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Characterization of *S*. Typhimurium, *S*. Enteritidis and *S*. Albany Isolated from Chickens and Ducks using Repetitive Extragenic Palindromic (Rep) - PCR

Frederick Adzitey[*1], Saba Courage Setsoafia Kosi[2], and Raja Arief Deli[3]

[1]University for Development Studies, Faculty of Agriculture, Animal Science Department, Box TL 1882, Tamale, Ghana.
[2]University for Development Studies, Department of Biotechnology, Faculty of Agriculture, , P.O. Box TL 1882, Tamale, Ghana.
[3]UniversitiSains Malaysia, School of Industrial Technology, Food Technology Division, 11800 Pulau Pinang, Malaysia.

Corresponding author: adzitey@yahoo.co.uk

Abstract

The objective of this study was to characterize Salmonella Typhimurium, *Salmonella* Enteritidis and *Salmonella* Albany strains isolated from chickens and ducks to determine their relatedness using Repetitive Extragenic Palindromic (REP)-PCR. REP-PCR analysis of the *Salmonella* serovars produced DNA bands that ranged from 253 to 7517 bp for *Salmonella* Typhimurium, 302 to 6746 bp for *Salmonella* Enteritidis and 241 to 7550bp 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 3 clusters and 3 singletons at a discriminatory index of 0.85. *Salmonella* Enteritidis were grouped into 3 clusters and 1 singleton at a discriminatory index of 0.75. *Salmonella* Albany were grouped into 1 cluster and 8 singletons at a discriminatory index of 0.91. One each of *Salmonella* Typhimurium and *Salmonella* Enteritidis isolated from chicken carcass and mature egg, respectively was not characterized as the REP-PCR employed failed to produce any DNA band from that isolate. Characterizing *Salmonella* serovars from different sources is important to determine their relatedness, and source of contamination and spread.

Keywords: Chickens, Ducks, *Salmonella* Albany, *Salmonella* Enteritidis, *Salmonella* Typhimurium,

INTRODUCTION

Various *Salmonella* serovars have been implicated in human foodborne infections. In the United States, a total of 195 persons infected with the outbreak strains of *Salmonella* serotypes. Infantis, Newport, and Lille were reported from 27 states between March to September, 2012 (Centers for Disease Control and Prevention, CDC, 2013a). CDC (2013b) also reported of *Salmonella* outbreaks resulting from consumption or contact with tahini sesame paste (*Salmonella* Montevideoand *Salmonella* Mbandaka), cucumbers (*Salmonella* Saintpaul), small turtles (*Salmonella* Sandiego, *Salmonella* Pomona, and *Salmonella* Poona), peanut butter

(*Salmonella* Bradeney), mangoes (*Salmonella* Braenderup), restaurant chain A and Turkish pine nuts (*Salmonella* Enteritidis) etc.

Apart from the sources of infection mentioned above, *Salmonella* serovars have been isolated from a variety of sources including ducks, their rearing and processing environment (Adzitey *et al.,* 2012a), live poultry, ground turkey, ground beef, raw scraped ground tuna product, kosher broiled chicken livers and cantaloupe (CDC, 2013b), soil/sediment, water, wildlife (birds, coyotes, deer, elk, wild pig, skunk) and cattle faeces (Gorski*et al*., 2011), lactating cows and in contact humans in dairy farms (Addis *et al.,* 2011), pigs and the environment (Fedorka-Cray *et al*., 1997), beef (Adzitey *et al*., 2011), chevon and mutton (Adzitey *et al*., 2010) and many more.

The use of molecular methods to characterize *Salmonella* serovars serve a lot of purposes including determining their genetic relatedness, tracing their primary source, elucidating their route of spread, understanding the mechanisms by which they cause infection and many more. These molecular methods included 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) (Versalovic*et al*., 1991; Adzitey *et al*., 2013a, b, c, d; Chansiripornchai*et al*., 2000; Lim *et al*., 2005; Albufera*et al*., 2009). This study was carried out to characterize *Salmonella* serovars isolated from chickens and ducks isolated from Penang using REP-PCR to determine their genetic relatedness.

