Genotyping of Salmonella strains isolated from ducks, their rearing and processing environments in Penang, Malaysia, using RAPD

Frederick Adzitey • Gulam Rusul Rahmat Ali • Nurul Huda • Rosma Ahmad

Received: 15 November 2012 / Accepted: 2 January 2013 / Published online: 18 January 2013 © The Author(s) 2013. This article is published with open access at Springerlink.com

Abstract Salmonella species are important foodborne pathogens that can cause illness and death in humans. The objective of this study was to determine the genetic relatedness of 115 Salmonella strains isolated from ducks and their environment using random amplified polymorphic deoxyribonucleic acid (RAPD). The analysis of Salmonella strains by RAPD produced DNA fingerprints of different sizes for differentiation purposes, and cluster analysis at a coefficient of 0.85 grouped the Salmonella strains into various clusters and singletons. S. Typhimurium were grouped into nine clusters and ten singletons, S. Hadar were grouped into seven clusters and nine singletons, S. Enteritidis were grouped into four clusters and five singletons, S. Braenderup were grouped into five clusters and four singletons, S. Albany were grouped into two clusters and seven singletons, and S. Derby were grouped into two clusters and four singletons at a coefficient of 0.85 with discriminatory index (D) ranging from 0.879 to 0.957. With the exception of S. Typhimurium strains which were grouped into three major groups (genotypes) by RAPD analysis, the rest were grouped into two major genotypes. RAPD was a useful genotyping tool for determining the genetic relatedness of the duck Salmonella strains. Comparison of the genetic relatedness among foodborne pathogens and their sources of isolation are important to trace their source and possibly the source of human infection.

Keywords Ducks • Genetic relatedness • Salmonella strains • RAPD

Introduction

Salmonella species together with other foodborne pathogens are a threat to public health. They are responsible for a number of foodborne outbreaks or sporadic cases that have resulted in foodborne illnesses, hospitalizations and/or deaths. In the United States, Scallan et al. (2011) estimated the pathogens to be a leading cause of human illnesses to be 58 % for norovirus and 11 % for nontyphoidal Salmonella spp. Hospitalization-related bacterial foodborne illnesses were estimated to be 35 % for nontyphoidal Salmonella spp., 26 % for norovirus and 15 % for Campylobacter spp., and that of deaths were estimated to be 28 % for nontyphoidal Salmonella spp., 24 % for T. gondii and 19 % for L. monocytogenes (Scallan et al. 2011). In the UK, 70,298, 9,685 and 176 cases of human campylobacteriosis, salmonellosis and listeriosis, respectively, were reported in 2010 (Defra 2010). In Malaysia, it is cumbersome to estimate the rate of salmonellosis due to inadequately detailed and well-collated epidemiological data from researchers, the veterinary and public health sectors. Salmonellae are widely distributed in food animals, pets, plants, the environment and many other samples (Adams and Moss 2008; Adzitey et al. 2010, 2011; Defra 2010; Frederick and Huda 2011; Abley et al. 2012). The consumption of foods contaminated with foodborne pathogens is responsible for human infections,
while contact with surfaces contaminated with foodborne pathogens poses and increases the risk of human infections. Ducks, duck eggs and duck environmental samples in recent times have been reported as very important sources of Salmonella (Pan et al. 2010; Adzitey et al. 2012a, b); and contact with ducklings or the consumption of duck eggs, meats or products contaminated with Salmonella have been associated with salmonellosis, hospitalization or death of affected persons (Merritt and Herlihy 2003; Noble et al. 2012).

Effective control of Salmonella spp. and their outbreak investigations largely depend on efficient isolation, detection and typing methods. A wide range of molecular typing methods have been used to study the genetic relatedness or diversity of bacterial pathogens and even plants (Wassenaar and Newell 2000; Elmeer et al. 2011; Adzitey et al. 2012c; Rezk et al. 2012; Tripathi et al. 2012). Genotyping methods such as pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), random amplified polymorphic deoxyribonucleic acid (RAPD), enterobacterial repetitive intergenic consensus (ERIC) and repetitive extragenic palindromic (REP), are among those routinely used to type Salmonellae to study their genetic diversity or relatedness (Chansiripornchai et al. 2000; Khoodoo et al. 2002; Lim et al. 2005; Albufera et al. 2009; Smith et al. 2011; Noble et al. 2012). However, published data on the use of RAPD analysis to determine the genetic relatedness of foodborne bacterial pathogens isolated from ducks are rare.

The objective of this study was therefore to ascertain the genetic relatedness of 115 Salmonella strains isolated from ducks, and their rearing and processing environments in Penang, Malaysia.

