Epidemiology of *Enterobacteriaceae* resistance and prevailing conditions in pig farms in Ashanti region, Ghana.

A thesis submitted to the Department of Pharmaceutics, Kwame Nkrumah University of Science and Technology in partial fulfilment of the requirements for the award of

MASTER OF PHILOSOPHY

IN THE DEPARTMENT OF PHARMACEUTICS,

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

COLLEGE OF HEALTH SCIENCES

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September, 2013

DECLARATION

I hereby declare that this submission is my own work toward the M. Phil and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

I dedicate this work to my dear wife Mrs Dora Osei Sekyere, my daughter Adwoa B.-D. Serwaa Sekyere and all my beautiful children yet unborn.

ACKNOWLEDGEMENTS

I am grateful to God almighty for His kindness and mercy in granting me the strength, knowledge and wisdom to design and complete this research work. This work would not have been possible without financial support from **ADMER** whose funding aided me to complete this work. My very good friend, Bernard Keraita of University of Copenhagen, Department of Global Health, was very instrumental in securing the funding from ADMER and in guiding the research and analysis.

I thank my Uncle Mr Kwaku Duah for his constant support and training throughout my life. My parents, Mr and Mrs Osei Kwadwo have been very supportive in my education and upbringing. Mrs Dora Osei Sekyere, my very good wife, has suffered with me during these periods of stress and hard work.

My supervisor, Mr Francis Adu and the Department's research coordinator, Dr Christian Agyare have been a sine qua non to the success and completion of this noble project—their contributions and systematic critiquing of this work is invaluable. Mrs Vivian Etsiapa Boamah, a senior colleague and fellow student, has been very resourceful as a consultant throughout the 'Antibiotic Susceptibility Testing' phase of this work.

Finally, I am grateful to all teaching and non-teaching staff of the Department of Pharmaceutics, including my fellow postgraduate students for their contributions, directly or indirectly, towards the successful completion of this work.

ABSTRACT

Pig production in Ghana is increasing at an unprecedented pace under intensive farming conditions. With this shift, farmers have adopted the use of antibiotics for the prophylaxis and treatment of pig diseases; creating a concern that their handling practices could affect the susceptibilities of pathogens to these antibiotics. Due to the public health hazard posed by the antibiotic handling practices of these farmers, a total of 110 pig farms from five districts within the Ashanti Region of Ghana, were surveyed using validated questionnaires and observations to assess the husbandry practices and prevalence of resistance among microbes isolated from the pigs. Interviews were held with veterinarians and animal scientists. Enterobacteria isolates from collected pig faecal samples were analysed for their susceptibility. Tests showed that more than 80% of all isolates were susceptible to the fluoroquinolones and gentamicin (which have less patronage among the farmers). Susceptibility to amoxicillin and streptomycin was observed among at least 25% of all isolates whiles 40-50% of the isolates were susceptible to the tetracyclines and Sulphamethoxazole-trimethoprim (antibiotics commonly patronised by the farmers). Most (78%) of the resistant organisms were multi-drug resistant. Bosomtwe and Atwima Kwanwoma districts had the greatest prevalence of resistant isolates, followed by Ejisu Juaben district. *E. coli* and *Salmonellae* from all the districts showed resistance to clinically important antibiotics. Educational level of farmers, the type of farm manager, antibiotic storage site, the use of protection, body washing after antibiotic handling, routes of antibiotic administration and dosage form, type of antibiotics used and source of farm water were all found to significantly impact on antibiotic resistance. However, types of protection used during antibiotic handling had no effect on bacterial resistance to antibiotics. Consequently, pig farms are harbouring resistant bacteria which are a threat to public health. Stricter regulations and supervision regarding the sales and use of antibiotics, promotion of probiotics and vaccinations, periodic education of farmers through workshops and seminars and periodic surveillance studies to follow the trend in antibiotic resistance in pig farms are necessary measures to check the development and spread of resistant bacteria.

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CHAPTER ONE

1. INTRODUCTION

1.1 Antibiotic Resistance

Antibiotics are used in livestock production for prevention and treatment of infectious diseases besides their sub therapeutic use as growth promoters (Chee-Sanford *et al*., 2009). Since the introduction of concentrated animal feeding operations (CAFOs), larger amounts of antimicrobials are employed in livestock production than in human medicine (Silbergeld *et al*., 2008). Antimicrobials are mainly used in the production of pig, cattle, poultry and recently, in aquaculture (Silbergeld *et al*., 2008). The antibiotics used in livestock fall into all the major classes of antibiotics used in clinical practice; there have even been cases in which antimicrobials were licensed for livestock use before their subsequent use in humans (Silbergeld *et al*., 2008).

In nature, there are microorganisms that are resistant to a wide variety of antibiotics; these microorganisms have not acquired this resistance but are innately resistant. It is estimated that such naturally resistant bacteria form 2% of the 'wild type' bacterial population (Novick 1981). In nature, microorganisms produce antibiotics to inhibit the growth of other microorganisms to prevent competition (Davies and Davies, 2010). The microorganisms that produce these antibiotics tend to have the natural ability to evade the lethal effect of its own antibiotic and all other antibiotics that have similar mechanisms of action or similar chemical structures (Guilfoile and Hutchinson, 1991; Piddock, 2006a).

Several studies have shown that long-term exposure of bacteria to a particular antibiotic will cause resistance not only to that particular antibiotic but to several others (Levy and Marshall, 2004); this phenomenon has been implicated as the cause for multi-drug resistant bacterial species. The actual mechanism for such an occurrence is unknown, but it suggests the linkage between different resistance genes on the same plasmids or transposons. Studies have shown that resistant bacteria

easily acquire resistance determinants from other bacteria within their environment (Levy and Marshall, 2004).

Bacteria can become resistant to antibiotics in gradual or sequential mutations that occur in their chromosomes. The progression from susceptibility to resistance through mutation is step-wise and not as fast as that of resistance genes acquisition from the environment and is not dependent on the presence of transposons or plasmids. In the presence of antibiotics, resistance species are selected and multiplied whiles susceptible species are repressed (Levy and Marshall, 2004).

Antibiotic resistance is also acquired by bacteria through the natural ability to share genetic material between themselves, even among bacteria of different species (Levy and Marshall, 2004; Chee-Sanford *et al*., 2009). Bacteria that do not have the natural ability to produce or become resistant to the lethal effect of antibiotics can acquire it from naturally resistant bacteria or bacteria that have acquired resistance through transfer of genetic material via three modes: conjugation, transduction and transformation (Davies and Davies, 2010). Consequently, bacteria can acquire resistance whenever they come into contact with sub therapeutic doses of an antibiotic (Gilchrist *et al*., 2007), a resistant bacteria or gene. This acquisition of resistance can occur in the intestines of farm animals, farm litter, dumping site of antibiotic-containing waste, soils amended by animal manure, ground and surface waters contaminated with farm waste etc., resulting in the selection and proliferation of resistant species (Novick, 1981; Gilchrist *et al*., 2007). These resistant bacteria are a threat to public health when they infect people as they make the antibiotics used in clinical medicine ineffective. This increases the mortality and morbidity rates of infectious diseases, health care costs, duration of antibiotic treatment, the resort to more expensive and toxic antibiotic alternatives and protracted hospitalisation.

Antibiotics tend to break down with time through radioactive degradation (i.e. half decomposition of the active ingredient), hydrolysis (when in the presence of water, acids or bases), biodegradation (through the biochemical processes of soil micro organims) or photo degradation (when they absorb particular wavelengths of electromagnetic waves) (Halling-Sorensen, 2000; Chen *et al*., 1997). Such

processes reduce the concentration of the drug and their ability to kill the bacteria they come in contact with, allowing the exposed bacteria the time to develop resistance. Antibiotic degradation is accelerated or decelerated by environmental factors, making storage conditions of antimicrobials very important. In an extensive study of antimicrobials' quality from shops to consumers in Nigeria, substandard antimicrobials, counterfeit and expired antimicrobials and antibiotics degraded by poor storage and transportation conditions were also found floating freely in several Nigerian markets (Okeke *et al*. 1999)

The inappropriate use of antibiotics in agriculture has been found to contribute to increased antibiotic resistance in human pathogens through the consumption of antibiotic residues in animal products (Novick, 1981; Richter *et al*., 1996). Human exposure to these antibiotics and their resistant microorganisms during animal care and the contamination of ground and surface waters, soils and crops by farm wastes (Borgen *et al*., 2000; Jindal *et al*., 2006) are also implicated. A concomitant increase in resistant *enterococci* from human faecal samples in the Netherlands with the introduction of vancomycin and pristinamycin in pig production have been reported (Van den Bogaard *et al*. 2000). Various other studies, especially by Nachamkin (2000), have documented a direct relationship between the increase in resistance to particular antibiotics in humans before and after their introduction into livestock production (Norstrom *et al*., 2006; Gupta *et al*., 2004; Endtz *et al*., 1991).

1.2 Pig farming in Ghana

There is currently little or no data on the types of antibiotics used by pig farmers on pig health in Ghana. Available literature on pig farming mainly focuses on their nutrition, management and challenges. This stems from the relatively unpopular nature of pigs and pork products in the Ghana due to cultural, religious, and sanitary reasons which have seriously affected the pig industry, stifling its growth to give poultry a better advantage (Okai, 2010).

1.2.1 Public health threat of commercial pig production

The use of antibiotics in commercial livestock production farms (also known as Concentrated Animal Feeding Operations—CAFOs) for treatment, prophylaxis and growth promotion has been implicated

by several studies as some of the major sources and causes of antibiotic resistance development and spread into the environment and through the food chain (Gilchrist *et al*., 2007; Silbergeld *et al*., 2008; Chee-Sanford *et al*., 2009). Resistant bacteria isolated from farmers and farm workers, surface and groundwater, animal products, farm crops, manure and animal wastes have all been traced to commercial animal food production farms. Consequently, the use, abuse and misuse of antibiotics in veterinary medicine have become a means of fostering the development and spread of antibiotic resistant bacteria with its attendant public health implications (Gilchrist *et al*., 2007; WHO, 2011).

Such an awareness of the potential risks of antibiotic use in pigs on human health is essential to advise policy makers, regulatory bodies, veterinarians and farmers on the need to address the issue of appropriate use of antibiotics in pig production to contain the development and spread of antibiotic resistance which threatens to make the treatment of bacterial infections with common and inexpensive antibiotics impossible (WHO, 2011).

1.3 Justification

1. Piggery is currently the fastest growing animal industry in the Ashanti region.
2. It is the second most organised commercial animal production after poultry in Ghana.
3. There is currently no study carried out on the health status, types and user practices of antibiotics in the Pig industry.
4. Such a data will inform policy makers and regulatory bodies in their policies and decisions on antibiotics use in Ghana.
5. It will provide a basis for further study into the role antibiotics use in piggery plays in the development and spread of resistance through the food chain.

1.4 Aim of study

The overall objective is to determine the different types of antibiotics and their handling practices in pig production in Ashanti Region and the prevalence of bacteria resistance.

1.4.1 Specific objectives

1. To identify main sources and types of antibiotics used for prophylaxis and treatment of infections by pig farmers in Ashanti Region.
2. To assess the antibiotic handling practices of the farmers.
3. To evaluate the susceptibilities of isolated enterobacteria (*E. coli*, *Enterobacter*, *Salmonellae* and *Proteus vulgaris*) to different antibiotics.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Antibiotics

Antibiotics are natural or semi-synthetic compounds that inhibit or kill bacteria by specific interactions with bacterial targets (Davies *et al*., 2010; WHO, 2011). They have become important in veterinary medicine, aquaculture and agriculture as growth promoters, prophylactic and therapeutic agents (Chee-Sanford *et al*., 2009). Though initially produced for clinical use, it is now estimated that the largest use of antibiotics worldwide occurs in the production of food animals (Silbergeld *et al*., 2008). The Union of Concerned Scientists in 2001 indicated that 87% of all antibiotics produced in the United States are used for animals whiles 13% are used clinically (Gilchrist *et al*., 2007).

The era of antibiotics began in 1928 when Sir Alexander Fleming discovered penicillin (WHO, 2011). They became medically important in the 1940s when they were used to treat serious bacterial infections like pneumonia, sepsis, meningitis, severe wound infections etc. (WHO, 2011).

Antibiotics are chemical compounds produced naturally by fungi (for example, penicillin), and bacteria (for example, tetracycline). However, there are currently synthetic and semi-synthetics ones (fluoroquinolones and amoxicillin respectively). Nevertheless, Nobel laureate Selman Waksman, the discoverer of Streptomycin and pioneer in screening soils for antibiotics, limits the term 'antibiotic' to only compounds of natural origin (Davies and Davies, 2010). The term antibiotic and antimicrobials are synonymous and are used interchangeably in literature to refer to agents of all sorts that inhibit or kill microorganisms. Agents specifically used for inhibiting or killing bacteria are termed antibacterial agents (WHO, 2011).

