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 while 64.4% 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*


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. flexneri 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 equipment 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 in abattoirs. 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-plated on 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 tube. 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 MgCl2, 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>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ – 3’)</th>
<th>Target Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh16S-F</td>
<td>AGACTGCTACGGAGGACGACGAT</td>
<td>16 rRNA gene fragment</td>
</tr>
<tr>
<td>Sh16S-R</td>
<td>GTTGCGCTCTGTCGGAGGACTTAA</td>
<td></td>
</tr>
<tr>
<td>IpaH-F</td>
<td>GCTGGAAAAAACCTGACTTCT</td>
<td>IpaH virulence gene for Shigella species</td>
</tr>
<tr>
<td>IpaH-R</td>
<td>GCTGGAAAAAACCTGACTTCT</td>
<td></td>
</tr>
<tr>
<td>IpaBCD-F</td>
<td>GCTATAGCAGTGAGCATG</td>
<td>IpaBCD virulence gene for Shigella species</td>
</tr>
<tr>
<td>IpaBCD-R</td>
<td>ACGAGTTCGAAGCACTC</td>
<td></td>
</tr>
</tbody>
</table>
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 ampiclons 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 ampiclons.

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>
<thead>
<tr>
<th>Sample site</th>
<th>GS (+ve rod)</th>
<th>OT (+ve)</th>
<th>TSI LFT (+ve)</th>
<th>GFT (+ve)</th>
<th>H2S (+ve)</th>
<th>Gas (+ve)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rustenburg</td>
<td>21</td>
<td>16</td>
<td>14</td>
<td>21</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>23</td>
<td>20</td>
<td>25</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>Total No.</td>
<td>46</td>
<td>39</td>
<td>34</td>
<td>46</td>
<td>46</td>
<td>39</td>
</tr>
<tr>
<td>Zeerust</td>
<td>23</td>
<td>23</td>
<td>18</td>
<td>23</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>28</td>
<td>22</td>
<td>28</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>Total No.</td>
<td>51</td>
<td>51</td>
<td>40</td>
<td>51</td>
<td>51</td>
<td>46</td>
</tr>
</tbody>
</table>

*Inside the carcass; 1Outside 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>
<thead>
<tr>
<th>Sample site</th>
<th>Sampling No. / Position</th>
<th>No. of isolates positively identified</th>
<th>% of isolates positively identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rustenburg</td>
<td>Inside (NT=21)</td>
<td>14</td>
<td>19.2%</td>
</tr>
<tr>
<td></td>
<td>Outside (NT=25)</td>
<td>19</td>
<td>26.0%</td>
</tr>
<tr>
<td>Zeerust</td>
<td>Inside (NT=23)</td>
<td>18</td>
<td>24.7%</td>
</tr>
<tr>
<td></td>
<td>Outside (NT=28)</td>
<td>22</td>
<td>30.1%</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>73</td>
<td>100%</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Sample site</th>
<th>Sampling No. / Position</th>
<th>No. of isolates positively identified</th>
<th>% of isolates positively identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rustenburg</td>
<td>Inside (NT=14)</td>
<td>12</td>
<td>22.2%</td>
</tr>
<tr>
<td></td>
<td>Outside (NT=19)</td>
<td>17</td>
<td>31.5%</td>
</tr>
<tr>
<td>Zeerust</td>
<td>Inside (NT=18)</td>
<td>7</td>
<td>13.0%</td>
</tr>
<tr>
<td></td>
<td>Outside (NT=22)</td>
<td>18</td>
<td>33.3%</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>54</td>
<td>100%</td>
</tr>
</tbody>
</table>

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.

Acknowledgement

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References


Sharma, A., Singh, S.K., Bajpai, D., “Phenotypic and genotypic characterization of Shigella spp. with reference to its virulence genes and anti-biogram analysis from river Narmada”. Microbiol Res 165. 33-42. 2010


