PREVALENCE AND CHARACTERIZATION OF BACTERIA OF GENUS *SALMONELLA* IN RETAIL PORK AND RAW VEGETABLES, BUSIA COUNTY

DR. CHRISTINE MAKENA MBABU

(B.V.M, UON)

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF REQUIREMENTS FOR MASTER'S DEGREE OF UNIVERSITY OF NAIROBI (VETERINARY PUBLIC HEALTH)

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF NAIROBI

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Dr. Christine Makena Mbabu

Signature…… **Date.…23rd March 2022……**

This thesis has been submitted for examination with our approval as University supervisors

Prof. James Mbaria (Ph.D)

Chairman of the Department of Public Health, Pharmacology and Toxicology

Signature…… **Date……1st…April 2022……**

Dr. Peter Baaro Gathura (Ph.D.)

Department of Public Health, Pharmacology and Toxicology

Signature…… **Date…29th …March 2022…….**

Dr. Lian Thomas (Ph.D.)

International Livestock Research Institute (ILRI), Nairobi

Signature…… **Date……..23rd March 2022……..**

DEDICATION

To my loving husband (Dr. Kevin Miheso), my babies, my parents, my brothers and to all my friends. Thank you for always loving and supporting me throughout this journey.

ACKNOWLEDGEMENTS

I have taken efforts in this project. However, this project would not have been possible without the kind support and help of many individuals and organisations. I would like to extend my sincere thanks to all of them**.**

I would like to thank first and foremost the lord God Almighty for his never ending grace and providing me with everything that I required in completing this project. I have been immeasurably enriched by working under the supervision of Dr. Lian Thomas, Prof James Mbaria and Dr. Peter Baaro Gathura for their guidance, constant supervision and also for their support which made me finish this project with ease. I express my deep gratitude and appreciation to my husband Dr. Kevin Miheso for not only his financial and moral support throughout my project but also for his time expended and courage in sharing insights, with a fledging student.

I would like to express my special gratitude and thanks to Dr. Mercy Cianjoka who has always been my immediate supervisor and for her guidance throughout the project. We have worked tirelessly together in this project back in Busia where this project was undertaken. In the same breath, I would like to extend my many thanks to other individuals that helped in one way or another during this project " - the Busia ZooLinK team" to mention but a few Josiah Obadia, Masinde, Lucy Mungai, Hannah Kamau and Samuel Njoroge.

I sincerely wish to thank the University of Nairobi for admitting me as a Master's student. Special thanks goes to the Department of Public Health, Phamarcology and Toxicology where I carried some part of this project. I am highly indebted to Gitahi Nduhiu, the principal technologist in the department for allowing me in the university laboratories and for his guidance and support. I would also like to give many thank to miss Beatrice Wandia, laboratory technician who helped me do my laboratory work with ease.

Above all, this project would not have been possible without the support of the Biotechnology and Biological Sciences Research Council, the Department for International Development, the Economic and Social Research Council, the Medical Research Council, the Natural Enviroment Research Council and the Defense Science and the Technology Laboratory under the Zoonoses and Emerging Livestock Systems (ZELS) programme, grant reference BB/L019019/1. It also received support from the CGIAR Research Program on Agriculture for Nutrition and Health (ANH), led by the International Food Policy Research Institute (IFRI) and also I acknowledge the CGIAR fund Donors (http://www.cgiar.org/funders). Many thanks to Prof Eric Ferve for facilitating the funding of my projects. I would also like to thank Christine Mosoti and Victoria Kyalo, the program managers that made it possible for the availability of cash whenever required. Every need was communicated through Dr. Lian Thomas, my supervisor, who would never rest until the problem was solved, many many thanks to you Dr. Lian Thomas.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPINDICES

LIST OF ABBREVIATIONS AND ACRONYMS

AMC	Amoxicilin/clavulanic acid
AMP	Ampicillin
AMR	Antimicrobial resistant
C	Chloramphenicol
CDC	Center for Disease Control and Prevention
CIP	Ciprofloxacin
CLSI	Clinical and laboratory standards institute
CN	Gentamicin
CRO	Ceftriaxone
CXM	Cefuroxime
EFSA	European Food Standards Authority
FAO	Food and Agriulture Organization of the United Nations
FDA	Food and Drug Administration
GPS	Global positionimg system
MDR	Multidrug resistant
NA	Nalidixic acid
NAP	National action plan
SPP	Species
SXT	Trimethoprim/sulfamethoxazole
TE	Tetracycline
XDR	Extended drug resistant

XLT-4	Xylose Lysine Tergitol-4
ILRI	International Livestock Research Institute
UoN	University of Nairobi

ABSTRACT

Salmonellosis is a major global threat to public health and causes emergence and spread of antimicrobial-resistant strains. This study aimed to determine the prevalence and characterization of *Salmonella* species (spp.) in retail pork and raw vegetables commonly known as Kachumbari(tomatoes, onions, pepper, dhania, avocados) served alongside cooked pork in Busia County, Kenya. Samples collected from selected butcheries were 451 consisting of 262 raw pork, 108 cooked pork and 81 side salads served alongside cooked pork. Samples were cultured in Salmonella selective media in Busia ILRI laboratories. Isolated Salmonella spp. were afterwards identified using genus antiserum at the UoN laboratories . Serotyping was done using Kauffmann-Whyte scheme and antimicrobial sensitivity was determined using disc diffusion method. The overall *Salmonella* prevalence of retail pork was 32.59% (147/451 95% CI 32.40% - 32.80%). Raw pork recorded the highest prevalence of 49.24% (129/262 95% CI 48.86% - 49.61%), followed by raw vegetables with 19.75% (16/81 95% CI 18.79% - 20.72%) and cooked pork recorded the least with 1.85% (2/108 95% CI 1.65% - 2.10%). *Salmonella*-positive isolates were highly resistant to the antibiotics used with an overall resistance of 135/147 (91.84%). 67/147 (45.58%) of the samples had multidrug resistance while 99/147 (67.35%) had extended drug resistance. Overall, the isolates had the highest resistance to Gentamicin (63.94%) followed by Ampicillin (59.86%). The highest intermediate resistance was found in ciprofloxacin (76.19%) and nalidixic acid (74.15%). Seven isolates were resistant to third-generation cephalosporins, ceftriaxone.

This study highlights a risk of exposure to *Salmonella* spp. from retail pork and raw vegetables sold at pork butcheries in Busia. This is evidenced by the high prevalence of *Salmonella* spp. from the raw pork and raw vegetables served alongside cooked pork from butcheries in the four sub-

counties in Busia that were being investigated. In this setting, cooking of pork mitigated much of the risk of exposure to *Salmonella* spp. from pork, though the practice of plating pork with raw vegetable side-salads (Kachumbari) with a high prevalence of *Salmonella* spp. is thought to re-introduce the exposure risk. This study is not able to determine if the vegetables entered the butcheries while contaminated or whether they were cross-contaminated within the butchery setting. This is the first study reporting on salmonella prevalence in retail pork in this country. Worryingly the majority of the isolates were resistant to one or more antimicrobials tested and more than half of them had multidrug-resistant to three or more classes of antimicrobials tested, creating an additional potential health burden. This study highlights risks that may be present in other butchers in the country where hygiene and sanitation are not strictly followed hence necessitating more investigation and action to be taken.

Keywords: *Salmonella* spp.; pork; retail pork; antimicrobial-resistant; Busia

CHAPTER ONE: INTRODUCTION

1.1: Introduction

Foodborne illnesses especially those caused by bacterial pathogens like *Salmonella* spp. are one of the leading public health problems globally with a plurality of cases and deaths reported as a result of consumption of contaminated food (Hendriksen *et al*., 2011; Majowicz *et al.*, 2010). *Salmonella* is considered one of the most prevalent pathogens mainly from the food of animal origin and is responsible for causing zoonotic infections in humans and animals (Sánchez-Vargas *et al*., 2011; Carrasco 2012). Swine infected with Salmonella presents prostration, fever, diarrhoea and death. Nevertheless, most infected swine commonly harbor *Salmonella* spp. and remain as healthy carriers, and are one of the main avenues of contamination in the pork value chain (Bornardi, 2017). Pork contamination can occur in the slaughter due to cross-cntamination with other carcasses as well as presence of *Salmonella* in the environment. In fact, pork is a potential source of foodborne diseases attributed to its favorability for the multiplication of various microbial organisms. Raw and undercooked pork if consumed often represents a source of non-typhoidal *Salmonella* strains to humans (Doulgeraki *et al.*, 2012). Recently, over 2,500 *Salmonella* serotypes have been identified and among the serotypes that are significantly associated with human and animal disease are ; Typhimurium, Newport, Heidelberg and Enteritidis (Foley and Lynne, 2008)

The emergency of antimicrobial resistant (AMR) and potentially more pathogenic *Salmonella* strains has resulted in serious public health issues. Some *Salmonella* spp. have developed Multidrug resistance (MDR) such as resistance towards clinically important antimicrobials like fluoroquinolones and third-generation cephalosporins which is worrisome worldwide (Jajere, 2019). This presents a public health risk because the resistance strains end up in humans through

contaminated food, unfortunately, the sources and transmission routes are poorly understood in the developing countries due to poorly resourced surveillance systems within veterinary and public health systems.

Pork consumption in Kenya has increased in the recent past and is expected to continue that way due to population increase as well as people become more affluent (Thomas *et al.*, 2013; McGlone, J.J., 2013). Western Kenya is ranked second-most in pig population in Kenya (FAO, 2012) and consumption of pork is common. Retail outlets of pork in Busia are the butchers' shop. Butchers buy their animals and slaughter them at either their home or at slaughter with poor hygienic conditions (Cook *et al*., 2017; Levy, 2014). The majority of pork consumers buy pork either cooked or raw from butchers' shop (Levy, 2014) at which food hygiene and safety condition are not assured. There is no information on the prevalence of *Salmonella* species in retail pork in Busia or elsewhere in Kenya to compare. Although *Salmonella* species have been isolated from pigs at slaughter in Busia (Wilson *et al*.,2018) no study has been done on *Salmonella* species from retail pork and raw side vegetable served alongside cooked pork. There is also no information on *Salmonella* serovars from *Salmonella* contaminated pork at the point of retail likely to be transferred to the consumers as well their AMR. Elsewhere studies have shown retail pork contaminated with *Salmonella* hence presents a significant public health issue.

This study is aiming to determine *Salmonella* prevalence in retail pork and raw vegetables served alongside cooked, characterize the *Salmonella* species isolated from retail pork and raw vegetables served alongside cooked pork as well as determine their antimicrobial resistance profiles which will help in surveillance of zoonotic diseases that originate from the food of animal origin in particular *Salmonella* species (Spp.) that is one of the major foodborne pathogens.

1.2 Objectives

1.2.1 Overall objective

The overall objective of this study was to determine the prevalence and characterize *Salmonella* species in retail pork and side salads in Busia County, Western Kenya

1.2.2 Specific objectives

1. To determine and compare the prevalence of *Salmonella* species in pork and side salads at the point of retail.

2. To characterize the *Salmonella* species isolated.

3. To determine the antimicrobial resistance profile of the identified *Salmonella* species

1.3 Justification

Salmonella species are a major foodborne pathogen and associated with several outbreaks due to contaminated food and pork is understood to be a common source of infection. In Kenya, there is a paucity of data on *Salmonella* species contamination in retail pork and side salads hence the need for this research**.** The determination of antibiotic resistance in retail pork and side salads will be important as the Kenyan National Action Plan on Antimicrobial resistance requires a greater understanding of the Antimicrobial resistance in the food of animal origin across the country.

CHAPTER TWO: LITERATURE REVIEW

2.1 Salmonella spp.

Genus *Salmonella* is a gram-negative, facultative anaerobe and rod-shaped that belong to the Enterobacteria family and lives in the intestinal tract of animals and humans (Su and Chiu□□□□7; Sterzenbach *et al*., 2013; Keeble and Koterwas, 2020). There are two species assigned to this genus: *S. enterica* and *S. bongori*. *S. enterica* itself is divided into six subspecies; *enterica*, *salamae*, *arizonae*, *diarizonae*, *indica*, *houtenae* , also known as subspecies I, II, IIIa, IIIb, IV, and VI, respectively (Brenner *et al*., 2000; Tindall *et al*., 2005). Warm-blooded animals are associated with S. enterica subspecies enterica consisting of about 99% of clinical isolates which cause infections ranging from mild gastroenteritis to life-threatening systemic infections (Fierer and Guiney, 2001; Lamas *et al*., 2018) while the remaining subspecies, S. bongori are isolated from cold-blooded animals and account for less than 1% clinical isolates (Pui *et al.,* 2011). *Salmonella* serotypes are determined by the immunoreactivity of three surface antigens "O" (lipopolysaccharides), "H" (flagellin protein), and "Vi" (capsule) (Grimont and Weill, 2007) and between the two species of *Salmonella*, over 2,500 unique serotypes have been described and new serotypes are described regularly. However, only some of these serotypes have been frequently associated with food-borne illness.

Salmonella serotypes can be divided into host restricted and generalist serotypes/ host-adapted. Paratyphi A and Typhi are examples of hosts specific serotypes that only cause disease in one host species. Generalist serotypes such as *Salmonella* Typhimurium exhibit a promiscuous phenotype in that they maintain the ability to colonize and potentially cause infections in more than one host species (Thomson *et al*., 2008□Langridge *et al*., 2015). Loss of genetic materials and mutation are some of the ways that have enabled *Salmonella* serotypes to become adapted to their hosts (Kisiela

et al., 2012). *Salmonella* serotypes that cause diseases in humans are; typhoidal serotypes and non-typhoidal *Salmonella* serotypes. Typhoidal serotypes, that have humans as their only reservoir are; Typhi and Paratyphi A. They can only be transmitted from humans to humans and can cause foodborne infections, typhoid fever, and paratyphoid fever also known as enteric fever (Eng *et al*., 2015). Nontyphoidal serotypes on the other hand are predominantly found in animals as their reservoir and can be transferred from animals to humans as well as humans to humans (Eng *et al*., 2015). Nontyphoidal *salmonella*, particularly those associated with pork are the main concern of this thesis. These serotypes exhibit a promiscuous phenotype in that they maintain the ability to colonize and potentially cause infections in more than one host species (Thomson *et al*., 2008). Hendriksen *et al*., 2011 established that there is a difference in commonly isolated serovars among regions. *S. typhimurium* for instance is the most dominant serovar worldwide and is associated with foodborne outbreaks in both high-income countries and developing ones. Besides, *S. Infantis* occur world. However, others such as *S. Newport, S. Agona, S. Virchow, and S. Hadar* have regional differences (Hendriksen *et al*., 2011)

2.2 *Salmonella* spp. foodborne disease burden

Salmonellosis is a common foodborne disease in animals and people around the globe, besides, the virulent *salmonella* serotypes are widely spread. The majority of human infection of *Salmonella* is related to the ingestion of contaminated foods such as poultry, beef, pork, egg, milk, cheese, seafood, fruits, juices, and vegetables (Zhao *et al.,* 2008). The main reservoir of *Salmonella* spp. is the gastrointestinal tract of warm-blooded animals, in particular food-producing animals, which lead to foodstuffs contamination (Crump *et al.,* 2015; Arya *et al.,* 2017). Ingestion of contaminated food, particularly foods of animal origin, is recognized as the most relevant source of transmission of *Salmonella* to humans, with a high global impact on human

health (Arya *et al.,* 2017)**.** An estimated 93.8 million cases (90% CI, 61.8-131.6 million) of gastroenteritis caused by *Salmonella* spp. occur globally each year and of these, nearly 80.3 million cases are foodborne (Majowicz *et al.,* 2010). Five pathogens account for over 90% of estimated food-related deaths: *Salmonella* (31%), Listeria (28%), Toxoplasma (21%), Norwalk-like viruses (7%), Campylobacter (5%), and Escherichia coli O157:H7 (3%) (White *et al.,* 2002)

According to (Havelaar *et al*., 2015), foodborne pathogens cause about 600 million illnesses and 420,000 deaths worldwide and NTS are among the major causative agent. Globally, 33 million DALYs (disability-adjusted life years) are due to foodborne illnesses. Nontyphoidal *salmonella* enterica ranked 5th out of the 31 hazard that causes global DALYs FBD burden and about 90% of the burden is due to years of life lost due to premature mortality and the remainder is due to the years lived with disabilities (Li et al., 2019). Nontyphoidal *Salmonella enterica* are reported to cause diarrhea in children under the age of five resulting in several deaths (Havelaar *et al*., 2015). According to Li *et al*., 2019 NTS has a global distribution. NTS is associated with mild gastroenteritis illness in developed countries that mainly is self-limiting and treatment with antimicrobial is not necessary (Kariuki *et al*, 2006). Whereas nontyphoidal serotypes present mostly as a gastrointestinal disease in developed countries, it was estimated that sub-Saharan Africa ranks the highest in cases of invasive NTS infections in the world (Feasy *et al*., 2015). Besides, these NTS infections occur endemically in sub-Sahara Africa causing bacteremia which results in 4100 deaths mostly in children (Majowicz *et al*., 2010).

Nontyphoidal *salmonella* spp. are transmitted to humans when food and water contaminated with animal waste is ingested (Eng *et al*., 2015). Infection with Nontyphoidal salmonella serotypes can result in noninvasive forms leading to gastrointestinal diseases and invasive forms causing bloodstream infections (Feasy *et al*., 2012). Gastroenteritis in humans develops 6 to 12 hours after

ingestion of salmonella organisms, it is associated with nausea and cramping abdominal pain followed by inflammatory diarrhea, fever, and sometimes vomiting. The disease is mild and lasts for a few days although at times specific strains can cause bacteremia mostly in young children and patients that are immunocompromised (Glynn and Pander, 1992; Gal-mor *et al*., 2014). Besides reactive arthritis develops for some weeks to months after diarrhea stops. NTS bloodstream infections affect those with underlying hemolytic conditions such as individuals suffering from HIV, and children suffering from malaria infection as well as those with malnutrition (Faesy *et al*., 2015).

The proportion of food of animal origin foodborne burden for NTS in Africa was reported to be 84% in 2010. The burden of disease attributable to consumption of pork was reported highest in Africa where T. solium was the major causative agent followed by salmonella. Food animals are reported to be reservoirs of NTS, however, NTS is spread by other means such as waterborne, direct contact with animals, human to human contact (Li *et al*., 2019).

NTS strains such as *S.* Choleraesuis, *S.* Typhimurium, and *S.* Heidelberg, among others are capable of causing sustained and frequently lethal bacteremv0ic syndrome with prolonged fever, malaise, chills, headache but rarely diarrhea. Patients may have recurrent episodes of blood infection or other invasive infections for instance septic arthritis. The evolution of the genetic makeup of invasive NTS into a more typhoid-like bacterium makes it possible for it to efficiently spread around the human body (Gal-mor, 2018).

The economic development of a country has been linked to effective food of animal origin safety systems. Therefore, the resource-poor countries suffer the highest-burden of FBD due to inadequate systems to regulate the safety of foods. Additionally, there is a paucity of data on the

burden of foodborne illnesses. There is hence a need for a multisector approach to improving the safety of food, particularly of animal origin through One Health initiatives.