MATERIALS AND METHODS

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 a wet market and a variety of farms 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.

DNA Extraction

A single colony of pure *Salmonella* was inoculated into 10 ml Trypticase-Soy Broth and incubated at a temperature of 37 ^{o}C overnight. One ml of the overnight culture was centrifuged for 2 min 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.

REP analysis of *Salmonella* isolates

The (18-mer) primer REP1R-I (5'-IIIICGICGICATCIGGC-3') and REP2-I (5'-ICGICTTATCIGGCCTAC-3) was used for the REP-PCR (Versalovic*et al*., 1991). 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 25mM $MgCl_2$, 2.5µl template DNA (10 µM concentration) and 1.25 µl primer (5µM concentration). Amplification was performed with the following PCR conditions: initial denaturation 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; terminating at 65 °C for 16 min. Amplifications were performed using Biometra® Tprofesssional thermocycler. Amplicons (10 µl) were stained with EZ-Vision® One DNA Dye (2µl), loaded on a 1.5% agarose gel and electrophoresed at 90 V for 1h 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 for the various *Salmonella* serovars. 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 (*D* value) was calculated according to Hunter and Gaston (1988) based on the number of clusters and singletons identified.

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

S. Typhimurium		*S*. Enteritidis		*S*. 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

RESULTS AND DISCUSSION

Repetitive Extragenic Palindromic (REP) - PCR was used to analyse 36 *Salmonella* serovars isolated from chickens and ducks in Penang, Malaysia. REP analysis of the *Salmonella* strains produced DNA bands of different sizes for differentiation purposes. The reproducibility of the REP-PCR was checked and confirmed by repeating 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 dendograms (Figures 1 to 3) were constructed from these scores based on simple matching coefficient and UPGMA (Unweighted Pair-Group Arithmetic Average Clustering) using NTSYSpc Version 2.2 computer software. Dendrograms were constructed separately for *Salmonella* Typhimirium, *Salmonella* Enteritidis, and *Salmonella* Albany. 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 (2 or more isolates) and singletons (single isolates). REP-PCR analysis and clustering of the *Salmonella* strains at a coefficient of 0.85 produced 3 clusters and 3 singletons for *Salmonella* Typhimirium at a *D* value of 0.85, 3 clusters and 1 singleton for *Salmonella* Enteritidis at a *D* value of 0.75, and 1 cluster and 8 singletons for *Salmonella* Albany at a *D* value of 0.91.

Clusters consisted of 2 or more *Salmonella* strains and include *Salmonella* Typhimurium cluster 1 (TRep1), *Salmonella* Albany cluster 1 (ARep1), and *Salmonella* Enteritidis cluster 1 (ERep1) etc. (Figures 1 to 3). *Salmonella* strains in the same cluster are genetically more closely related (Adzitey *et al*., 2013a, b, c). Singletons were also observed for all groups of *Salmonella* serovars (Figures 1 to 3), e.g. *Salmonella* Typhimurium assigned with the code S3F (TRepi), *Salmonella* Enteritidis assigned with the code S8F (ERepi), and *Salmonella* Albany assigned with the code A35 (ARepi) and so on. Singleton *Salmonella* strains are more distant in relation to other *Salmonella* strains (Adzitey *et al*., 2013a, b, c).

