cluster 1 (ERapd1), *Salmonella* Enteritidis cluster 2 (ERapd2), *Salmonella* Albany cluster 1 (ARapd1), *Salmonella* Albany cluster 2 (ARapd2) and *Salmonella* Albany cluster 3 (ARapd3) (Fig 1 to 3).

Salmonella strains in the same cluster are genetically more closely related (Adzitey *et al*., 2012b; Adzitey *et al*., 2013a, b). Thus *Salmonella* strains A12, A19, A3 and Z (TRapd1) are genetically more closely related than S1 and S5R (TRapd2). Similarly, *Salmonella* strains in TRapd1 are more related to -

Table 1: *Salmonella serovars*, designated code and source of isolation

Salmonella Typhimurium		*Salmonella* Enteritidis		*Salmonella* Albany	
Code	Sample origin	Code	Sample origin	Code	Sample origin
Chickens					
G	Feed	A	Immature egg	A35	Carcass
Z	Cloacal swab	B	Feed	A53	Carcass
A3	Faeces and litter	A13	Mature egg	A62	Carcass
A19	Carcass	A33	Egg wash water	A65	Carcass
A12	Feed	E	Feed	A66	Carcass
A26	Carcass	A11	Feed	A75	Immature egg
Ducks					
S1	Intestines	S6	Intestines	S20F	Faeces
S2F	Faeces	S7	Intestines	S23F	Faeces
S5R	Carcass rinse	S13	Intestines	S24F	Faeces
S3F	Faeces	S7.1	Intestines	S2Fe	Feed
S23	Intestines	S13F	Faeces	S1CR	Crate
S1T	Table	S8F	Faeces	S2CR	Crate

TRapd2 than TRapd3. Singletons were also observed for all groups of *Salmonella* serovars (Fig 1 to 3), for example *Salmonella* Typhimurium assigned with the code G (TRapdi), *Salmonella* Enteritidis assigned with the codes S13F (ERapdi) andS8F (ERapdii), and *Salmonella* Albany assigned with the code A66 (ARapdi). Singleton *Salmonella* strains are more distant in relation to other *Salmonella* strains (Adzitey *et al*., 2012b; Adzitey *et al*., 2013a, b).

Figs 1 to 3 also show that *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Albany can generally be grouped into three major genotypes (Group I, Group II and Group III). Some of these groups include *Salmonella* strains isolated from both chickens and ducks and this indicates that *Salmonella* strains of similar genotypes were circulating within chickens, ducks and their environmental samples in Penang, Malaysia between 2009 to 2010. This is expected since the *Salmonella* strains characterized were isolated from similar animal species (poultry), similar environment and geographical area. This finding is agrees with work done by Adzitey *et al*. (2013a, b). Generally, there was the tendency of *Salmonella* serovars isolated from ducks to be closely related to each other than those isolated from chickens. There were very few exceptions notably *Salmonella* Typhimurium with the code G (TRapdi) and *Salmonella* Enteritidis with the code S6 (ERapd1). G was isolated from chickens and was rather close to the duck isolates. S6 was also isolated from ducks but rather close to chicken isolates. The RAPD-PCR adapted was unable to characterize one *Salmonella* Typhimurium designated as A27 (Table 1) isolated from chicken carcass since no DNA band was produce for this isolate.

Adzitey *et al*. (2012b) reported that *Salmonella* serovars in the same cluster but obtained from different origin suggests possible cross contamination. In this study, *Salmonella* Typhimurium Cluster 2 (TRapd2), *Salmonella* Typhimurium Cluster 3 (TRapd3), *Salmonella* Albany cluster 3 (ARapd3) etc include *Salmonella* isolates from different sources. *Salmonella* Typhimurium Cluster 2 consists of one isolate each from intestines (S1) and carcass rinse (S5R), *Salmonella* Typhimurium Cluster 3 consists of one isolate each from intestines (S23) and Table (S1T), and *Salmonella* Albany cluster 3 consists of two isolates from crates (S1CR and S2CR) and one from faeces (S24F). The intestines of farm animals are known to be primary reservoirs of *Salmonella* rather than carcass rinses and table samples (Adams and Moss, 2008; EFSA, 2012). Therefore intestinal and faecal samples might have contaminated carcass rinse, tables and crates.