2.3 Pork value chain in Western Kenya

Pork consumption is projected to increase as demand for livestock products increases in Kenya tied to growth in human population, development, increased income among the middle class as well as changing food preferences (FAO, 2010). In Western Kenya, pig keeping serves as a crucial source of income (Mutua *et al.,* 2011), and pigs are kept/reared under traditional management (FAO,2010). During the purchase of pigs, it is the butchers or middlemen and at times traders who go looking for pigs from homes or pig farms (Kagira *et al.,* 2010; Mutua *et al.,*2011) and then transport them to slaughter by trekking or using Motorcycles and bicycles (Levy *et al.,* 2013). Pigs are slaughtered in private slaughter facilities, before being inspected by government veterinarians, and then sold in butcheries in local markets (Kagira *et al.,* 2010; FAO, 2012**).** However, some pigs are slaughtered on-farm, and consumption of uninspected pork has been reported (Githigia *et al*. 2005, Levy, 2014). Additionally, the hygiene status of slaughterhouses in Western Kenya does not follow what is required by the Meat Control Act of Kenya pigs (Levy, 2014; Cook *et al.,* 2017). Raw meat is transported with nonrefrigerated metallic containers to the butcheries and it is displayed in the pork butcheries at ambient temperature often with no protection from environmental contamination. Butcheries serve as the main source of retail pork where the majority of pork consumers buy pork either raw or cooked pork from butchers' shops (Levy, 2014). It is uncertain whether the raw pork sold to the public is contaminated with *Salmonella* pathogens, yet some study has shown that pork at a retail point is more likely to be contaminated than at slaughter (Heilmann *et al.,* 2016). Butcheries in the low-end market like Busia offer little to no product differentiation and their customers are low to medium-income earners who buy meat on

borne and meat is openly displayed without refrigeration. Customers also buy meat at the point-of-sale eatery inform of roasted, fried, or boiled. A plate of cooked pork is served optionally with a cornmeal starch made into a thick paste locally known as ugali (Levy, 2014) and for those who like with some side vegetables which are either raw or cooked. The number of staff employed at the pork butcheries are lower, mainly the butcher himself with the help of an extra person (Levy, 2014) hence you find one person doing the role of cutting raw pork, cooking and preparing the raw salads. Few workers at the pork butchery are one of the contributors to contamination according to Dang-Xuan *et al*., 2018. Besides, when the same hands, knives, and chopping boards are used, there is a high likelihood of cross-contamination. There are great chances of cross-contamination of foodstuffs in these butcheries due to unhygienic conditions.

2.4 Sources of Pork contamination with *Salmonella* spp.

Pigs serve as Salmonella spp. reservoirs (Kikuvi *et al*., 2010). *Salmonella* spp. have the potential to colonize the pig's gut, however, the majority of pigs do not show clinical signs of the disease. Nevertheless, some *salmonella* serotypes such as S. Choleraesuis are reported to cause disease whenever they infect pigs (Fedorka-Gray *et al.,* 2000). All the stages of the pork production chain have shown to be avenues of *Salmonella* **(**Baer *et al.,* 2013). When the environment is contaminated with *Salmonella* spp., swine end up inhaling or feeding on then especially it is in their feed and they end up being infected (Fedorka-Gray *et al.,* 2000) and carry it during the transfer from the farrowing farm to the finishing farm or the slaughterhouse (Kranker *et al.,* 2003). Whenever the pig is infected with *Salmonella* spp., there is a high likelihood to end up with an infected/ contaminated carcass according to Berends *et al.,*1997, compared to when the pig was not infected. During stressful events, pigs shed *Salmonella* which leads to pork contamination via feces during processing (Rostagno *et al.,* 2009; Arguello *et al.,* 2013). The biggest issue

with *Salmonella* spp. and food safety is cross-contamination. This means the bugs from one food are passively transferred to another food, where they grow. Slaughterhouse cross-contamination of pork with *Salmonella* spp. maybe a food safety risk and without proper hygienic control, the environment in the abattoir area can act an as important source of *Salmonella* contamination of raw pork (Carrasco *et al.,* 2009). During slaughter, carcasses are dressed by first scalding, followed by dehairing, singeing, and lastly polishing. The scalding process has been reported to reduce the number of *Salmonella* spp. (Tadee *et al*., 2014). However, during the scalding process, the water temperature may drop to below 62^{o}C and or with enough organic materials to protect the bacteria against heat this may result in to increase in the bacteria, hence the scalding process becomes a critical site of contamination (Letellier *et al*., 2009; Tadee *et al*., 2014). The dehairing process may also act as a recontamination site for scalded carcass (Borch *et al*.,1996; Bolton *et al*., 2002) facilitated by the rotating flails that may press the anus thus leading to feces coming out, thus potentially contaminating the equipment with feces that may contain *Salmonella* spp. The evisceration of the carcasses, bung dropping, and the removal of pluck set act as a source of contamination of the carcass with *Salmonella* spp. besides contamination of equipment used for splitting the carcass (Swart *et al*., 2016). Although equipment plays more role in carcass contamination due to the build-up of the bacteria in or on the equipment during working hours, workers handling the carcass as well as those equipment act as sources of contamination (Nyamakwere *et al*., 2016). Carcasses, either contaminated or clean end up at the retail level. Handling, temperature, and time are the three main factors that influence Salmonella spp. contamination of the carcasses at the point of retail (Carrasco *et al*., 2012). The retail display of the carcass acts as the weakest link (Wong *et al*., 2002) leading to the proliferation of *Salmonella* spp. to the hazardous number during periods of temperature abuse in the display

area. When the carcasses are being cut into smaller pieces, they are usually placed on different surfaces that might be harboring *Salmonella* spp. which results in contaminated carcasses (Borch *et al.,* 1996; Arguello *et al.,* 2013). Todd *et al*., 2010 established that contamination of the carcass can further be accelerated by the handling of contaminated equipment as well as utensils such as hooks, tables, and logs among others. Processing of the ready-to-eat products is the last step for carcass decontamination at the retail level, consequently, the amount of contaminated fresh products will at best remain the same (Wong *et al*., 2002).

Butchers shop that serves both raw pork and cooked pork have a high prevalence of Salmonella spp. due to hygiene levels or cross-contamination (Hansen *et al*., 2010). Salmonella spp. already endemic in butchers' shops can also act as a source of contamination and it is believed they originate from carcasses that were bought contaminated into the butchers' shop (Berend 1998). It is advisable to prepare different foodstuff in different working areas, for instance, preparing raw pork and raw vegetable in different working areas to reduce the risk of cross-contamination (Anderson *et al.*, 2000). The significant risk was reported highest when the same hands, same cutting board, and same knives were used for both raw pork and cooked pork during preparation (Dang-Xuan *et al*.,2018). Cooking pork for a temperature of 65^{o}C for 10 minutes has been reported to kill almost all the Salmonella, however, contamination and re-contamination with *Salmonella* spp. is likely to occur due to under-cooking of pork, contamination from raw materials, food handlers, or animals inside the facility, such as insects and using same utensils for raw and cooked pork (Mamber 2010; Dang-Xuan *et al*.,2018). Ready to eat vegetable salads prepared with unwashed or inadequately washed and dried chopping boards and or knives previously used for raw meat have been reported to be contaminated with *Salmonella* (Redmond *et al*., 2004). Outbreaks of infectious gastrointestinal disease associated with the consumption of

salad as well as vegetables have been reported, Sagoo *et al*., 2003. Vegetables grown in the natural environment are certainly contaminated by microbiological agents existing in the soil; water used for irrigation; wild animals; personnel; harvesting equipment and post-harvesting handling and distribution (Goodburn and Wallace, 2013). Besides, the use of contaminated irrigation water and manual from animals used as fertilizers leads to contamination of the vegetables. Abadias *et al*.,2006 stated that to have safe food it is necessary to do thorough disinfection and decontamination while producing foods.

2.5 Food safety interventions for pork contamination at the retail and household level

A lot of research has been done on the factors that affect pork contamination with *Salmonella* during pre-slaughter stages (Wong *et al*., 2002; Rostagno and Callaway, 2012; Arguello et al., 2013; Bonardi, 2017). However, post-slaughter processes, which affect the value of pork carcasses are neglected. Transportation of live hogs to the slaughterhouse and the delivery of carcasses to the retail are crucial steps in pork distribution since it determines the quality of pork before it reaches the shelves (Rani *et al*., 2017). Generally, meat is highly perishable due to its biological composition (Gul *et al*., 2016), hence spoilage bacterial growth might be accelerated when the meat is not properly handled during transportation. During Pork distribution the aim should be; retardation of spoilage bacteria; retain an attractive and fresh appearance of the product achieved through good manufacturing practices, good hygiene practices, temperature control (Nychas *et al*., 2008). Handling the carcass post-slaughter contributes significantly to the meat quality which in turn affects eating quality, acceptability of the meat by consumers as well as profits (Adzitey & Huda, 2012). According to (Adzitey & Huda, 2012), the carcass is likely to be contaminated at the slaughter, during processing, when being transported to the retail, as well as when being handled poorly by the consumers. Proper meat handling together with good hygiene practices is crucial for

the quality of the final product that ends up in the retail since the higher the initial spoilage potential bacteria, the faster the meat deterioration especially when the temperature is not controlled (Koutsoumanis *et al*., 2008). Meat display at the retail point is affected by the temperature that plays a significant role in the final quality of the product. A longer display period of pork at the retail especially when not refrigerated or frozen enables the proliferation of spoilage bacteria. It is therefore important to display meat unwrapped in chilled display cabinets. Microorganisms transferred from meat to consumers which are the 'fork' in the farm to fork continuum results in foodborne illness. Noteworthy, most consumers only consider the appearance and other perceived qualities when buying pork at the retail shop due to limited knowledge of food safety (Rani *et al*., 2017). Forks hence have a role to play in making sure they consume quality pork free from potential pathogens by properly cooking meat to destroy bacteria. Promoting better meat handling, particularly in the informal sector would result in safer meat for the consumers. Inspection of the carcass is important to enabling condemnation of unfit carcasses since consumers only consider meat attributes such as color, tenderness, flavor, and juiciness as their most important intrinsic cues to judge meat quality (Glitsch, 2000).

Transportation plays a crucial role in the delivery of meat after the slaughter. Before transportation, carcasses need to be chilled for hygiene purposes, nutrition qualities, and safety to reduce the rate of meat deterioration and prevent the proliferation of micro-organisms just before distribution (Rani *et al* 2017; Addis, 2015). However, in remote places, especially in small abattoirs or slaughter slabs where this study was done, refrigeration may not be available and carcasses are transported without the initial chilling and throughout the transportation. Maintaining proper refrigeration temperatures is a challenge, especially while keeping the cold chain from breaking during loading, unloading, and storing the carcass. It is therefore important to adhere to

temperature and handling conditions in all the stages of the cold chain to prevent the multiplication of bacteria. Food safety is the main concern for consumers worldwide. According to Aymerich *et al*., 2008, consumers demand high quality, fresh appearing, natural, long shelf life, and tasty meat. Once the swine has been slaughtered, techniques such as decontamination and antimicrobial additive of the carcass help to reduce human foodborne infections. According to Omer *et al*., 2015, spraying-washing the carcass using water, nonacid organic solutions help decontaminate the red meat carcass, besides, dehairing through chemicals, live animal washing before slaughter, and spot cleaning through hot water.

In a retail establishment, prevention of contamination and bacteria proliferation is attributable to maintaining good hygiene practices. Ready-to-eat vegetable contaminated vegetables may occur at any stage from the growing of the vegetables on the farm to the fork. To ensure safe ready-to-eat products, safe production methods, proper decontamination, and disinfection practices are very important, besides effective washing and decontamination, and proper storage at a controlled temperature. Washing of the vegetables will enable the removal of soil particles from the vegetables. Combining water with other ingredients such as organic acids make washing more effective and minimizes the amount of water that will be needed to gain the same level of microbial reduction (Mir *et al*., 2018).

Forks are considered the last line of defense against foodborne illness. It is of great significance to educate consumers about high-risk foods by first identifying their food handling practices that might place the public at risk for contact with foodborne pathogens enable. Proper food-handling skills by the consumers are necessary from the time of purchase of the food products to processing and making them available for themselves as well as for others to prevent the risk of pathogen proliferation, cross-contamination, and ensure thorough cooking procedures. Consumers should

consider cleaning and disinfection of working area after preparing raw meat, have separate chopping boards for raw meat and other foods, ensure doneness of meat by use of a thermometer, always ensure leftover foods are refrigerated within two hours after cooking and thorough and frequent hand washing using warm water and soap (Murray *et al*., 2017). According to Cogan *et al* 2015, thorough hand washing and rinsing under running water after handling Salmonella-containing chicken reduced the occurrence of Salmonella contamination from 40% to 16.7%. Sorry to say, most consumers are vaguely aware of hygiene practices when handling especially in developing countries. Therefore, it is of great significance to educate consumers on how pathogens cause foodborne diseases, and what is required of them to control foodborne illnesses (Anderson *et al*., 2004)

2.6 Antimicrobial resistance

2.6.1 Introduction to Antimicrobial resistance

Antimicrobial utilization in livestock production includes illnesses treatment in aid of promoting health and general welfare, disease control, and prevention in flocks (Sneeringer *et al.,* 2015). The excessive use of antimicrobials in growth promotion, feed proficiency enhancement, and prophylaxis is likely to accelerate the development of AMR in both pathogens and commensal organisms (Lekshmi *et al.,* 2017). The development of multidrug-resistant bacteria has been reported globally and this poses a risk to animals, humans, and (Agyare *et al.,* 2018).

Antimicrobial resistance is a vital cause of death around the globe, with the most burden borne by the low- and middle-income countries which experience weak infrastructure in terms of clean water and sanitation. Antimicrobial resistance (AMR) comes about when microorganisms that cause infections such as bacteria revolve with time and no longer respond to drugs hence making infections trickier to treat. One of the leading threats to public health in the 21st century is bacterial

antimicrobial resistance due to changes in the bacteria. According to O' Neill 2014, AMR is estimated to account for more than 700,000 mortalities yearly and is expected to reach 10 million by the year 2050 given that the current trends in antimicrobial use do not cease.

Consequently, the spread of AMR is an urgent issue that needs a coordinated action plan globally according to WHO. Misuse and overuse of antimicrobials is reported to be the main causes of the development of drug-resistant pathogens (Antonelli *et al.*, 2019) as it allows for the selection of resistant isolates (Davies and Davies, 2010). The widespread use of antibiotics has led to an increase in the selection of bacterial strains resistant to pressure from the various antimicrobial compounds (Meek *et al*., 2015). Antimicrobials are misused and /or used inappropriately when diseases and infections are poorly prevented and controlled, when there is weakness in legislation enforcement for antimicrobial use, use of counterfeits drugs as a result of poor access to quality medicines, and scarcity of clean water, sanitation, and hygiene (Om *et al*., 2017).

Bacterial AMR has resulted in the prompt spread of superbugs; multi and pan resistance bacteria that cause infections that are not treatable with available antimicrobials is a threat to public health worldwide (Engström., 2021 WHO issued a priority list for the development of new and more effective antibiotics (Tacconelli *et al*., 2018)with the most emphasis put on the pathogens with multidrug resistance. Nevertheless, the manufacturing of new antimicrobials is absent and hence there is a lack of access to quality antimicrobials which poses a major problem and this is due to the absence of a global assessment of the burden of bacterial AMR (Laxminarayan et al., 2016 Medical procedures such as cancer chemotherapy and surgeries are likely to be very risky. Besides, AMR has led to a longer stay in the hospitals and increased healthcare costs.

Antimicrobial resistance organisms exist in people, animals, and the environment. Humans can contract infections caused by antibiotic-resistant pathogens by preparing or consuming food

contaminated by those pathogens. On the other hand, humans also contract the resistant pathogens when they come in contact with animal waste via direct contact with animals themselves, and animal environments (Chang *et al*., 2015). Animals contain bacteria in their guts and the antibiotic-resistant bacteria present in animals' guts can get into food during slaughter and processing of food, therefore contaminating foods derived from animals. Animal wastes containing antibiotic-resistant bacteria get into the immediate environment, consequently contaminating the environment which pools all sorts of resistant genes. According to Van *et al* 2007, there is a rising trend in the bacteria originating from foods of animal origin or retail meat which is attributable to the improper use of antibiotics in farming practices. Antibiotics used during food animal production account for the antimicrobial consumption by mass (Schmidt *et al*., 2021), so it was established that there is a strong relationship between antibiotic consumption level and the proliferation of antibiotic resistance. Manyi-loh *et al*., 2018 reported that heightened consumption of antibiotics and resistance is a result of their extensive use in agriculture.

This increasing level of risk for the development of AMR presents a potential negative impact on livestock production due to potential failures in treatment outcomes resulting in higher cost of disease management on farms, increased economic losses (reduced farm productivity). This can act as a source for the accumulation of resistance genes that are transmitted to humans who are in contact with various products. The presence of antimicrobial residues in meat, eggs, and other animal products affects human health (Mikecz *et al.*,2020). Meat and meat products contaminated with antibiotic-resistant pathogens are reported to result in high mortality hence are of great concern globally (Uzeh *et al*.,2021). Transfer of resistant pathogens to humans via food of animal origin results in complications such as not being curable, prolonged infections, and sometimes death.

More than 60% of all antibacterial that is manufactured are used in animal production (Agyare *et al.,* 2018). Misuse of antimicrobials by the farmers especially without prescriptions, as well as engaging in non-prudent practices, for instance, violating antimicrobial withdrawal periods, suboptimal treatments and drug nonadherence is a common practice in low- and middle-income countries (LMICs) (Caudell *et al.,* 2017*).* This is compounded by the fact that the burden of infectious disease is highest (LMICs), due to the poor sanitation and hygiene combined with limited health and veterinary systems, which further increases misuse of antimicrobials and thereby AMR (Afakye *et al.,* 2020).

The occurrences of animal diseases cause major economic losses in the livestock industry worldwide. The economic losses of animals attributable to diseases can be categorized as either direct or indirect costs. Direct costs can be described as the total losses from the first confirmation of a disease outbreak until disease freedom is declared (Porphyre *et al.,* 2018), while indirect costs are those economic losses incurred due to affected commodities in markets after disease freedom is declared. Livestock illnesses contribute to losses via direct productivity losses on affected farms which include increased mortality, weight loss, growth retardation, reproductive losses, premature culling, reduced slaughter value, and indirect productivity losses due to prevention and control costs (vaccination, improved biosecurity, and management cost) and treatment costs, loss in trade, decreased market value, and food insecurity. According to (Gilbert and Rushton, 2018).

There is a need for data on both production losses and the costs of interventions to disease presence or risks which will allow economics to guide resource prioritization and allocation to improve the health and welfare of animals (Gilbert and Rushton, 2018).

2.6.2 Antimicrobial resistance of *Salmonella* species isolated from pigs

The UN's Food and Agriculture organization forecast strong growth in pig farming, and the increase is expected to reach 12.7% by the year 2050 (Magnusson *et al*., 2019). In the future, it is expected to have noticeable growth in antimicrobial application by 2030 (Tiseo *et al*., 2020). Pigs are mostly raised in intensive conditions that are conducive to the spread of infectious infection, hence antimicrobials are frequently used in pig farms which have resulted in antimicrobial resistance (Monger *et al*., 2021). A study conducted in Quebec province in Canada which produces pigs and pork in large quantities has shown that majority of salmonella spp. isolates are resistant to more than three antibiotics classes, however, there has been a marked decrease in resistance to trimethoprim/sulfamethoxazole, florfenicol, and tetracycline (Monger *et al*., 2021). Tetracycline is the most common antibiotic used worldwide for pigs and in the pig microbiome, the tetracycline-resistant genes are the most abundant (Lekagul *et al*., 2019). (Græsbøll *et al*., 2017) reported that using tetracycline in pigs prompted co-selection for resistance genes for aminoglycosides and tetracycline. Burrow *et al*., 2019 established that there is naturally an elevated resistance to tetracycline even when pigs are raised without the antibiotics and this is attributable to facilitated co-selection for tetracycline resistance when there is the use of another antibiotic, for instance, trimethoprim.

There has been a disturbing trend of antimicrobial-resistant phenotypes among *Salmonella* spp. such as *S. typhimurium, S. enteritidis, newport* around the globe recently which has raised serious concern (Hur *et al*., 2012). In the same light, increased resistance is reported in fluoroquinolones, quinolones, and extended-spectrum beta-lactamases (ESBL) such as ceftriaxone. The transmission of pig-related multidrug-resistant *Salmonella* serotypes carrying clinically-relevant antibiotic resistance genes, from pigs and pork meat to humans, has been reported and highlights the

contribution of different drivers to the antibiotic resistance burden (Campos *et al*., 2019). Antimicrobial-resistant *Salmonella* strains have been detected in many serotypes, such as *S.* Typhimurium, *S.* Saintpaul, *S.* Derby, *S.* Choleraesuis, *S.* Braenderup, *S.* Heidelberg, *S.* Had ar, *S.* Newport, *S.* Stanleyville, *S.* Fulica, and *S.* Uganda, among others from pigs (Hur *et al*., 2011). S. typhimurium has been studied widely and the most common MDR pattern has been a pattern of resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT) (Welch *et al*., 2007).