Materials and methods

Bacterial strains

A total of 115 Salmonella serovars comprising of 37 S. Typhimurium, 26 S. Hadar, 15 S. Enteritidis, 15 S. Braenderup, 14 S. Albany, and 8 S. Derby, isolated from ducks and their environmental sources in Penang, Malaysia, between 2009 and 2010 were used for this study (Adzitey et al. 2012a). Salmonella serovars were isolated from duck faeces (n = 39), intestines (n = 25), cloaca swab (n = 14), soil (n = 14), wash water (n = 8), pond water (4), feed (n = 2), drinking water (n = 2), carcass rinse (n = 3), transport crate swab (n = 2), floor swab (n = 2) and table swab (n = 1) (Adzitey et al. 2012a).

DNA extraction

A single colony of pure Salmonella was inoculated into 10 ml Trypticase Soy Broth (Merck, Germany) and incubated at a temperature of 37 °C overnight. One millilitre of the overnight culture was centrifuged for 2 min at 14,000×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/0Wizard%20Genomic%20DNA%20Purification%20Kit%20Protocol.pdf.

RAPD analysis of duck Salmonella isolates

The C-05 (10-mer) primer 5′-GATGACCGCC-3′ was selected for RAPD-PCR after a panel of eight random primers (designed and manufactured by 1st BASE) had been screened. The PCR was performed in a 25 μl volume containing 12.5 μl GoTaq mastermix (M5132, Promega, USA), 6.25 μl nuclease-free water, 2.5 μl 25 mM MgCl₂, 2.5 μl template DNA (10 μM concentration) and 1.25 μl primer (5 μM concentration). Amplification was performed with the following PCR conditions: initial denaturation at 95 °C for 2 min, followed by 35 cycles at 95 °C for 30 s, 45 °C for 30 s, and 72 °C for 1 min; terminating at 72 °C for 7 min.

All amplifications were performed using Biometra® Tprofessional 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 for 1 h 30 min. VC 1 kb and VC 100 bp 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).

Cluster analysis and calculation of discriminatory index

Cluster analysis and calculation of discriminatory index was done individually for the various Salmonella serovars. DNA fingerprint positions were determined as described by Adzitey et al. (2012c). 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 (D value) was calculated according to Hunter and Gaston (1988) based on the number of clusters and singletons identified.

Results and discussion

Random amplified polymorphic deoxyribonucleic acid was used to analyse 115 Salmonellae isolated from ducks
in Penang, Malaysia. RAPD analysis of the _Salmonella_ strains produced DNA bands for differentiation purposes. Figure 1 is a representative RAPD PCR agarose gel showing DNA fingerprints of _Salmonella_ strains. The reproducibility of the RAPD PCR was verified and confirmed by running the same experiment twice, and the results of both experiments were the same. DNA bands were scored as presence (a score of 1) or absence (a score of 0), and dendrograms (Figs. 1, 2, 3, 4, 5, 6) were constructed from these scores based on simple matching coefficient and Unweighted Pair-Group Arithmetic Average Clustering (UPGMA) using NTSYSpc Version 2.2 computer software. Dendrograms were constructed separately for the various _Salmonella_ serovars, and thus, _S. Typhimurium_, _S. Hadar_, _S. Enteritidis_, _S. Braenderup_, _S. Albany_, and _S. Derby_. Clustering was defined at a coefficient of 0.85, and discriminatory index (D value) calculated according to Hunter and Gaston (1988) based on the number of clusters and singletons (single isolates). RAPD analysis and clustering of the _Salmonella_ strains at a coefficient of 0.85 produced nine clusters and ten singletons for _S. Typhimurium_ at a D value of 0.943, seven clusters and nine singletons for _S. Hadar_ at a D value of 0.957, four clusters and five singletons for _S. Enteritidis_ at a D value of 0.924, five clusters and four singletons for _S. Braenderup_ at a D value of 0.933, two clusters and seven singletons for _S. Albany_ at a D value of 0.879, and two clusters and seven singletons for _S. Derby_ at a D value of 0.929.