RAPD-PCR has been employed by other researchers to successfully characterize *Salmonella* isolates to determine their genetic relatedness and to trace the source of foodborne infections (Khoodoo *et al*., 2002; Albufera *et al*., 2009). Khoodoo *et al*. (2002) examined 19 clinical and 7 local broiler chicken *Salmonella* isolates by RAPD and reported that *Salmonella* isolates from Mauritius were genetically diverse. Albufera *et al*. (2009) reported that RAPD-PCR analysis of *Salmonella* isolates from human and food sources (fish and poultry) generated different profiles for isolates of the same serogroup for differentiation purposes. Adzitey *et al*. (2013a) analysed 115 *Salmonella* strains isolated from ducks, their rearing and processing environment using RAPD

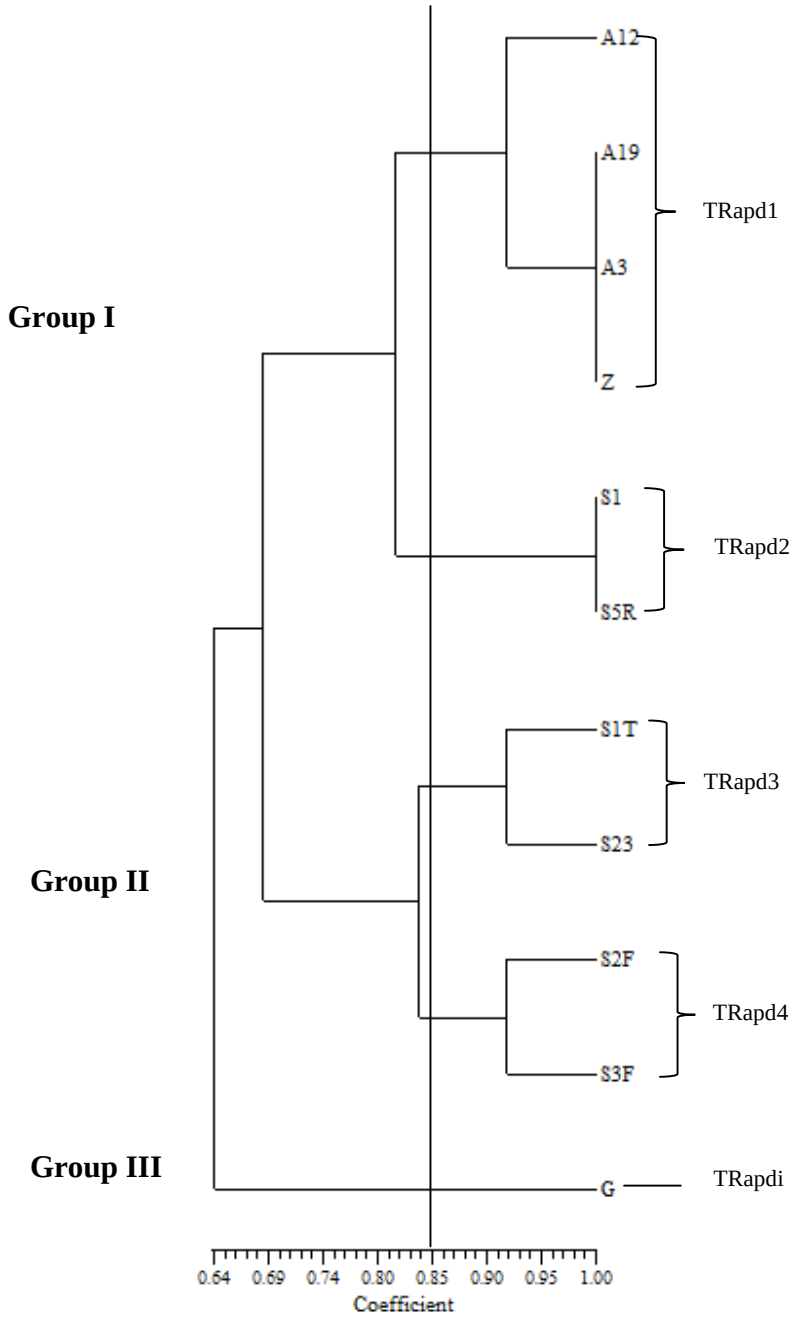

Fig 1: Dendrogram showing the genetic relatedness of *Salmonella* Typhimurium isolated from chickens and ducks performed by RAPD-PCR. TRapd1-TRapd4 = *Salmonella* Typhimurium cluster 1-4; TRapdi = *Salmonella* Typhimurium singleton i.