Salmonella spp. resistance to antimicrobials is attributable to various means such as enzyme production that enables inactivation of antimicrobial agents via degradation or structure modification, reduction of bacterial cell permeability to antibiotics, modification of cellular drug target, and activation of antimicrobial efflux pumps (Sefton, 2002). According to (Bush, 2003), *Salmonella* spp. have developed resistance to penicillin and cephalosporins and this can be attributable to the acquired ability of the strains to produce beta-lactamases that can degrade the chemical structure of antimicrobial agents. Resistance to extended-spectrum beta-lactams such as third and fourth-generation cephalosporins is of special concern due to their critical importance in humans and veterinary medicine (Rodríguez *et al*., 2009).

Salmonella serovars are significant reservoirs of antimicrobial resistance. There is a disturbing concern for the health of the public due to the emergency of *Salmonella* spp. that are resistant to antimicrobials commonly used in pig farming. According to (Campos *et al.,* 2019), there high risk of transmitting these *Salmonella* serotypes that are resistant to humans. Antibiotic-resistant bacteria in the pigs may contaminate carcass tissues and may ultimately contaminate consumable products found at retail (White *et al*., 2001). Developing countries especially sub-Saharan countries are still far behind high-resource settings in terms of curbing the spread of AMR

generally, and via the food chain specifically. Besides, the true burden of AMR in food animals is only partially documented and its threat via the food chain is under-estimated in low resource settings.

2.6.3 Drivers of AMR in Kenya

In Kenya, as it is in other developing countries, inappropriate application of antibiotics has resulted in antibiotic resistance. Some of the factors that have contributed to inappropriate use are; poverty (this limits their access to available antibiotics), unauthorized prescription of drugs, unregulated sale, the heavy burden of diseases, and dearth of hygienic and clean water availability (Byarugaba, 2004; Nepal *et al*., 2021)

In Kenya like in other developing countries, farming is expected to be intensified due to the increased demand for animal protein which results in excessive use of antibiotics which end up becoming residues in food of animal origin (Manyi-loh *et al*., 2018). However, livestock production is faced with the challenging lack of qualified specialists like vets due to their high charges. Besides, most diseases are not tested in the laboratory and this has led to the purchase of antimicrobial over the counter (Kagira *et al*., 2010). Additionally, over-the-counter antimicrobials are also added to animal feeds with no prescription. Recently, Muloi *et al*., 2019 found that there was an overlap between antibiotics classes sold for use both in veterinary and human medicine, and a majority of veterinary drugs are sold without prescription. A study by Mitema *et al*., 2001 established that sulfonamides, trimethoprim nitrofuran, and tetracyclines are the most common antibiotics used to treat animals in Kenya. Since then, these commonly used drugs have shown resistance in livestock (Kariuki *et al*., 2013).

There is paucity in developing countries as was highlighted by the World Organization for Animal Health stating that 94% of these countries do not have an official surveillance system. With the

little data available, it is evident that the majority of farmers prescribe drugs on their own, there is a lot of over-the-counter purchase of drugs as well as poor compliance to antimicrobial use such as withdrawal periods and use of counterfeit drugs (Queenan *et al*., 2016).

Inappropriate use of antimicrobial in the country which has led to antimicrobial resistance is attributable to the dearth of functioning antibiotic policies. Lack of antimicrobial stewardship that encompasses the choice, dosing, route, and duration of antibiotic administration led to incongruous use of antibiotics (Van Boeckel *et al*., 2015). The country also lacks stringent and comprehensive surveillance systems by the government to monitor the use and resistance of antimicrobials, to evaluate the knowledge of food animal handlers, those producing animal feeds, and the veterinary and paraprofessionals which would help circumvent antibiotic resistance. There is hence the need to strengthen policies that monitor antibiotic manufacture, distribution, dispensing, and prescription, hence fostering antibiotic stewardship worldwide.

2.6.4 Control and prevention of AMR in Kenya

The Government of Kenya has prioritized the prevention and containment of AMR, through a One Health platform by launching a National Action Plan (NAP). The government aims to implement the NAP by creating awareness through training and research, optimizing the use of antibiotics, and developing a sustainable investment in new medicines, diagnostic tools, and vaccines (Government of Kenya, 2017). Consequently, a coordinated network of involvement veterinarians, pharmaceutical industries, and regulatory authorities is critical to enforce prudent antibiotic use. Therefore, the coordination of various stakeholders set realistic and achievable targets to minimize the consumption of antibiotics (O' Neill, 2016) and this will enable intensified farming to produce more animal-sourced protein which is crucial for food safety or food security while reducing the amount of antimicrobial used in food animal.

between minimizing antibiotic use in food animals, while meeting the unprecedented rise of animal protein demands, which is a fundamental food security/food safety challenge that must be considered when defining target goals for reducing antibiotic use.

The majority of farmers need to be educated on antimicrobial use and the consequences of misuse to public health and the environment since lack of knowledge on antibiotics is a general weakness observed in farmers (Islam *et al*., 2016). A sensitization campaign should be conducted to enlighten people on the dangers of misusing antibiotics and subsequent antibiotic resistance, with its consequences on public health. Besides, to evade therapeutic antibiotic use in animals, proper hygiene on farms should be strongly recommended.

Motivating veterinary officials by paying them well would lead to better animal healthcare since most poorly paid veterinarians in Kenya as well as other developing countries seek surplus income from selling drugs, hence the more they dispense the drugs the more they supplementary money (Guetiya *et al*.,2016). Noteworthy, veterinarians supervise the prescription of antibiotics and their administration which has been established to influence the attitude of farmers to antibiotic use is. Thus, to optimize antibiotic use by farmers, veterinarians need to change the way they prescribe antibiotics.

Therefore, to control antimicrobial resistance, there is a need for more research focusing on novel antimicrobials. The public need also to be made aware of the antimicrobial and their resistance through campaigns and training. There is also a need for infection testing through the establishment of more antimicrobial laboratories which will enable the prescription of the right antimicrobial. Improvement of animal husbandry is the key to reducing the usage of antimicrobial in animals and educating farmers on the dangers of antimicrobial resistance to their animals, humans, and environment.

CHAPTER THREE: MATERIALS AND METHODS

3. 1 Study area

The field and initial lab-work for this study was conducted between April and October 2019 in Busia County. Busia County is situated in Western Kenya and it borders the counties of Kakamega to the east, Bungoma to the north, Siaya to the south, and border Uganda to the west (Wikipedia, 2019). It is made up of seven sub-counties, which are Budalangi, Funyula, Matayos, Teso-North, Teso-South, Nambale, and Butula. However, the data was sampled from four sub-counties; Funyula, Butula, Matayos, and Teso-south due to their higher numbers of pork butcheries and ease of accessibility from the County headquarter situated in Matayos

1

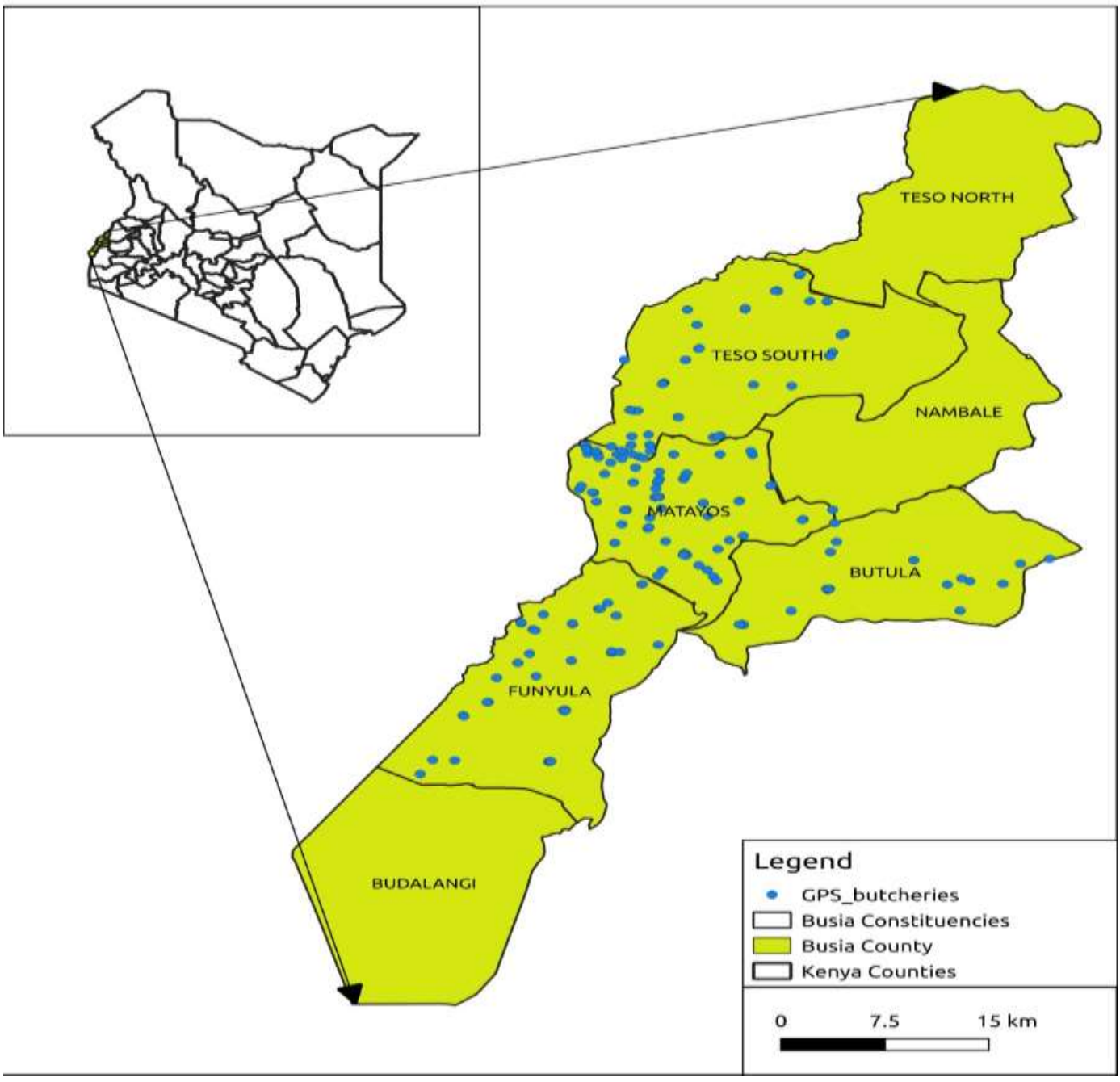


Figure 1. A map of Busia County with GPS (global positioning system) codes for the pork butcheries in the four sub Counties where the food samples were collected

[1] Mapped using QGIS (Quantum geographical information system) version 3.12

3.2 Ethical Consent

Ethical approval for this study was obtained from the International Livestock Research Institute (ILRI) Institutional Research Ethics Committee (IREC), ILRI-IREC2018-13. ILRI-IREC is registered and accredited by the National Commission for Science, Technology, and Innovation (NACOSTI) in Kenya.

3.3 Sample size

This was a cross-section kind of study aiming to determine the prevalence of contaminated pork with Salmonella spp. sold to consumers.

3.3.1 Estimated sample size of *Salmonella* in raw pork

Heilmann *et al.*, 2016 found 53.2% prevalence of *Salmonella* in pork butcheries. Sample size was determined using the Dohoo *et al.*, (2012) formula; $N= Z\ \alpha/2^2\ p\ (1-p)/\ d^2$ (Where N= sample size; Z α/2=critical value at 95% confidence interval and a probability of type 1 error 0.05(2 sided) =1.96; P = estimated prevalence; proportion= (1-prevalence); d= margin of error 0.05). Therefore; $N=1.96^2*0.532(1-0.532)/0.05^2$=383 pork butcheries.

The estimated sample size (383) was more than the number of pork butcheries in sub-counties of interest in Busia which was 170 in total (Butula 28, Teso-south 45, Funyula 37, and 60 inMatayos), therefore the sample size for pork butcheries was adjusted to 118 using the finite population correction factor as;

$N=n_0 * N / n_0 + (N-1)$, where n_0 is the sample size without considering the finite population correction factor (Levine *et al.*, 2017)

N= 383*170 / 383 + (170 – 1) =118

Therefore 118 pork butcheries were to be sampled from the four sub-counties and were proportionally allocated as (number of pork butcheries in each sub-county divide by the total number of the pork butcheries in the four sub-counties multiplied by adjusted pork butcheries sample size). Therefore 19 butcheries were sampled from Butula, 31 from Teso south, 26 from Funyula, and 42 from Matayos. For each butchery, 2 raw pork samples were to be collected hence the total number of raw pork to be collected was (118 * 2) = 236.

Figure 2. Example of pork butchery from one of the four sub Counties in Busia where samples were collected.

3.3.2 Estimated sample size of *Salmonella* in cooked pork samples

The estimated prevalence in cooked pork by Heilmann *et al.*, 2016 was 1%. A precision of 5% would give a very wide estimate of around 1% (0-6%). It was, therefore, decided a precision of 2% would be more appropriate to estimate the sample size which was calculated as below;

$N=1.96^2*0.01*(1-0.01)/0.02^2=95$ cooked pork samples. I decided

It was therefore decided to collect 1 cooked pork per retail unit.

3. 3. 3 Estimated sample size of *Salmonella* in raw vegetables samples

The estimated prevalence of raw vegetables was 5% by Heilmann *et al.,* 2016. Hence; $N=1.96^2*0.05*(1-0.05)/0.05^2=$ 73 raw vegetable samples. The study was designed in a way that for every cooked pork sampled from a retail shop, there will be raw vegetable sampled hence the number of raw vegetables was sampled was expected to be equal to that of cooked pork given that all cooked pork was accompanied by raw vegetable.

Figure 3. Raw pork and raw vegetables from one of the pork butcheries ready to be prepared as cooked pork and side salad for consumer

3.4 Sample collection from the Field

Retail pork and raw vegetable (n= 451) samples, consisting of 108 cooked pork, 262 raw pork, and 81 vegetables were collected from pork retailers in Western Kenya, Busia county. At the pork retailer one would start with introducing themselves, 250 grams of raw pork was then bought, cut into small pieces by the pork retailer, and packed normally as for a usual customer. Additionally, 100 grams of cooked pork and 100 grams of raw vegetables were bought from the same pork

retailers which were served and packed as for a usual customer. In this part of the country, the majority of pork retailers served as sources for both raw and cooked pork as well as side vegetables. Each foodstuff was put in a sterile whirl pack, a field barcode was given, and transported to the laboratory in cool boxes maintained at 4^{o} Celsius using ice packs

3.5 Samples reception and processing in the laboratory

Samples were transported to the Busia ILRI laboratory within the hours of collection. The samples for that day would be recorded manually in the laboratory book with these details; laboratory number, field barcode, sample type, and the sub-county they were from was recorded.

3.6 Bacterial isolation

Isolation and identification of *Salmonella* spp. was conducted following the guidelines given by the International Organization for Standardization. The process of isolation and identification was done at ILRI Busia laboratory. For each food sample type, 25 gm was weighed and put in sterile bag containing 225 ml of buffered peptone water pre- enrichment media then the mix was homogenized for 2 minutes. Following thorough mix of the food sample and nonselective buffered peptone water, the broth was incubated at 37°C overnight. After incubation, the broth was mixed with the first selective enrichment broth by transferring 1 ml of pre-enrichment broth to 10 ml of tetrathionate broth which was to enhance *Salmonella* growth while other microorganisms were inhibited to grow. This was done simultaneously with mixing 0.1 ml of pre-enrichment broth with 10 ml of Rappaport-Vassiliadis which was the second selective enrichment broth and this was followed by incubation for 18 to 24 hours at 37°C. Following growth on selective broth, each suspect colony was streaked onto selective xylose lysine Tergitol 4 (XLT-4) agar and incubated for 18 – 24 hours at 37°C. Following 18-24 hours incubation Presumptive *Salmonella* colonies (red with black centers) on an XLT4 plate were sub-cultured on an XLT-4 agar plate to purify then

incubated at 37°C for 18–24 hours. After incubation, (2-3) pure *Salmonella* colonies were streaked on Muller-Hinton a general-purpose nutrient agar which were then incubated again at 37 degrees Celsius for 18 to 24 hours. Following incubation at 18-24 hours, growth was transferred to Tryptone soy broth containing 15% glycerol and stored at -40 degrees Celsius freezer to be used later.

Figure 2 below shows the details

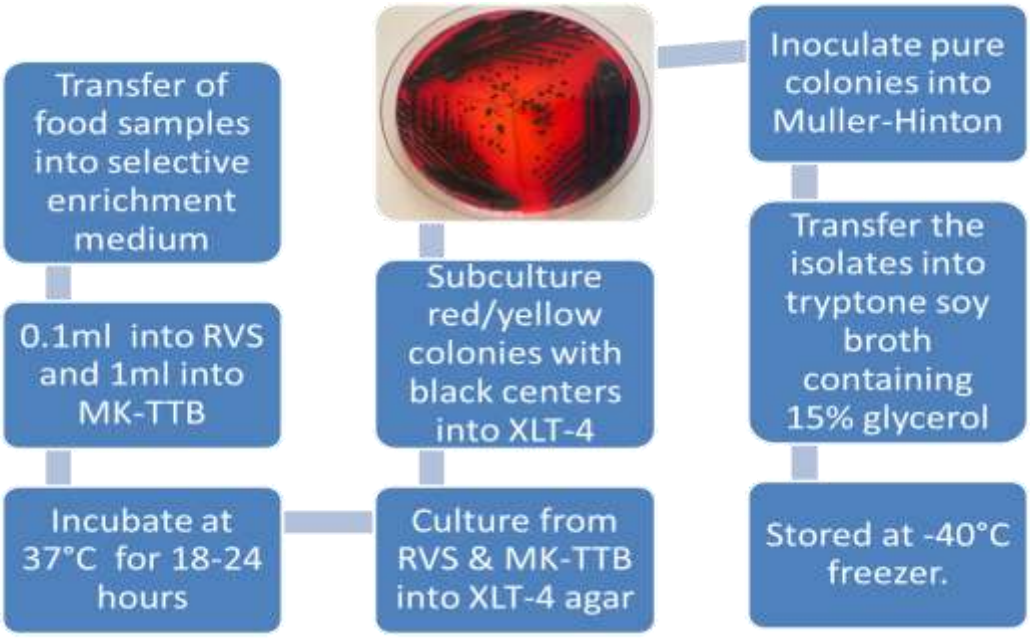


Figure 4: A flow chart diagram showing isolation of *Salmonella* spp. from the retail pork and raw vegetables sampled in Busia.

3.7 Characterization of *Salmonella* isolates

3.7.1 Polyvalent-O testing to confirm to confirm that an isolate is *Salmonella* species

The frozen *Salmonella* isolates were thawed for about 30 minutes. They were then revived on XLT-4 agar and incubated for 18-24 hours at 37^{o}C. After the incubation period, typical *Salmonella* colonies were cultured on Muller-Hinton agar then incubated again at 37^{o}C for

18-24 hours. The growth from Muller- Hinton agar was ready for slide agglutination test using polyvalent O antisera to confirm for *Salmonella*.

First testing for auto-agglutination was done as follows; on a glass slide, a drop of normal saline was carefully added followed by adding a growth from Muller-Hinton agar and then mixing them thoroughly. If there was no auto-agglutination, the colony proceeded to test using polyvalent O antisera. Testing with polyvalent O antisera proceeded as follows; placing a drop of saline solution on a glass slide and adding a loop full of the colony and mix well, then polyvalent O antisera (A-S) was added followed by mixing with a loop for one minute.

