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Genotypic Characterization of *Shigella* Species Isolated from Abattoirs in the North West Province, South Africa Using PCR Analysis

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Abstract Foodborne pathogens pose a serious threat to food safety especially in developing countries where hygiene facilities are not well developed and operational practices in abattoirs and retail shops are often poor. *Shigella* species are known to cause foodborne complications in humans including shigellosis that is not only characterized by destruction of the epithelium of the colon but usually results to an inflammatory response. The transmission of *Shigella* species to humans most often results through the consumption of contaminated food, meat and water. The aim of this study was to isolate and identify *Shigella* species from carcass of cattle in some abattoirs in the North West Province, South Africa and determine the virulence gene profiles of the isolates using PCR assays. A total of 97 carcass swabs were obtained from the abattoirs that were sampled. Swabs were properly labeled and transported on ice to the laboratory for analysis. The swabs were washed in 2% (w/v) peptone water and plated on *Salmonella-Shigella* agar. Standard identification tests (Gram staining, oxidase test, TSI test and 16S rRNA) were used to confirm the identities of 97 (one from each sample) presumptive isolates. Large proportions (85% to 100%) of the isolates from Rustenburg and Zeerust were oxidase positive. None of the isolates produced hydrogen sulphide gas on TSI medium but utilize glucose as a source of carbon. A large proportion (75.3%) of the isolates was positively identified as *Shigella* species based on PCR analysis. The number of isolates confirmed as *Shigella* species was higher in Zeerust (54.8%) than in Rustenburg (45.2%). *Shigella* species were most often isolated from samples that were collected outside than inside the carcass. Generally a large proportion (74.0%) of the isolates possessed the *IpaH* gene while64.4% of these isolates were positive for the *IpaBCD* gene that encodes for the invasion plasmid antigen. An analysis of the isolates from the different sampling sections indicated that 46.3% and 55.3% of the isolates from Zeerust possessed the *IpaH* and the *IpaBCD* genes, respectively while 53.7% and 44.7% of the isolates from Rustenburg possessed these genes. The detection of virulent *Shigella* species in beef carcasses demonstrates the need for a continued surveillance of this pathogen in meat in order to ensure the implementation of improved food safety measures.

Keywords: *Shigella species, abattoir, carcass, beef, PCR analysis, IpaH, IpaBCD*

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1. Introduction

Foodborne pathogens pose a serious threat to food safety especially in developing countries where hygiene facilities and operational practices in abattoirs and retail shops are often properly implemented. Recently, the incidence of foodborne infections have greatly increased worldwide and it is estimated that nearly a quarter of the population is at risk [1,2]. In most cases where foodborne infections and outbreaks occur in humans, meat and its related products are usually identified as the sources of contamination [3,4,5,6,7]. *Shigella* species are Gram-negative, non-spore forming, non-motile, non-lactose fermenting, facultative anaerobic bacteria that inhabit the gastrointestinal tract of humans and warm blooded animals. These organisms are amongst the major foodborne pathogens currently known and are therefore frequently isolated from infected patients [8,9,10]. Infections caused by *Shigella* species include bacillary dysentery which is endemic throughout the world and it is responsible for approximately 165 million cases annually, of which 163 million occur in developing countries and 1.5 million in industrialized ones [11]. In addition 1812

cases of both invasive and non-invasive shigellosis has been reported in South Africa and the incidence was high among children who below 5 years of age [12]. Therefore foodborne infections caused by these organisms are of major international health concern [8]. The differentiation of *Shigella* species is mainly based on serotyping and biochemical assays [9]. *S. dysenteriae*, *S. flexineri* and *S. boydii* are physiologically similar but *S. sonnei* can be differentiated using metabolic assays [13]. These three *Shigella* species are most often implicated as agents of human disease worldwide [14]. In addition *S. dysenteriae* is known to cause epidemics of dysentery, particularly in confined populations such as refugee camps [14].

Several PCR protocols using different molecular markers have been used for the detection of *Shigella* species in environmental, faecal, and food samples [15-22]. The invasion plasmid antigen H (*IpaH*) and the *IpaABCD* genes are virulence gene markers whose protein products are necessary for invasion of colonic epithelial cells. These target genes are reliable for the detection of *Shigella* species in food and environmental samples [15,17,19]. These genetic markers are carried by all four *Shigella* species [15].