Antibiotics come in various classes. This classification is based on their chemical structure, mechanism of action and or their biological source. Some antibiotic classes are specific for gram positive or gram negative bacteria or both; however, they differ in their strengths against bacteria within these groups of gram negatives and gram positives. The extent to which an antibiotic is

effective against a number of bacterial species is referred to as its spectrum of activity (Rang *et al*., 2003).

2.1.1 Antibiotics in food animal production and agriculture

After the successful application of antibiotics in clinical medicine to treat bacterial infections in the 1940s, they were introduced into veterinary medicine in the 1950s. Subsequently, antibiotics have become an important part in industrialised or intensive production of livestock (WHO, 2011; Cromwell, 2001). In fact, the introduction of antibiotics into livestock production has been a driving factor in the growth of concentrated animal food operations (CAFOs) across Europe and America as they are employed in the treatment and prevention of livestock infections and for growth promotion (Gilchrist *et al*., 2007). In order to prevent the outbreak of major diseases that can wipe out an entire population of animal stock in a farm, farmers employ sub-therapeutic doses of antibiotics for prophylaxis. The use of sub-therapeutic doses of antibiotics as growth promoters has become a normal practice among most farmers in the western world (Gilchrist *et al*., 2007). Though the scientific basis for such practices is unclear, farmers use large volumes of antibiotics to boost and promote the growth of their animals (WHO, 2011).

As a result of the comparatively larger populations of livestock of all forms and the frequent use of antibiotics on livestock farms compared to clinical medicine, large quantities of antibiotics are employed in farm animal production than in human medicine (Gilchrist *et al*., 2007;Silbergeld *et al*., 2008; WHO, 2011). Moreover, antibiotics employed in farm animal production encompass all antibiotic classes important in clinical medicine (see Table 1) in some cases, new antibiotics have been registered for agricultural use before approval for clinical use. The FDA's approval of Virginiamycin (quinupristin-dalfopristin) for instance, resulted in the isolation of resistant bacterial species from humans though Virginiamycin has not been approved for clinical use; demonstrating the effects of antibiotic use in veterinary medicine on public health (Silbergeld *et al*., 2008). The penicillins, macrolides, polypeptides, Streptogramins and tetracyclines are used for diseases prevention, treatment and growth promotion; other classes like the quinolones, lincosamides and aminoglycosides are basically used for disease treatment or prevention. According to the Union of

Concerned Scientists (UCS, 2001), the use of antibiotics as growth promoters far outweighs their use in disease treatment. After the introduction of antibiotics in farm animal production as growth promoters (AGP-Antibiotic Growth Promoters) irrespective of the health status of the animal, antibiotic use exploded in many countries by exponential proportions; the use of antibiotics as AGPs in the United States, increased fiftyfold between 1951 and 1978 (from 110 tonnes to 5580 tonnes), while there was only a tenfold increase in the use of antibiotics to treat infections in people and animals (World Organisation for Animal Health, 2011a and 2011b).

Antibiotics are also employed in agriculture as biocides (in crop and fruit production) but in relatively lower quantities than that used in livestock (Silbergeld *et al.*, 2008). With the increase in fish farming (aquaculture) the world over, the use of antibiotics has seen a tremendous rise in fish feed for treatment and prevention of bacterial diseases (WHO, 2011). The amount of antibiotics used in aquaculture is as substantial as that of intensive food animal production (WHO, 2011).

Table 1: Common antibiotics used in pig, poultry and beef cattle production industries (USGAO, 1999; USDA, 2007)

Antibiotic Class	**Industry**
Aminoglycosides	Pig, poultry, beef cattle
B-Lactams	Pig, poultry, beef cattle
Chloramphenicol	Beef cattle
Ionophores	Poultry, beef cattle
Lincosamides	Pig, poultry
Macrolides	Pig, poultry, beef cattle
Polypeptides	Pig, poultry
Quinolones (and fluoroquinolones)	Poultry, beef cattle
Streptogramins	Pig, poultry, beef cattle
Sulphonamides	Pig, poultry, beef cattle
Tetracyclines	Pig, poultry, beef cattle
Glycolipids(Bambermycin)	Pig, poultry, beef cattle
Carbadox	Pig
Aminocoumarins (Novobiocin)	Poultry
Aminocyclitols (Spectinomycin)	Pig, poultry

2.1.2 Antibiotic resistance

The immeasurable benefits of antibiotics are waning due to the emergence of resistance among almost all species of microorganisms (Davies and Davies, 2010; WHO, 2011). Antibiotic resistance is the phenomenon in which bacteria heretofore susceptible to particular antibiotics are able to survive and proliferate in the presence of the antibiotic (WHO, 2011). All species of bacteria have the innate ability to evolve or adapt to its environment in order to survive. By so doing, they develop or change their genetic make up to produce new enzymes, proteins and or other phenotypic characters that enable them to survive in their environments. Consequently, the beneficial effects of antibiotics have become short-lived as antibiotic resistance threatens to return the world to the pre-antibiotic era (Davies and Davies, 2010). The introduction of every new antibiotic has been plagued by the rapid development of species resistant to that antibiotic. Within few months after the introduction of Sulphonamides (1937), resistant species were reported in 1939 (WHO, 2011; Davies and Davies, 2010).

Antibiotic resistance has become a global menace and a threat to public health as it renders our current antibiotic arsenals useless, prolongs the stay of patients affected by resistant bacteria in hospitals, increases healthcare costs following protracted hospitalisation and the consequent resort to more expensive but more toxic alternative antibiotics and increases the morbidity and mortality rates of infectious diseases (Levy and Marshall, 2004; WHO, 2011). Research to produce new antibiotics is no more attractive as pharmaceutical companies are now focusing on the production of medicines for chronic diseases which fetch them more money over a longer time period than antibiotics; subsequently, there are few new antibiotics in the pipeline (Projan S. J., 2003). Moreover, the research to find and produce new antibiotics is expensive and lengthy and the rapid development of resistance to any new antibiotic discourages manufacturers from investing therein [Infectious Diseases Society of America, 2005; Institute of Medicine (IOM) 1998; WHO, 2011]

This threat is not limited to just a person or community. The use of antibiotics by any person, plant or animal selects for resistant species which multiplies to occupy the region of the annihilated species. These resistant species are later spread from individual to individual, traversing whole communities

and the whole globe through person to person contact, the food chain, water, commerce and international travel (Church D. L., 2004; WHO 2011). Thus, the use of antibiotics by any individual, farm etc. affects the whole world.

2.1.3 Mechanisms of antibiotic resistance

Certain soil bacterial species have been found to produce antibiotics; Streptomycetes, for instance, is well known to produce a variety of B-lactamases (Forsman *et al*., 1990; Ogawara *et al*., 1999). These species have the intrinsic ability to protect themselves against the antibiotics they produce or antibiotics with similar structure and mechanisms of action (Davies and Davies, 2010). Though the natural functions of the antibiotics are not well established, various studies have shown that the antibiotics help the producing-bacteria to overcome competition by inhibiting or killing other species (Davies and Davies, 2010). Such bacterial species, like the the Actinomycetes, are naturally multidrug resistant and can transfer these resistant genes to other bacteria; *S. pneumoniae* became resistant to penicillin by the acquisition of genes from the naturally occurring penicillin-resistant commensal *Streptococcus viridans* and the formation of penicillin binding proteins that are penicillin-insensitive (Spratt, 1994; Dowson and Spratt, 1994; Petkovic *et al*., 2006; Davies and Davies, 2010). Some antibiotic-producing organisms express resistance to their products through the use of efflux systems (Piddock, 2006b; Guilfoile and Hutchinson, 1991). In one study (Dantas *et al*. 2008), several soil bacteria from different environments were grown on 18 antibiotics and surprisingly, many were profusely growing on common antibiotics, including aminoglycosides and fluoroquinolones. These are species that contain enzymes capable of catabolising xenobiotics in the environment, raising questions about the potential of these enzymes to serve as resistance determinants. Resistance genes have been found in areas far away from human settlements and activities (Barlow and Hall, 2002; Allen *et al*., 2009; Bartoloni *et al*., 2009), posing the question of what came first: antibiotics or antibiotic resistant genes?

Upon exposure of bacteria to lethal doses of antibiotics, all susceptible species are eliminated, leaving resistant species behind to multiply and occupy the whole region of antibiotic application (Levy and Marshall, 2004), a process called natural selection. Through natural selection, resistant species

proliferate and dominate whole niches as there are no other bacteria to compete with them for space and nutrients or inhibit them with their secretions. These resistant species then serve as a pool for the dissemination of resistant species to other individuals in the environment Levy and Marshall, 2004). This phenomenon has been shown to breed multiple drug resistance among exposed bacteria (Levy, 1985; Levy, 2002).

1. Sequential mutations in the genome of bacteria occur upon exposure to sub-lethal antibiotic doses resulting in changes in the antibiotic target or binding sites (Wang *et al*., 2001; Levy, 2002; Schneiders *et al*., 2003). These changes in the target sites make the antibiotic ineffective in the bacteria, making the bacteria proliferate in the presence of the antibiotic.
2. Certain bacterial species respond to antibiotic exposure by producing enzymes that inactivate the antibiotic (Wang *et al*., 2001; Schneiders *et al*., 2003; Long *et al*., 2006; Piddock, 2006a).
3. The use of efflux pumps to pump out antibiotics from the bacterial cytosol, reducing the antibiotic concentration within the bacteria, is another means adopted by certain species to thwart the effects of antibiotics. By making their cell walls impermeable, some bacterial species are also able to keep out antibiotics, thus evading their toxic effects. Additionally, some ingenious species of bacteria naturally produce a lot of the antibiotic binding sites or proteins that bind with the antibiotic to reduce the antibiotic concentration within the cell (Davies and Davies, 2010). Through this process, called gene amplification, microbes developed resistance to the sulphonamides and trimethoprim (Brochet *et al*., 2008; Kashmiri and Hotchkiss, 1975).
4. Bacteria have the natural ability to transfer or receive genes from cells of the same species or from different genera (Levy and Marshall, 2004; Dowson and Spratt, 1994). This natural phenomenon can be advantageous for bacteria as they can acquire genes that are resistant to an antibiotic or series of antibiotics, making them multi-drug resistant (Levy and Marshall, 2004). Genes resistant to an antibiotic, called resistance genes, are found on extra chromosomal genetic elements like plasmids, integrons, transposons or gene cassettes (Levy

and Marshall, 2004). Transfer of resistance genes with virulent characters have occurred through this means (Norman *et al*., 2009).

2.1.4 Driving factors of Antibiotic resistance

The development and spread of resistant bacteria has been fuelled by the use, abuse and misuse of antibiotics in agriculture, aquaculture, human and veterinary medicine (Silbergeld *et al*., 2008; Chee-Sanford *et al*., 2009). One of the major instances of antibiotic use—for treatment, prevention or growth promotion—that selects resistant species and totally annihilate susceptible species is the use of a single type of antibiotic over a longer period of time (at least for more than ten days) (Moller *et al*., 1977; Levy, 1985; Levy, 2002;). Upon prolonged use, susceptible populations are wiped out to the extent that resistant ones occupy their niches, establishing a pool of resistant bacteria and resistance genes. Moreover, resistant species lose their resistance slowly (Seppala *et al*., 1997).

Antibiotics are excreted unchanged or partially or totally metabolised in human or animal faeces and urine after administration (Thiele-Bruhn, 2003; Boxall *et al*., 2004). These antibiotics persist in the faeces for longer periods of time to continually exert selective pressures on the enteric bacteria in the faeces, thus continuing the process of resistance development extra-corporally (Loke *et al*., 2000). Resistance can develop de novo in soil bacteria upon exposure to antibiotics in manure (Chander *et al*., 2005).

Farm workers have been implicated as an important means of spreading antibiotics and resistant bacteria through their homes into the communities (Gilchrist *et al*., 2007). This is as a result of their exposure to antibiotics and resistant species during farming activities (Van den Bogaard *et al*., 2000; Price *et al*., 2007). Consequently, exposure of farmers and farm workers to resistant microbes is a serious public health concern as they become carriers and disseminators of resistance via person-to-person contact (Smith *et al*., 2005; Saenz *et al*., 2006).

Disposal of farm waste releases resistant bacteria and antibiotics into air, water and soils (Silbergeld *et al*., 2008). There are no standards or limits to the amount of antibiotics or pathogens in animal waste in several countries though animal waste contains more pathogens and resistant species than

human faeces (Silbergeld *et al*., 2008). Coupled with farm waste management is the disposal of antibiotic containers and unused drugs (Buchberger, 2007; Utah Department of Health, 2007). Intensive food animal farms use ventilators to regulate heat and humidity. These high-volume fans blow considerable volumes of antibiotics, and bacteria through expelled dust into the atmosphere and surrounding environment (Silbergeld *et al*., 2008). Resistant bacteria and antibiotics have been detected in air around pig farms as high as 30m upwind and 150m downwind (Hamscher *et al*., 2003; Gibbs *et al*., 2006)

Animal products have been found to harbour many resistant bacterial species and have been pointed as a source of antibiotic residues which engender resistance among the consumers' microbial flora (White *et al*., 2001; NAS, 2003). Meat consumers may become colonised with resistant species through mishandling or insufficient cooking (Gilchrist *et al*., 2007).