The slide was tilted for 5- 10 seconds. If an isolate was positive, agglutination was observed and from this point onwards those isolates that tested positive for polyvalent O antisera will be referred to as confirmed *Salmonella* species isolates. Figure 3 below shows the steps taken.

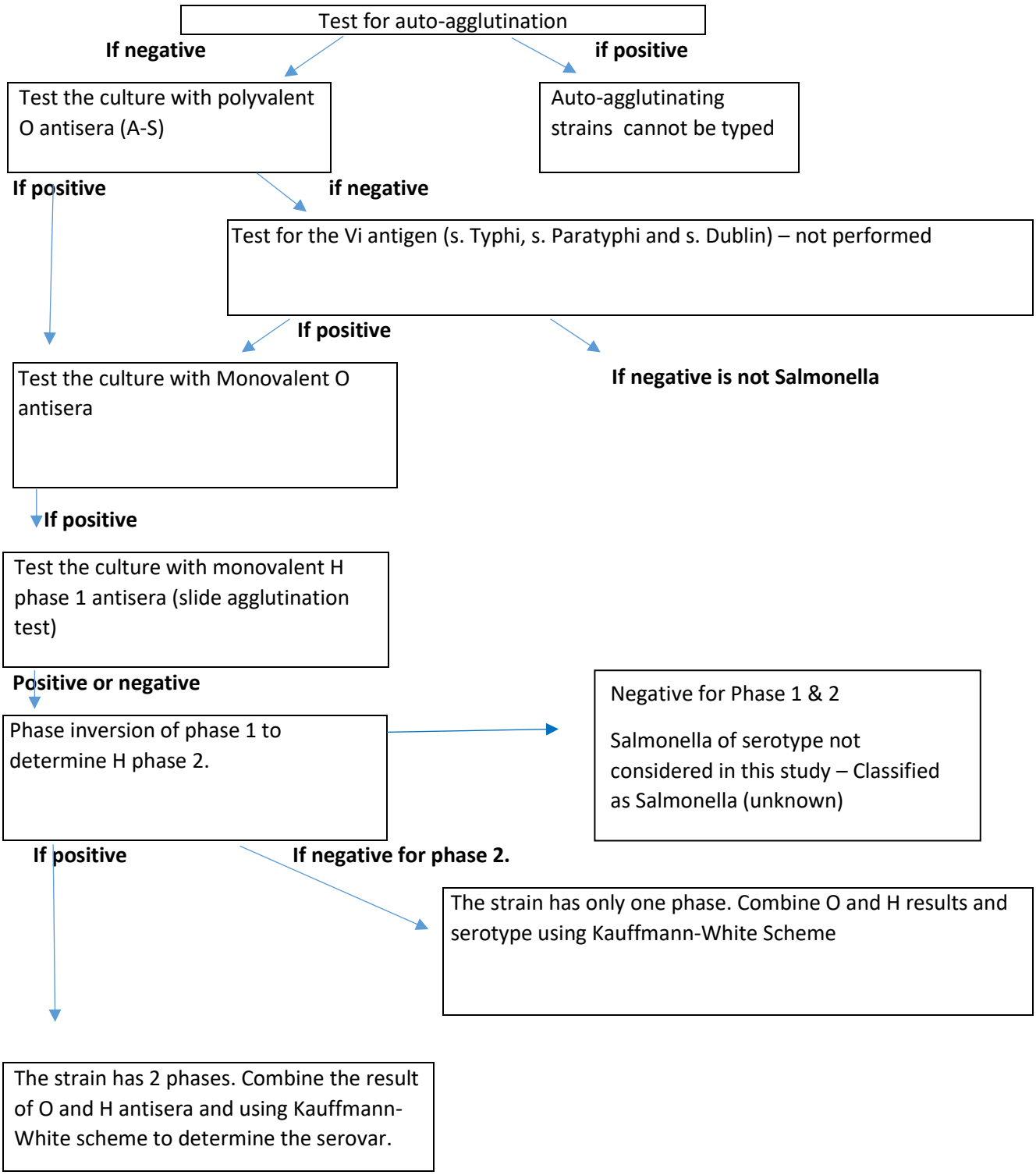


Figure 5. Salmonella identification using slide agglutination method for the three food stuffs sampled in Busia County.

3.7.2 Testing for somatic O groups using somatic O antisera

The confirmed *Salmonella* species isolates were then tested to confirm the somatic O groups they belonged to. The somatic O groups were chosen based on *Salmonella* serotypes commonly isolated from Busia County and its environs (Wilson, 2018) as outlined in table 1 below.

Table 1: The 8 somatic O groups with individual serovars that were tested for samples collected from four sub Counties in Busia.

Somatic O groups	Individual serovars of interest for this study			
Group 3	S. Uganda			
Group 4	S. Typhimurium	S. Heidelberg	S. Stanleyville	S. Fulica
Group 7	S. Orangeburg			
Group 8	S. Newport	S. Hadar	S. Bovismorbificans	
Group 9	S. Enteritidis			
Group 11	S. Aberdeen			
Group 28	S. Guildford			
Group 41	S. Offa			

The confirmed *Salmonella* species isolates were revived on Muller-Hinton agar followed by incubation at 37^{o}C for 18 to 24 hours. Each sample was tested against the 8 groups of somatic O antisera by placing a drop of normal saline on a glass slide followed by adding a loop full of growth from Muller- Hinton agar. The somatic O group antisera was added then thorough mixing. Results were observed with an unaided eye and the presence of agglutination represented a positive result for the group being tested.

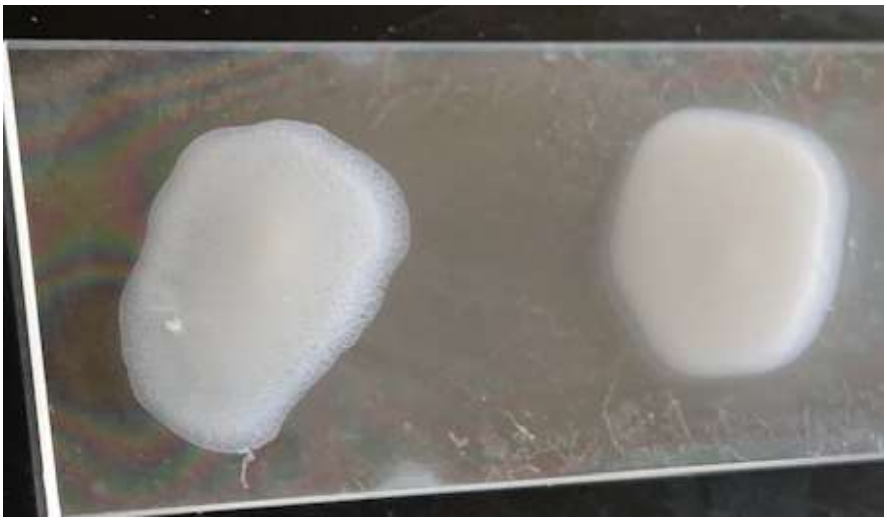


Figure 6. Example of slide agglutination result that was from one of the food stuff sample tested using somatic O antisera.

The results were recorded on the Open data kit. Samples that typed for more than one group of O antisera were purified by; culturing the isolates on XLT-4 agar followed by incubation at 37 degrees for 18- 24 hours then culturing them onto M-Ha. A single well-isolated colony was then retested with somatic O antisera and the data for the purified samples was recorded in an excel sheet. As it was not possible to test for every possible somatic O group due to a large number of O groups (67 groups), it was found that some isolates did not test positive for an O group, additionally, we found occasionally isolates which tested positive to more than one O group. It was therefore decided to utilize the analytical profile index (API) 20 E to characterize the isolates further.

3.8 Antimicrobial susceptibility test

Salmonella spp. isolates that were positive to poly O antisera were tested against antibiotics of interest using disc diffusion method as per guidelines proven by the Clinical and Laboratory Standards Institute (CLSI), 2018. First step was standardization of the inoculum using McFarland

standards ensuring a turbidity of 0.5 using a pure isolated colony to make bacterial suspension. Using a sterile cotton swab, a bacterial suspension was then transferred to a plate containing Muller-Hinton agar making sure the swab was rubbed in the entire agar surface in order to have a uniformly seeded bacterial suspension and this was followed by incubation for 24 hours. Following the drying of the inoculum, a disk containing an antibiotic compound was placed onto surface of inoculated agar with the aid of a dispenser. The antibiotic impregnated discs were arranged as follows in a set of two plates; plate one had Ampicillin (10µg), Cefuroxime (30µg), ceftriaxone (30 µg), and amoxicillin-clavulanic acid (20/10 µg ratio) was placed at the center. The second plate had Nalidixic acid (30µg), Ciprofloxacin (5µg), Sulfamethoxazole/Trimethoprim (1.25/ 23.75 µg), Gentamicin (10µg), Chloramphenicol (30µg) and Tetracycline (30µg) then the plates were incubated at 37^{o}C for 24 hours. Control strain was *E. coli* ATCC 25922 as it is susceptible to virtually all antimicrobials. Following the 24 hours, there was distinct zones of inhibition which appeared as clear zones around each antibiotic disc was measured.

3.9 Data handling and analysis

Data obtained was analyzed in R software version 3.0.2. Descriptive tables were created using proportions for the three food samples (cooked pork, raw pork, and raw vegetables). The prevalence of *Salmonella* species in retail pork and raw vegetables was calculated by dividing the total number of positives samples for polyvalent O antisera with the total number of samples collected at a 95% confidence interval and a p-value of 5%.

A homogeneity test was applied to assess whether the difference in the prevalence of the three food samples was significant using the formula; Chi-squared = sum of (observed – expected) 2 expected. The null hypothesis stated that there is no significant difference in the prevalence of *Salmonella* contamination of the three food types, over that would be observed by chance and

vice versa for the alternative hypothesis. Post hoc result was based on a method called “fdr” in R under the “rcompanion” package

CHAPTER FOUR: RESULTS

4.1. Contaminated samples differences across sub Counties

In Butula a total of 75 foodstuff samples (43 raw pork, 17 cooked pork, and 15 raw salads) were collected and 22/75 (19 raw pork, 1 cooked pork, and 2 raw vegetables) of these samples were contaminated with *Salmonella* spp. 130 foodstuff samples (71 raw pork, 35 cooked pork, and 24 raw vegetables) were collected from Teso- South and 45/130 of these samples (42 raw pork and 3 raw vegetables) were contaminated with *Salmonella* spp. In Funyula, a total of 73 foodstuff samples (49 raw pork, 15cooked pork, and 9 raw vegetables) samples were collected. 34/73 of these foodstuff samples (32 raw pork, 1 cooked pork, and 1 raw vegetable) were contaminated with *Salmonella* spp. In Matayos, 173 foodstuff samples (99 raw pork, 41 cooked pork, and 33 raw vegetables) samples were collected. 46/173 of these foodstuff samples (36 raw pork and 10 raw vegetables) were contaminated with *Salmonella* spp

4.2 Prevalence of *Salmonella* infection in retail pork and raw vegetables samples

A total number of 118 pork retailers were visited (Butula 19, Teso-south 31, Funyula 26 and Matayos 42), and 451 samples (262 raw pork, 108 cooked pork, and 81 raw vegetables served alongside cooked pork) were collected. 222 of these samples had at least one presumptive *Salmonella* species isolated and stored. 147 samples 32.59% (147/451 95% C.I 32.40% - 32.80%) had at least one presumptive *Salmonella* species confirmed by polyvalent O antisera and the data analysis going forward uses these confirmed *Salmonella* species isolates. Table 2 below shows the prevalence of *Salmonella* in the three food types.

Table 2: The prevalence of Salmonella species in three food samples collected from pork butcheries in Busia County

Sample types	Number sampled	Number positive	Prevalence of *Salmonella* spp.	95% CI	
				Lower class	Upper class
Raw pork	262	129	(49.24%)	48. 86%	49.61%
Cooked pork	108	2	(1.85%)	1. 61%	2. 10%
Raw vegetables	81	16	(19.75%)	18. 79%	20. 72%
Totals	451	147	(32.59%)	32.40%	32.80%

A chi-square test of homogeneity was done for 451 samples collected using RStudio. It was concluded that the difference in the prevalence of *Salmonella* contamination in raw pork, cooked pork and raw vegetables was significant. The post hoc test shows that all the comparison categories are significantly different. See table 2 below for details.

4.2.1 Homogeneity test

A chi-square test of homogeneity was done for 451 samples (262 raw pork, 108 cooked pork and 81 raw vegetables) collected in R. studio. 147/451 samples (129/262 raw pork, 2/108 cooked pork and 16/ 81 raw vegetables) were contaminated with *Salmonella* spp. Table 3 below illustrated summarized data of the three-food type's contamination with *Salmonella* in a two-way table

Table 3. Summarized data of the three food types in a two- way table representing the observed and the expected values used for the chi square statistics

Observed			
	Salmonella contamination		
	Yes	no	Totals
Raw pork	129	133	262
Cooked pork	2	106	108
Vegetables	16	65	81
Totals	147	304	451
Expected values			
	Salmonella contamination		
	Yes	no	Totals
Raw pork	85.39690	176.60310	262
Cooked pork	35.20177	72.79823	108
Vegetables	26.40133	54.59867	81
Totals	147	304	451

In conclusion, there is a significant difference in the prevalence of *Salmonella* contamination in r aw pork, cooked pork and raw vegetables. Table 4 below shows post hoc results.

Table 4. Post hoc test

Comparison	P value chi square	P value adjusted Chi square
1 raw pork: cooked pork	1.28E-17	3.84E-17
2 raw pork: vegetables	4.97E-06	7.46E-06
3 cooked pork: vegetables	9.68E-05	9.68E-05

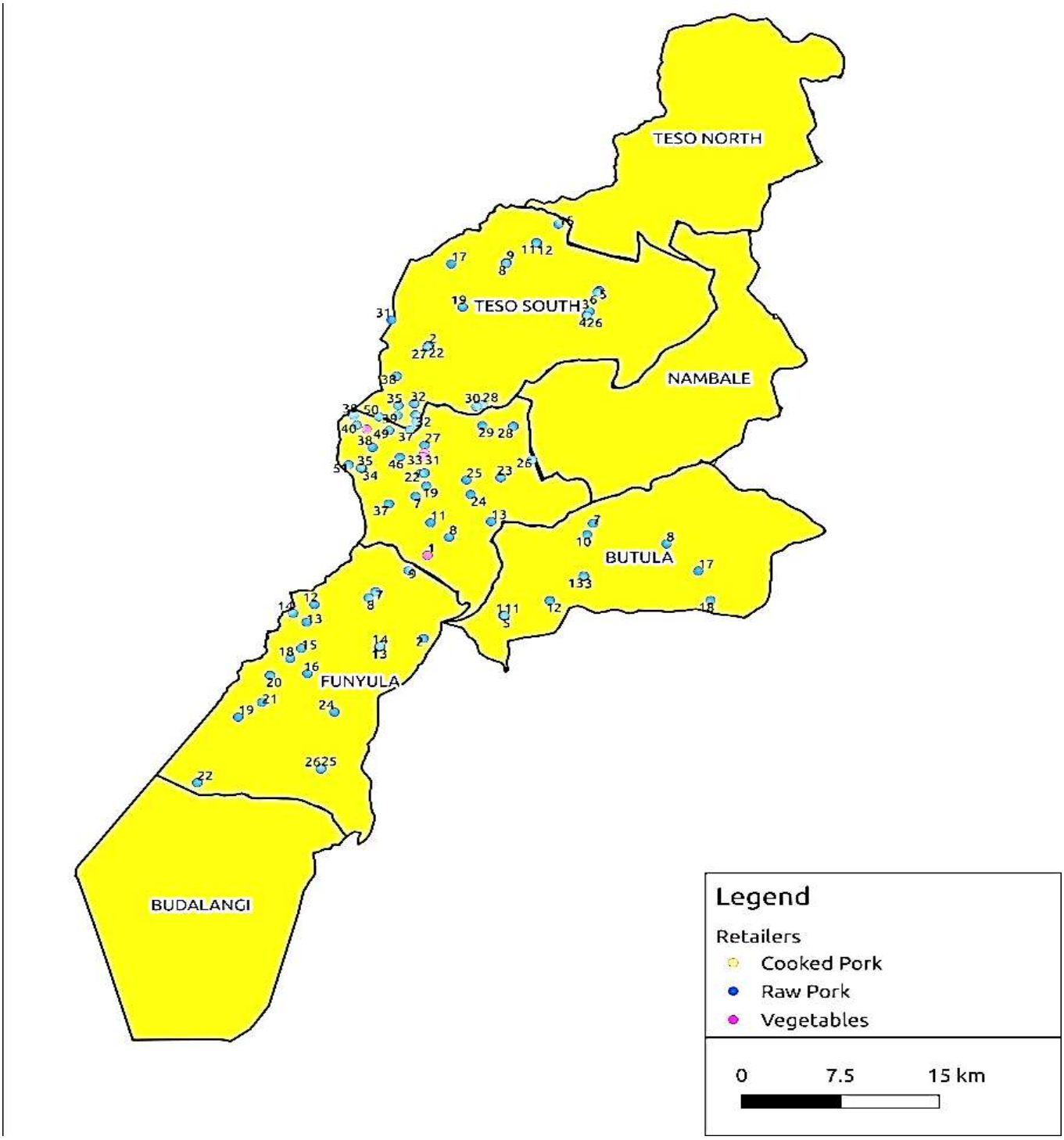


Figure 7 A map of busia with GPS codes for pork butcheries that sold salmonella contaminated food samples.

4.3 Characterization of *Salmonella* isolates from retail pork and raw vegetables

4.3.1 Somatic O groups identified

144 out of 147 (128 raw pork, 14 raw vegetables, and 2 cooked pork) samples that were confirmed to be *Salmonella* species were tested for somatic O groups using somatic O group antisera. 103 out of 144 samples (93 raw pork, 2 cooked pork and 8 raw vegetables) were positive for somatic O groups. 84 out of the total O group positive samples tested for just a single group of the eight O groups that were being tested while 19 samples tested for more than one group being tested even after purification of the isolates as described in the methods as illustrated below.

Table 5. Result for the somatic O groups

Serogroups	N	Raw pork (%)	Cooked Pork (%)	Raw vegetable (%)
Group 3	9	8 (88.89%)	1 (11.11%)	0
Group 4	17	14 (82.35%)	1 (5.88%)	2 (11.76%)
Group 7	18	16 (88.89%)	0	2 (11.11%)
Group 8	26	22 (84.62%)	0	4 (15.34%)
Group 9	9	8 (88.89%)	0	1 (11.11%)
Group 11	2	2 (100%)	0	0
Group 28	2	2 (100%)	0	0
Group 41	1	1 (100%)	0	0
Group (7 and 8)	15	13 (86.67%)	0	2 (13.33%)
Group (3,7 and 8)	1	1 (100%)	0	0
Group (3,8 and 9)	1	1 (100%)	0	0
Group (7 and 9)	1	1 (100%)	0	0
Total	102	89 (87.25%)	2 (1.96%)	11 (10.78%)

4.3.2 Antimicrobial susceptibility test (AST) for *Salmonella* isolates.

135/147 (91.84%) isolates (118 raw pork, 15 raw vegetables and 2 cooked pork) were resistant to one or more antibiotics tested. 67/147 (45.58%) isolates had multidrug resistance (resistant to three or more classes of antibiotics). while 99 (67.35%) of the isolates had extended drug resistant (XDR). Extended drug resistant in this case were the isolates that were resistant to one or two first line drugs (Ampicillin, Chloramphenicol, Trimethoprim/sulfamethoxazole, fluoroquinolones and third generation cephalosporins. The highest level of resistance was observed for Gentamicin (63.94%), Ampicillin (59.86%), Cefuroxime (34.69%) and Amoxicillin/Clavulanic acid (25.17%). Most isolates were susceptible to Sulfamethoxazole/ Trimethoprim (86.39%) followed by Chloramphenicol (80.95%) then Ceftriaxone (61.22%) and Tetracycline (52.38%). There were no isolates susceptible to Ciprofloxacin (0%) as shown in (Table 6 and 7).