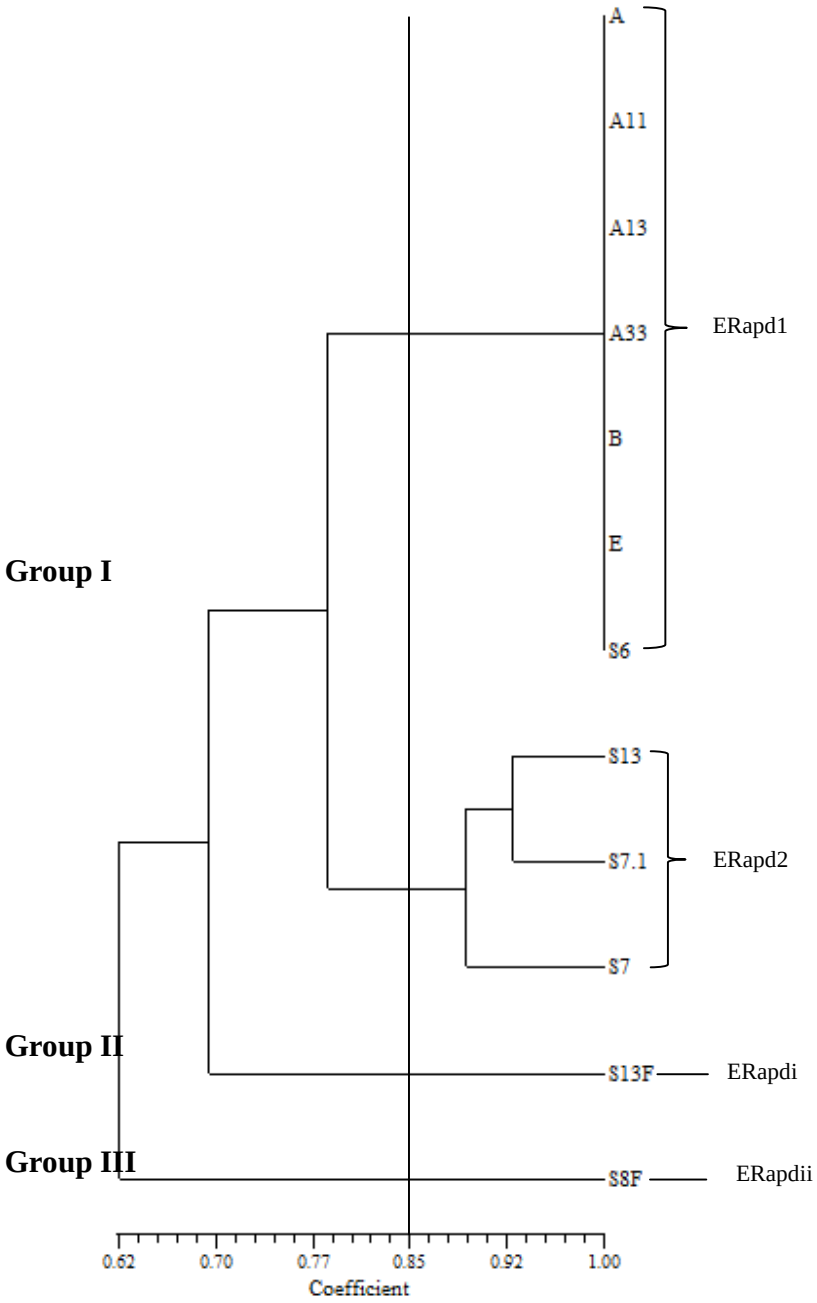

Fig 2: Dendogram showing the genetic relatedness of *Salmonella* Enteritidis isolated from ducks and chickens performed by RAPD-PCR. ERapd1-TRapd2 = *Salmonella* Enteritidis cluster 1-2; ERapdi-ERapdii = *Salmonella* Enteritidis singleton i-ii.