Antibiotic resistant bacteria can be transferred from farms into the environment through animals which roam between the farm and the outside environment (Silbergeld *et al*., 2008). Insects and flies are usually found around farms hovering on feed or faeces. These flies or insects have been implicated in the transmission of *Campylobacter* infections in communities around farms (Nichols, 2005). Rodents have also been found to transfer pathogens to and fro food animal farms (Henzler and Opitz., 1992). Wild birds visit concentrated animal food farms to ravage through discarded farm waste for spilled feed. Contact between these animals and domestic animals or humans can transfer resistant bacteria into the environment.

Due to the ability of sub-lethal doses of antibiotics to trigger mutations that result in resistance genes (Gavalchin and Katz, 1994), substandard or poor quality antibiotics are a potential cause for the selection and spread of resistant species in vivo. Low quality antibiotics are common in developing countries due to poor drug regulations and lack of well-resourced analytical laboratories. As a result of higher tropical temperatures, antibiotics which can degrade at temperatures above 25°C can break down into less active metabolites, reducing the concentrations of the antibiotics in these formulations. Higher temperatures reduce the shelf lives of antibiotics due to its effect on the half-life of the drug

ingredients. These are common during transportation, distribution and storage of the medicines. Antibiotic peddlers in the streets of developing countries are unskilled in their handling, exposing them to direct sunlight and heat that can degrade them (Okeke *et al*., 1999).

Many international donors and pharmaceutical companies ship expired or almost to expire drugs to developing countries under new labels or unchanged labels (Berckmans *et al*., 1972; Gustafson and Wide, 1981; Ali *et al*., 1988).

Counterfeit drugs that contain a lower concentration of antibiotics or substandard excipients are common in developing countries where cheaper prices are used as a means to obtain larger shares of the antibiotic market. Most of these drugs are undetected due to the unavailability of analytical facilities to check such medicines and the prevalence of many generic drugs on the market, many of these counterfeits go undetected. Substituting the original excipients with cheaper alternatives affect the bioavailability of the antibiotics *in vivo*, allowing the antibiotic to stay in the stomach or intestines longer than necessary to select for resistant phenotypes (Ogunbona and Akanni, 1985; Okeke *et al*., 1995; Okeke *et al*., 1999). Many herbal preparations in the developing countries have been found to contain orthodox medicines, some of which are antibiotics, in order to augment the effect of the herbal preparations. Such practices have been reported in Taiwan of Chinese herbal preparations marketed there (Huang *et al*., 1997)

Antibiotic susceptibility testing (AST)

The development of resistance to antibiotics by microbes prompted clinicians to demand a test of the causal organism against varying concentrations of different antibiotics to determine the resistance or susceptibility of the pathogen (Hudzicki, 2010). Though the original method of determining bacterial susceptibility to antimicrobials was the broth dilution methods (Kirby *et al*., 1957; Jorgensen and Turnridge, 2007), which still remains the gold standard, the relative difficulty associated with this method prompted the development of the disc diffusion method. Most clinical microbiologist had accepted the disc diffusion method by the 1950s albeit different methods and standards including different inoculum sizes and concentrations, media, incubation temperature, incubation time and

antibiotic concentrations (Bauer *et al*., 1959; Hudzicki, 2010). Conflicting publications by various researchers using different techniques made the WHO, based on a publication from Kirby and Bauer, to form a committee that lay the groundwork for standardized procedures to be used for antibiotic disc susceptibility tests called the Kirby-Bauer method (Bauer *et al*., 1959; Bauer *et al*., 1966; Jorgensen and Turnridge, 2007). The Clinical Laboratory Standards Institute (CLSI), responsible for setting standards and uniformity in antibiotic susceptibility testing, publishes interpretative guidelines for zone sizes which are used by clinical laboratories (CLSI, 2006).

Antibiotic sensibility testing using the Kirby-Bauer's method is carried out using Mueller-Hinton (MH) agar due to its acceptable batch-to-batch reproducibility, low sulphonamide, trimethoprim and tetracycline inhibitors, and its support for the satisfactory growth of non-fastidious pathogens and the already voluminous research data and experience gathered from susceptibility tests performed with this medium (Winn *et al*., 2006). Using any other media aside MH agar will result in erroneous zones which cannot be compared with the CLSI breakpoints interpretation. The antibiotic discs containing standard concentrations of the antibiotic are placed on the surface of the MH agar already inoculated with the test organism. Fresh overnight cultures made from a single-colony isolate is suspended into sterile saline water to attain a concentration commensurate with a 0.5 McFarland's standard; 0.5 McFarland's standard is equivalent to a suspension containing $1 - 2 \times 10^8$ CFU/mL of *E. coli.* Sterile cotton swabs dipped into the 0.5 McFarland's standard saline suspension are used to inoculate the surface of the MH agar (Hudzicki, 2010).

Antimicrobials in the susceptibility discs diffuse through the agar in three dimensions. Hence, the extent of diffusion and zone sizes are affected by the depth of the agar in the petri dish; shallow layers result in larger inhibition zones and vice versa. The discs draw water from the surface of the media to diffuse the antibiotic into the agar slowly depending on the diffusion and solubility properties of the drug in the MH agar (Bauer *et al*., 1966). As a result, a logarithmic reduction in drug concentration occurs farther away from the disc; the immediate surroundings of the disc are subsequently of a higher concentration (Jorgensen and Turnridge, 2007). Molecules with smaller molecular weights and

structures diffuse faster than molecules with larger ones. Consequently, these factors affect the breakpoint zone size, giving every antibiotic a different zone diameter (Hudzicki, 2010)

The breakpoint zones or margins around the discs do not indicate the extent of drug diffusion. However, they represent the extent or concentration at which the organisms are able to overcome the effects of the antibiotic. This is equivalent to the minimum inhibitory concentration obtained in broth dilution susceptibility tests (Hudzicki, 2010). Bacteria are able to grow in the presence of antimicrobials when the concentration is not able to totally kill or inhibit the organisms, allowing them to reach a critical mass that is able to overcome the lethal action of the antibiotic. The time taken to reach this critical mass depends on the organism and is affected by temperature and the media (Jorgensen and Turnridge, 2007). The zones are in themselves meaningless (Jorgensen and Turnridge, 2007). The interpretation of resistance or susceptibility is done through in vivo blood and urine test to establish the obtainable level of an antibiotic that is capable of resolving an infection. The CLSI correlates this data with zone sizes to define interpretive standards. These standards are reviewed and published periodically by the CLSI.

2.2 Some protocols in isolating and identifying selected Enterobacteriaceae

MacConkey agar contains bile salts and crystal violet to inhibit gram-positive bacteria. Due to lactose and neutral red dye (pH indicator), it can differentiate rapid lactose fermenters from slow or non-lactose fermenting bacteria. Coliforms show red to pink colonies due to the accumulation of acid acting on the neutral red indicator; non- coliforms are colourless (Talaro and Talaro, 2002).

Levine's EMB agar contains bile salts, eosin and methylene blue dyes to make lactose fermenters develop a dark nucleus and or a metallic sheen over the surface. Non-lactose fermenters are pale lavender and non-nucleated (Talaro and Talaro, 2002).

Indole production demonstrates the ability of an organism to cleave a compound called indole off the amino acid tryptophan. If cleaving occurs, Kovac's reagent reacts with the indole to produce a bright red ring at the surface of the media; absence of indole production will result in a yellow coloration of the medium even in the presence of Kovac's reagent (Talaro and Talaro, 2002).

Citrate media contains citrate as the only usable carbon source and bromothymol blue, a pH indicator. An organism that can utilize citrate as a carbon source grows and produces alkaline by-products that turn the medium from green (neutral) to blue (alkaline) [Talaro and Talaro, 2002].

The **Methyl red (MR) test** shows glucose fermentation with the accumulation of large amounts of mixed acids which lower the pH so that methyl red dye remains red upon its addition in the medium. MR negative organisms do not lower the pH and the medium remains yellow to orange after the addition of the methyl red dye (Talaro and Talaro, 2002).

The **Voges-Proskauer (VP) test** indicates if the product of glucose fermentation is a neutral metabolite called acetylmethylcarbinol (acetoin). Barritt's reagent reacts with acetoin to form a rosy-red or pink tinge in the medium. The tube remains brown to yellow if the result is negative (Talaro and Talaro, 2002).

TSI agar contains three sugars, namely glucose, lactose and sucrose and iron and phenol red indicator dye; consequently, it shows a combination of sugar fermentation reactions. It again shows the production of gas, hydrogen sulphide and acids; hydrogen sulphide is produced from the reduction of sulphur and can react with iron salts to form a black precipitate of ferric sulphide (Talaro and Talaro, 2002).

Table 2: Biochemical tests for differentiating common enteric bacteria (Talaro and Talaro, 2002)

Genus	Indole	Methyl Red	Voges-Proskauer	Citrate
Escherichia	+	+	-	-
Citrobacter	+	+	-	-
Klebsiella/Enterobacter	-	-	Species specific	+
Serratia	-	Species specific	+	+
Proteus	+	-	-	+
Providencia	+	+	-	-
Pseudomonas	-	-	-	Species specific

Key: '-'= Negative; '+' = Positive

2.3 Pig farming

Pig farming is not new in Ghana, but the system and practice has changed overtime. It has existed since pre-colonial times, of course under widely different methods from today. Several traditional and religious beliefs have deterred its progress into full scale commercial production, making the industry lag behind its enviable rival, poultry (Okai, 2010).

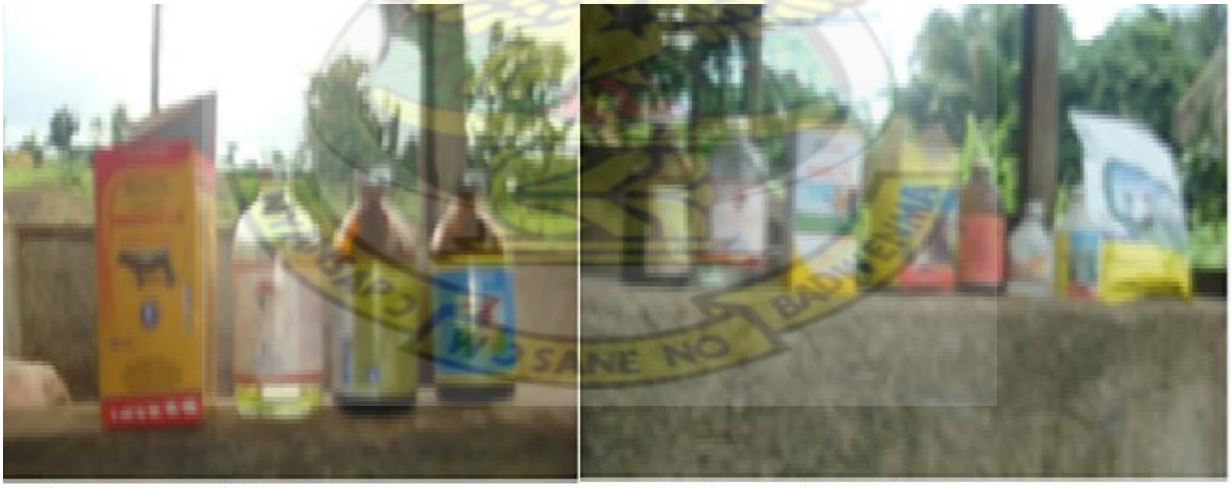
Pig farming is shifting from the free range subsistence form to a confined and commercial system. One of the major reasons underlying the dislike for pork in Ghanaian markets, aside the religious and cultural influences, is the unhygienic environments in which they are raised (Okai, 2010).

Figure 1: view of modern pig houses under intensive pig farming in Ashanti Region, Ghana.

In order to make pork more marketable and appealing to a larger section of the population, efforts were made by the Government to organise pork shows to increase public interest and awareness in the potential of pork as a source of meat and protein (Okai, 2007; Okai, 2010). These efforts among others have had an enormous influence in reshaping the pig industry in Ghana. Pork is quickly and

increasingly becoming accepted in Ghanaian markets. There are persons by the road sides who sell roasted and barbecued pork to the public on a daily basis and during occasions (Okai, 2010). Pig feet in brine, which used to be imported, are in most cases now obtained from Ghanaian abattoirs where pigs are butchered and sold per pounds basis (Okai., 2007).

The drift towards commercialised pig farming however, is not without public health implications. Whereas the new drive towards intensive pig farming has far better advantages, it has introduced relatively new and hitherto unconsidered challenges. All pig farmers use disinfectants to ensure the sanity and health of the stys. The use of antibiotics by pig farmers, as a result of commercialisation of the pig industry, has raised brows about the possibilities of having their residues in the pork and of causing antibiotic resistance among bacteria. These fears are not unwarranted as antibiotic residues can lead to antibiotic build up in the consumer; a situation which can have dire consequences on the antibiotic resistance levels of the consumer's internal microbial flora exposed to these residues (Kobe *et al*., 1995; Richter *et al*., 1996). The use of antibiotics in pigs and the consequent possibility of causing resistance among bacteria in the pigs could endanger our food chain and lead to epidemics of difficult-to-treat infections.