Table 6. Prevalence of a drug and multidrug resistance in retail pork and raw vegetables

Food sample types	**Salmonella positive isolates**	**No. resistant to antibiotics tested**	**drug resistant prevalence**	**95% CI**		**No. resistant to 3 or more antimicrobial class**	**Multidrug resistant prevalence**	**95%CI**	
				Upper class	Lower class			Upper class	Lower class
Raw pork	130	119	91.54%	91.96%	91.12%	63	48.46%	49.22%	47.71 %
Cooked pork	2	2	1%	1%	1%	0	0%	0%	0%
Raw vegetable	15	14	93.33%	96.59%	90.07%	5	33.33%	39.49%	27.17 %
Totals	147	135	135/147 (91.84%)	92.20%	91.47%	68	68/147 (46.26%)	46.92%	45.59 %

Table 7: Antimicrobial antibiogram assay of Salmonela contaminted food samples from the pork butcheries in Busia County

Antimicrobials	Salmonella isolates (N = 147)		
	Resistance (N (%))	Susceptible (N (%))	Intermediate (N (%))
AMP	88 (59.86%)	33 (22.45%)	26 (17.69%)
CRO	7 (4.76%)	90 (61.22%)	50 (34.01%)
AMC	37 (25.17%)	73 (49.66%)	37 (25.17%)
CXM	51 (34.69%)	52 (35.37%)	44 (29.93%)
NA	30 (20.41%)	8 (5.44%)	109 (74.15%)
CIP	35 (23.81%)	0 (0%)	112 (76.19%)
SXT	14 (9.52%)	127 (86.39%)	6 ((4.08%)
CN	94 (63.95%)	14 (9.52%)	39 (26.53%)
C	5 (3.40%)	119 (80.95%)	23 (15.65%)
TE	14 (9.52%)	77 (52.38%)	56 (38.10%)

Key: AMC = Amoxicillin/Clavulanic acid, AMP = Ampicillin, TE = Tetracycline, CN = Gentamycin, SXT = Sulfamethoxazole-Trimethoprim, NA = Nalidixic Acid, CRO = Ceftriaxone, CXM=Cefuroxime, C=Chloramphenicol, CN=Gentamicin, MDR=Multidrug resistant, DR=Drug resistant

A number of antimicrobial resistant patterns for the three food samples are shown in the table 8 below. In this case MDR was considered to be resistant to three or more different classes of antibiotic. One raw pork isolates was resistant to seven antibiotics out the ten antibiotics tested which was the highest recorded.

Table 8. Drug resistant profile for the three food samples collected in Busia County

Resistance profile	No. of isolates with resistance profile (N (%)	Resistant category
AMP-CRO-AMC-CXM-NA-CIP-CN	1(0.68)	MDR
AMP-AMC-NA-CIP-CN-	1(0.68)	MDR
AMP-AMC-CIP-SXT-CN-TE	1(0.68)	MDR
AMP-AMC- TE CXM-NA-CIP-CN	5(3.40)	MDR
AMP-AMC-SXT-CN-TE	1(0.68)	MDR
AMP-CXM-NA-CN-TE	2(1.36)	MDR
AMP-AMC-CXM-SXT-TE	1(0.68)	MDR
AMP-AMC-CXM-NA-CN	1(0.68)	MDR
AMP-NA-CIP-SXT-CN	1(0.68)	MDR
AMP-AMC-NA-CIP-CN	2(1.36)	MDR
NA-SXT-CN-TE	1(0.68)	MDR
AMP-SXT-CN-TE	1(0.68)	MDR
AMP-CXM-CN-TE	1(0.68)	MDR
AMP-CRO-CIP-SXT	1(0.68)	MDR
AMP-AMC-NA-CN	2(1.36)	MDR
AMP-AMC-CXM-CN	2(1.36)	MDR
CXM-NA-CIP-CN	1(0.68)	MDR
AMP-CXM-CIP-CN	1(0.68)	MDR
AMP-AMC-CIP-CN	3(2.04)	MDR
AMP-AMC-NA-CIP	2(1.36)	MDR
AMP-AMC-SXT-C	1(0.68)	MDR
AMC-NA-CN-C	1(0.68)	MDR
AMP-NA-CN-C	1(0.68)	MDR
AMP-CRO-CN-AMC	1(0.68)	MDR
AMP-CN-C	1(0.68)	MDR
AMP-AMC-CIP	2(1.36)	DR
AMP-CRO-CIP	1(0.68)	MDR
AMP-AMC-CXM	2(1.36)	DR
AMP-AMC-CN	4(2.72)	DR
CXM-CIP-CN	4(2.72)	DR
NA-CIP-CN	2(1.36)	MDR
AMP-CIP-CN	1(0.68)	MDR
AMP-CRO-CN	1(0.68)	MDR
AMP-CXM-CN	8(5.44)	MDR
CXM-NA-CN	1(0.68)	MDR
AMP-NA-CN	1(0.68)	MDR
AMP-CRO-SXT	1(0.68)	MDR
CXM-NA-CIP	1(0.68)	MDR
AMP-AMC	2(1.36)	DR

AMP-C	1(0.68)	DR
AMP-CIP	5(3.40)	DR
AMP-CRO	1(0.68)	DR
AMP-CXM	1(0.68)	DR
CXM-CN	14(9.52)	DR
AMP-CN	9(6.12)	DR
NA-CN	1(0.68)	DR
SXT-CN	1(0.68)	DR
AMP-NA	1(0.68)	DR
CXM-TE	1(0.68)	DR
CXM	2(1.36)	DR
AMP	9(6.12)	DR
CN	14(9.52)	DR
NA	1(0.68)	DR
TE	2(1.36)	DR

4.4 Contaminated samples differences across Sub Counties

In Butula a total of 75 food stuff samples (43 raw pork, 17 cooked pork and 15 raw salads) were collected and 22/75 (19 raw pork, 1 cooked pork and 2 raw vegetables) of these samples were contaminated with *Salmonella* spp. Out of 19 retail pork butcheries sampled, 6 of them had each two or more samples collected that tested positive for *Salmonella.* In Teso-South, 130 food stuff samples (71 raw pork, 35 cooked pork and 24 raw vegetables) were collected and 45/130 of these samples (42 raw pork and 3 raw vegetables) were contaminated with *Salmonella* spp. 31 butcheries were sampled and 11 of them had each two or more food stuff samples contaminated.

In Funyula, 73 food stuff samples (49 raw pork, 15cooked pork and 9 raw vegetables) samples were collected. 34/73 of these food stuff samples (32 raw pork, 1 cooked pork and 1 raw vegetable) were contaminated with *Salmonella* spp. 26 pork retail shops were sampled and 12 of the had each two or more-food stuff contaminated. In Matayos, 173 food stuff samples (99 raw pork, 41 cooked pork and 33 raw vegetables) samples were collected. 46/173 of these food stuff samples (36 raw pork and 10 raw vegetable) were contaminated with *Salmonella* spp. Out of the 42pork retail shop

sampled, 13 of them had each two or more food stuff samples contaminated with *Salmonella* spp.

See details in (Table 9) below

Table 9. cross contamination across the 4 sub-Counties with antibiotics resistant result for every sample

Butula	**Food sample type**	**Antibiotic resistant profile**
BTL, R no. 1	Raw pork	AMP-AMC-NA-CN
	Raw pork	CXM-CN
	Raw pork	CXM-CN
BTL, R no.5	Raw pork	AMP-CIP
	Raw vegetable	CXM-CN
BTL, R no.9	Raw pork	AMP
	Raw pork	AMP-CN
BTL, R no.12	Raw pork	NA-CIP-CN
	Raw pork	CXM-CIP-CN
	Raw vegetables	CXM-CN
BTL, R no.13	Raw pork	AMP-AMC-CXM-NA-CIP-CN
	Raw pork	CXM-CIP-CN
Teso- South		
TS, R no.5	Raw pork	AMP-NA
	Raw pork	AMP-AMC-CIP-CN
	Raw vegetables	CN
TS, R no.9	Raw vegetables	AMP-CXM
	Raw pork	AMP-CN
TS, R no.11	Raw pork	CN
	Raw pork	AMP-AMC-CN
TS, R no.19	Raw pork	NA-CN
	Raw pork	CN
TS, R no.22	Raw pork	AMP-CN-C
	Raw pork	AMP-CIP
TS, R no.27	Raw pork	AMP-CRO-CN
	Raw pork	AMP-CRO
TS, R no.28	Raw pork	AMP-CRO
	Raw pork	AMP-CXM-CN
TS, R no.32	Raw pork	AMP-CRO-CIP
	Raw vegetable	AMP-AMC-NA-CIP
	Raw pork	AMP-AMC-CXM-NA-CIP-CN
	Raw pork	AMP-AMC-CIP
	Raw pork	AMP-NA-CN-C
TS, R no.34	Raw pork	AMP-AMC-CN
	Raw pork	AMP-AMC-CIP-CN
TS, R no.35	Raw pork	AMP

	Raw pork	AMP-CN
TS, R no.38	Raw pork	AMP-CIP-CN
	Raw pork	AMP-C
Funyula		
FYL, R no.1	Raw pork	CXM-NA-CIP
	Raw pork	AMP-CXM-NA-CN-TE
FYL, R no.2	Raw pork	AMP
	Raw pork	AMP-AMC-CXM-CN
FYL, R no.4	Raw pork	AMP-CXM-CN-TE
	Raw pork	AMP
FYL, R no.7	Raw pork	AMP-CRO-AMC-CXM-NA-CIP-CN
	Raw pork	AMP-AMC-CXM-NA-SXT-CN
	Raw pork	AMP-CN
FYL, R no.8	Raw pork	AMP
	Raw pork	AMP-AMC-NA-CIP-CN
FYL, R no.9	Raw pork	CXM-CN
	Raw pork	AMP-AMC-NA-CIP-CN
FYL, R no.13	Raw pork	CXM-NA-CN
	Raw pork	CN
FYL, R no.14	Raw pork	AMP-AMC-CIP-SXT-CN-TE
	Raw pork	AMC-NA-CN-C
FYL, R no.20	Raw pork	AMP-NA-CN
	Raw pork	SXT-CN
FYL, R no.22	Raw pork	AMP-SXT-CN-TE
	Raw pork	AMP-AMC-CXM-NA-CIP-CN
FYL, R no.24	Raw pork	AMP-AMC-CXM-NA-CN
	Raw pork	AMP-AMC-NA-CIP-CN-TE
FYL, R no.26	Raw pork	-
	Raw pork	NA-SXT-CN-TE
Matayos		
MTY, R no.17	Raw vegetable	CXM
	Raw pork	AMP-CXM-NA-CN-TE
	Raw vegetable	CN
	Raw pork	NA
MTY, R no.19	Raw pork	AMP-SXT
	Raw pork	AMP-SXT
MTY, R no.21	Raw pork	CN
	Raw pork	CXM-CN
MTY, R no.23	Raw vegetable	AMP
	Raw pork	Susceptible
MTY, R no.28	Raw pork	CN
	Raw pork	Susceptible
MTY, R no.31	Raw pork	AMP-CN
	Raw vegetable	CXM-CN

MTY, R no.34	Raw vegetable	AMP-CIP
	Raw pork	Susceptible
MTY, R no.35	Raw pork	CXM-TE
	Raw pork	CN
MTY, R no.37	Raw vegetable	AMP
	Raw pork	AMP-AMC-CXM-CN
MTY, R no.38	Raw vegetable	AMP-AMC-SXT-CN-TE
	Raw pork	AMP-AMC-CXM-SXT-TE
MTY, R no.40	Raw pork	CXM-CN
	Raw pork	AMP-AMC-CXM-NA-CIP-CN
MTY, R no.49	Raw pork	AMP-AMC-CXM
	Raw pork	Susceptible
MTY, R no.51	Raw pork	Susceptible
	Raw pork	AMP-CIP

Key: BTL (Butula), FYL (Funyula), MTY (Matayos), TS (Teso South), R (Retailer), no. (Number)

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1: Discussion

In the present study, the overall prevalence of *Salmonella* contamination in pork samples and raw vegetables served alongside cooked pork from four cities in Busia county was 32.59% (147/451 95% C.I 32.40% - 32.80%). Considering the hygiene status of butcheries in Busia operates, a prevalence of 32.59% is not surprisingly high. Poor sanitation, hygiene and inadequate quality water supply in retail shops contributes to *Salmonella* spp. contamination of pork (Kariuki *et al.*, 2013). There is a high likelihood of pork butcheries not meeting minimum sanitation and hygiene standards in Busia, however this cannot be confirmed from this study. Besides, a study conducted by Chepkemoi *et al*., 2015 reviewed those butcheries in Kenya do not meet minimum sanitation and hygienic standards. Though there are no hard data to substantiate this in the present study, it can be suspected that these pork butcheries where the present study was conducted may be poorly cleaned and disinfected, inadequate water supply in the pork butchery, lack of training to the meat handlers at the butcheries, and unhygienic handling of pork and other raw vegetables may have contributed to a great extent to the high prevalence of *Salmonella* at the retail level in this study. Unfortunately, there is no similar study to compare with in the country, however, in other part of the world, similar studies have shown comparable results. Heilmann (2016) reported a prevalence of 8.8% of 693 samples from Kampala pork joints in Uganda, Yang et al., 2019 reported a prevalence of 37.3% of 287 samples from retail pork in China.

This study presented a significant difference in the prevalence of *Salmonella* in the three food stuffs; raw pork at 49.24% (129/262 95% C.I 49.61% - 48.86%) followed by raw vegetables at 19.75% (16/81 95% C.I 20.72% - 18.79%) then cooked pork at 1.85% (2/108 95% C.I 2.10% - 1.61%) which correlates with that which was established by (Heilmann, 2016). In another study

conducted on pigs at slaughter by (Wilson *et al*., 2018) that was carried out in the same place as the present study indicated a higher prevalence 67% (39/61 C.I 51.9-76.0%) of *Salmonella* spp. recorded a higher prevalence of pork carcass at the slaughter compared to that at the butchery. The lower prevalence of *salmonella* spp. at the pork butcheries may indicate reduced spread of *salmonella* spp. On the other hand, the high prevalence of raw pork contamination suggests that there is a likelihood of slaughtered pigs being infected with *Salmonella* spp. from other carcasses. Infected carcasses act as avenues for infection of other slaughtered pigs if strict hygienic practices are not followed (Baer *et al*., 2013).

Prevalence of *Salmonella* spp. in cooked pork 1.85% (2/108 CI 1.61%- 2.10%) compared to that of raw pork (49.24% (129/262 95% C.I 49.61% - 48.86%) in this study was significantly low. This is a clear indication that cooking helps to reduce the amount *Salmonella* spp., however, to make sure most of *Salmonella* spp. are destroyed cooking should be done for at least 65^{o}C for ten minutes (Doyle and Mazzotta, 2000). Undercooked pork could be the reason for the 1.85% prevalence observed in this study, however, cross contamination and recontamination may have occurred while handling the cooked pork, for instance, using the same utensils for both raw and cooked pork and lack proper hand washing. Thoroughly cooked pork is safer for the consumers.

Prevalence of *Salmonella* spp. in raw vegetables of 19.75% (16/81 CI 18.79%-20.72%) could suggest that there is a likelihood of cross-contamination with juices from contaminated raw pork especially when the same hands, utensils, and surfaces were used to handle raw vegetables and raw pork. Raw vegetables intended to be consumed as salads can be of great risk to the consumers if they are contaminated with *Salmonella* spp. since they are not intended to be washed. Therefore, raw vegetables should be handled separately and should be cleaned thoroughly to avoid cross-contamination.

In the present study, the vast majority 92% (135/147) of the samples in this study were resistant to at least one of the ten antimicrobials tested with the highest resistance recorded in gentamicin (63.94%) followed by ampicillin (59.86%) and cefuroxime (34.69%). These drugs are commonly used for treatment or prophylaxis in Kenya and are readily available over the counter. These findings were consistent with what of Ndoboli *et al.,* 2018 of 98% antibiotic resistance. The closeness of the results of the present study and that of Ndoboli et al., 2018 could be due to similar sample types (samples from pork retail point). This suggests that retail outlets are import in the pork value chain since as evidenced by these studies, they serve as the source of AMR *Salmonella* isolates that ends up to the consumers. However, the results of this study were much higher than 10% reported by (Wilson, 2018) and 36% reported by Kikuvi *et al.,* 2010 from pigs at the slaughter. This suggests that the pork retail outlets are highly contaminated with *Salmonella* spp. with drug resistant genes than pigs at slaughter. It has been reported that *Salmonella* spp. differ from experiment to experiment or from place to place and within the same place with different experiment or samples, this justifies the reason for the above statement.

Most of the resistance was observed in raw pork at 87.41% (118/135), followed by raw vegetables with 11.11% (15/135), and lastly with cooked pork with a resistance of 1.48% (2/135). Without proper hygienic measures in the pork butcheries that serve raw pork and cooked pork as well raw salads can lead to the transfer of AMR *Salmonella* spp. to consumers which can be of great risk to the health of the public. In this study, the least resistance was observed in cooked pork which could suggest with proper cooking, most of the AMR *Salmonella* spp. were destroyed. Raw vegetables consumed as salads can transfer AMR Salmonella spp. to the consumers and hence those handling foods need to practice good hygienic measures when handling ready-to-eat salads.

The highest resistant were observed in Gentamicin (63.94%), Ampicillin (59.86%) and Cefuroxime (34.69%). These findings correlate with those of Ndoboli *et al.,* 2018 where they gentamicin, ampicillin and cefuroxime among the most resistant antibiotics. Interestingly, low resistant to tetracycline and trimethoprim/sulfamethoxazole was similar to Ndoboli *et al.,* 2018 conducted in Uganda. In contract, kikuvi *et al*., 2010 reported no resistant to gentamicin and highest resistant was observed in tetracycline and streptomycin and ampicillin which was similar to what was reported by (Wilson, 2018) both of which were conducted in Kenya. In Kenya almost all the drugs that are used for veterinary medicine are the same ones that are used in humans to a large extent signifying misuse of antimicrobials. Ampicillin, tetracyclines and streptomycin are readily available and sold over the counter to anyone buying not necessarily to a vet or animal health practitioner hence encouraging unscrupulous practices. This may explain the findings of this study although there is paucity of studies to show information on antimicrobial use by farmers. In the present study, a vegetable sample was resistant to seven different antimicrobials (AMP, CRO, AMC, CXM, NA, CIP and CN) which was the highest number from a single sample and a raw pork from the same butchery was resistant to six antimicrobials (AMP, AMC, CXM, NA, SXT, CN).

Recently, *Salmonella* serotypes especially S. typhimurium have been found to have XDR (Eng *et al*., 2015), in this study, resistant to the first line drugs (AMP, C, SXT, CIP, CRO was observed in 67.35 % samples. This means that bacterial infections will have to be treated with other antibiotics that are more potent such as fourth generation cephalosporins. In most cases, especially in Kenya, use of antimicrobials depends on the cost and availability of the drugs (Mangesho *et al*., 2021). Most people buy cheap and most available drugs for their own use and that of the animals hence resistant to these drugs is worrisome for the majority of the people that cannot afford expensive

drugs. One of the remedies to this will be to reduce antimicrobial use through adoption of preventative measures such as prophylactic use of antibiotics in animals and good hygienic practices. Some basic food safety measures, such as convenient hand-washing with water and soap especially before and after meal preparation, after usage of toilets, effective vegetable-washing, adequate cooking temperatures, and food storage are important to reduce the spread of antibiotic-resistant bacteria and the prevalence of antimicrobial resistant foodborne infections.

5.2 Conclusions

This study presents *Salmonella* prevalence, characterization, and antimicrobial-resistant from pork butcheries in Busia, Kenya. This study generated evidence for the first time on the high prevalence of *Salmonella* in pork butcheries and the raw pork recorded the highest prevalence of *Salmonella* spp. followed by raw vegetables and lastly the cooked pork. It is evidence in this study that retail pork and raw vegetables are able to carry a wide variety of non-typhoidal salmonella that are capable of causing diarrheal diseases in people. The study highlights that retail pork and raw vegetables served alongsided cooked pork have increased resistance to commonly used antibiotics such as ampicillin, hence the humans are at risk of consuming the resistant strains.