Figures 1 to 3 also show that *Salmonella* Typhimurium and *Salmonella* Enteritidis can generally be grouped into two major genotypes. *Salmonella* Albany can be grouped into three main genotypes. This is not surprising since the *Salmonella* strains were isolated from similar animal species (poultry), similar environment and geographical area. This suggested that *Salmonella* strains of similar genotypes were circulating within chickens and ducks and their environmental samples in Penang, Malaysia between 2009 to 2010. This finding is consistent with work done by Adzitey *et al.* (2013a, b, c). Except *Salmonella* Enteritidis which did not show a clear pattern, there was the tendency of the other *Salmonell*a serovars (*Salmonella* Typhimurium and *Salmonella* Albany) isolated from chickens or ducks to be grouped together or more closely related to each other (Figure 1 to 3). Also the REP-PCR adapted was unable to characterize one each of *Salmonella* Typhimurium (A19) and *Salmonella* Enteritidis (A13), isolated from chicken carcass and mature egg respectively since no DNA band was produce for these isolates.

Salmonella serovars in the same cluster but obtained from different origin suggested possible cross contamination (Adzitey *et al*., 2012b; Adzitey *et al*., 2013a, b, c). Examples of such a cluster include

Salmonella Typhimurium cluster 3 (TRep3) which consisted of one isolate each from intestines and carcass rinse (Figure 1); *Salmonella* Enteritits cluster 2 (ERep2) which consists of two isolates from intestines, one isolated from feed and one from egg wash water (Figure 2); and *Salmonella* Albany cluster 1 (ARep1) which consisted of two isolates from carcass, one from immature egg and one faeces (Figure 3). Intestinal and faecal samples are potential sources of contamination for feed, egg wash water, and carcass samples due to the fact that intestines and faeces of farm animals are known to be primary reservoirs of foodborne pathogens rather than feed, egg wash water, and carcass samples (Adams and Moss, 2008; Adzitey *et al*., 2012a; EFSA, 2012).

REP depends on repetitive DNA elements present in *salmonellae* which are amplified during PCR process and when electrophoresed produce DNA bands of different sizes and numbers that can be analysed to determine the genetic relatedness Salmonellae (Adzitey *et al*., 2013). REP has been used to successfully determine the genetic relatedness and for epidemiological studies of *Salmonella* spp. (Bennasar*et al*., 2000; Albufera*et al.,* 2009; Adzitey *et al*., 2013a). Bennasar*et al.* (2000) analysed *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Virchow strains and reported that these three serovars gave reproducible and distinguishable profiles using REP, ERIC or ITS, and the conserved patterns in each serovar allowed for easy differentiation from other serovars of *Salmonella*. REP-PCR analysis of *Salmonella* isolates from human and food sources generated different profiles for isolates of the same serogroup for differentiation purposes (Albufera*et al*., 2009). Adzitey *et al*. (2013a) analysed 107 *Salmonella* strains isolated from ducks, their rearing and processing environment using REP and reported that the REP-PCR successfully typed all the *Salmonella* strains and proved to be a useful typing tool for determining the genetic diversity of the duck *Salmonella* strains. They also use REP to show that cross contamination might have occurred among the samples they examined and *Salmonella* serovars of similar genotypes were circulating within the samples examined.

CONCLUSION

We have compared the genetic relatedness of chicken and duck *Salmonella* serovars for the first time in our study area. REP-PCR analysis of the 36 *Salmonella* serovars resulted in strain characterization of the various *Salmonella* strains isolated from chickens and ducks and provided a means of determining the genetic relatedness of the various serovars. Chickens and ducks (eggs, meats and products) in recent years have received much attention because of their involvement in a number of foodborne outbreaks. Knowing the genetic relatedness among *Salmonella* strains is important to know their primary source and possibly their association with foodborne infections. The REP-PCR adapted was a useful tool for characterizing the *Salmonella* strains isolated from chickens and ducks.