Behinase, Bosomtwe district | Aboaso, Kwabre east district

Left and right pictures were taken in Behinase (Bosomtwe district) and Aboaso (Kwabre East district) respectively on October 2012. Both pictures show drugs typically used in pigs as consisting mainly of liquid dosage forms for injections; only one antibiotic product (last item in right picture) is a water soluble powder for oral administration.

Figure 2: Antibiotics used in pigs' health management.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1.1 Materials and equipment

i. Sterile sample tubes
ii. Cotton wool
iii. Inoculating loops
iv. Reciprocal Water bath Shaker (Model R76; New Brunswick Scientific, Edison, N. J., USA)
v. Test tubes
vi. Autoclave ('Express Equipment' Arnold and sons Ltd., Basildon, UK)
vii. Petri dishes (100mL)
viii. Spatula
ix. Antibiotic cartridges/discs (Oxoid, UK)
x. Incubator (Gallenkamp plus II, UK: SN: SG93/07/695)
xi. Antibiotic disc dispenser (Oxoid, UK)
xii. Conical flasks
xiii. Electronic balance (Sartorius electrical balance, Germany)
xiv. Ruler
xv. Micropipette (Eppendorf, Germany)
xvi. GPS mapping instrument (Etrex from Garmin, USA: 16Q446957)
xvii. Forceps
xviii. 0.5 McFarland's standard
xix. Sterile cotton wool swabs
xx. SANYO Microwave, Japan (EM-S1055S)

3.1.2 Reagents and media

i. Bismuth sulphite agar (CM 0201; Oxoid, UK)
ii. MacConkey agar (Scharlau-01-118; Germany)
iii. X.L.D. agar (LAB-LAB032; Oxoid, UK)
iv. Mueller-Hinton agar (CM 0337; Oxoid, UK)
v. Soya peptone broth (N. L44; Oxoid, UK)
vi. TSI agar (CM0277; Oxoid, UK)
vii. Sterile water
viii. 0.85% saline
ix. Isopropyl alcohol

3.1.3 Organisms

i. *E. coli* ATCC 29212

ii. *Pseudomonas aeruginosa* ATCC 27853

iii. Pig faecal *E. coli, Proteus vulgaris, Proteus mirabilis, Salmonella spp. ,Enterobacter spp.* isolates

3.2 Methods: Pig farms Survey

3.2.1 Study Area

The study was conducted in five districts within the Ashanti Region of Ghana: Ejisu Juaben district (12 settlements), Atwima Nwabiagya district (12 settlements), Bosomtwe and Atwima Kwanwoma districts (5 settlements) and Kwabre-East districts (10 settlements). The study covered 108 farms in the five districts i.e. 43 in Ejisu-Juaben, 20 in Atwima Nwabiagya, 24 in Bosomtwe and Atwima Kwanwoma and 21 in Kwabre-East district. The main language spoken in these areas is Asante Twi.

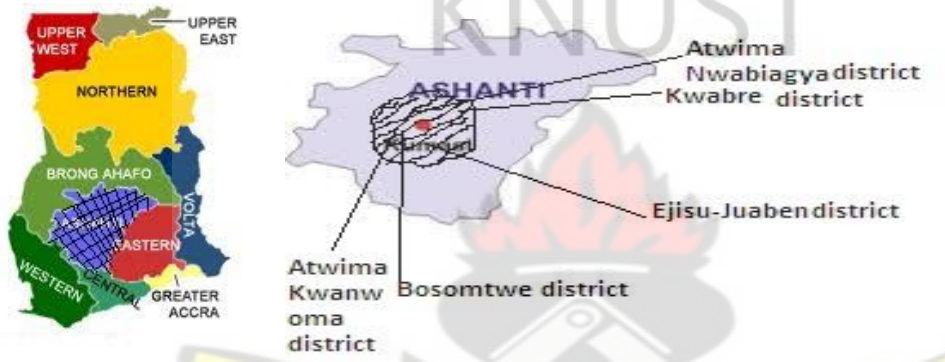


Figure 3: A map of Ghana showing Ashanti region (shaded region), where the study was carried out (left picture).

The circled and shaded area in the map on the right shows the areas/districts where the study was carried out within the region

3.2.2 Data collection

A pilot study was first conducted with forty farmers in the Ejisu-Juaben district to validate and develop questionnaires (Appendix A).

Farmer surveys: An elaborate structured questionnaire (Appendix A) was used to collect data from farmers. 200 questionnaires were sent out in all to farmers within five districts in Ashanti region: Ejisu-Juaben, Atwima Nwabiagya, Bosomtwe, Atwima Kwanwoma and Kwabre east. These districts are the recommended and recognised pig farming areas by the regional Veterinary Services Department of Ashanti region. Sampling of farmers for interviews was done using the list of farmers provided by various farmers associations in the respective districts. The questionnaire was designed to collect data on the storage conditions, hand washing practices after antibiotics administration, protection used during antibiotic handling and means of discarding waste water contaminated with

antibiotics. Questionnaire administration was done by trained field assistants in the local language, Asante-Twi.

3.2.3 Global Positioning System (G.P.S.) mapping

G.P.S coordinates of all the farms were recorded using a G.P.S. instrument [Etrex from Garmin, USA: 16Q446957] to produce a GPS map of all farms surveyed within the region.

3.2.4 Interviews with key informants

Interviews were conducted with ten key informants in the pig industry. They included veterinarians from the districts and at the regional offices, executives of the pig farmers' associations as well as leading researchers in veterinary medicine and animal sciences from Kwame Nkrumah University of Science and Technology (KNUST). The interviews focused on getting their views on antibiotics use by pig farmers, antibiotic residues and antibiotic resistance, withdrawal period of antibiotics and questions addressed in the questionnaires. Based on these interviews and that of a few farmers within the Ejisu-Juaben district, as a pilot study, the questionnaires were reconstructed, modified and validated.

3.2.5 Observations

Personal visits were made to each farm to observe farmers' practices. Observations were made on antibiotic administration practices, storage of antibiotics and disposal of waste effluents from the farms.

3.2.6 Analysis

All interviews with farm key informants were in English and were recorded in notes. The questionnaires, observations and interviews were structured into subthemes that guided the analysis. Quantitative data from questionnaires were entered into Microsoft Excel software (version 2010 from Microsoft Corporation registered to KNUST) and descriptive answers computed in verbatim text.

3.2.7 Ethical clearance/approval, collection and sampling of faecal material

Ethical approval was applied for and obtained from the KNUST ethical clearance committee through the department of Pharmaceutics. The work was commenced after the ethical clearance was obtained.

Fresh pig faeces were collected from 108 pig farms from the five districts, namely Ejisu-Juaben, Bosomtwe, Atwima Kwanwoma, Atwima Nwabiagya and Kwabre East districts, into sterile sample tubes. The tubes were dated and coded.

In order to be able to work on all five bacterial species within time, a representation of ten (10) faecal samples were chosen from each district, making a total of fifty (50) samples representing all the surveyed farms and districts.

3.3 Isolation and Identification of Enterobacteria

3.3.1 Preparation of pig faeces' suspensions

Fifty (50) tubes of soya peptone broths (Oxoid) were prepared and sterilised. Samples (100mg) from each of the fifty (50) selected faecal samples were suspended into the broths (50mls). The suspensions (Batch A) were incubated for 24 hours at 37°C. 1mL of each suspension from Batch A was transferred into 9mL of soya peptone broths; these were mixed and incubated at 37°C for 24 hours (Batch B).

Figure 4: Sample tubes containing collected pig faeces for bacterial isolation

3.3.2 Isolation of pure enterobacteria colonies

Fifty (50) tubes each of MacConkey, Bismuth sulphite and Xylose Lysine Deoxycholate (XLD) agars (20mL each) were poured and allowed to set. The bacteria to be isolated were *Escherichia coli, Proteus vulgaris, Salmonellae* and *Enterobacter spp.* These *Enterobacteriaceae* were used because they are in direct contact with administered antibiotics, offering an effective means of measuring antibiotics effects; there are several reports of their sensitivities in already published works, allowing easy comparability; *E. coli, Salmonellae* and *Enterobacter spp.* are WHO recommended indicator species for resistance surveillance studies (WHO, 2011)

1mL each of the soya peptone broth suspensions (Batch B) were diluted 10 fold with sterile water and single bacteriological loopfuls were uniformly spread on the surface of solidified agar media using sterile cotton swabs. The petri dishes were incubated at 37°C for 24 hours (NB: bismuth sulphite plates were incubated for 18 hours) (BP, 2007).

Pure or distinct violet-red colonies (coliforms or lactose fermenters) were isolated from the MacConkey agar plates (see previous paragraph) using a sterile needle and sub-cultured in fresh soya peptone broths (Batch C). The isolates were incubated at 37°C for 24 hours (BP, 2007).

Pure or distinct red colonies with or without black centres on the XLD plates and black or brown rabbit-eyed distinct colonies with a surrounding metallic sheen on the bismuth sulphite plates were likewise isolated with a sterile needle and sub-cultured in fresh soya peptone broths (Batch C). The isolates were incubated at 37°C for 24 hours (BP, 2007).

3.4 Biochemical tests

3.4.1 Indole production and Citrate utilisation

The pure isolates (10 µL each) in broth (Batch C; see paragraph 3.4.2) were inoculated into freshly prepared Tryptone water and Koser's Citrate medium and incubated at 37°C for 24 hours. Drops of Kovac's reagent were added to the Tryptone water after overnight incubation to determine organisms that were able to produce indole from Tryptone. Citrate utilizers (with blue cultures) were distinguished from non-citrate utilizers (with green cultures).

3.4.2 Triple sugar (glucose, lactose, sucrose) fermentation and sulphur reduction

Standard loopful of Batch C isolates (see paragraph 3.3.2) was streaked on the surface and deeply into the bottom of TSI (Triple sugar, iron) agar slants with an inoculating loop. The agar slants were incubated at 37°C for 24 hours (BP, 2007).

3.4.3 Antibiotic sensitivity testing

Quality control tests were carried out on Mueller-Hinton agar, *Pseudomonas aeruginosa* (ATCC 27853) with Gentamycin (an aminoglycoside) and *Enterococcus faecalis* (ATCC 29212) with Trimethoprim-Sulphamethoxazole respectively. The zones were measured and compared with the standard breakpoint values to verify the quality of the Mueller-Hinton agar (Hudzicki, 2010; EUCAST 2012).

Inoculum from the already prepared pure-colony suspensions (Batch C; see paragraph 3.4.2) were streaked on nutrient agar plates and incubated overnight at 37°C for 24 hours. Single bacterial colonies were isolated and transferred into 2mL sterile saline (0.85%w/v) until its turbidity equalled 0.5 McFarland's standard. The sterile saline was shaken vigorously (vortexed) and sterile cotton wool swabs were dipped into them and pressed on the sides of the tubes to remove excess fluid. The swabs were streaked on the surface of Mueller Hinton agar plates (20mL each) in all three directions and allowed to dry. The inoculation and streaking for each bacterial isolate was done within fifteen minutes (Hudzicki, 2010; EUCAST, 2012).

Table 3: Antibiotics used in sensitivity tests

Antibiotic discs used	Concentration (µg)
Gentamicin	10
Erythromycin	15
Streptomycin	10
Norfloxacin	10
Benzyl Penicillin	10 IU
Enrofloxacin	5
Ciprofloxacin	5
Tetracycline	30
Doxycycline	30
Amoxicillin	10
Suphamthoxazole-Trimethoprim (SXT)	25

Antibiotic sensitivity testing discs (table 3) from Oxoid and a semi-automated multi disc dispenser (Oxoid) were used to place the antibiotic-impregnated discs on the surfaces of the various plates. The plates were incubated at 37°C for 24 hours within fifteen minutes after disc placement (EUCAST, 2012).

Positive controls were set up for every batch of plates tested using *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 for *Pseudomonas aeruginosa* and Enterobacteriaceae respectively. Batches that failed the control test were repeated.

The zones of inhibition produced by the antibiotics were measured and compared with the CLSI tables to determine the susceptibility levels of the various bacterial isolates.

3.4.4 Data analysis

In testing for resistance to antibiotics, the antibiotics commonly used for pig production and also important in clinical medicine were chosen: Benzyl penicillin, Amoxicillin, Streptomycin, Ciprofloxacin, Enrofloxacin, Norfloxacin, Gentamicin, Tetracycline, Doxycycline, Sulphamethoxazole-Trimethoprim and Erythromycin. However, CLSI and EUCAST do not have breakpoint averages or ranges for Erythromycin, Benzyl penicillin and Enrofloxacin, making it difficult to determine their resistance or susceptibility except by comparison. Their inhibition zones

however (see Appendix C 1.3) are similar to those of their classes: benzyl penicillin with amoxicillin, enrofloxacin with the fluoroquinolones, erythromycin with amoxicillin (no macrolide breakpoint is available).