In the meat industry public health regulatory policies are designed and proposed to be implemented to ensure food safety [23]. Safe food products will greatly meet the high expectations of consumers [23]. Unfortunately, meat can be contaminated through contact with the working surfaces and the equipments at the time of slaughter and if processed in unhygienic conditions [24]. Contamination of carcasses and the environment in abattoirs with intestinal contents of cattle is known to be a critical risk factor for the transmission of pathogens to humans [25]. It is therefore important to ensure that measures are put in place to prevent contamination of meat with foodborne pathogens including *Shigella* species. This can also be achieved through the implementation of surveillance network programs. The present study was therefore designed to investigate the level of contamination of carcasses in selected abattoirs by *Shigella* species.

2. Materials and Methods

2.1. Area of the Study

The research was conducted in the North-West University (Mafikeng campus) – North-West Province, South Africa. Meat samples were obtained from different abattoirs in the North-West Province. This consisted of 97 samples both from Rustenburg and Zeerust.

2.2. Sample Collection

The samples were collected by swabbing meat carcasses inabattoirs. Prior to collection of samples, hand cloves were worn and swaps were used to collect samples from inside and outside the carcasses. Swabs were placed in transport media, labeled properly and immediately transported on ice to the laboratory for analysis.

2.3. Selective Isolation of Shigella Species

Swabs were washed in 5mL of 2% (w/v) peptone water, and 100µL of the sample mixture was spread-platedon the *Salmonella-Shigella* agar (SSA) and incubated aerobically at 37°C for 24 hours. Two to three characteristic colonies were sub-cultured on SSA plates and the plates were incubated at 37°C for 24 hours. Pure isolates were retained for identification using specific biochemical tests.

2.4. Bacterial Identification

Cellular morphology

The isolates were Gram stained using a standard method [26]. All Gram-negative rods were subjected to primary and secondary identification tests.

2.5. Preliminary Biochemical Identification Tests for Shigella Species

2.5.1. Oxidase Test

Oxidase test was performed on all isolates using the oxidase test reagent from Pro-Lab Diagnostics – United Kingdom.

2.5.2. Triple Sugar Iron Agar Test

The Triple sugar iron (TSI) agar (Bio-Lab) obtained from Merck, South Africa was used to assay the potential of isolates in utilizing the three sugars; glucose, sucrose and lactose present at final concentrations 0.1, 1.0 and 1.0%, respectively. Results were interpreted using previously recommended procedure [27].

2.5.3. Extraction of Genomic DNA

Bacterial genomic DNA was extracted using the Boiling method [28]. Aliquots of 500µL distilled water were placed in Eppendorf tubes and pure colonies of isolates were used to prepare bacteria suspensions. The tubes were vortexed and heated in a dry bath at 100 °C for 15 minutes. The contents were centrifuged at 13500 rpm for 2 minutes and the supernatant was transferred into a new Eppendorf tubes. An aliquot of 5 µL from each supernatant was used for the PCR assay.

2.6. PCR for the Identification of Shigella Species and Their Associated Virulence Genes

Standard 25 µL PCR reactions were performed to amplify bacteria 16S rRNA [29], *IpaH* gene fragments [16] and *Shigella* species virulence gene [16] fragments using oligonucleotide primer sequences that occur in Table 1. The reaction mixture consisted of 5 µL of template DNA, 12.5 µL of master-mix, 1.5 µL of reaction buffer, 1.5 µL of the $MgCl_2$, 0.5 µL of both oligonucleotide primers, and 4 µL of nuclease free water. The primers were synthesized by Inqaba Biotec Pty Ltd, Pretoria, South Africa and all PCR reagents were obtained from Fermentas, USA.

