OCCURRENCE, DISTRIBUTION, SEROTYPES AND ANTIMICROBIAL RESISTANCE AMONG *SALMONELLA* ISOLATED FROM CATTLE AND ENVIRONMENTAL SAMPLES IN VHEMBE DISTRICT, SOUTH AFRICA

By

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Submitted in partial fulfilment of the academic requirements for the degree of

Masters of Science in Agriculture

College of Agriculture and Environmental sciences

Department of Agriculture and Animal Health

University of South Africa

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September 2017
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September 2017
Dedication

This dissertation is devoted to my lovely wife Madeleine Lubikamba Kapeta for your unconditional love that helps me to stay motivated and made me look forward to the realization of my dissertation.

To my beloved children: Jeriel Lumbala Kapeta and Asriel Ndemba Kapeta this is the inspiration for your future education accomplishment.
Acknowledgments

It has been a long and interesting journey of my life. I sincerely want to thank my supervisor: Dr. Evelyn Madoroba for her laboratory technical assistance, the constructive ideas and positive criticism throughout this project. If it was not for your assistance, none of this study would have happened. I have learnt so much from you.

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Furthermore, my honest appreciation to all my friends for their valuable input and support.

Finally, I would like to thank God for his everlasting support and protection during the entire period of my research.

Sincerely,

Daniel Kapeta
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## Acronyms and abbreviations

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<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AMR</td>
<td>Antimicrobial resistance</td>
</tr>
<tr>
<td>ARC</td>
<td>Agricultural Research Council</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>BGA</td>
<td>Brilliant Green agar</td>
</tr>
<tr>
<td>BTA</td>
<td>Blood tryptose agar</td>
</tr>
<tr>
<td>Bp</td>
<td>Base pair(s)</td>
</tr>
<tr>
<td>BPW</td>
<td>Buffered peptone water</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical and Laboratory Standards Institute</td>
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<tr>
<td>Cm</td>
<td>Centimeter</td>
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<tr>
<td>Diam</td>
<td>Diameter</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DT</td>
<td>Definitive phage type</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>I</td>
<td>Intermediate</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
</tr>
<tr>
<td>Kb</td>
<td>Kilo base</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>MAR</td>
<td>multiple antibiotic resistances</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug resistance</td>
</tr>
<tr>
<td>MHA</td>
<td>Mueller-Hinton agar</td>
</tr>
<tr>
<td>NARMS</td>
<td>National Antimicrobial Resistance Monitoring System</td>
</tr>
<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards</td>
</tr>
<tr>
<td>OD</td>
<td>Optical Density</td>
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<tr>
<td>OVR</td>
<td>Ondersterpoort Veterinary Research</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>R</td>
<td>Resistant</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>-------------</td>
</tr>
<tr>
<td>Rpm</td>
<td>Revolutions per minute</td>
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<tr>
<td>RMRDT</td>
<td>Red Meat Research and Development Trust</td>
</tr>
<tr>
<td>RVS</td>
<td>Rappaport–Vassiliadis soy broth</td>
</tr>
<tr>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>ST</td>
<td>Sequence type</td>
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<tr>
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<td>Unity</td>
</tr>
<tr>
<td>UTIs</td>
<td>Urinary Tract Infections</td>
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<tr>
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<td>Tris-Acetate-EDTA</td>
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<tr>
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<td>World Health Organization</td>
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<td>XLD</td>
<td>Xylose Lysine Desoxycholate agar</td>
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<td>µg</td>
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<tr>
<td>µl</td>
<td>Micro liter</td>
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<td>Percentage</td>
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Declaration

The study has not been submitted in any form to another university and it represents the original work by the author. Information and ideas from other sources have been referenced and cited accordingly.

Student: Daniel Kapeta Djabintu

Date............. Day of ............... 2017

Signature of the candidate.............................................
ABSTRACT

Background: *Salmonella* species is the etiologic agent of salmonellosis, which is a zoonotic infection that is characterized by diarrhea and systemic infection. Contaminated foods are usually the vehicles of *Salmonella* transmission along the food supply chain. Asymptomatic food production animals and effluents also contribute to contamination of meat. Antimicrobials have contributed significantly to treatment of salmonellosis. However, uncontrolled antimicrobial use is among the causes of antibiotic resistance, which results in treatment failure.

Aim and Objectives: The aim of this research study was to determine the extent of *Salmonella* spp contamination during the cattle slaughtering process in South African rural abattoirs (n = 23), water and cattle feaces. In addition, the aim was to determine antimicrobial resistance profiles of the *Salmonella* spp isolates. The specific objectives were: i) to establish the occurrence and distribution of *Salmonella* spp on cattle carcasses, hides, and intestinal contents and environmental samples using classical microbiology and molecular techniques; ii) to determine the *Salmonella* serovars using serotyping; and iii) to determine antimicrobial resistance patterns and multidrug resistance among the *Salmonella* isolates using the Kirby-Bauer disc diffusion method.

Materials and Methods: Classical microbiology techniques were used to analyze cattle faeces (n = 400), hides (n = 67), intestinal contents (n = 62), carcass sponges (n = 100), and water from the abattoirs (n = 75) for the presence of *Salmonella* spp. Further confirmation of the *Salmonella* isolates was done using Polymerase Chain Reaction whereby the *invA* gene was targeted. A total of 92 *Salmonella* spp isolates were recuperated. The 92 *Salmonella* spp isolates were serotyped as described in the White-Kauffmann-Le Minor scheme. The 92 *Salmonella* spp isolates were further subjected to antimicrobial susceptibility examination towards the following antimicrobials: ampicillin (10µg), cefotaxine (30µg), kanamycin (30µg), oxytetracycline (30µg), and enrofloxacin (5µg) by using the Kirby-Bauer disk diffusion procedure.
Results and Discussion: All the isolates carried the *invA* genes. The average *Salmonella* spp occurrence on carcasses, hides, and intestinal contents was 35.37% (n = 81). Eleven of the faecal samples (2.75%) tested positive for *Salmonella* spp.

The *Salmonella* serovar that occurred more frequently was S. Enteritidis. Different serovars that were recognized on carcasses were not automatically found on the hides and intestinal contents. The incompatible frequency of the different *Salmonella* serovars on carcasses, intestinal contents and hides means that in addition to carriage on hides and in intestinal contents, new external causes that did not form part of this study also play a vital role concerning carcass contamination. Most *Salmonella* spp (n = 66; 71.7%) isolates were resistant to a minimum of one antimicrobial with main resistance detected towards oxytetracycline (51.90%). This emphasizes on the call for wise antimicrobial use at some stage in animal production and strict sanitation for the duration of slaughtering.

Conclusion and Recommendation: Briefly, cattle slaughtered in South African rural abattoirs harboured different types of *Salmonella* serovars that were resistant to antimicrobials, which could be a public health risk and danger. The outcome should support policymakers with determining the effectiveness of existing sanitary measures during cattle slaughtering in rural abattoirs, which is vital from socio-economic, public health, and epidemiological perspectives.

Key words: *Salmonella*, antimicrobial susceptibility and resistance, carcass contamination, polymerase chain reaction (PCR), rural abattoirs.
Publications arising from this thesis