The zones of inhibition due to erythromycin, enrofloxacin and penicillin were analysed by comparing them to that of other antibiotics in the same class since there are no breakpoint tables for them (both from CLSI and EUCAST). Enrofloxacin is basically a veterinary antibiotic; hence CLSI and EUCAST, which deal with clinical antibiotics, do not provide breakpoints for it. CLSI and EUCAST do not provide breakpoints for benzyl penicillin and erythromycin (and other macrolides) because most organisms are assumed to be resistant to these agents. Consequently, benzyl penicillin and erythromycin zones are discussed by comparing them with amoxicillin; because penicillin and ampicillin are in one class with the same mechanism of action and macrolides (like erythromycin) were developed to treat infections resistant to methicillin (Davies and Davies, 2010).

The analysis of the data was generally done per organisms, antibiotics used and districts basis using SPSS (16.0) and Graph Pad Prism 5 software. The patterns and discrepancies resulting from the results of these categories were compared and contrasted.

CHAPTER FOUR

4.0 RESULTS

4.1 Response from questionnaires

A total of 200 questionnaires were distributed to farmers within five districts in the Ashanti region. 108 farmers responded to the questionnaires. Below is a summary of the number of farmers who responded to the various questions.

Table 4: Response from questionnaires

Question	Number of responding farmers (n)	Per cent of total (%)
Who manages the farm?	108	100
What is the educational level of farm manager?	38	35.19
Farm environment and farm locations	48	44.44
What is the source of farm water	81	75.00
What diseases on the farm are treated with antibiotics?	96	88.89
Dosage form of antibiotics	86	79.63
Routes of antibiotic administration	86	79.63
Storage sites of antibiotics	78	72.22
Disposal site of farm waste water	108	100.00
Do you wash yourself after antibiotic use?	68	62.96
Types of antibiotics used	86	79.63
Do you use protection during antibiotic handling?	62	57.41
Types of protection used	53	49.07
Is there direct contact with antibiotics during antibiotic handling?	70	64.81

4.2 Global Positioning system (GPS) Map of Pig Farms in Ashanti Region

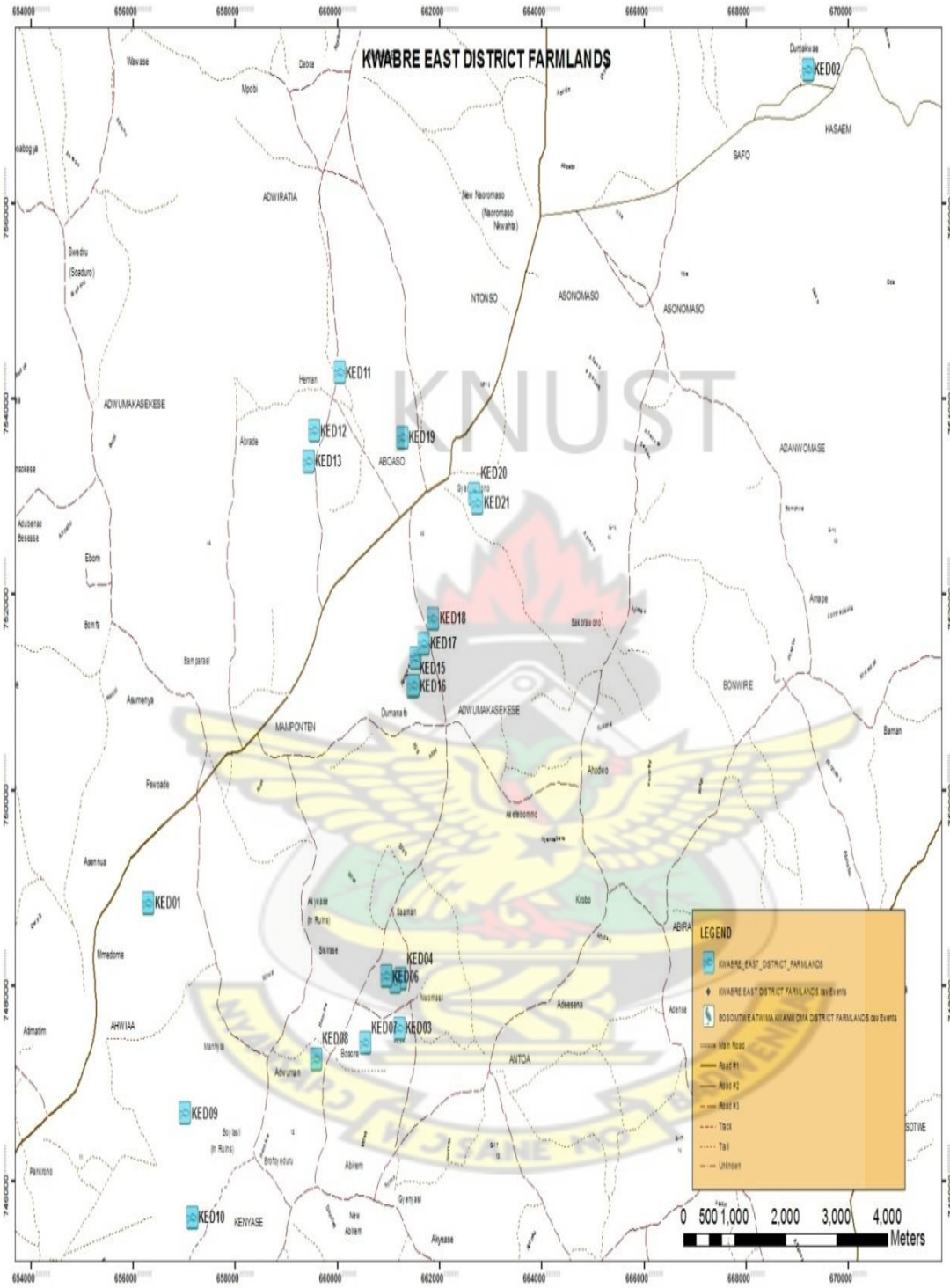


Figure 5: GPS map of pig farms in Kwabre East district. Each square shape represents a farm.

Only farms visited and used for the analysis are shown here. Hence, there are more farms within the district that are not appearing here.

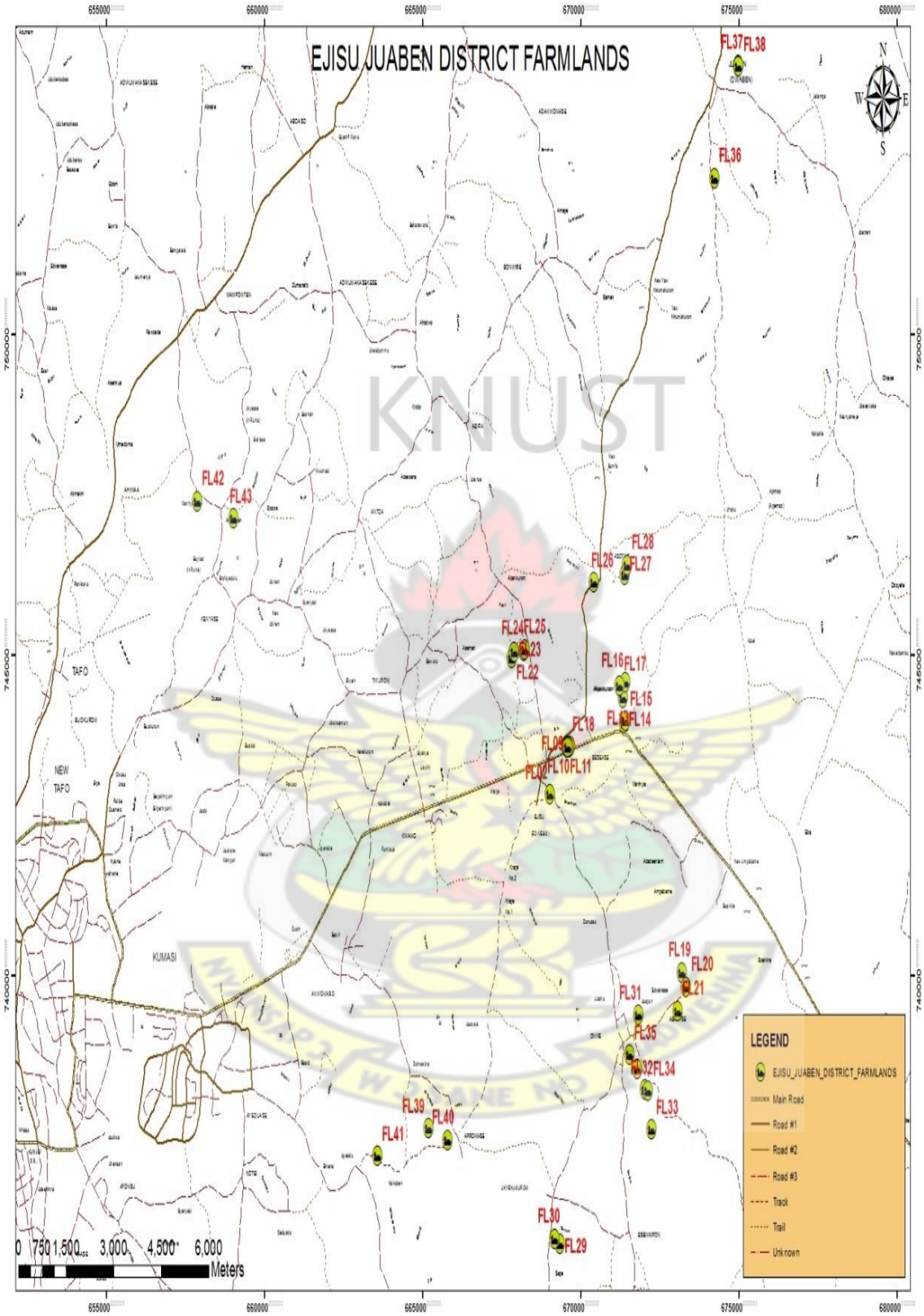


Figure 6: GPS map of Ejisu-Juaben pig farms showing only farms visited as round ribbons. Farms not visited are not shown here.

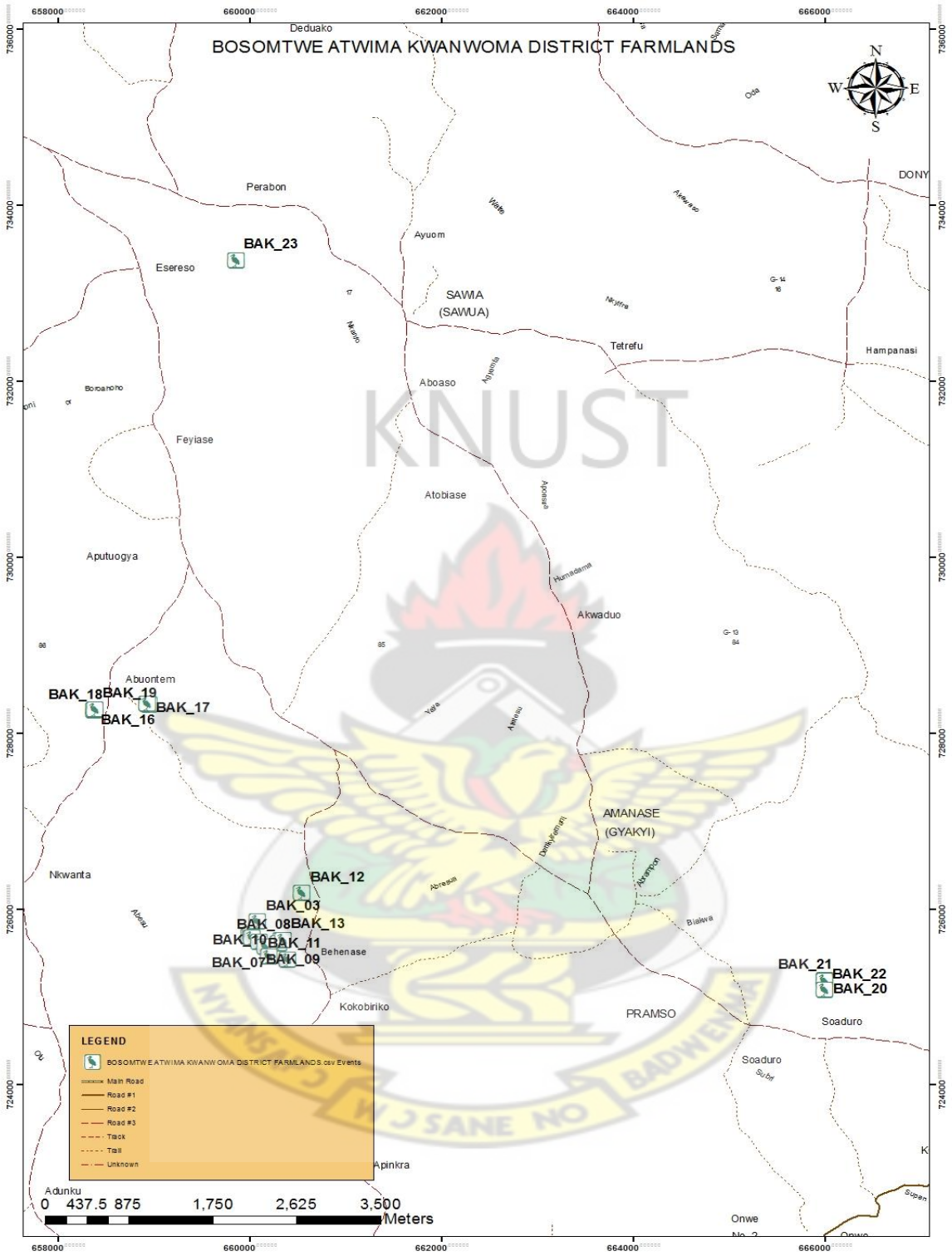


Figure 7: GPS map of Bosomtwe & Atwima Kwanwoma districts pig farms that were visited. Square symbols represent individual farms