Table 1. Oligonucleotide primer sequences used for PCR analysis of *Shigella* species

Primer	Sequence (5' – 3')	Target Gene
Sh16S-F	AGACTGCTACGGGAGGCAGCAGT	16 rRNA gene fragment
Sh16S-R	GTTGCGCTCGTTGCGGGACTTAA	
IpaH-F	GCTGGAAAAACTCAGTCCT	*IpaH* virulence gene for *Shigella* species
IpaH-R	GCTGGAAAAACTCAGTCCT	
IpaBCD-F	GCTATAGCAGTGACATG	*IpaBCD* virulence gene *Shigella* species
IpaBCD-R	ACGAGTTCGAAGCACTC	

2.7. Electrophoresis of PCR Products

The PCR products were resolved by electrophoresis on a 2% (w/v) agarose gel. A horizontal Pharmacia biotech equipment system (model Hoefer HE 99X; Amersham Pharmacia biotech, Sweden) was used to carry out electrophoresis and this was run for 2 hours at 80 V using 1X TAE buffer (40mM Tris, 1mM EDTA and 20mM glacial acetic acid, PH 8.0). Each gel contained a 100 bp DNA molecular weight marker (Fermentas, USA). The gels were stained in ethidium bromide (0.001µg/ml) for 15 minutes and the amplicons were visualized under UV light [30]. A Gene Genius Bio Imaging System (Syngene, Synoptics; UK) was used to capture the images using GeneSnap (version 6.00.22) software. GeneTools (version 3.07.01) software (Syngene, Synoptics; UK) was used to analyze the images in order to determine the relative sizes of the amplicons.

3. Results and Interpretation

3.1. Occurrence of Shigella Species in Meat Carcasses from the Abattoirs

A total number of 97 beef carcass swab samples that comprised 46 and 51 from abattoirs in Rustenburg and Zeerust respectively were analysed for the characters of *Shigella* species using preliminary identification tests that are specific for the Enterobacteriaceae (Gram staining, oxidase test and the triple sugar iron agar test). The proportion of isolates that satisfied the preliminary identification criteria for *Shigella* species are shown in Table 2. As shown in Table 2, all the isolates from Zeerust and Rustenburg were Gram negative rods. All the isolates from Zeerust and a large proportion (85%) of those from Rustenburg were oxidase positive. None of these isolates was able to produce hydrogen sulphide gas from the TSI medium but they were all able to utilize glucose as a source of carbon. These results indicate the possibility of cross contamination during the processing of meat. However, the contamination of the beef carcass may have resulted through contact of the carcass with the hides of animals. This therefore amplifies the need to improve on farm management techniques in the area.

Table 2. Proportion of isolates that were positive for preliminary identification tests of *Shigella* species

Sample site	GS (-ve rod)	OT (+ve)	TSI			
			LFT (+ve)	GFT (+ve)	H_2S (-ve)	Gas (+ve)
Rustenburg	21[a]	16	14	21	21	18
	25[b]	23	20	25	25	21
Total No.	**46**	**39**	**34**	**46**	**46**	**39**
Zeerust	23[a]	23	18	23	23	20
	28[b]	28	22	28	28	26
Total No.	**51**	**51**	**40**	**51**	**51**	**46**

[a]Inside the carcass; [b] =Outside the carcass

3.2 Confirmatory Identification Presumptive Shigella Species Using Specific 16S rDNA PCR Analysis

A total number of 97 isolates that comprised 46 and 51 from beef carcass in abattoirs in Rustenburg and Zeerust, respectively were subjected to specific PCR analysis for characters of *Shigella* species using specific primer sequences. The number of isolates tested and those that were positive for the 16S rDNA gene fragment are shown in Table 3. As shown in Table 3, a large proportion (75.3%) of the isolates was positively identified. The proportion of isolates was generally higher in samples obtained from outside than inside the carcass of animals. Moreover, the number of isolates confirmed as *Shigella* species was higher in samples obtained from abattoirs in Zeerust (54.8%) than in Rustenburg (45.2%).