Clusters consisted of two or more _Salmonella_ strains and include _S. Typhimurium_ cluster 1 (TRapd1), _S. Hadar_ cluster 1 (HRapd1), _S. Enteritidis_ cluster 1 (ERapd1), _S. Braenderup_ cluster 1 (BRapd1), _S. Albany_ cluster 1 (ARapd1) and _S. Derby_ cluster 1 (DRapd1), etc. (Figs. 2, 3, 4, 5, 6, 7). _Salmonella_ strains in the same cluster are genetically more closely related. Singletons were also observed for all groups of _Salmonella_ serovars (Figs. 2, 3, 4, 5, 6, 7), e.g., _S. Typhimurium_ T1ln (TRapdi), _S. Hadar_ H1Cl (HRapdi), _S. Enteritidis_ E1ln (ERapdi), _S. Braenderup_ B1So (BRapdi), _S. Albany_ A2Ca (ARapdi) and _S. Derby_ D3So (DRapdi) and so on. _Salmonella_ strains

---

**Fig. 1** Representative RAPD agarose gel showing DNA fingerprints of _Salmonella_ strains. Lane 1, 1 kb DNA ladder, Vivantis; lanes 2–19, _Salmonella_ strains isolated from ducks and their environmental samples; lane 20, 100 bp DNA ladder, Vivantis

**Fig. 2** Dendrogram showing the genetic relatedness of _S. Typhimurium_ isolated from ducks and their environmental sources performed by RAPD-PCR. TRapd1–TRapd9, _S. Typhimurium_ cluster 1–9; TRapd1–TRapdx, _S. Typhimurium_ singleton i–x; In, intestines; Fa, faeces; Wa, wash water; So, soil; Fl, floor swab; Ta, table swab
belonging to these groups have more distant relationships to other *Salmonella* strains.

Figures 2, 3, 4, 5, 6 and 7 also show that the various *Salmonella* serovars were generally grouped into two major genotypes, except for *S*. Typhimurium, which was grouped into three major genotypes. This is expected since the *Salmonella* strains were isolated from the same animal species, similar environment and geographical area. Our results strongly suggest that *Salmonella* strains of similar genotypes were circulating within ducks and their environmental samples in Penang, Malaysia, between 2009 and 2010. *Salmonella* serovars in the same cluster but isolated from different sources could suggest possible cross-contamination. Two examples of such clusters are *S*. Hadar cluster 1–7 and *S*. Albany cluster 2 (ARapd2) which consists of four isolates from faeces and one from soil (Fig. 6). Wash water and soil *Salmonella* isolates could have been contaminated by intestinal and faecal isolates, respectively. This is because the intestines and faeces of farm animals are known to be primary reservoirs of foodborne pathogens instead of wash water and soil samples (Adams and Moss 2008; Defra 2010; Pan et al. 2010; Adzitey et al. 2012a; EFSA 2012).

Random amplified polymorphic deoxyribonucleic acid analysis depends on the DNA polymorphisms within *Salmonella* strains which are amplified during PCR process to produce DNA fingerprints of different sizes for differentiation purposes. This technique has been used to successfully determine the genetic relatedness and for epidemiological studies of *Salmonella* spp. (Khodoo et al. 2002; Albufera et al. 2009, Smith et al. 2011). Khodoo et al. (2002) analysed 19 clinical and 7 local broiler chicken *Salmonella* isolates by RAPD using four arbitrary primers (OPA-10, OPR-03, OPI-06 and OPJ-09) and reported that *Salmonella* isolates from Mauritius were genetically diverse. Albufera

---

![Fig. 3 Dendrogram showing the genetic relatedness of *S*. Hadar isolated from ducks and their environmental sources performed by RAPD-PCR. HRapd1–HRapd7, *S*. Hadar cluster 1–7; HRapdi–HRapdix, *S*. Hadar singleton i–ix; Cl, cloaca swab; Po, pond water; So, soil; Fa, faeces; Dr, drinking water](image)

![Fig. 4 Dendrogram showing the genetic relatedness of *S*. Enteritidis isolated from ducks and their environmental sources performed by RAPD-PCR. ERapd1–ERapd4, *S*. Enteritidis cluster 1–4; ERapdi–ERapdv, *S*. Enteritidis singleton i–v; Fa, faeces; In, intestines](image)
et al. (2009) showed that RAPD-PCR analysis of Salmonella isolates from human and food sources (fish meat and poultry) generated different profiles for isolates of the same serogroup for differentiation purposes. Smith et al. (2011) screened 61 Salmonella spp. (26 clinical, 20 food handlers and 15 animal isolates) by RAPD using four primers and reported that RAPD1 and 2 primers were useful for epidemiological typing of their Salmonella spp. The limitation of this study was that only one primer was used after a panel of eight arbitrary primers was screened and the isolates also originated from a very similar source. Nonetheless, our aim was to find out how Salmonella species within ducks and their environments relate or diverse from each other genetically using RAPD-PCR.