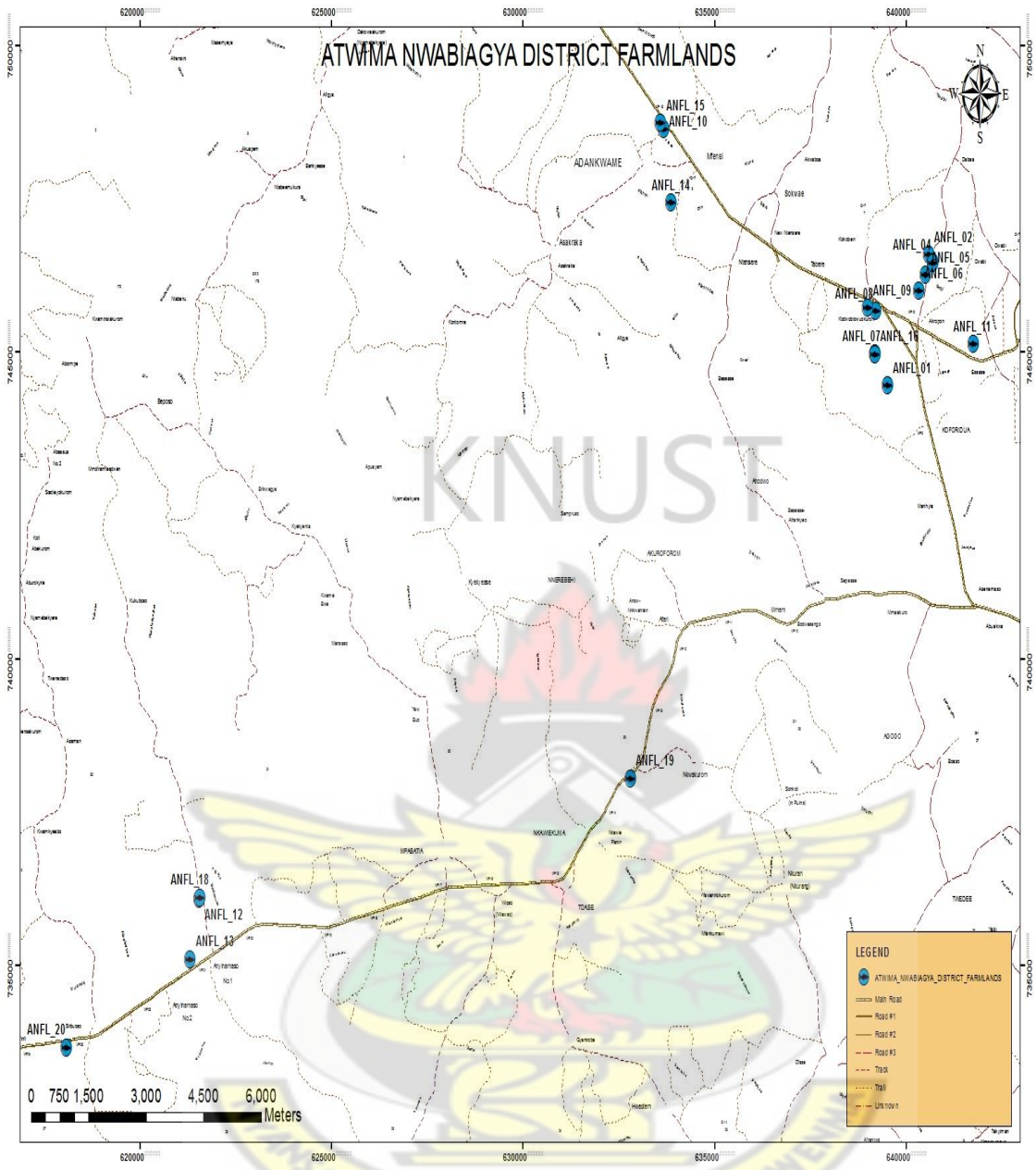


Figure 8: GPS map of Atwima Kwanwoma pig farms that were visited. Farms are shown as round ribbons with a dash in the centre.

94.8% of the farmers were males. They were mostly Christians with an average age of 30 years. Wealthier farmers kept an average of 150 to 500 animals and poorer farmers kept an average of 50 animals. More than half of the famers had a post-basic education/secondary education.

The maps of five districts (Bosomtwe and Atwima Kwanwoma have been merged into one map because they used to be under one district until recently) within Ashanti region where pig rearing is prominent are shown in figures 4 to 8. The location of the farms on the map is demonstrated with coloured balloons and farm codes which are explained in tables 3 to 6. It must be noted that the farms shown on the map are not exhaustive. This is because not all farmers were available at the time of visitation and some of these farmers were not part of the pig farmers' associations present in all the districts; the data of the pig farmers were provided by these associations. In such circumstances, the association members were not willing to disclose the location of such farms as a punishment for their decision to stay away from the association. Consequently, the maps are representative of mostly the farms which are part of the pig farmers' associations and the farms which farmers were available during the time of visitation; however, there is also a minority of farms on the map which farmers were either absent or not part of the afore mentioned associations.

Most of the pig farms visited was sited outside the towns in bushes and marshes. Such was the case in all the districts visited. This is because of the destructive nature of the pigs, their foul smells, their noise and the general dislike for pigs by Muslim communities, some Christians and the existence of cultural taboos that forbid their presence (Okai, 2010). Consequently, the farms are forced to relocate as the residences expand towards the outskirts of the town where the farms are located. Such conditions have forced many farmers to acquire their own land and to move towards intensive pig farming, thus increasing the number of commercial pig farmers in the region (See appendix C).

The move towards commercial pig farming has not only made the industry and pork attractive to investors and consumers respectively, but also increased the financial commitments involved in their housing, feeding, healthcare, security etc. But for these factors, there would be more pig farms than represented on the maps—more farms have been forced to close down due to their inability to live up to the financial pressure of the fast-growing industry. It is worth noting however, that such close associations of pigs together in one farm, is an easy means of spreading resistant genes and bacteria among all the animals in a given farm through the pigs' interactions and the farm hands (workers).

From the maps (figures 4-7) and tables (3-6), the representation of farms in the Ejisu-Juaben district far outnumbers those in the other districts. This is true not only on the maps but in reality. It is the only district with two competing pig farmers' associations. With years of interactions and knowledge sharing between the numerous farms within this district, the farmers have a much developed and well-honed expertise in pig rearing vis-à-vis the other districts. Through such interactions, very false information can easily spread and become established among the farmers within a district and beyond. Atwima Nwabiagya district also have a well-developed farmers' association though not so much than Bosomtwe, Atwima Kwanwoma and Kwabre east districts.

It can also be seen that some towns have a much concentrated number of pig farms than others. This was true in all the districts. Some towns within the districts had no pig farms at all whereas some were famous for the high number of farms. Ejisu and Onwe (in the Ejisu-Juaben district), Behinase (in the Bosomtwe district), Wadie Adwumakase (in the Kwabre east district) and Akropong (in the Atwima Nwabiagya district) are towns with very close and highly concentrated pig farms. Such a high density of farms can be a recipe for disaster in cases of disease outbreak or spread of resistance genes between farms. Such a threat is precipitated by the fact that some farmers borrow or hire boars with good pedigrees to mate their sows. Farmers also buy and sell piglets among themselves. In the course of the farm visitations, one farmer in Onwe (Ejisu-Juaben district) reported that there was an outbreak of pig flu and H1N1 in his farm and in all the surrounding farms (even in farms several towns away) some few years back. He believed it was from a nearby farm.

Though many of the pig farms visited were sited far away from residential areas, such was not the case in Ejisu where rapid settlement has brought the pig farms within the neighbourhood of communities. Such will be the case in the other areas as the settlements increase. The towns and communities near such farming areas are at risk of contracting zoonosis from these farms. This possibility is now more concerning with the increasing incidences of resistance due to antibiotic use among pig farmers. The practices of the farmers with waste management (Appendix C 1.1.5, 1.1.14) can also be a communal threat due to the closeness of these farms to water bodies (54% of the visited

farms: see section 4.2.7). These foreseen dangers make the need to tackle antibiotic resistance and regulate the use of antibiotics among food animal farmers more pressing.

4.3 Survey results

4.3.1 Types of antibiotics used

Pig farmers resort to antibiotics for prophylaxis and therapeutics. Whereas very few farmers (about 3%) occasionally use clinical antibiotics, all the farmers on the average use veterinary antibiotics. All the antibiotics used by the farmers fall into the classes used for clinical medicine. They were the same antibiotics or different ones of the same class. The tetracycline class of antibiotics was the most highly 36atronized among farmers in all the districts. The penicillins and combinations of penicillin and streptomycin were also common among the pig farmers (table 5); note that penicillin-streptomycin (in table 1) includes medicines containing only penicillins, only streptomycin and those containing both combinations.

Table 5: Number of farmers using particular antibiotics for pig farming per district

Antibiotics	Ejisu-Juaben (n=43)	Bosomtwe &Atwima Kwanwoma (n=24)	Atwima Nwabiagya (n=20)	Kwabre East (n=21)	Total (n=108)
Penicillin-streptomycin	25	3	10	10	48
Tetracyclines (Oxytetracycline, Doxycycline, Remacycline, tetracycline)	31	9	16	8	64
Tylosin	4		2		6
Fluoroquinolones (Enrofloxacin Norfloxacin)	3		2	5	10
Sulfadimidine	9	2	13	7	31
Amoxicillin			1		1
Metronidazole (clinical)	6				6
Erythromycin	1			4	5
Trimethoprim	2		2		4
Gentamycin	2		2		4

Key: n is number of farmers per district

Table 6: Percentage of farmers using common antibiotics and their brands throughout the districts

Antibiotics used among pig farmers	Number of brands commonly used by farmers	Number of users/farmers	Percentage of user farmers
Procaine benzyl penicillin	6	45	20.45%
Dihydrostreptomycin	8	50	22.73%
Chlortetracycline	1	9	4.09%
Gentamicin	1	4	1.82%
Sulphadimidine	8	33	15%
Trimethoprim	1	4	1.82%
Oxytetracycline	21	58	26.36%
Enrofloxacin	4	9	4.09%
Erythromycin	2	5	2.27%
Norfloxacin	1	2	0.91%
Amoxicillin	1	1	0.46%

Tables 5 and 6 show the types of antibiotics and the frequency of their brands commonly used by pig farmers in the region respectively. A close observation shows a direct relationship between the most patronized antibiotics and their respective number of brands on the market: the higher the patronage of an antibiotic among the farmers, the higher its number of brands containing and vice versa.

The antibiotics commonly used among the pig farmers are the tetracyclines, benzyl penicillin, streptomycin and sulfadimidine. Benzyl penicillin and streptomycin are mostly combined into one product. Brands containing only benzyl penicillin or streptomycin are few compared to those containing both. Next to the tetracyclines, penicillin-streptomycin brands enjoy the highest patronage in Ejisu-Juaben district and the highest in the Kwabre East district. Streptomycin is always in the form of dihydrostreptomycin in all veterinary preparations. In table 8, preparations containing both benzyl penicillin and dihydrostreptomycin as a combination are counted separately under procaine benzyl penicillin and dihydrostreptomycin and not together under the same heading. Though gentamicin is also an aminoglycoside, it has a negligible use in the pig industry; there's currently only one brand containing gentamicin. Amoxicillin is also one of the least used penicillin antibiotic among the farmers. Benzyl penicillin is the veterinary penicillin antibiotic of choice. Amoxicillin is more

common in poultry than in piggery where the antibiotics are soluble powders; the amoxicillin seen during the farm visit was a clinical antibiotic being used with the pig water to treat and prevent diarrhea at a farm in Onwe (Ejisu-Juaben district).

The tetracyclines enjoy the highest patronage in all the districts except in Kwabre east where they are only second to benzyl penicillin and dihydrostreptomycin preparations. As shown in tables 5 and 6, all antibiotics falling within the tetracycline class are put under the same heading. Even among the tetracyclines, oxytetracycline happens to be the ubiquitous tetracycline in all veterinary tetracycline brands. In a few number of cases, doxycycline, remacycline, tetracycline and chlortetracycline are found in veterinary antibiotic preparations. Chlortetracycline, however, is the tetracycline of choice for antibiotic aerosols; it is difficult to find an antibiotic aerosol without chlortetracycline.