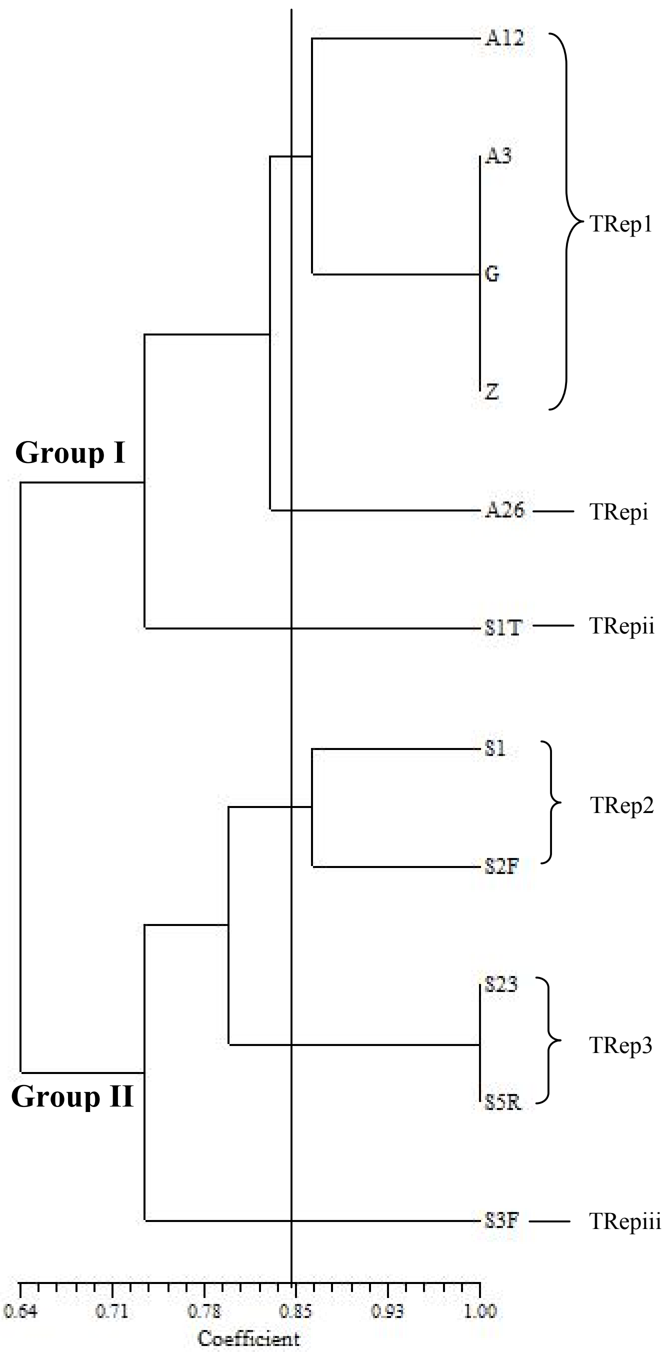

Figure 1: Dendrogram showing the genetic relatedness of *Salmonella* Typhimurium isolated from chickens and ducks performed by REP-PCR. TRep1-TRep3 = *Salmonella* Typhimurium cluster 1-3; TRepi-TRepiii = *Salmonella* Typhimurium singleton i-iii.