5.3 Recommendations

Educating and training food handlers and consumers on food safety is therefore essential to enhance their knowledge on foodborne infections and hazards. More research is required in future on specific MDR serotypes or clones that are related to pig/pork to help reduce their introduction in the food of animal origin which will further prevent them from being transmitted to humans.

REFERENCES

Abadias, M., Cañamas, T. P., Asensio, A., Angueram, M., & Viñas, I. (2006). Microbial quality of commercial 'Golden Delicious' apples throughout production and shelf-life in Lleida (Catalonia, Spain). International Journal of Food Microbiology, 108, 404e409.

Acar, J. F., and Moulin, G. (2006). Antimicrobial resistance at farm level. *Rev. Sci. Tech.* 25, 775–792. doi: 10.20506/rst.25.2.1695

Addis, M., 2015. Major Causes Of Meat Spoilage and Preservation Techniques: A. *changes*, *41*.

Adelaide, O.A., Bii, C. and Okemo, P., 2008. Antibiotic resistance and virulence factors in Escherichia coli from broiler chicken slaughtered at Tigoni processing plant in Limuru, Kenya. *East Afr Med J*, *85*(12), pp.597-606.

Adesiji, Y.O., Deekshit, V.K. and Karunasagar, I., 2014. Antimicrobial-resistant genes associated with *Salmonella* spp. isolated from human, poultry, and seafood sources. *Food science & nutrition*, *2*(4), pp.436-442.

Adzitey, F. and Huda, N., 2012. Effects of post-slaughter carcass handling on meat quality. *Pakistan Veterinary Journal*, *32*(2).

Afakye, K., Kiambi, S., Koka, E., Kabali, E., Dorado-Garcia, A., Amoah, A., Kimani, T., Adjei, B. and Caudell, M.A., 2020. The impacts of animal health service providers on antimicrobial use attitudes and practices: An examination of poultry layer farmers in Ghana and Kenya. *Antibiotics*, *9*(9), p.554.

Agustin, A.I., Carraminana, J.J., Rota, C. and Herrera, A., 2005. Antimicrobial resistance of *Salmonella* spp. from pigs at slaughter in Spain in 1993 and 2001. *Letters in applied microbiology*, *41*(1), pp.39-44.

Agyare, C., Boamah, V.E., Zumbi, C.N. and Osei, F.B., 2018. Antibiotic use in poultry production and its effects on bacterial resistance. *Antimicrobial resistance-A global threat*, pp.1-20.

Anderson, A.D. Nelson, J.M., Rossiter, S and Angulo, F.J. (2003). Public health consequences of use of antimicrobial agents in foods in the United States. *Microb Drug Resist* 9, 373-379.

Anderson, J.B., Shuster, T.A., Hansen, K.E., Levy, A.S. and Volk, A., 2004. A camera's view of consumer food-handling behaviors. *Journal of the american dietetic association*, *104*(2), pp.186-191.

Andreas JB, 2004. Foodborne *Salmonella* Infection. In: Beier RC, Pillai SD, Phillips TD, editors. Preharvest and postharvest Food Safety, Contemporary Issues and Future Directions. Malden, United States: Blackwell publishing; 2004. p. /3–12

Anon (2003). Commission regulation 2003/1831/ EC (September 22, 2003). *Off J Eur Commun* (no. L 268), 18.10.2003, 29-43

Antonelli, P., Belluco, S., Mancin, M., Losasso, C. and Ricci, A., 2019. Genes conferring resistance to critically important antimicrobials in *Salmonella* enterica isolated from animals and food: a systematic review of the literature, 2013–2017. *Research in veterinary science*, *126*, pp.59-67.

Apata, D. F. (2009). Antibiotic resistance in poultry. *Int. J. Poult. Sci.* 8, 404–408. doi: 10.3923/ijps.2009.404.408

Arguello, H., Alvarez-Ordonez, A., Carvajal, A., Rubio, P. and Prieto, M., 2013. Role of slaughtering in Salmonella spreading and control in pork production. *Journal of food protection*, *76*(5), pp.899-911.

Arya, G.; Holtslander, R.; Robertson, J.; Yoshida, C.; Harris, J.; Parmley, J.; Nichani, A.; Johnson, R.; Poppe, C, 2017. Epidemiology, Pathogenesis, Genoserotyping, Antimicrobial Resistance, and Prevention and Control of Non-Typhoidal *Salmonella* Serovars. Curr. Clin. Microbiol. Rep. 2017, 4, 43–53. [CrossRef]

Ashley, E. A., Lubell, Y., White, N. J., & Turner, P. (2011). Antimicrobial susceptibility of bacterial isolates from community acquired infections in Sub-Saharan Africa and Asian low and middle income countries. *Tropical Medicine & International Health*, *16*(9), 1167-1179.

Aymerich, T., Picouet, P.A. and Monfort, J.M., 2008. Decontamination technologies for meat products. *Meat science*, *78*(1-2), pp.114-129.

Baer, A.A., Miller, M.J. and Dilger, A.C., 2013. Pathogens of interest to the pork industry: a review of research on interventions to assure food safety. *Comprehensive Reviews in Food Science and Food Safety*, *12*(2), pp.183-217.

Barker, J.; Naeeni, M.; Bloomfield, S.F, 2003. The effects of cleaning and disinfection in reducing *Salmonella* contamination in a laboratory model kitchen. J. Appl. Microbiol. 2003, 95, 1351–1360. [CrossRef] [PubMed]

Berends, B. R., F. Van Knapen, D. A. A. Mossel, and J. M. Snijders, 1997: Identification and quantification of risk factors regarding *Salmonella* spp. on pork carcasses. Int. J. Food Microbiol. 36, 199–206.

Berends,B.R., Urlings,H.A.P., Snijders,J.M.A., Van Knapen,F. 1996. Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. International Journal of Food Microbiology, v.30, p.37-53.

Berends,B.R.; Van Knapen,F.; Snijders,J.M.; Mossel,D.A. 1997. Identification and quantification of risk factors regarding *Salmonella* spp. on pork carcasses. International Journal of Food Microbiology, v.20, n.36, p.199-206.

Berendsen, B., Stolker, L., de Jong, J., Nielen, M., Tserendorj, E., Sodnomdarjaa, R., Cannavan, A. and Elliott, C., 2010. Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. *Analytical and Bioanalytical Chemistry*, *397*(5), pp.1955-1963.

Bloomfield, S.F.; Aiello, A.E.; Cookson, B.; O'Boyle, C.; Larson, E.L, 2007. The effectiveness of hand hygiene procedures in reducing the risks of infections in home and community settings including handwashing and alcohol-based hand sanitizers. Am. J. Infect. Control 2007, 35, S27–S64. [CrossRef]

Bolton, D.J., Pearce, R.A., Sheridan, J.J., Blair, I.S., McDowell, D.A. and Harrington, D., 2002. Washing and chilling as critical control points in pork slaughter hazard analysis and critical control point (HACCP) systems. *Journal of Applied Microbiology*, *92*(5), pp.893-902.

Bonardi, S., 2017. Salmonella in the pork production chain and its impact on human health in the European Union. *Epidemiology & Infection*, *145*(8), pp.1513-1526.

Borch, E., Nesbakken, T. and Christensen, H. (1996) Hazard identification in swine slaughter with respect to foodborne bacteria. Int J Food Microbiol 30, 9–25

Boyen F, Haesebrouck F, Maes D, Van Immerseel F, Ducatelle R, Pasmans F. 2008. Non-typhoidal *Salmonella* infections in pigs: a closer look at epidemiology, pathogenesis and control. Vet. Microbiol. 130:1–19. 10.1016/j.vetmic.2007.12.017 [PubMed] [CrossRef] [Google Scholar]

Brenner, F.W., Villar, R.G., Angulo, F.J., Tauxe, R. and Swaminathan, B., 2000. Salmonella nomenclature. *Journal of clinical microbiology*, *38*(7), pp.2465-2467.

Breuil J, Brisabois A, Casin I, Armand-Lefèvre L, Frémy S, Collatz E, 2000. Antibiotic resistance in *Salmonella*e isolated from humans and animals in France: comparative data from 1994 and 1997. J Antimicrob Chemother. 2000;46:965–971. [PubMed] [Google Scholar]

Burow, E., Rostalski, A., Harlizius, J., Gangl, A., Simoneit, C., Grobbel, M., Kollas, C., Tenhagen, B.A. and Käsbohrer, A., 2019. Antibiotic resistance in Escherichia coli from pigs from birth to slaughter and its association with antibiotic treatment. *Preventive veterinary medicine*, *165*, pp.52-62.

Burton,M.;Cobb,E.;Donachie,P.;Judah,G.;Curtis,V.;Schmidt,W.P,2011.Theeffectofhandwashingwithwateror soaponbacterialcontaminationofhands.Int. J.Environ. Res. PublicHealth2011,8,97–104. [CrossRef][PubMed]

Bush, K., 2003. β-lactam antibiotics: Penicillin, and other β-lactam antibiotics. *Antibiotic and chemotherapy: anti-infective agents and their use in therapy. Philadelphia: Churchill Livingstone*, pp.p224-278.

Butaye, P., Cloeckaert, A. and Schwarz, S., 2003. Mobile genes coding for efflux-mediated antimicrobial resistance in Gram-positive and Gram-negative bacteria. *International journal of antimicrobial agents*, *22*(3), pp.205-210.

Byarugaba, D.K., 2004. Antimicrobial resistance in developing countries and responsible risk factors. *International journal of antimicrobial agents*, *24*(2), pp.105-110.

Campos, J., Mourão, J., Peixe, L. and Antunes, P., 2019. Non-typhoidal Salmonella in the pig production chain: a comprehensive analysis of its impact on human health. *Pathogens*, *8*(1), p.19.

Carrasco, E., Morales-Rueda, A. and García-Gimeno, R.M., 2012. Cross-contamination and recontamination by Salmonella in foods: a review. *Food Research International*, *45*(2), pp.545-556.

Caudell, M.A., Quinlan, M.B., Subbiah, M., Call, D.R., Roulette, C.J., Roulette, J.W., Roth, A., Matthews, L. and Quinlan, R.J., 2017. Antimicrobial use and veterinary care among agro-pastoralists in Northern Tanzania. *PloS one*, *12*(1), p.e0170328.

Chang, Q., Wang, W., Regev-Yochay, G., Lipsitch, M., and Hanage, W. P. (2015). Antibiotics in agriculture and the risk to human health: how worried should we be? *Evol. Appl.* 8, 240–245. doi: 10.1111/eva.12185

Chepkemoi, S., Lamuka, P.O., Abong, G.O. and Matofari, J., 2015. Sanitation and hygiene meat handling practices in small and medium enterprise butcheries in Kenya-case study of Nairobi and Isiolo Counties. [Google Scholar]

Coetzee, J., Corcoran, C., Prentice, E., Moodley, M., Mendelson, M., Poirel, L., *et al*. (2016). Emergence of plasmid-mediated colistin resistance (MCR-1) among *Escherichia coli* isolated from South African patients. *S. Afr. Med. J.* 106, 449–450. doi: 10.7196/SAMJ.2016.v106i5.10710

Cook, E. A. J., de Glanville, W. A., Thomas, L. F., Kariuki, S., Bronsvoort, B. M. de C., & Fèvre, E. M., 2017. Working conditions and public health risks in slaughterhouses in western Kenya. *BMC Public Health*, *17*(14), 1–12. https://doi.org/10.1186/s12889-016-3923-y

Cosgrove, S.E, 2006. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. Clin Infect Dis. 2006 Jan 15;42 Suppl 2: S82-9

Crump, J.A.; Sjölund-Karlsson, M.; Gordon, M.A.; Parry, C.M. 2015. Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive *Salmonella* Infections. Clin. Microbiol. Rev. 2015, 28, 901–937. [CrossRef] [PubMed]

Dang-Xuan, S., Nguyen-Viet, H., Pham-Duc, P., Grace, D., Unger, F., Nguyen-Hai, N., Nguyen-Tien, T. and Makita, K., 2018. Simulating cross-contamination of cooked pork with Salmonella enterica from raw pork through home kitchen preparation in Vietnam. *International journal of environmental research and public health*, *15*(10), p.2324. [Google scholar]

Davies, J. and Davies, D., 2010. Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews*, *74*(3), pp.417-433.

Davis, M. A., D. D. Hancock, and T. E. Besser. 2002. Multiresistant clones of *Salmonella* enterica : the importance of dissemination. J. Lab. Clin. Med. 140:135–141.

De Busser, E.V., Maes, D., Houf, K., Dewulf, J., Imberechts, H., Bertrand, S. and De Zutter, L. (2011). Detection and characterization of *Salmonella* in lairage, on pig carcasses and intestines in five slaughterhouses. Int J Food Microbiol 145, 279–286

Donabedian, S.M., Thal, L.A., Hershberger, E., Perri, M.B., Chow, J.W., Bartlett, P., Jones, R., Joyce, K., Rossiter, S., Gay, K. and Johnson, J., 2003. Molecular characterization of gentamicin-resistant enterococci in the United States: evidence of spread from animals to humans through food. *Journal of Clinical Microbiology*, *41*(3), pp.1109-1113.

Doulgeraki, A.I., Ercolini, D., Villani, F. and Nychas, G.J.E., 2012. Spoilage microbiota associated to the storage of raw meat in different conditions. *International journal of food microbiology*, *157*(2), pp.130-141.

Doyle, M.E. and Mazzotta, A.S., 2000. Review of studies on the thermal resistance of Salmonellae. *Journal of food protection*, *63*(6), pp.779-795.

E. Carrasco, A. Morales-Rueda, and R. M. 2012. Garc´ıa-Gimeno, "Cross-contamination and recontamination by *Salmonella* in foods: a review," Food Research International, vol. 45, no. 2, pp. 545–556.

E.J. Threlfall, B. Rowe, L.R. Ward. 1993. A comparison of multiple drug resistance in *Salmonella*s from humans and food animals in England and Wales, 1981 and 1990, Epidemiol. Infect. 111 (1993) 189–197

Eaves, Deborah J., Luke Randall, Douglas T. Gray, Antony Buckley, Martin J. Woodward, Allan P. White, and Laura JV Piddock, 2004. "Prevalence of mutations within the quinolone resistance-determining region of gyrA, gyrB, parC, and parE and association with antibiotic resistance in quinolone-resistant *Salmonella* enterica." *Antimicrobial agents and chemotherapy* 48, no. 10 (2004): 4012-4015.

Economou, V. and Gousia, P., 2015. Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and drug resistance*, *8*, p.49.

Eng, S.K., Pusparajah, P., Ab Mutalib, N.S., Ser, H.L., Chan, K.G. and Lee, L.H., 2015. Salmonella: a review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science*, *8*(3), pp.284-293.

Engström, A., 2021. Antimicrobial Resistance as a creeping crisis. In *Understanding the Creeping Crisis* (pp. 19-36). Palgrave Macmillan, Cham.

F. Kaferstein. 2003. "Foodborne diseases in developing countries: ¨ aetiology, epidemiology and strategies for prevention," International Journal of Environmental Health Research, vol. 13, no. 1, pp. S161–S168

F. M. Sanchez-Vargas, M. A. Abu-El-Haija, and O. G. G ´ omez- ´ Duarte, 2011. "*Salmonella* infections: an update on epidemiology, management, and prevention," Travel Medicine and Infectious Disease, vol. 9, no. 6, pp. 263–277, 2011.

FAO (2012). Pig Sector Kenya, FAO Animal Production and Health Livestock Country Reviews, No. 3. Rome, FAO.

Feasey, N.A., Dougan, G., Kingsley, R.A., Heyderman, R.S. and Gordon, M.A., 2012. Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *The Lancet*, *379*(9835), pp.2489-2499. [Google scholar]

Fedorka-Cray, P.J., Gray, J.T. and Wray, C., 2000. *Salmonella* infections in pigs. *Salmonella in domestic animals*, pp.191-207.

Foley, S.L. and Lynne, A.M., 2008. Food animal-associated Salmonella challenges: pathogenicity and antimicrobial resistance. *Journal of animal science*, *86*(suppl_14), pp.E173-E187.

Food and Drug Administration (2010) CVM Updates - CVM Reports on Antimicrobials Sold or Distributed for Food-Producing Animals (Food Drug Admin, Silver Spring. MD). Available at www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm236143.htm. Accessed March 10, 2015

Food and Drug Administration, 2010. National Antimicrobial Resistance Monitoring System-Enteric Bacteria (NARMS): 2007 Executive Report. *Rockville, MD, US: Department of Health and Human Services, US FDA.*

Founou, L.L., Founou, R.C. and Essack, S.Y., 2016. Antibiotic resistance in the food chain: a developing country-perspective. *Frontiers in microbiology*, *7*, p.1881.

G. Prats, B. Mirelis, T. Llovet, C. Munoz, E. Miro, F. Navarro, 2000. Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985–1987 and 1995–1998 in Barcelona, Antimicrob. Agents Chemother. 44 (2000) 1140–1145

Gal-Mor, O., 2019. Persistent infection and long-term carriage of typhoidal and nontyphoidal Salmonellae. *Clinical microbiology reviews*, *32*(1), pp.e00088-18.

Gal-Mor, O., Boyle, E.C. and Grassl, G.A., 2014. Same species, different diseases: how and why typhoidal and non-typhoidal Salmonella enterica serovars differ. *Frontiers in microbiology*, *5*, p.391.

Gilbert, W. and Rushton, J., 2018. Incentive perception in livestock disease control. *Journal of agricultural economics*, *69*(1), pp.243-261.

Gillings, M.R., 2013. Evolutionary consequences of antibiotic use for the resistome, mobilome and microbial pangenome. *Frontiers in microbiology*, *4*, p.4.

Githigia, S.M., Murekefu, A. and Otieno, R.O., 2005. Prevalence of porcine cysticercosis and risk factors for Taenia solium taeniosis in Funyula Division of Busia District, Kenya. *Kenya Veterinarian*, *29*, pp.37-39.

Glitsch, K., 2000. Consumer perceptions of fresh meat quality: cross-national comparison. *British Food Journal*.

Glynn, J.R. and Palmer, S.R., 1992. Incubation period, severity of disease, and infecting dose: evidence from a Salmonella outbreak. *American journal of epidemiology*, *136*(11), pp.1369-1377.

Goodburn, C. and Wallace, C.A., 2013. The microbiological efficacy of decontamination methodologies for fresh produce: a review. *Food Control*, *32*(2), pp.418-427.

Government of Kenya, June 2017. National Action Plan for the Prevention and Containment of Antimicrobial Resistance, Nairobi, Kenya.

Græsbøll, K., Damborg, P., Mellerup, A., Herrero-Fresno, A., Larsen, I., Holm, A., Nielsen, J.P., Christiansen, L.E., Angen, Ø., Ahmed, S. and Folkesson, A., 2017. Effect of tetracycline dose and treatment mode on selection of resistant coliform bacteria in nursery pigs. *Applied and environmental microbiology*, *83*(12), pp.e00538-17.

Grimont, P.A. and Weill, F.X., 2007. Antigenic formulae of the Salmonella serovars. *WHO collaborating centre for reference and research on Salmonella*, *9*, pp.1-166.

Guetiya Wadoum, R.E., Zambou, N.F., Anyangwe,F.F., Njimou, J.R., Coman, M.M., Verdenelli, M.C., Cecchini, C., Silvi, S., Orpianesi, C., Cresci, A. and Colizzi, V., 2016. Abusive use of antibiotics in poultry farming in Cameroon and the public health implications. *British poultry science*, *57*(4), pp.483-493.

Gul, K., Singh, P. and Wani, A.A., 2016. Safety of Meat and Poultry. In *Regulating Safety of Traditional and Ethnic Foods* (pp. 63-77). Academic Press.