Sulfadimidine is the most common sulfonamide antibiotic used in liquid veterinary antibiotics. Other sulfonamides are like sulfaquinoxaline, sulfadimethoxine, sulfadiarine, sulfadiazine etc. are common in powdered antibiotic preparations than in liquid ones. Hence, in piggery where most of the antibiotics are liquid for injection (see Appendix C 1.1.12), sulfadimidine is the commonest. Sulfadimidine is one of the commonest antibiotics used among pig farmers, especially in the Atwima Nwabiagya district where they are the highest used antibiotic. Together with dihydrostreptomycin, it has the second highest number of brands. Sulfamethoxazole is not used by pig farmers.

The fluoroquinolones, macrolides and trimethoprim are rare antibiotic classes in Ghanaian piggery. The major fluoroquinolones used in pigs are enrofloxacin and norfloxacin; no other fluoroquinolone was seen beside these. Erythromycin and tylosin are the commonest macrolide antibiotics, but tylosin is easy to find than erythromycin which is rather common in poultry as a water soluble powder in combination with other antibiotics. Trimethoprim containing brands are always combination products containing other antibiotics like erythromycin, streptomycin and gentamycin.

There are a few farmers who usually use clinical metronidazole for treatment and prevention of diarrhea. There is hardly a veterinary antibiotic for pigs containing metronidazole. All metronidazoles seen during the farm visits were clinical brands.

4.3.2 Farm owners and farm managers

The pig farms surveyed for this study were not wholly managed by the owners themselves. In estimation, 69.23%, representing 72 farms were directly supervised by the owners themselves. Only 25.00% were supervised by hired or rented labourers who managed the farms for the owners. In some cases, the management of the farms was done by both the farm owner and the hired labour.

Table 7: Number of farms managed by their owners, hired labour or both

Farm managed by	Number of farms	Percentage (%) of farms
Farm owner	72	23
Farm manager	26	25
Both	6	5.77
Total	104	100

4.3.3 Educational level of farmers

The educational level of the farmers (in this case the farm managers), did not greatly influence the farming practices. The farm owners on the average were secondary school or A-level certificate graduates whiles the farm managers were in most cases Junior high school graduates or school drop outs. Ejisu-Juaben district recorded the highest number of responding farmers and highest number of farmers with tertiary education which is 11% of all the respondents. There was no respondent from Bosomtwe and Atwima Nwabiagya districts.

Table 8: Educational level of farm managers per district

Educational level	Ejisu-Juaben (n=24)	Bosomtwe and Atwima Nwabiagya (n=0)	Kwabre East (n=2)	Atwima Nwabiagya (n=12)	Total (n=38)
Basic-secondary	20 (83%)	-	2 (100%)	12 (100%)	34 (89%)
Tertiary	4 (17%)	-	-	-	4 (11%)
Grand total					**38 (100%)**

4.3.4 Source and level of farming knowledge

Most of the farmers or farm managers had learned about the practice of pig farming from their friends who had recommended the practice to them. A minority of these farmers had learned about the industry from their parents, teachers, neighbours or relatives who also kept pigs. Of this class, there were those who worked as farm managers in their youth for their neighbours, teachers, parents or relatives. A smaller number of the farmers had actually learnt about the industry from school. These fell within the highly educated section of the farmers. The highly educated farmers who had learnt about the business from school were more informed about the business and followed a more systematic method of husbandry; these understood the reason behind every practice. The majority of the farmers who learnt the farming from experience and from friends knew little about the rationale behind every practice. They had mastered the practice over time and were very skilful, though less informed. These farmers tended to depend more on the veterinarians than their highly educated colleagues.

4.3.5 Farm structure

The pioneer investors in the pig industry did not invest into very expensive housing. In this study, several farmers were encountered who still use very simple and inexpensive housing for their pigs due to the huge financial burden involved in modern housing units.

The type of housing used for housing the pigs was on the average, similar within the districts and reflected the financial status of the farmer; i.e. farmers within a particular district used common housing units. For instance, within the Ejisu-Juaben district, more than half (more than 60%) of the farmers housed their pigs in stys with cemented floors, wooden walls and palm roofs. This housing type however, was found in other districts in smaller numbers especially among poorer farmers. Upon enquiry, the farmers who adopted the simpler housing system argued that the wooden walls and palm-fronds roof was better suited to the pigs due to the tropical environmental conditions; this allowed greater circulation of air and better reduction of heat and odour. However, farmers who became rich from the sales of their animals moved towards improved and modern housing for their pigs with a

different reason that the latter provided better security from harsher environmental conditions and thieves. The wealthy farmers in all the districts used blocks for the walls and aluminium roofing sheets for the roofing.

4.3.6 Farm hygiene and waste treatment

Each type of housing unit has sloping floors and gutters that run from the inside of the sty to the outside. The farm hands clean up the stys with water and periodically with disinfectants in the morning and in the evening before feeding the pigs. Consequently, the stys are cleaner just before the pigs are fed and dirty at all other times; this is especially so as the animals are always defecating and urinating. The faeces are cleared with shovels and dumped behind the stys. Some of the farmers sweep the faeces through the gutters with the water they use to clean the sty floors. The latter practice leads to choked gutters behind the stys. A few farmers dump their faeces a distance from the stys either on the soils or in dug out pits. The liquid waste is left to run out into the soil behind the pig house. The faeces are thus left to rot and add up to the soil. Hence, it is not uncommon to find a hill of decaying pig faeces behind every pig house, filling the air with stench; one of the reasons why they are located far from residential areas.

There is no better waste management practice among the pig farmers surveyed. Because they only produce organic waste, they depend on nature to recycle their waste for them. One in four stys would be found clean and attractive at all times. This is as a result of the diligence of the farmers in cleaning up the stys often.

One rare practice which was only observed within the Ejisu-Juaben district was the use of water to bath the pigs continually. This made them look very healthy and hygienic with shining skin and lustrous hair. A considerable number of farmers within this district and other districts had water ponds within which the animals wallowed. The habit of the pigs to be dirty was thus checked by such practises. Farmers who carried out better hygienic practises had nicer and healthier animals and cleaner stys. A few farmers covered the whole pig house with insecticide treated nets to ward-off insects.

4.3.7 Farm environment and locations

58 (54%) of the 108 farms surveyed were located near water bodies vis-à-vis 50 (46%) farms not situated near water bodies.

Table 9: Water bodies around farms per district

Water body	Ejisu-Juaben (n=20)	Bosomtwe and Atwima Kwanwoma (n= 7)	Kwabre East (n=11)	Atwima Nwabiagya (n=10)	Total (n=48)
Stream	13 (65%)	1 (14%)	9 (82%)	6 (60%)	29 (60%)
River	3 (15%)	5 (72%)	1 (9%)	4 (40%)	13 (27%)
Lagoon	4 (20%)	1 (14%)	1 (9%)		6(13%)
Grand total	20	7	11	10	**48**

Pig farms are not located near residential areas, except in a few areas. The Asante's traditional view on pigs and the religious belief of many Christians and Muslims deter many from rearing pigs. The fatty content of pork makes it abhorred by most people as a health risk. The squalid nature of pigs and their association with putrefying matter and unhygienic environments make them objects of scorn. Being noisy animals and destructive animals, they tend to eat almost anything they find. Their strong stench quickly fills the air with unbearable odour. These among several factors, make them intolerable as domestic animals and abominable to be reared near people's houses. Consequently, pig farms are almost always located near swampy or marshy areas that are not suitable for residential buildings or most agricultural practices. In other places, they are cited near refuse dumps which are normally situated at a distance from homes. Currently, more and more of these farms are being situated on farm lands located within bushes or forests far away from human residence.

4.3.8 Sources of water and feed

There are no commercially-prepared pig feed in Ghana. Farmers make their own feed by collecting home and school food left overs, buying fish, soya beans, oyster shells, rice bran, biscuit waste, malt, maize bran and maize. They mix these in their own proportions and feed it to the animals. Some

farmers occasionally gave their animals grass, plantain and or cassava leaves. All the farms visited fed their pigs twice daily.

Feeding the pigs is a major problem for all farms. This is due to the gluttonous nature of the animals coupled with the difficulty in obtaining enough feed for them. The staple food of the pigs is malt and cereal products; but these however, are difficult to obtain continually in larger proportions due to competition from other pig farmers and other livestock farmers. These circumstances have made the raw materials used by the farmers to formulate feed for the pigs very expensive and few farmers are able to afford them continually. The rate and amount of food pigs consume weighs heavily on the farmers' pockets. It is not uncommon therefore, to see some pigs starving to emaciation in some farms. The number of pigs kept on every pig farm has therefore become a function of the farmers' wealth or ability to afford feed. Farmers with money to afford feed keep larger numbers of animals and vice versa. Farmers therefore regulate their animal population by determining when to impregnate the sows and when not to and when to sell off some of the animals and when not to.

The pig farmers associations (within the various districts) invite specialists from the University of Science and Technology and the district veterinaries to advise them on the formulation of feed and other management practices. But these recommended feed are quixotic for most farmers as it boils down to affordability.

Table 10: Sources of water for Pig farms per district

Water source	Ejisu-Juaben (n=36)	Bosomtwe & A. Kwanwoma (n=11)	Kwabre East (n=14)	A. Nwabiagya (n=20)	Total (n=81)
Well	32(88%)	8 (73%)	11 (79%)	11 (55%)	62 (77%)
Stream	2 (6%)	1 (9%)	3 (21%)	-	6 (7%)
Rain water	-	-	-	1 (5%)	1 (1%)
Pipe	2 (2%)	2 (18%)	-	8 (40%)	12 (15%)
Grand total	36	11	14	20	**81**

Every farm visited had its own water supply system. This was necessary for the constant hygiene of the farm and well-being of the animals. Being situated at the outskirts of town, most pig farms had no

access to water used by the community and had to find their own. Among the pig farmers surveyed, well water or bore holes was used by almost all the farms (76.54%). A few of them used pipe water, stream and rain water. The wells were more reliable than the other water sources due to its unfailing water supply. A few farmers went further to use pumps to force water from the wells into storage tanks which supplied the whole farm. The water from the wells, streams and rains were used exclusively for the pigs; human use was limited to domestic purposes except drinking.

4.3.9 Funding

Generally, pig farmers financed their farms from their own savings. Very few farmers solicited the help of relatives or resorted to loans in starting their farms. After their establishment however, many farmers try to access loans to aid them feed their animals. Sourcing for and obtaining loans is difficult for the farmers due to collateral requirements and the exorbitant interest rates. Farmers within the district's associations are better able to source for some loans through the aid of the association; but this source is not always reliable due to the large numbers of applicants. More and more farms are closing down due to insufficient funds to continue the business.

4.3.10 Distribution of the farms and their concentrations

Within the Ashanti Region, pig farming is predominant and ever increasing. The pig farms are distributed and concentrated within certain towns and districts (figures 5 to 8). According to the Regional veterinarian (personal interview), the Pig farmers are concentrated within five districts namely, Atwima Nwabiagya, Atwima Kwanwoma, Bosomtwe, Ejisu-Juaben and Kwabre East districts and the Obuasi Municipality. The farmers within these districts and municipality are organised into associations to see to their mutual interest. According to the farmers, the main reason for these associations is the feasibility in obtaining brewers malt from the Guinness Brewery in Kumasi and loans. Hence, farmers who feel they do not need the associations' help to access feed or who find it difficult to spare time, do not join or attend their meetings.

A critical analysis of the maps (figures 5-8) below will show that most of the pig farms within the Ashanti region are located within the Ejisu-Juaben district. It is the only district within the region with

two pig farm associations, each association being vibrant, well organised and composed of substantial and dedicated members. There are a few persons who belong to both associations in order to benefit from both. The concentration of farmers within Besease Gyamaase and Onwe are substantial, following after Ejisu though not so closely.

Within the Atwima Nwabiagya district, the concentration of farmers within Akropong far outweighs that of the other towns. A similar trend occurs in the Bosomtwe district where there are a far greater number of farmers than in the other towns. The distribution is well spread in the Kwabre East district where the farms are not concentrated in one town.

4.3.11 Common diseases affecting pigs

Table 11: Common diseases affecting pigs per district

Disease	Ejisu-Juaben (n=32)	Bosomtwe and Atwima Kwanwoma (n=6)	Kwabre East (n=11)	Atwima Nwabiagya (20)	Total (n=96)
Skin rashes	11	1	3	4	19
Cough	5	-	-	-	5
Diarrhoea	30	6	7	8	51
Worms infestations	6	-	-	2	8
anorexia	3	-	1	9	13
Pneumonia	2				

Diarrhoea happens to be the most prevalent condition or symptom affecting pigs within all the districts, being most prevalent within Ejisu-Juaben district. Skin rashes are the second most prevalent infection in Ejisu and Kwabre East districts. Pneumonia has the highest incidence in Atwima Nwabiagya district, a little higher than diarrhoea. On the whole, cough, pneumonia, skin rashes, worm and anorexia were not common or prevalent conditions affecting many farms. They occurred in few farms.