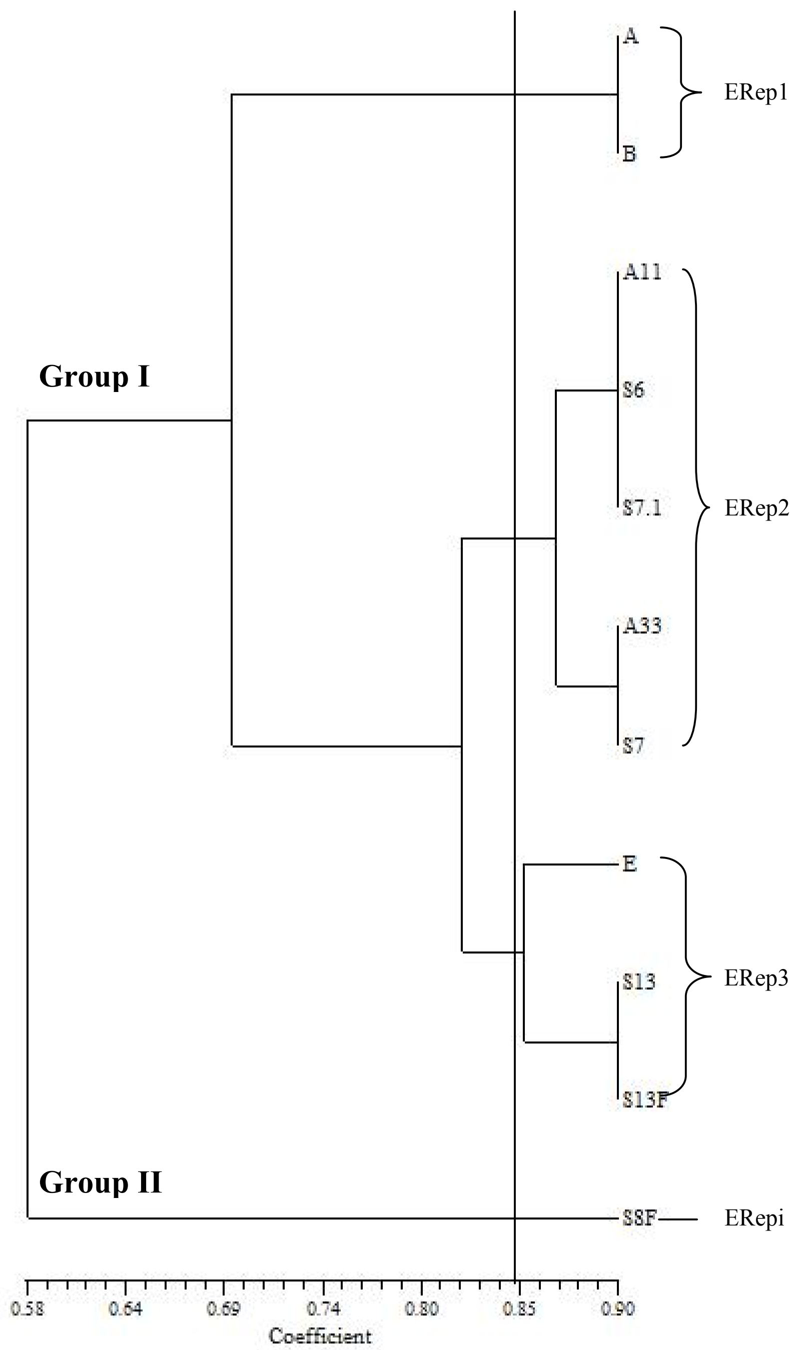

Figure 2: Dendrogram showing the genetic relatedness of *Salmonella* Enteritidis isolated from chickens and ducks performed by REP-PCR. ERep1-ERep3 = *Salmonella* Enteritidis cluster 1-3; ERepi = *Salmonella* Enteritidis singleton i.