Hammerum, A.M., Heuer, O.E., 2009. Human health hazards from antimicrobial-resistant Escherichia coli of animal origin. Clin. Infect. Dis. 48, 916–921. https://doi.org/10. 1086/597292.

Hansen TB,Christensen BB, Aabo S. 2010. *Salmonella* in pork cuttings in supermarkets and butcher shops in Denmark in 2002 and 2006. Zoonoses Public Health 57:23–9.

Havelaar, A.H., Kirk, M.D., Torgerson, P.R., Gibb, H.J., Hald, T., Lake, R.J., Praet, N., Bellinger, D.C., De Silva, N.R., Gargouri, N. and Speybroeck, N., 2015. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS medicine*, *12*(12), p.e1001923.

Heider, L. C., J. A. Funk, A. E. Hoet, R. W. Meiring, W. A. Gebreyes, and T. E. Wittum. 2009. Identification of Escherichia coli and *Salmonella* enterica organisms with reduced susceptibility to ceftriaxone from fecal samples of cows in dairy herds. Am. J. Vet. Res. 70:389–393.

Heilmann, M., 2016. Flies as vectors for Salmonella spp. and their control in pork butcheries in Kampala, Uganda–A contribution to improve public health (Doctoral dissertation). [Google scholar]

Hendriksen, R.S., Vieira, A.R., Karlsmose, S., Lo Fo Wong, D.M., Jensen, A.B., Wegener, H.C. and Aarestrup, F.M., 2011. Global monitoring of Salmonella serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. *Foodborne pathogens and disease*, *8*(8), pp.887-900.

Høg, B.B., Bager, F., Korsgaard, H.B., Ellis-Iversen, J., Pedersen, K., Jensen Lars Bogøand Hendriksen, R.S., Bortolaia, V., Larsen, A.R., Petersen, A., *et al.*, 2018. DANMAP 2017-Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria From Food Animals, Food and Humans in Denmark

Holmberg, S.D., M.T. Osterholm, K.A. Senger, and M.L. Cohen. 1984. Drug-resistant *Salmonella* from animals fed antimicrobials. N. Engl. J. Med. 311:617–622

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karley, A., *et al*. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* 387, 176–187. doi: 10.1016/S0140-6736(15)00473-0

Hoque, B.A.; Mahalanabis, D.; Pelto, B.; Alam, M.J. 1995. Research methodology for developing efficient handwashing options: An example from Bangladesh. J. Trop. Med. Hyg. 1995, 98, 469–475. [PubMed]

Hur, J., Jawale, C. and Lee, J.H., 2011. Antimicrobial resistance of *Salmonella* isolated from food animals: A review. *Food Research International*, *45*(2), pp.819-830.

Islam, K.S., Shiraj-Um-Mahmuda, S. and Hazzaz-Bin-Kabir, M., 2016. Antibiotic usage patterns in selected broiler farms of Bangladesh and their public health implications. *Journal of Public Health in Developing Countries*, *2*(3), pp.276-284.

Issenhuth-Jeanjean S, Roggentin P, Mikoleit M, Guibourdenche M, de Pinna E, Nair S, *et al*., 2014 . Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme. Res Microbiol. (2014) 165:526–30. 10.1016/j.resmic. [PubMed] [CrossRef] [Google Scholar]

Jajere, S.M., 2019. A review of Salmonella enterica with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Veterinary world*, *12*(4), p.504.

Jimenéz, S.M., Tiburzi, M.C., Salsi, M.S., Moguilevsky, M.A., Pirovani, M.E., 2009. Survival of *Salmonella* on refrigerated chicken carcasses and subsequent transfer to cutting board. Letters in Applied Microbiology 48, 687-691

Kagira, J.M., Kanyari, P.W., Maingi, N., Githigia, S.M., Ng'Ang'A, J.C. and Karuga, J.W., 2010. Characteristics of the smallholder free-range pig production system in western Kenya. *Tropical animal health and production*, *42*(5), pp.865-873.

Kariuki *et al*., 2013 . FAO/WHO Project Report: Improving Food Safety in Meat Value Chains in Kenya. Food Protection Trends 2013; 33:172-175

Kariuki, S., Revathi, G., Kariuki, N., Kiiru, J., Mwituria, J., Muyodi, J., Githinji, J.W., Kagendo, D., Munyalo, A. and Hart, C.A., 2006. Invasive multidrug-resistant non-typhoidal Salmonella infections in Africa: zoonotic or anthroponotic transmission?. *Journal of medical microbiology*, *55*(5), pp.585-591.

Keeble, E. and Koterwas, B., 2020. Salmonellosis in hedgehogs. *Veterinary Clinics: Exotic Animal Practice*, *23*(2), pp.459-470.

Kikuvi, G.M., Ombui, J.N. and Mitema, E.S., 2010. Serotypes and antimicrobial resistance profiles of Salmonella isolates from pigs at slaughter in Kenya.

Kisiela, D.I., Chattopadhyay, S., Libby, S.J., Karlinsey, J.E., Fang, F.C., Tchesnokova, V., Kramer, J.J., Beskhlebnaya, V., Samadpour, M., Grzymajlo, K. and Ugorski, M., 2012. Evolution of Salmonella enterica virulence via point mutations in the fimbrial adhesin. *PLoS pathogens*, *8*(6), p.e1002733.

Koutsoumanis, K.P., Stamatiou, A.P., Drosinos, E.H. and Nychas, G.J., 2008. Control of spoilage microorganisms in minced pork by a self-developed modified atmosphere induced by the respiratory activity of meat microflora. *Food microbiology*, *25*(7), pp.915-921.

Kraemer, S.A., Ramachandran, A. and Perron, G.G., 2019. Antibiotic pollution in the environment: from microbial ecology to public policy. *Microorganisms*, *7*(6), p.180.

Kranker, S., Alban, L., Boes, J. and Dahl, J., 2003. Longitudinal study of Salmonella enterica serotype Typhimurium infection in three Danish farrow-to-finish swine herds. *Journal of Clinical microbiology*, *41*(6), pp.2282-2288.

L.A. Lee, N.D. Puhr, E.K. Maloney, N.H. Bean, R.V. 1994. Tauxe, Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989–1990, J. Infect. Dis. 170 (1994) 128–134.

Lamas, A., Miranda, J.M., Regal, P., Vázquez, B., Franco, C.M. and Cepeda, A., 2018. A comprehensive review of non-enterica subspecies of Salmonella enterica. *Microbiological research*, *206*, pp.60-73.

Langridge, G.C., Fookes, M., Connor, T.R., Feltwell, T., Feasey, N., Parsons, B.N., Seth-Smith, H.M., Barquist, L., Stedman, A., Humphrey, T. and Wigley, P., 2015. Patterns of genome evolution that have accompanied host adaptation in Salmonella. *Proceedings of the National Academy of Sciences*, *112*(3), pp.863-868. Langridge, G.C., Fookes, M., Connor, T.R., **Feltwell, T., Feasey, N., Parsons, B.N., Seth-Smith, H.M., Barquist, L., Stedman, A., Humphrey, T. and Wigley, P., 2015**. Patterns of genome evolution that have accompanied host adaptation in Salmonella. *Proceedings of the National Academy of Sciences*, *112*(3), pp.863-868.

Langridge, G.C., Nair, S. & Wain, J. Nontyphoidal Salmonella serovars cause different degrees of invasive disease globally. J. Infect. Dis. 199, 602–603 (2009).

Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Røttingen, J.A., Klugman, K. and Davies, S., 2016. Access to effective antimicrobials: a worldwide challenge. *The Lancet*, *387*(10014), pp.168-175.

Lekagul, A., Tangcharoensathien, V. and Yeung, S., 2019. Patterns of antibiotic use in global pig production: a systematic review. *Veterinary and animal science*, *7*, p.100058.

Lekshmi, M., Ammini, P., Kumar, S. and Varela, M.F., 2017. The food production environment and the development of antimicrobial resistance in human pathogens of animal origin. *Microorganisms*, *5*(1), p.11.

Letellier, A., Beauchamp, G., Guévremont, E., D'ALLAIRE, S.Y.L.V.I.E., Hurnik, D. and Quessy, S., 2009. Risk factors at slaughter associated with presence of Salmonella on hog carcasses in Canada. *Journal of food protection*, *72*(11), pp.2326-2331.

Levy, M., 2014. Challenges and opportunities of small-holder pig production and marketing in Western Kenya (Doctoral dissertation).

Li, M., Havelaar, A.H., Hoffmann, S., Hald, T., Kirk, M.D., Torgerson, P.R. and Devleesschauwer, B., 2019. Global disease burden of pathogens in animal source foods, 2010. *PloS one*, *14*(6), p.e0216545.

Liu, Y.-Y., Wang, Y., Walsh, T. R., Yi, L.-X., Zhang, R., Spencer, J., *et al*. (2016). Emergence of plasmid-mediated colistin resistance mechanism mcr-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* 16, 161–168. doi: 10.1016/S1473-3099(15)00424-7

Losasso, C., DiCesare, A., Mastrorilli, E., Patuzzi, I., Cibin, V., Eckert, E.M., Fontaneto, D., Vanzo, A., Ricci, A., Corno, G., 2018. Assessing antimicrobial resistance gene load in

vegan, vegetarian and omnivore human gut microbiota. Int. J. Antimicrob. Agents 52, 702–705.

Luvsansharav, U.O., Wakhungu, J., Grass, J., Oneko, M., Nguyen, V., Bigogo, G., Ogola, E., Audi, A., Onyango, D., Hamel, M.J. and Montgomery, J.M., 2020. Exploration of risk factors for ceftriaxone resistance in invasive non-typhoidal Salmonella infections in western Kenya. *Plos one*, *15*(3), p.e0229581.

Magnusson, U., Sternberg Lewerin, S., Eklund, G. and Rozstalnyy, A., 2019. *Prudent and efficient use of antimicrobials in pigs and poultry*. FAO.

Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A., Hoekstra, R.M. and International Collaboration on Enteric Disease "Burden of Illness" Studies, 2010. The global burden of nontyphoidal Salmonella gastroenteritis. *Clinical infectious diseases*, *50*(6), pp.882-889.

Mamber SW. 2010. Analysis of ALLRTE and RTE001 sampling results for *Salmonella* species, calendar years 2005 through 2008. Available from: http://www.fsis.usda.gov/PDF/Analysis_ALLRTE_RTE001_Sampling_*Salmonella* _2005–2008.pdf FSIS-USDA. Accessed October 6, 2011.

Manyi-Loh, C., Mamphweli, S., Meyer, E. and Okoh, A., 2018. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules*, *23*(4), p.795.

Maragakis, L.L., Perencevich, E.N., Cosgrove, S.E, 2008. Clinical and economic burden of antimicrobial resistance. Expert Rev Anti Infect Ther. 2008 Oct;6(5):751-63. doi: 10.1586/14787210.6.5.751.

Marshall, B. M., and Levy, S. B. (2011). Food animals and antimicrobials: impacts on human health. *Clin. Microbiol. Rev.* 24, 718–733. doi: 10.1128/CMR.00002-11

Maynard, C., J. M. Fairbrother, S. Bekal, F. Sanschagrin, R. C. Levesque, R. Brousseau, *et al*. 2003. Antimicrobial resistance genes in enterotoxigenic Escherichia coli O149:K91 isolates obtained over a 23-year period from pigs. Antimicrob. Agents Chemother. 47:3214–3221.

McEwen, S. A. & Fedorka-Cray, P. J. Antimicrobial use and resistance in animals. *Clin. Infect. Dis.* **34**, S93–S106 (2002).

McGlone, J.J., 2013. The future of pork production in the world: towards sustainable, welfare-positive systems. *Animals*, *3*(2), pp.401-415.

Meek, R.W., Vyas, H. and Piddock, L.J.V., 2015. Nonmedical uses of antibiotics: time to restrict their use?. *PLoS biology*, *13*(10), p.e1002266.

Mensah, S. E., Koudande, O. D., Sanders, P., Laurentie, M., Mensah, G. A., and Abiola, F. A. (2014). Antimicrobial residues in foods of animal origin in Africa: public health risks. *Rev. Sci. Tech.* 33, 987–996.

Mikecz, O., Pica-Ciamarra, U., Felis, A., Nizeyimana, G., Okello, P. and Brunelli, C., 2020. Data on antimicrobial use in livestock: Lessons from Uganda. *One Health*, *10*, p.100165.

Mir, S.A., Shah, M.A., Mir, M.M., Dar, B.N., Greiner, R. and Roohinejad, S., 2018. Microbiological contamination of ready-to-eat vegetable salads in developing countries and potential solutions in the supply chain to control microbial pathogens. *Food Control*, *85*, pp.235-244.

Mitema, E.S., Kikuvi, G.M., Wegener, H.C. and Stohr, K., 2001. An assessment of antimicrobial consumption in food producing animals in Kenya. *Journal of veterinary pharmacology and therapeutics*, *24*(6), pp.385-390.

Monger, X.C., Gilbert, A.A., Saucier, L. and Vincent, A.T., 2021. Antibiotic resistance: From pig to meat. *Antibiotics*, *10*(10), p.1209.

Morosini,M.-I.,Cantón,R.,2011.Emergenceandspreadofantibioticresistancefollowing exposuretoantibiotics. FEMSMicrobiol. Rev.35,977–991.https://doi.org/10.1111/ j.1574-6976.2011.00295.x.

Muloi, D., Fevre, E.M., Bettridge, J., Rono, R., Ong'are, D., Hassell, J.M., Karani, M.K., Muinde, P., van Bunnik, B., Street, A. and Chase-Topping, M., 2019. A cross-sectional survey of practices and knowledge among antibiotic retailers in Nairobi, Kenya. *Journal of global health*, *9*(2).

Murray, I.A. and Shaw, W.V., 1997. O-Acetyltransferases for chloramphenicol and other natural products. *Antimicrobial agents and chemotherapy*, *41*(1), p.1.

Murray, R., Glass-Kaastra, S., Gardhouse, C., Marshall, B., Ciampa, N., Franklin, K., Hurst, M., Thomas, M.K. and Nesbitt, A., 2017. Canadian consumer food safety practices and knowledge: Foodbook study. *Journal of Food Protection*, *80*(10), pp.1711-1718.

Mutua, F.K., Dewey, C.E., Arimi, S.M., Ogara, W.O., Githigia, S.M., Levy, M. and Schelling, E., 2011. Indigenous pig management practices in rural villages of Western Kenya. *Livestock Research for Rural Development*.

Muyanja, C., Nayiga, L., Brenda, N. and Nasinyama, G., 2011. Practices, knowledge and risk factors of street food vendors in Uganda. *Food control*, *22*(10), pp.1551-1558.

Ndoboli, D., Roesel, K., Heilmann, M., Edward, T.A.P.H.C. and Huehn, W.D.G.S., 2018. Serotypes and antimicrobial resistance patterns of *Salmonella* enterica subsp. enterica in pork and related fresh-vegetable servings among pork outlets in Kampala, Uganda. *Revue d'élevage et de médecine vétérinaire des pays tropicaux*, *71*(1-2), pp.103-109.

Nepal, A., Hendrie, D., Selvey, L.A. and Robinson, S., 2021. Factors influencing the inappropriate use of antibiotics in the Rupandehi district of Nepal. *The International Journal of Health Planning and Management*, *36*(1), pp.42-59.

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F. and van der Giessen, J., 2010. Food-borne diseases—the challenges of 20 years ago still persist while new ones continue to emerge. *International journal of food microbiology*, *139*, pp.S3-S15.

Nyamakwere, F., Muchenje, V., Mushonga, B., Makepe, M. and Mutero, G., 2016. Assessment of Salmonella, Escherichia Coli, Enterobacteriaceae and Aerobic Colony Counts contamination levels during the beef slaughter process. *Journal of Food Safety*, *36*(4), pp.548-556.

Nychas, G.J.E., Skandamis, P.N., Tassou, C.C. and Koutsoumanis, K.P., 2008. Meat spoilage during distribution. *Meat science*, *78*(1-2), pp.77-89.

Oliveira, C.J.B., Carvalho, L.F.O.S., Fernandes, S.A., Tavechio, A.T., Menezes, C.C.P. and Domingues Jr, F.J., 2002. Antimicrobial resistance of *Salmonella* serotypes isolated from slaughter-age pigs and environmental samples. *Microbial Drug Resistance*, *8*(4), pp.407-411.

Om, C., Daily, F., Vlieghe, E., McLaughlin, J.C. and McLaws, M.L., 2017. Pervasive antibiotic misuse in the Cambodian community: antibiotic-seeking behaviour with unrestricted access. *Antimicrobial Resistance & Infection Control*, *6*(1), pp.1-8.

Omer MK, Hauge SJ, Ostensvik O, Moen B, Alvseike O, Rotterud OJ, Prieto M, Dommersnes S, Nesteng OH, Nesbakken T, 2015. Effects of hygienic treatments during slaughtering on microbial dynamics and contamination of sheep meat. *Int J Food Microbiol.*;2:194–197

O'Neill O.J., 2014: Antimicrobial Resistance: tackling a crisis for the health and wealth of nations. The Review on Antimicrobial Resistance.

Orman B, Pineiro SA, Arduino S, Galas M, Melano R, Caffer MI, Sordelli DO, Centron,D (2002). Evolution of multiresistance in nontyphoid *Salmonella* serovars from 1984 in Argentina. Antimicrob Agents Chemother 46: 396370

P.D. Fey, T.J. Safranek, M.E. Rupp, E.F. Dunne, E. Ribot, P.C. Iwen, *et al.* (2000). Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle, N. Engl. J. Med. 342 (2000) 1242–1249.

P.L. Winokur, A. Brueggemann, D.L. DeSalvo, L. Hoffmann, M.D. Apley, E.K. Uhlenhopp, *et al.* (2000). Animal and human multidrugresistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC beta-lactamase, Antimicrob. Agents Chemother. 44 (2000) 2777–2783

Partridge, S.R., Kwong, S.M., Firth, N., Jensen, S.O., 2018. Mobile genetic elements associated with antimicrobial resistance. Clin. Microbiol. Rev. 31. https://doi.org/10. 1128/CMR.00088-17.

Poole, K., 2005. Aminoglycoside resistance in Pseudomonas aeruginosa. *Antimicrobial agents and Chemotherapy*, *49*(2), pp.479-487.

Porphyre, T., Rich, K.M. and Auty, H.K., 2018. Assessing the economic impact of vaccine availability when controlling foot and mouth disease outbreaks. *Frontiers in veterinary science*, *5*, p.47.

Prendergast, D.M., Duggan, S.J., Gonzales-Barron, U., Fanning, S., Butler, F., Cormican, M. and Duffy, G., 2009. Prevalence, numbers and characteristics of *Salmonella* spp. on Irish retail pork. *International journal of food microbiology*, *131*(2-3), pp.233-239.

Price, L. B., Stegger, M., Hasman, H., Aziz, M., Larsen, J., Andersen, P. S., *et al*. (2012). *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *MBio* 3, e00305–e00311. doi: 10.1128/mBio.00305-11

Pui, C.F., Wong, W.C., Chai, L.C., Tunung, R., Jeyaletchumi, P., Hidayah, N., Ubong, A., Farinazleen, M.G., Cheah, Y.K. and Son, R., 2011. *Salmonella*: A foodborne pathogen. *International Food Research Journal*, *18*(2).

Queenan, K., Häsler, B. and Rushton, J., 2016. A One Health approach to antimicrobial resistance surveillance: is there a business case for it?. *International journal of antimicrobial agents*, *48*(4), pp.422-427.

R. S. Hendriksen, A. R. Vieira, S. Karlsmose *et al.* 2011. "Global monitoring of *Salmonella* serovar distribution from the world health organization global foodborne infections network country data bank: results of quality assured laboratories from 2001 to 2007," Foodborne Pathogens and Disease, vol. 8, no. 8, pp. 887–900.

Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock, L.J.V. and Woodward, M.J., 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five

serotypes of *Salmonella* enterica isolated from humans and animals in the UK. *Journal of Antimicrobial Chemotherapy*, *53*(2), pp.208-216.