The RAPD successfully typed all the duck Salmonella isolates, showed that Salmonella strains of similar genotypes were circulating within ducks/environments in Penang and could also differentiate within some serovarants. This work agrees with findings by Chansiripornmachai et al. (2000) who reported that RAPD provided a simple, rapid and cheap typing tool and may be a valuable tool for studying molecular genetic epidemiology of both inter- and intra-serovars of Salmonella enterica spp. enterica. Hunter and Gaston (1988) reported that a discriminatory index (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 mostly >0.900 except S. Albany and thus the RAPD PCR was a valuable genotyping tool for the duck Salmonella isolates. RAPD can be used together with PFGE, MLST or DNA sequencing to study the genetic diversity or outbreak investigations of Salmonella spp. This is because PFGE, MLST and DNA sequencing are generally known to have better discriminatory power and/or reproducibility, which can sometimes be lacking in RAPD (Wassenaar and Newell 2000; Adzitey et al. 2012d). Furthermore, factors such as mutation, horizontal transfer of non-homologous DNA sequences, insertion and deletion of DNA and/or any kind

![Figure 5](image1)

![Figure 6](image2)

**Fig. 5** Dendrogram showing the genetic relatedness of S. Braenderup isolated from ducks and their environmental sources performed by RAPD-PCR. BRapd1–BRapd5, S. Braenderup cluster 1–5; BRapd1–BRapdiv, S. Braenderup singleton i–iv; Cl, cloaca swab; So, soil; In, intestines; Fa, faeces; Wa, wash water

**Fig. 6** Dendrogram showing the genetic relatedness of S. Albany isolated from ducks and their environmental sources performed by RAPD-PCR. ARapd1–ARapd2, S. Albany cluster 1–2; ARapd1–ARapd11, S. Albany singleton i–vii; Ca, carcass rinse; So, soil; Cr, crate swab; Cl, cloaca swab; Fe, feed; In, intestines; Fa, faeces
of genetic recombination could contribute to or alter DNA fingerprint patterns and subsequently the genetic diversity of *Salmonella* isolates (Luz et al. 1998; Shabarinath et al. 2007). Lim et al. (2005) compared four molecular typing methods (RAPD, ERIC, ribotyping and single-strand confirmation polymorphism) for the differentiation of 57 strains of *Salmonella* spp. and reported that organisms showing identical fingerprinting patterns are considered to be genetically related, but such relationships depend on the technique applied and no direct correlation existed between the four typing methods used.

**Conclusion**

To our knowledge, this is the first extensive report on the use of RAPD PCR to determine the genetic relatedness of *Salmonella* spp. isolated from ducks and their environmental samples in Malaysia. RAPD analysis of the 115 *Salmonella* spp. resulted in strain differentiations and similarities among *Salmonella* strains isolated from the same or different sources. Ducks (eggs, meats and products) and ducklings in recent years have received much attention because of the emergence of *S. Typhimurium* DT8, which has been responsible for a number of foodborne outbreaks. This equally suggests the potential for duck eggs, ducklings and ducks to be important reservoirs for other foodborne pathogens. Knowing the genetic relatedness among *Salmonella* strains is important to know their primary source and possibly their involvement in foodborne infections. The RAPD PCR we adapted was a useful tool for determining the genetic relatedness or diversity of the *Salmonella* strains isolated from ducks and their environmental samples.

**Acknowledgments**

F. Adzitey is grateful to IPS-USM, Dr. Janet Corry and Dr. Tristan Cogan for their financial support and/or advice toward running this project. This project was supported by Grants from the Postgraduate Research Grant Scheme (1001/PTK1ND/843007) of the Universiti Sains Malaysia.

**Conflict of interest**

We the authors declare that we have no conflict of interest, and this work is also our original work, unpublished, and not under consideration by another journal.

**Open Access**

This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

**References**

Abley MJ, Wittum TE, Zerby HN, Funk JA (2012) Quantification of *Campylobacter* and *Salmonella* in cattle before, during, and after the slaughter process. Foodborne Pathog Dis 9:113–119

Adams R, Moss MO (2008) Food microbiology. RSC Publishing. ISBN 978-0-85404-284-5

Adzitey F, Teye GA, Ayim AG, Adday S (2010) Microbial quality of chevon and mutton sold in Tamale Metropolis of Northern Ghana. J Appl Sci Environ Manag 14:53–55

Adzitey F, Huda N, Gulam R (2011) Comparison of media for the isolation of *Salmonella* (XLD and Rambach) and *Listeria* species (ALOA and Palcam) in naturally contaminated duck samples. Int J Food Saf 13:20–25