Table 3. Proportion of isolates that were positive for 16S rDNA species specific PCR for *Shigella*

Sample site	Sampling No. / Position	No. of isolates positively identified	% of isolates positively identified
Rustenburg (NT=46)	Inside (NT=21)	14	19.2%
	Outside (NT=25)	19	26.0%
Zeerust (NT=51)	Inside (NT=23)	18	24.7%
	Outside (NT=28)	22	30.1%
Total	**97**	**73**	**100%**

3.3. PCR Analysis of Virulence Genes of Shigella Species

All the 73 isolates that were positively identified as *Shigella* species were subjected to PCR for the detection of *IpaH* gene and the *IpaBCD* virulence genes. Results for the *IpaH* and *IpaBCD* PCR assays are shown on Tables 4. Large proportions (74.0%) of the isolates from Rustenburg and Zeerust possessed the invasion gene *IpaH*. Moreover, a similarly large proportion (64.4%) of the isolates harboured the *IpaBCD* gene that encodes for the invasion plasmid antigen. An analysis of the isolates from both sampling sites indicated that 46.3% and 55.3% of the isolates from Zeerust possessed the *IpaH* and the *IpaBCD* genes, respectively while 53.7% and 44.7% of the isolates from Rustenburg possessed these genes.

Table 4. Proportion of isolates that were positive for *IpaH* and *IpaBCD* virulence gene specific PCR analysis

Sample site	Sampling No. / Position	No. of isolates positively identified		% of isolates positively identified	
		IpaH	*IpaBCD*	*IpaH*	*IpaBCD*
Rustenburg (NT=33)	Inside (NT=14)	12	8	22.2%	17.0%
	Outside (NT=19)	17	13	31.5%	27.7%
Zeerust (NT=42)	Inside (NT=18)	7	15	13.0%	31.9%
	Outside (NT=22)	18	11	33.3%	23.4%
Total	**73**	**54**	**47**	**100%**	**100%**

4. Discussions

Food products of animal origin including meat are currently known to be the main vehicle for the transmission of pathogens to humans [31]. Shigellosis is endemic in most developing countries and causing

hundreds of deaths yearly [32] and a number of outbreaks have resulted from the consumption of contaminated food even in countries with more advanced public health facilities [33,34,35]. Moreover, opportunities for the transmission of pathogens from contaminated food products to humans are further enhanced when these products are consumed undercooked [37]. By implication it is very crucial to determine the level of contamination of meat in abattoir settings. This may provide valuable information that could be used to prevent contamination of carcass and consequently meat products during processing.

The primary objective of this study was to isolate and identify *Shigella* species from meat carcasses obtained from abattoirs in the Zeerust and Rustenburg in the North West Province, South Africa. This was motivated from the fact that other pathogenic foodborne bacteria strains have been isolated from meat in the North West province [37]. Moreover, it is evident that the presence of these bacteria cells in carcass in abattoirs can serve as a potential source for the isolates to be transmitted to humans and cause gastrointestinal diseases if the meat products are not cooked properly before they are eaten [8,33,34,35,36]. In the present study, *Shigella* species were detected in 73/97 (75.3%) of the samples analysed. These finding are even higher than those reported in other countries [38,39,40]. In Egypt, 2.0% of meat products were positive for *Shigella* species [38] while only a 0.6% prevalence rate was detected in meat products analysed in Ethiopia [39]. On the contrary, none of the meat samples analysed in a study conducted in Turkey were positive for the pathogen [40]. However, live animals present in an abattoir at a particular time may not be the only reservoir of *Shigella* species and therefore during the processing of meat, bacteria that were present in the hides and faeces of previously processed animals may have contaminated the equipments in the abattoir [41]. These contaminants can then be transported to the carcass of subsequent animals. In addition the prevalence rate of *Shigella* species in the current study is very high and this suggests the need to strengthen food safety control systems in abattoirs.

In the present study, a genetic PCR assay was used to also detect *Shigella* species specific virulence genes in isolates obtained from beef carcasses in the North West Province. Results obtained were similar to a previous study in which the *IpaH* gene and the *IpaBCD* were used as indicators to assess the health impacts of *Shigella* species in humans [16]. Large proportions (64.4% and 74.0%) of the isolates from Rustenburg and Zeerust possessed the *IpaBCD* genes and the invasion gene *IpaH* respectively. It has been suggested that environmental *Shigella* isolates tend to lose some of their plasmid-encoded invasion genes since they may not have any known functions as compared to when they are found in the gastrointestinal tract of their hosts [16]. On the contrary, the chromosomally located multicopy virulence genes, *ipaH*, which is also known to play a role in producing bacteria invasive characteristics [16] is usually more stable and present in a large proportion of *Shigella* isolates. Similar findings were reported in the present study and the latter may account for this observation.