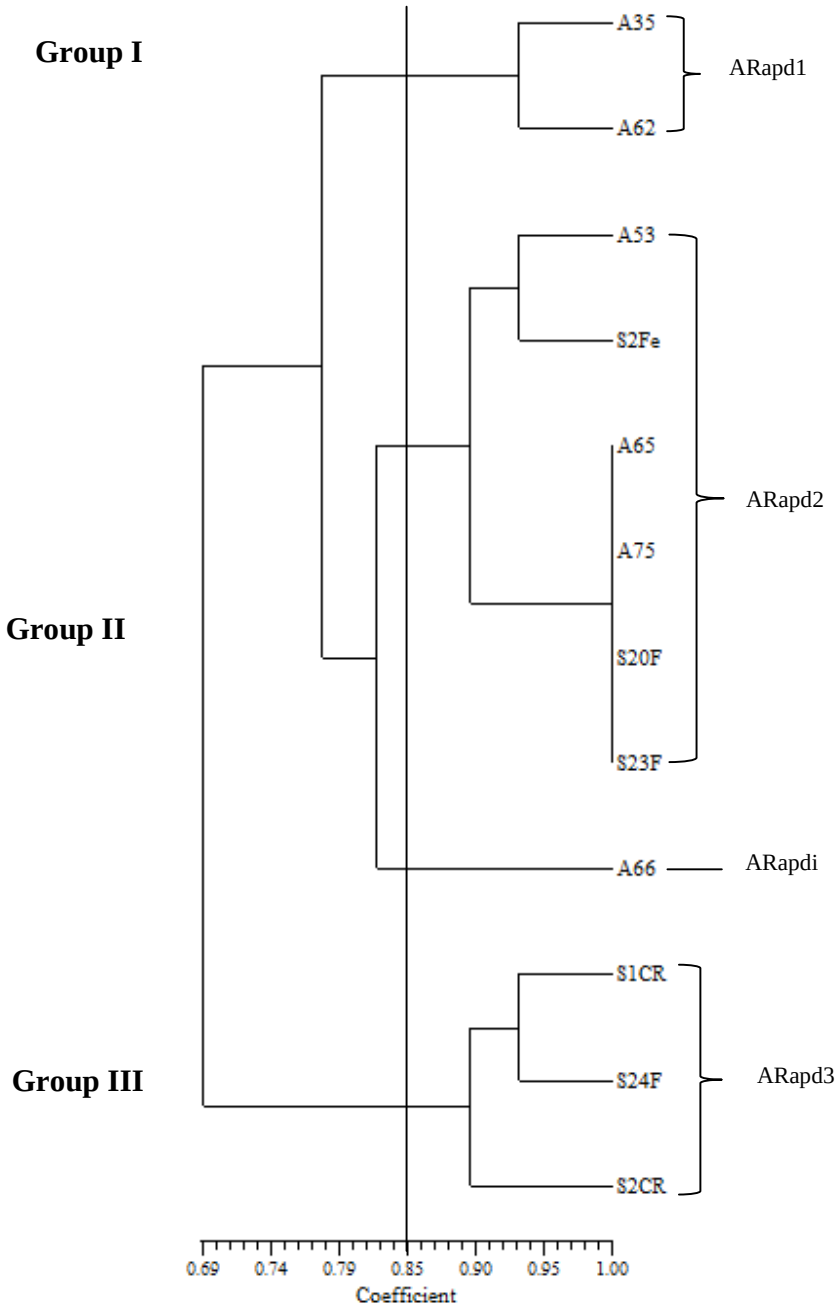

Fig 3: Dendrogram showing the genetic relatedness of *Salmonella* Albany isolated from chickens and ducks performed by RAPD-PCR. ARapd1-ARapd3 = *Salmonella* Albany cluster 1-3; ARapdi = *Salmonella* Albany singleton i.

and reported that the RAPD-PCR was a useful typing tool for determining the genetic diversity of the duck *Salmonella* strains.

4.0 Conclusion

This study compared the genetic relatedness of chicken and duck *Salmonella* serovars using RAPD-PCR. RAPD-PCR analysis of the 36 *Salmonella* serovars resulted in the characterization of the various *Salmonella* strains isolated from chickens and ducks and provided a means of determining the genetic relatedness among the serovars. Knowing the genetic relatedness among *Salmonella* strains is important to know their primary source and the source of cross contamination.

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