Adzitey F, Rusul G, Huda N (2012a) Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang, Malaysia. Food Res Int 45:947–952

Adzitey F, Huda N, Ali GRR (2012b) Prevalence and antibiotic resistance of *Campylobacter, Salmonella* and *L. monocytogenes* in ducks: a review. Foodborne Pathog Dis 6:498–505

Adzitey F, Ali GRR, Huda N, Cogan T, Corry J (2012c) Prevalence, antibiotic resistance and RAPD typing of *Campylobacter* species isolated from ducks, duck rearing and processing environments in Penang, Malaysia. Int J Food Microbiol 154:197–205

Adzitey F, Huda N, Ali GRR (2012d) Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks. 3 Biotech. doi: 10.1007/s13205-012-0074-4

Albufera U, Bhugaloo-Vial P, Issack MI, Jaufeerally-Fakim Y (2009) Molecular characterization of *Salmonella* isolates by REP-PCR and RAPD analysis. Infect Gen Evol 3:322–327
Chansiripornachai N, Ramasoota P, Bangtrakuknonth A, Sasipreeyajan J, Svenson SB (2000) Application of randomly amplified polymorphic DNA (RAPD) analysis for typing avian Salmonella enterica subsp. enterica. FEMS Immunol Med Microbiol 29:221–225

Department for Environment, Food and Rural Affairs (Defra) (2010) Zoonoses Report UK. http://www.defra.gov.uk/animal-diseases/zoonotic/. Accessed 23 Aug 2011

EFSA (2012) Scientific report of EFSA and ECDC the European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2010. EFSA J 10(3):2597

Elmeer K, Sarwath H, Malek J, Baum M, Hamwieh A (2011) New microsatellite markers for assessment of genetic diversity in date palm (Phoenix dactylifera L.). 3 Biotech 1:91–97

Frederick A, Hada N (2011) Salmonellas, poultry house environments and feeds: a review. J Anim Vet Adv 10:679–685

Hunter PR, Gaston MA (1988) Numerical index for the discriminatory ability of typing systems. An application of Simpson’s index of diversity. J Clin Microbiol 26:2465–2466

Khoodoo MH, Issack MI, Jaufeerally-Fakim Y (2002) Serotyping and RAPD profiles of Salmonella enterica isolates from Mauritius. Lett Appl Microbiol 35:146–152

Kung H, Lee KH, Hong CH, Bahk GJ, Choi WS (2005) Comparison of four molecular typing methods for the differentiation of Salmonella spp. Int J Food Microbiol 5:411–418

Luz SP, Rodriguez-Valera F, Lan R, Reeves PR (1998) Variations of the ribosomal operon 16S-23S gene spacer region in representatives of Salmonella enterica subspecies. J Bacteriol 180:2144–2151

Merritt TD, Herlihy C (2003) Salmonella outbreak associated with chicks and ducklings at childcare centres. Med J Aust 179:63–64

Noble DJ, Lane C, Little CL, Davies R, De Pinna E, Larkin L, Morgan D (2012) Revival of an old problem: an increase in Salmonella enterica serovar Typhimurium definitive phage type 8 infections in 2010 in England and Northern Ireland linked to duck eggs. Epidemiol Infect 140:146–149

Pan ZM, Geng SZ, Zhou YQ, Liu ZY, Fang Q, Liu BB, Jiao XA (2010) Prevalence and antimicrobial resistance of Salmonella sp. isolated from domestic animals in Eastern China. J Anim Vet Adv 9:2290–2294

Rezk NA, Mansour H, Ghoneim NH, Rifaiat MM (2012) Typing of Salmonella Typhi strains isolated from Egypt by RAPD PCR. 3 Biotech 2:17–25

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011) Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17:7–15

Shabarinath S, Kumar HS, Khushiramani R, Karunasagar I, Karunasagar I (2002) Detection and characterization of Salmonella associated with tropical seafood. Int J Food Microbiol 114:227–233

Smith SI, Fowora MA, Goodluck HA, Nwaokorie FO, Aboaba OO, Opere B (2011) Molecular typing of Salmonella spp. isolated from food handlers and animals in Nigeria. Int J Mol Epidemiol Genet 2:73–77

Tripathi N, Chouhan DS, Saini N, Tiwari S (2012) Assessment of genetic variations among highly endangered medicinal plant Bacopa monnieri (L.) from Central India using RAPD and ISSR analysis. 3 Biotech 2:327–336

Wassenaar TM, Newell DG (2000) Genotyping of Campylobacter spp.—a minireview. Appl Environ Microbiol 66:1–9