It is currently estimated that infections caused by *Shigella* species affect about 164.7 million people worldwide resulting to about 1.1 million death cases [42]. Given the easy with which *Shigella* species contaminate several types of food products, its transmission to humans is also easily achieved [43]. Similar to most foodborne pathogenic microorganisms the infectious dose of *Shigella* species is very low and epidemiological data reveal that approximately 10 bacteria cells can successfully cause disease [43]. Given that this amount of cells can be easily consumed in contaminated food it is therefore important to implement control strategies that would limit the transmission of these cells to food products.

5. Conclusion

The present study examined the level of contamination of beef carcasses in abattoirs with *Shigella* species. Results indicated the presence of pathogenic strains that can be transmitted to humans if the food products are consumed undercooked. This amplifies the need to implement proper regulatory policies in abattoirs to limit the health implications of these pathogens on consumers.

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References

[1] Oliver, S.P., Jayarao, B.M. and Almeida, R.A., "Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications". *Foodborne Pathog Dis* 2. 115-129. 2005.

[2] Centre for Disease Control., "Incidence and trends of infection with pathogens transmitted commonly through food – foodborne diseases active surveillance network, 10 U.S sites, 1996 – 2012". *Wkly Rep* 62(15). 283-287. 2013.

[3] DuPont, HL, *et al.*, "Inoculum Size in Shigellosis and Implications for expected mode of transmission," Journal of Infectious Diseases, Vol. 159, No. 6, pp. 1126-28. 1989.

[4] Wu, F.M., Doyle, M.P., Beuchat, L.R., Wells, J.G., Mintz, E.D. and Swaminathan, B., "Fate of *Shigella sonnei* on parsley and methods of disinfection". *J Food Prot*63. 568-572. 2000.

[5] Williams, R. C., Isaacs, S., Decou, M. L., Richardson, E. A., Buffet, M. C., Slinger, R. W., Brodsky, M. H., Ciebin, B. W., Ellis, A., and Hockin, J., "Illness outbreak associated with *Escherichia coli* O157:H7 in Genoa salami". *Can Med Assoc J* 162. 1409-1413. 2000.

[6] Sim, J., Hood, D., Finnie, L., Wilson, M., Graham, C., Brett, M. and Hudson, J.A., "Series of incidents of *Listeria monocytogenes* non-invasive febrile gastroenteritis involving ready-to-eat meats". *Lett Appl Microbiol* 35. 409-413. 2002.

[7] Kosmider,R.D., Nally, P´., Simons, R.R.L., Brouwer, A., Cheung, S., Snary, E.L. and Wooldridge, M., "Attribution of human VTEC O157 infection from meat products: a quantitative risk assessment approach. *Risk Anal* 30. 753-765. 2010

[8] Mensah, D. Yeboah-Manu, K. Owusu-Darko, A. Ablordey. "Street foods in Accra, Ghana: how safe are they?"*Bull World Health Org* 80. 546-553. 2002.

[9] Nakazato, A., Momoi, Y., Kadoya, M. and Iwasaki, T., "Measurement offeline serum interleukin-5 level". *J Vet Med Sci* 69. 843-846. 2007.

[10] Centre for Disease Control., "Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food –10 states, 2009'. *Morb Mortal Wkly Rep* 59. 418-422. 2010.

[11] Sharma, A., Singh, S.K., Bajpai, D., "Phenotypic and genotypic characterization of *Shigella* spp. with reference to its virulence genes and antibiogram analysis from river Narmada". *Microbiol Res* 165. 33-42. 2010

[12] Keddy, K.H., Sooka, A., Crowther-Gibson, P., Quan, V., Meiring, S., Cohen, C., Nana, T., Sriruttan, C., Seetharam, S., Hoosen, A., Naicker, P., Elliott, E., Haffejee, S., Whitelaw, A., Klugman, K.P., Group for Enteric, Respiratory, and Meningeal Disease Surveillance in South Africa (GERMS-SA)., "Systemic shigellosis in South Africa". *Clin Infect Dis* 54. 1448–54.2012

[13] Uyttendaele M., Bagamboula, C.F.,De Smet, E.,Van Wilder, S. andDebevere, J.,"Evaluation of culture media for enrichment and isolation of *Shigella sonnei* and *S. flexneri*". *IntJ Food Microbiol* 70. 255-265. 2001.