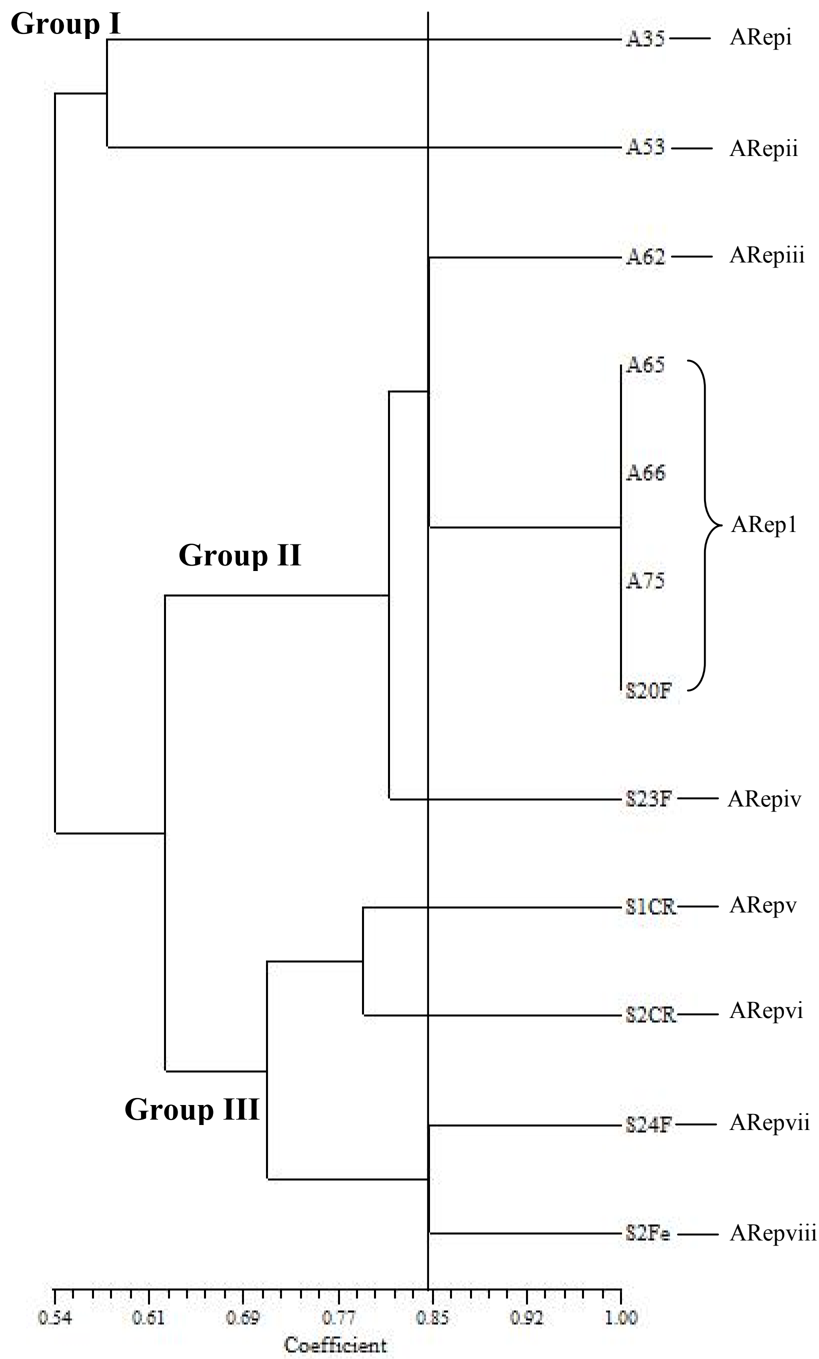

Figure 3: Dendrogram showing the genetic relatedness of *Salmonella* Albany isolated from chickens and ducks performed by REP-PCR. ARep1 = *Salmonella* Albany cluster 1; ERepi-ERepviii = *Salmonella* Albany singleton i-viii.

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REFERENCES

Adams. R. and Moss M.O. 2008. Food Microbiology. RSC Publishing, ISBN 978-0-85404-284-5.

Addis, Z., Kebede, N., Sisay, Z., Alemayehu, H., Wubetie, A. and Kassa, T. 2011. Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross sectional study. *BMC Infectious Diseases* 11:222.

Adzitey, F., Ali, G.R.R., Huda, N. and Rosma, A. 2013a. Genotyping of *Salmonella* strains isolated from ducks and their environments in Penang, Malaysia using repetitive extragenic palindromic (REP). *Journal Microbiology Biotechnology Food Science* 3: 87-93.

Adzitey, F., Ali, G.R.R., Huda, N. and Rosma, A. 2013b. Genetic diversity in *Salmonella* isolates from ducks and their environments in Penang, Malaysia using enterobacterial repetitive intergenic consensus. *Pakistan Veterinary Journal* 33:350-353.