Rani, Z.T., Hugo, A., Hugo, C.J., Vimiso, P. and Muchenje, V., 2017. Effect of post-slaughter handling during distribution on microbiological quality and safety of meat in the formal and informal sectors of South Africa: A review. *South African Journal of Animal Science*, *47*(3), pp.255-267.

Redmond, E.C., Griffith, C.J., Slader, J. and Humphrey, T.J., 2004. Microbiological and observational analysis of cross contamination risks during domestic food preparation. *British Food Journal*.

Rodríguez, I., Barownick, W., Helmuth, R., Mendoza, M.C., Rodicio, M.R., Schroeter, A. and Guerra, B., 2009. Extended-spectrum β-lactamases and AmpC β-lactamases in ceftiofur-resistant *Salmonella* enterica isolates from food and livestock obtained in Germany during 2003–07. *Journal of Antimicrobial Chemotherapy*, *64*(2), pp.301-309.

Rondón-Barragán, I.S., Arcos, E.C., Mora-Cardona, L. and Fandiño, C., 2015. Characterization of *Salmonella* species from pork meat in Tolima, Colombia. *Revista Colombiana de Ciencias Pecuarias*, *28*(1), pp.74-82.

Rostagno, M.H. and Callaway, T.R., 2012. Pre-harvest risk factors for Salmonella enterica in pork production. *Food Research International*, *45*(2), pp.634-640.

Rostagno, M.H., 2009. Can stress in farm animals increase food safety risk? *Foodborne pathogens and disease*, *6*(7), pp.767-776.

S. E. Majowicz, J. Musto, E. Scallan *et al., 2010*. "The global burden of nontyphoidal *Salmonella* gastroenteritis," Clinical Infectious Diseases, vol. 50, no. 6, pp. 882–889.

Sagoo, S.K., Little, C.L., Ward, L., Gillespie, I.A. and Mitchell, R.T., 2003. Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. *Journal of Food Protection*, *66*(3), pp.403-409.

Salisbury, J.G., Nicholls, T.J., Lammerding, A.M., Turnidge, I., Nunn, M.J., 2002. A risk analysis framework for the long-term management of antibiotic resistance in foodproducing animals. Int. J. Antimicrob. Agents 20, 153–164.

Sánchez-Vargas, F.M., Abu-El-Haija, M.A. and Gómez-Duarte, O.G., 2011. Salmonella infections: an update on epidemiology, management, and prevention. *Travel medicine and infectious disease*, *9*(6), pp.263-277.

Schmidt, J.W., Vikram, A., Doster, E., Thomas, K., Weinroth, M.D., Parker, J., Hanes, A., Geornaras, I., Morley, P.S., Belk, K.E. and Wheeler, T.L., 2021. Antimicrobial resistance in US retail ground beef with and without label claims regarding antibiotic use. *Journal of Food Protection*, *84*(5), pp.827-842.

Sefton, A.M., 2002. Mechanisms of antimicrobial resistance. *Drugs*, *62*(4), pp.557-566.

Slorach SA, 2002. Integrated approaches to the management of food safety throughout the food chain. Marrakesh, Morocco: FAO/WHO Global Forum of Food Safety Regulators.

Sneeringer, S., MacDonald, J.M., Key, N., McBride, W.D. and Mathews, K., 2015. Economics of antibiotic use in US livestock production. *USDA, Economic Research Report*, (200).

Soares, V.M.; Pereira, J.G.; Viana, C.; Izidoro, T.B.; Bersot, L.S.; Pinto, J.P, 2012. Transfer of *Salmonella* Enteritidis to four types of surfaces after cleaning procedures and cross-contamination to tomatoes. Food Microbiol. 30, 453–456. [CrossRef] [PubMed]

Sterzenbach, T., Crawford, R.W., Winter, S.E. and Bäumler, A.J., 2013. Salmonella virulence mechanisms and their genetic basis. *Salmonella in Domestic Animals*, p.81.

Su, L. and Chiu, C., 2007. Salmonella: clinical importance and evolution of nomenclature. *Chang Gung medical journal*, *30*(3), p.210.

Swart, A.N., Evers, E.G., Simons, R.L.L. and Swanenburg, M., 2016. Modeling of Salmonella contamination in the pig slaughterhouse. *Risk Analysis*, *36*(3), pp.498-515.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y. and Ouellette, M., 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases*, *18*(3), pp.318-327.

Tacconelli, E., Sifakis, F., Harbarth, S., Schrijver, R., van Mourik, M., Voss, A., Sharland, M., Rajendran, N.B., Rodríguez-Baño, J., Bielicki, J. and de Kraker, M., 2018. Surveillance for control of antimicrobial resistance. *The Lancet Infectious Diseases*, *18*(3), pp.e99-e106.

Tadee, P., Boonkhot, P. and Patchanee, P., 2014. Quantification of contamination levels and particular risk of Salmonella spp. in pigs in slaughterhouses in Chiang Mai and Lamphun provinces, Thailand. *Japanese Journal of Veterinary Research*, *62*(4), pp.171-179.

Thomas, L.F., de Glanville, W.A., Cook, E.A. and Fèvre, E.M., 2013. The spatial ecology of free-ranging domestic pigs (Sus scrofa) in western Kenya. *BMC veterinary research*, *9*(1), pp.1-12.

Thomson, N.R., Clayton, D.J., Windhorst, D., Vernikos, G., Davidson, S., Churcher, C., Quail, M.A., Stevens, M., Jones, M.A., Watson, M. and Barron, A., 2008. Comparative genome analysis of Salmonella Enteritidis PT4 and Salmonella Gallinarum

287/91 provides insights into evolutionary and host adaptation pathways. *Genome research*, *18*(10), pp.1624-1637.

Threlfall, E.J., Ward, L.R., Frost, J.A. and Willshaw, G.A (2000). The emergence and spread of antibiotic resistance in food-borne bacteria. *Int J Food Microbiol* 62, 1-5 throughout the Todd, E.C., 1997. Epidemiology of foodborne diseases: a worldwide review.

Tindall, B.J., Grimont, P.A.D., Garrity, G.M. and Euzeby, J.P., 2005. Nomenclature and taxonomy of the genus Salmonella. *International journal of systematic and evolutionary microbiology*, *55*(1), pp.521-524.

Tiseo, K., Huber, L., Gilbert, M., Robinson, T.P. and Van Boeckel, T.P., 2020. Global trends in antimicrobial use in food animals from 2017 to 2030. *Antibiotics*, *9*(12), p.918.

Todd, E.C., Michaels, B.S., Greig, J.D., Smith, D., Holah, J. and Bartleson, C.A., 2010. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 7. Barriers to reduce contamination of food by workers. *Journal of food protection*, *73*(8), pp.1552-1565.

Uzeh, R.E., Adewumi, F. and Odumosu, B.T., 2021. Antibiotic resistance and plasmid analysis of Enterobacteriaceae isolated from retail meat in Lagos Nigeria. *One Health Outlook*, *3*(1), pp.1-6.

Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A. and Laxminarayan, R., 2015. Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, *112*(18), pp.5649-5654.

Van Boeckel, T.P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N.G., Gilbert, M., Bonhoeffer, S. and Laxminarayan, R., 2019. Global trends in antimicrobial resistance in animals in low-and middle-income countries. *Science*, *365*(6459), p.eaaw1944.

Van, T. T., G. Moutafis, T. Istivan, L. T. Tran, and P. J. Coloe. 2007. Detection of *Salmonella* spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. Appl. Environ. Microbiol. 73: 6885–6890.

Van, T.T.H., Nguyen, H.N.K., Smooker, P.M. and Coloe, P.J., 2012. The antibiotic resistance characteristics of non-typhoidal Salmonella enterica isolated from food-producing animals, retail meat and humans in South East Asia. *International journal of food microbiology*, *154*(3), pp.98-106

Wang, B., Yao, M., Lv, L., Ling, Z., Li, L., 2017. The human microbiota in health and disease. Engineering 3, 71–82. https://doi.org/10.1016/J.ENG.2017.01.008.

Welch, T. J., W. F. Fricke, P. F. McDermott, D. G. White, M. L. Rosso, D. A. Rasko, M. K. Mammel, M. Eppinger, M. J. Rosovitz, D. Wagner, L. Rahalison, J. E. Leclerc, J. M. Hinshaw, L. E. Lindler, T. A. Cebula, E. Carniel, and J. Ravel. 2007. Multiple antimicrobial resistance in plague: an emerging public health risk. *PLoS One***2:**e309.

White, D.G., Zhao, S., Simjee, S., Wagner, D.D. and McDermott, P.F., 2002. Antimicrobial resistance of foodborne pathogens. *Microbes and infection*, *4*(4), pp.405-412.

WHO (World Health Organization), 2012. The Evolving Threat of Antimicrobial Resistance: Options for Action, WHO, Geneva, Switzerland.

WHO (World Health Organization), 2014. Antimicrobial Resistance: Global Report on Surveillance, WHO, Geneva, Switzerland.

Wilson, C.N., 2018. Prevalence and antimicrobial resistance profile of non-typhoidal Salmonella in pigs in Kenya and Malawi (Doctoral dissertation, The University of Liverpool (United Kingdom)).

Wilson, C.N., Pulford, C.V., Akoko, J., Perez Sepulveda, B., Predeus, A.V., Bevington, J., Duncan, P., Hall, N., Wigley, P., Feasey, N. and Pinchbeck, G., 2020. Salmonella identified in pigs in Kenya and Malawi reveals the potential for zoonotic transmission in emerging pork markets. *PLoS neglected tropical diseases*, *14*(11), p.e0008796.

Wong, D.L.F., Hald, T., Van Der Wolf, P.J. and Swanenburg, M., 2002. Epidemiology and control measures for Salmonella in pigs and pork. *Livestock Production Science*, *76*(3), pp.215-222.

World Health Organization (WHO), 2017. Critically Important Antimicrobials for Human Medicine—5th Revision 2016—Ranking of Medically Important Antimicrobials for Risk Management of Antimicrobial Resistance Due to Non-Human Use.

Yan, S.S., Pendrak, M.L., Abela-Ridder, B., Punderson, J.W., Fedorko, D.P. and Foley, S.L., 2004. An overview of Salmonella typing: public health perspectives. *Clinical and Applied Immunology Reviews*, *4*(3), pp.189-204.

Yang, X. and Wu, Q., 2019. Prevalence, bacterial load, and antimicrobial resistance of *Salmonella* serovars isolated from retail meat and meat products in China. *Frontiers in microbiology*, *10*, p.2121. [Google scholar]

Zhao S, White DG, Friedman SL, Glenn A, Blickenstaff K, Ayers SL, *et al*, 2008. Antimicrobial resistance in *Salmonella enterica* serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. Appl Environ Microbiol. 2008;74:6656–6662. [PMC free article] [PubMed] [Google Scholar]

Zhu, Z., Huang, Q., Hong, X., Chen, X., Lu, Y., Chen, Z., Wang, C., Meng, X., Xu, Q. and Li, S., 2020. Isolation and characterization of *Salmonella* in pork samples collected from retail and wholesale markets in each season from 2016 to 2018 in Wuhan, China. *Journal of Applied Microbiology*, *128*(3), pp.875-883.

APPENDICES

Appendix i: Ethical approval

ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

CGIAR

25th July 2018

Our Ref: ILRI-IREC2018-13

International Livestock Research Institute
P.O. Box 30709 00100
Nairobi, Kenya.

Dear Eric Fevre & Mercy Cianjoka,

REF: UNDERSTANDING THE PORK INDUSTRY IN WESTERN KENYA; THE VALUE CHAIN, PATHOGEN TRANSMISSION AND FUTURE TRENDS IN POPULATION GENETICS AND DISEASE BURDEN

Thank you for submitting your request for ethical approval to the International Livestock Research Institute (ILRI) Institutional Research Ethics Committee (IREC). ILRI IREC is registered and accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya.

I am pleased to inform you that ILRI IREC has reviewed and approved your study titled ***'Understanding the pork industry in western Kenya; the value chain, pathogen transmission and future trends in population genetics and disease burden'***. The approval period is 25th July 2018 to 24th July 2019 and is subject to compliance to the following requirements:

- Only approved documents will be used;
- All changes must be submitted for review and approval before implementation;
- Adverse events must be reported to ILRI IREC immediately;
- Access and Benefits Sharing (ABS) requirements, where applicable;
- Submission of a request for renewal of approval at least 30 days prior to expiry of approval period; and
- Submission of an executive summary report within 90 days upon completion of the study.

Please call on ILRI IREC on ILRIResearchcompliance@cgiar.org for any further clarification or information you may require.

Yours Sincerely,

Silvia Alonso, PhD (pp. Jane Poole, Statistician, ILRI IREC)
Chair, ILRI Institutional Research Ethics Committee

Documents received & reviewed:

- Research Compliance Form & IREC Form
- Research Proposal
- Consent Forms & Questionnaires (Butchers, Slaughter slab owners, Pig farmers, Pork consumers)
- Protocol – Field Lab and Data Analysis

9th September 2019

Our Ref: ILRI-IREC2018-13/3

International Livestock Research Institute

P.O. Box 30709 00100

Nairobi, Kenya.

Dear Prof. Eric Fevre & Mercy Cianjoka,

Ref: Renewal of Approval for ILRI-IREC2018-13

Thank you for submitting your request for renewal of ethical approval to the International Livestock Research Institute (ILRI) Institutional Research Ethics Committee (IREC). ILRI IREC is accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya, and approved by the Federalwide Assurance (FWA) for the Protection of Human Subjects in the United States of America.

I am pleased to inform you that ILRI IREC has approved your request for renewal of approval as per IREC Form 4 dated 6th September 2019 for the project titled *'Understanding the Pork Industry in Western Kenya; The Value Chain, Pathogen Transmission and Future Trends in Population Genetics and Disease Burden'*. Note that the approval period is 9th September 2019 to 8th September 2020. All other conditions as per IREC2018-13 and IREC2018-13/2 remain the same.

For further information or clarification, write to ILRI IREC on ILRIResearchcompliance@cgiar.org .

Yours Sincerely,

Silvia Alonso, PhD
Chair, ILRI Institutional Research Ethics Committee
Documents received & reviewed:

- IREC Form 4

22nd November 2018

Our Ref: ILRI-IREC2018-13/1

International Livestock Research Institute
P.O. Box 30709 00100
Nairobi, Kenya.

Dear Eric Fevre & Mercy Cianjoka,

REF: UNDERSTANDING THE PORK INDUSTRY IN WESTERN KENYA: THE VALUE CHAIN, PATHOGEN TRANSMISSION AND FUTURE TRENDS IN POPULATION GENETICS AND DISEASE BURDEN

Thank you for submitting your request for ethical approval to the International Livestock Research Institute (ILRI) Institutional Research Ethics Committee (IREC). ILRI IREC is registered and accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya, and approved by the Federalwide Assurance (FWA) for the Protection of Human Subjects in the United States of America.

I am pleased to inform you that ILRI IREC has reviewed and approved your request for minor amendment as per IREC Form 3 forms dated 1st November 2018 for the project titled *'Understanding the pork industry in western Kenya: the value chain, pathogen transmission and future trends in population genetics and disease burden'*. The approval is to include pork hotel owners and to change pathogens of interest to *Salmonella enterica, Escherichia coli* and

Campylobacter. Please note that all other approval conditions as per approval letter referenced ILRI-IREC2018-13 and dated 25th July 2018 remain the same.

Please call on ILRI IREC on ILRIResearchcompliance@cgiar.org for further clarification or information you may require.

Yours Sincerely,

Silvia Alonso, PhD

Chair, ILRI Institutional Research Ethics Committee

Documents received & reviewed:

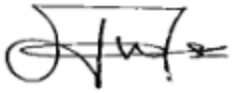
- IREC Form 3

Turnitin Originality Report

PREVALENCE AND CHARACTERIZATION OF BACTERIA OF GENUS SALMONELLA IN RETAIL PORK AND RAW VEGETABLES, BUSIA COUNTY by Dr. Christine Makena Mbabu

From Msc and PhD (Msc and PhD)

- Processed on 22-Aug-2022 12:38 EAT
- ID: 1885420890
- Word Count: 13468



Prof. James Mbaria
(Ph.D)

Similarity Index
10%
Similarity by Source

Internet Sources:
9%
Publications:
4%
Student Papers:
2%

sources:

1 1% match (Internet from 15-Jan-2022)
https://www.mdpi.com/1420-3049/23/4/795/html

2 1% match (Internet from 21-Oct-2021)
https://www.ncbi.nlm.nih.gov/sites/ppmc/articles/PMC5120092/

3 1% match (Internet from 09-Jan-2022)
https://www.coursehero.com/file/82839674/Untitled-documentedited-19docx/

4 < 1% match (Internet from 09-Jan-2020)
https://www.mdpi.com/2076-0817/8/1/19/html

5 < 1% match (Internet from 03-Jan-2022)
https://www.mdpi.com/2076-0817/8/1/19

6 < 1% match (publications)
Xavier C. Monger, Alex-An Gilbert, Linda Saucier, Antony T. Vincent. "Antibiotic Resistance: From Pig to Meat", Antibiotics, 2021

7 < 1% match (Internet from 27-Jul-2016)
http://docslide.us/documents/salmonella-a-dangerous-foodborne-pathogen.html

Appendix iii: Evidence of publication

Manuscript

Antimicrobial resistant *Salmonella* spp. identified from retail pork and raw side-salads in Busia County, Kenya.

Christine Makena[1,2], Eric M Fèvre[2,3], Mercy Cianjoka Gichuyia[1,2], Peter Gathura[1], James Mbaria[1], Gitahi Nduhiu[1], Lian F. Thomas[2,3*]*

[1]Department of Public Health, Pharmacology and toxicology; Faculty of Veterinary medicine, University of Nairobi, P O BOX 29053-00625, Nairobi, Kenya

[2]International Livestock Research Institute, PO BOX 30709, Nairobi 00100, Kenya

[3]Department of Livestock & One Health, Institute of Infection, Veterinary & Ecological Sciences, University of Liverpool, Liverpool, L69 3BX, UK

Emails;

CMM: christinemakena.m@gmail.com

EMF: eric.fevre@liverpool.ac.uk

MCG: gichuyiacianjo@gmail,com

PG: pgathura@uonbi.ac.ke

JM: james.mbaria@uonbi.ac.ke

NG: nduhiugitahi@gmail.com

LFT: lian.thomas@liverpool.ac.uk

These two authors contributed equally to this work.

Christinemakena.m@gmail.com (CMM); lian.thomas@liverpool.ac.uk (LFT)

Short running title; AMR *Salmonella* spp. in pork and salads sold in Kenya

Key Words; *Salmonella* spp.; foodborne disease; retail pork; cross-contamination; antimicrobial resistance, Kenya

ABSTRACT

Salmonella species are gram-negative bacteria and a major cause of foodborne burden that have the potential to be transmitted from food of animal origin to humans posing. This research aimed at determining the prevalence and characterization of *Salmonella* Spp. in raw pork, cooked pork and raw vegetables served alongside cooked pork from pork butcheries in four selected sub-Counties in Busia County, Western Kenya. 451 samples were collected and these consisted of 262

raw pork, 108 cooked pork and 81 side salads served alongside cooked pork. Samples were cultured in Salmonella selective media. Isolated Salmonella spp. were then identified using genus antiserum. Serotyping was done using Kauffmann-Whyte scheme and antimicrobial sensitivity was determined using disc diffusion method. High prevalence, 32.59% (147/451 95% CI 32.40% - 32.80%) of *Salmonella* spp. was detected from the three food stuffs. High antimicrobial resistant levels of 91.84% (135/147 95% CI 91.47%-92.20%) was recorded. 46.26% (68/147 95% CI 45.59%-46.92) of the samples were multidrug resistance. Highest resistant was recorded in Gentamicin and Ampicillin. This present study highlights worryingly high prevalence of antimicrobial resistant *Salmonella* spp. in retail pork and raw salads. This work presents first report work on Salmonella serotypes in retail pork in Kenya.

Keywords: Salmonella, antibiotic resistant, retail pork, isolates, serotyping.