[14] World Health Organisation, "Sixty-third World Health Assembly. Document WHA63/A63/11". 2012. Available at http://www.apps.who.int/gb/ebwha/pdf_files/WHA63/A63_11-en.pdf.

[15] Theron, J., Morar, D., Du Preez, M., Br¨ozel, V.S., and Venter, S.N., "A sensitive seminested PCR method for the detection of *Shigella* in spiked environmental water samples". *Water Res*35. 869-874. 2001.

[16] Faruque, S.M., Khan, R., Kammuzzaman, M., *et al*, "Isolation of *Shigella dysenteriae* type 1 and *S.flexneri* strains from surface water in Bangladesh; comparative molecular analysis of environmental *Shigella* versus clinical strains". *Appl Environ Microbiol*68(8). 3908-3913. 2002.

[17] Jin, Q., Yuan, Z., Xu, J.,Wang, Y., Shen, Y., Lu,W.,Wang, J., Liu, H., Yang, J., Yang, F., Zhang, X., Zhang, J., Yang, G., Wu, H., Qu, D., Dong, J., Sun, L., Xue, Y., Zhao, A., Gao, Y., Zhu, J., Kan, B., Ding, K., Chen, S., Cheng, H., Yao, Z., Bingkun, H., Chen, R., Ma, D., Qiang, B.,Wen, Y., Hou, Y. and Yu, J., "Genome sequence of *Shigella flexneri* 2a: insights into pathogenicity through comparison with genomes of *Escherichia coli* K12 and O157". *NucleicAcids Res*30. 4432-4441. 2002.

[18] Lampel, K.A. and Orlandi, P.A., "Polymerase chain reaction detection of invasive *Shigella* and *Salmonella enterica* in food". *Meth Mol Biol*179. 235-244. 2002.

[19] Peng, X., Luo, W., Zhang, J., Wang, S., and Lin, S., "Rapid detection of *Shigella* species in environmental sewage by an immunocapture PCR with universal primers". *Appl Environ Microbiol*68. 2580-2583. 2002.

[20] Sapsford, K.E., Rasooly, A., Taitt, C.R. and Ligler, F.S. "Detection of *Campylobacter* and *Shigella* species in food samples using an array biosensor". *Anal Chem*76. 433-440. 2004.

[21] Moganedi, K.L.M., Goyvaerts, E.M.A., Venter, S.N. and Sibara, M.M.,"Optimisation of the PCR-*inv*A primers for the detection of *Salmonella* in drinking and surface waters following a pre-cultivative step". *Water SA* 33(2).195-201. 2007.

[22] Penatti, M.P.A., Hollanda, L.M., Nakazato, G., Campos, T.A., Lancellotti, M., Angellini, M., Brocchi, M., Rocha, M.M.M. and Dias da Silveira, W., "Epidemiological characterization ofresistance and PCR typing of *Shigellaflexneri* and *Shigella sonnei* strainsisolated from bacillary dysentery casesin Southeast Brazil". *Braz J Med Biol Res* 40. 249-258. 2007

[23] Govindarajan, C.V.,"Maintenance of hygiene and sanitary conditions including personal hygiene in the meat factory. Technical paper in First National Seminar on Marketing of Meat Food Products in India, Aaligarh, India.1990.

[24] Lues, J.F.R, Theron, M.M, Venter, P. and Rasephei, M.H.R., "Microbial composition of bioaerosols of a high-throughput chicken slaughtering facility". *Poultry Sci*86. 142-149. 2007.

[25] Mead, P.S. and Griffin, P.M., "*Escherichia coli* O157:H7". *Lancet* 352. 1207-1212. 1998.

[26] Cruikshank, R., Duguid, J.P., Marmoin, B.P., Swain, R.H. (1975). Medical Microbiology, 12th ed. Longman, New York. (2): 3-4.