Adzitey, F., Ali, G.R.R., Huda, N. and Rosma, A. 2013c. Genotyping of *Salmonella* strains isolated from ducks, their rearing and processing environments in Penang, Malaysia, using RAPD. *3 Biotech* DOI 10.1007/s13205-013-0115-7.

Adzitey, F., Huda, N. and Ali, G.R.R. 2013d. Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks. *3 Biotech* 3: 97-107.

Adzitey, F., Ali, G.R.R. and Huda, N. 2012a. Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang, Malaysia. *Food Research International* 45:947-952.

Adzitey, F., Ali, G.R.R., Huda, N., Cogan, T. and Corry, J. 2012b. Prevalence, antibiotic resistance and RAPD typing of Campylobacter species isolated from ducks, duck rearing and processing environments in Penang, Malaysia. *International Journal of Food Microbiology* 154:197-205

Adzitey, F., Teye, G.A., Kutah, W.N. and Adday, S. 2011. Microbial quality of beef sold on selected markets in the Tamale Metropolis in the Northern Region of Ghana. *Livestock Research Rural Development* 23 (1): 2011.

Adzitey, F., Teye, G.A., Ayim, A.G. and Adday, S. 2010. Microbial quality of chevon and mutton sold in Tamale Metropolis of Northern Ghana. *Journal of Applied Science Environmental Management* 14: 53-55.

Albufera, U., Bhugaloo-Vial, P., Issack, M.I. and Jaufeerally-Fakim, Y. 2009. Molecular characterization of *Salmonella* isolates by REP-PCR and RAPD analysis. *Infectious Genetics and Evolution* 3: 322-327.

Bennasar, A., De Luna, G., Cabrer, B. and Lalucat, J. 2000. Rapid identification of *S.* Typhimurium, *S.* Enteritidis and *S.* Virchow isolates by polymerase chain reaction based fingerprinting

methods. *International Microbiology* 3:31-38.

Centers for Disease Control and Prevention (CDC), 2013a. Notes from the field: Multistate outbreak of *Salmonella*Infantis, Newport, and Lille Infections linked to live poultry from a single mail-order hatchery in Ohio - March–September, 2012. *MMWR* 62:213-213.

CDC, 2013b. Reports of selected *Salmonella* outbreak investigations 2013. Available at: http://www.cdc.gov/salmonella/outbreaks.html. Accessed on 01.10/2013

Chansiripornachai, N., Ramasoota, P., Bangtrakuknonth, A., Sasipreeyajan, J. and Svenson, S.B. 2000. Application of randomly amplified polymorphic DNA (RAPD) analysis for typing avian *Salmonella enterica* subsp. *enterica*.*FEMS Immunology Medicine and Microbiology* 29:221-225.

Fedorka-Cray, P., McKean, J.D. and Beran, G.W. 1997. Prevalence of *Salmonella* in swine and pork: A Farm to Consumer Study. Available at: http://www.extension.iastate.edu/Pages/ansci/swinereports/asl-1507.pdf. Accessed on 01.10/2013

Gorski, L., Parker, C.T., Liang, A., Cooley, M.B., Jay-Russell, M.T., Gordus, A.G., Atwill, E.R. and Mandrell, R.E. 2011. Prevalence, distribution, and diversity of *Salmonella enterica* in a major produce region of California. *Applied and Environmental Microbiology* 77: 8 2734-2748.

Hunter, P.R. and Gaston, M.A. 1988. Numerical index for the discriminatory ability of typing systems. An application of Simpson's index of diversity.*Journal of Clinical Microbiology* 26:2465-2466.

Lim, H., Lee, K.H., Hong, C.H., Bahk, G.J. and Choi, W.S. 2005. Comparison of four molecular typing methods for the differentiation of *Salmonella* spp. *International Journal of Food Microbiology* 5:411-418.

Versalovic J, Koeuth, T. and Lupski, J.R. 1991.Distribution of repetitive DNA sequences in Eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Research* 19:6823-6831.