[27] Forbes, A.B. and Weissfeld, A.S., "*Bailey and Scott's Diagnostic Microbiology*", 10th edn. Mosby, StLouis, MO. 1998.

[28] Tunung, R., Chai, L.C., Usha, M.R., Lee, H.Y., Fatimah, A.B., Farinazleen, M.G. and Son, R., "Characterization of *Salmonella enterica* isolated from street food and clinical samples in Malaysia". *ASEAN Food J* 14(3). 161-173. 2007.

[29] Villalobo, E., and Torres, A., "PCR for detection of *Shigella* spp. in mayonnaise". *Appl Environ Microbiol* 64. 1242-1245. 1998.

[30] Sambrook, J; Fritsch, E.F. and Maniatis, T. Molecular Cloning: a laboratory manual. 2nd ed. N.Y., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, 1989. 1659 p.

[31] European Food Safety Authority.,"The European Union summary report on trends and sources of zoonoses,zoonotic agents and food-borne outbreaks in 2011". *EFSA J*11(4). 3129. 2013.

[32] World Health Organization Shigellosis: disease burden, epidemiology and case management. *Wkly Epidemiol Rec* 80. 94-99. 2005

[33] Mead, P.S., L. Slutsker, V. Dietz, L.F. Mccaig, J.S. Bresee, C. Shapiro,*et al.*Food-related illness and death in the United States. *Emerg Infect Dis* 5. 607-625. 1999.

[34] Greig, J.D., Todd, E.C., Bartleson, C.A. and Michaels, B.S., "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 1 Description of the problems, methods, and agents involved". *J Food Prot* 70. 1752-1761. 2007.

[35] Anonymous., "*Shigella* – 2009. Travel and Migrant Health Section, Health Protection Agency, Colindale, UK (Published November 2011, available at http://www.hpa.org.uk/webc/HPAweb_C/1317131651978.

[36] Lampel, K.A., Jagow, J.A.,Trucksess, M. and Hill, W.E., "Polymerase chain reaction for detection of invasive *Shigella flexneri* in food". *Appl Environ Microbiol* 56.1536-1540. 1990.

[37] Ateba, C.N. and Mbewe, M.,"Detection of *E. coli* O157:H7 virulence genes in isolates from beef, pork, water, humans and animal species in the North West Province, South Africa: public health implications". *Res Microbiol* 128. 181-188.2011.

[38] Ahmed, A.M. and Shimamoto, T., "Isolation and molecular characterisation of *Salmonella enterica*, *Escherichia coli* O157:H7 and *Shigella* spp. from meat and dairy products in Egypt". *Int J Food Microbiol* 168-169. 57-62. 2014.

[39] Tassew, H., Abdissa, A., Beyene, G. and Gere-Selassie, S., "Microbial flora and foodborne pathogens on mince meat and their susceptibility to antimicrobial agents". *Ethiop J Health Sci* 20. 137-143. 2010.

[40] Centinkaya, F., Cibik, G., Soyuteniz, E., Ozkin, C., Kayali, R. and Levent, B., "*Shigella* and *Salmonella* contamination in various foodstuffs in Turkey". *Food Cont* 19. 1059-1063. 2008.

[41] Jackson, T.C., Marshall, D.L., Acuff, G.R. and Dickson, J.S., "Meat, poultry, and seafood, In Doyle, M.P., Beuchat, L.R., Montville, T.J. (Eds.), Food Microbiology: Fundamentals and Frontiers, 2nded. ASM Press, Washington DC, USA, pp 91-109.

[42] Thiem, V.D., Sethabutr, O., von Seidlein, L., Tung, T.V., Canh, D.G.,Chien, BT., Tho, L.H., Lee, H., Houng, H., Hale, T.L., Clemens, J.D.Mason, C. and Trach D.D.,"Detection of *Shigella* by a PCR assay targeting the *ipaH* gene suggests increased prevalence of shigellosis in Nha Trang, Vietnam". *J Clin Microbiol* 42. 2031-2035. 2004.

[43] Wachsmuth, K. and Morris G.K., "*Shigella*, pp 448-660. *In* M.P. Doyle (ed.), Foodborne becterial pathogens. Marcel Dekker, Inc., New York, USA.1989.