4.3.12 Knowledge base of the farmers concerning antibiotics

The farmers know very little about antibiotics except for their transfer and application of knowledge from clinical antibiotics to the veterinary ones. They are not very much informed about the uses of the medicines except through the veterinarians, experienced colleagues, veterinary shops and or years of experience. However, well-educated farmers are able to read the legends on the bottles and follow the instructions printed on the labels. Due to their limited knowledge and their dependence on experienced colleagues and veterinarians, most farmers are used to using one particular type of antibiotic. The transfer of knowledge from experienced colleagues to inexperienced ones through the farmers' association meetings and seminars help disseminate knowledge about specific antibiotics to the detriment of uncommon or new brands. It is therefore not uncommon to have most farmers within a district using a common antibiotic for a common problem or for every encountered disease. Hence, the antibiotics used by the farmers follow a predictable pattern.

100% of all respondent farmers used antibiotics for disease treatment. The extra cost involved in administering antibiotics to pigs for prophylaxis deterred farmers. Secondly, the farmers did not see the need to give medicines to healthy animals.

4.3.13 Dosage forms and routes of administration of the antibiotics

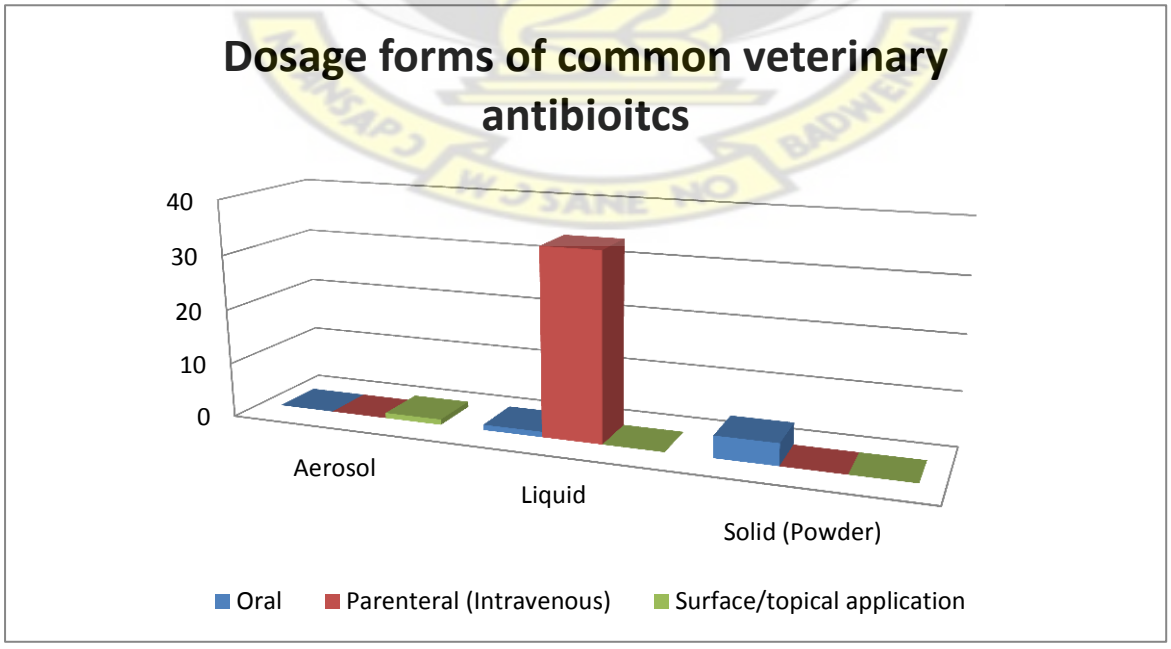


Figure 9: Dosage forms of veterinary antibiotics used by pig farmers.

Pig farmers obtain their antibiotics from veterinary shops in Kumasi and from the veterinarians stationed in the various districts. These veterinarians sell antibiotics to the farmers in their district offices or on the farm after seeing to their animals. Buying from the veterinarians is solely under prescription or after laboratory tests; such is not the case with the veterinary shops where veterinarians are in most cases absent. In the veterinary shops, off-label use and sales of antibiotics without prescription is common. In order to contain the spread of antibiotic resistance, it is imperative to regulate the activities of the veterinary shops where most farmers obtain their drugs and drug information. The WHO has mentioned off-label use, use without prescriptions and abuse of antibiotics as major causes in the spread of resistance to antibiotics (WHO, 2011).

Most of the antibiotics used in piggery were liquid dosage forms given via injection (fig. 17 and 18). These were mostly given through the ear intramuscularly or intravenously (Page and Gautier, 2012). The farmers did not understand the rationale behind injecting the antibiotics through the ear instead of through the skin (intracutaneously or intraperitoneally); they observed the veterinarians and also do likewise. Applying the medicines intramuscularly or intravenously ensured prolonged release circulation and onset of action respectively compared to the other injection methods since there is less fat in the ear to hinder the circulation and bioavailability of the medicine; the skin contains so much fat and poor blood circulation (Rang *et al.*, 2003)

Table 12: Dosage forms and routes of administration of antibiotics per district

		Ejisu-Juaben	Atwima Nwabiagya	Bosomtwe & Atwima Kwanwoma	Kwabre East
Dosage forms	Solid	18	1	-	11
	Liquid	63	41	13	23
	Aerosol	2	6	1	-
Routes of drug administration	Parenteral	57	37	13	23
	Surface/Topical	2	6	1	-
	Oral	24	5	-	14

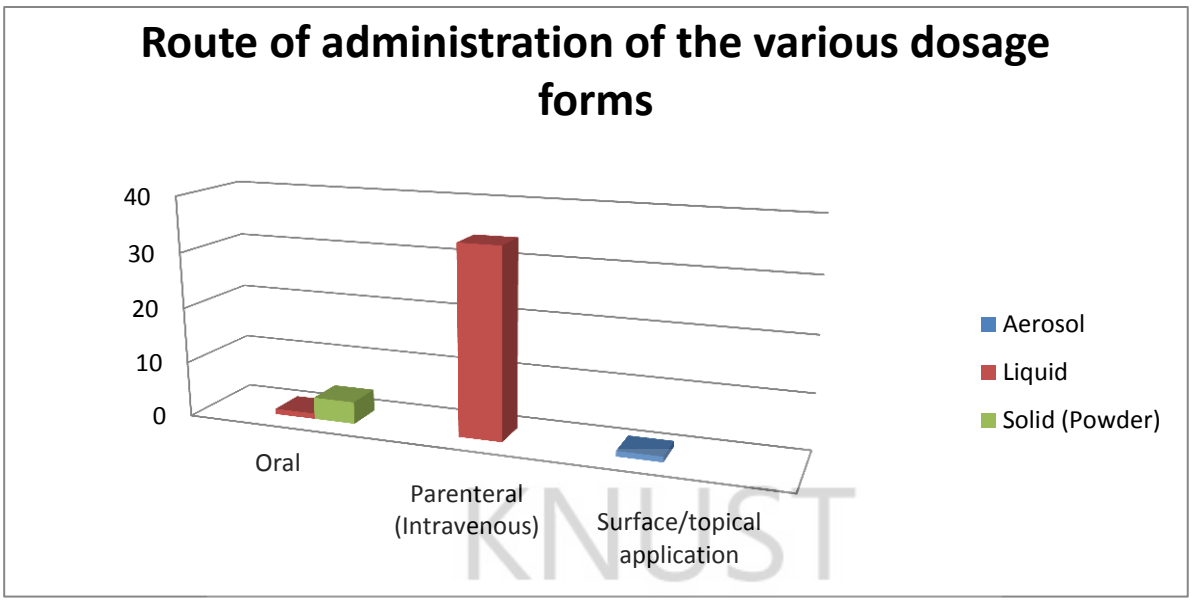


Figure 10: The routes of administration of the various antibiotic dosage forms used by the pig farmers.

A substantial number of medicines are administered orally in Ejisu-Juaben and Kwabre East districts. Atwima Nwabiagya has the highest number of aerosol antibiotics applied topically (table 14) though they are generally the least patronised dosage forms in all the districts. Liquid (dosage forms) antibiotics are mostly administered by injection. All the districts use more liquid antibiotics than other dosage-forms, depicting that most antibiotics are administered by injection.

4.3.14 Storage conditions of antibiotics

Figure 19 and table 15 show that most farmers keep their medicines on the floors of their rooms on the farms or in neglected stys and in shelves in all the districts; these two sites together form 61% of all the storage sites. One out of five farmers keep their medicines in paper or polystyrene boxes and 13% keep them in plastic or polythene bags. These storage sites do not pose a danger to the medicines but the immediate environments of these sites can. The number of farmers who store their medicines in boxes, cupboards and polythene bags are very low in all the districts compared to those kept on floors and shelves.

Figure 11: Common antibiotic storage sites

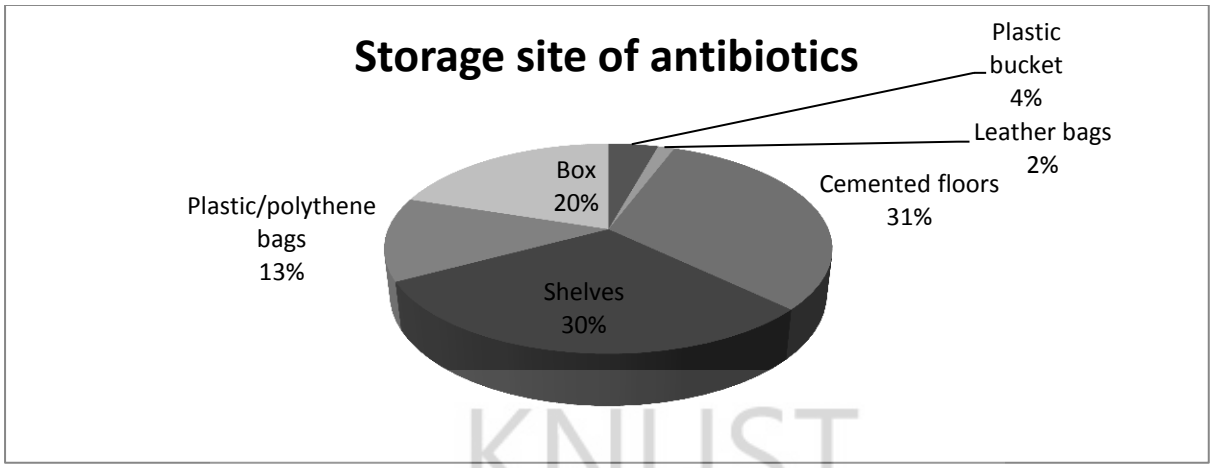


Table 13: Storage sites of antibiotics per district

Storage sites	Ejisu-Juaben (n=36)	Bosomtwe Atwima Kwanwoma (n=15)	Kwabre East (n=12)	Atwima Nwabiagya (n=15)	Total (78)
Cemented floor	8 (22%)	9 (60%)	3 (25%)	9 (60%)	29
Boxes	8 (22%)	-	2 (17%)	-	10
Shelves	10 (28%)	5 (33%)	5 (41%)	5 (33%)	25
Polythene bags	8 (22%)	-	2 (17%)	-	10
Cupboard	2 (6%)	1 (7%)	-	1 (7%)	4

4.3.15 Disposal sites of used antibiotics

Table 14: Antibiotics dumping sites per district

Dumping sites of antibiotics	Ejisu-Juaben (n=43)	Atwima Nwabiagya (n=20)	Bosomtwe & Atwima Kwanwoma (n=24)	Kwabre East (n=21)
Refuse dump	-	6	4	4
Sewage/ drains	23	17	10	24
Courtyard soil	53	22	-	5

Compared to the other districts, Ejisu-Juaben farmers dump most of their antibiotics on the soil or bare ground. In Atwima Nwabiagya, the number of antibiotics disposed through the sewage is very

close to that disposed unto the soil. Most farmers in Bosomtwe Atwima Nwabiagya and Kwabre East districts dispose their medicines through the canal or drains. Obviously, the dumping practices of the farmers vary from one district to another.

4.3.16 Body washing and protective measures

Table 15: Body washing and contact with antibiotics during and after antibiotic handling

		Ejisu-Juaben (n=37)	Atwima Nwabiagya (n=11)	Bosomtwe & Atwima Kwanwoma (n=5)	Kwabre East (17)	Total (70)
Direct contact with antibiotics during handling	Yes	13 (35%)	-	2 (40%)	4 (24%)	19 (27%)
	No	24 (65%)	11 (100%)	3 (60%)	14 (76%)	51 (73%)
		Ejisu-Juaben (n=27)	**Atwima Nwabiagya (n=19)**	**Bosomtwe & Atwima Kwanwoma (n=2)**	**Kwabre East (14)**	**Total (62)**
Farmers use some kind of protection during antibiotic handling	Yes	20 (74%)	13 (68%)	2 (100%)	7 (50%)	42 (68%)
	No	7 (26%)	6 (32%)		7 (50%)	20 (32%)

Seventy (70) out of seventy three (73) respondent farmers representing 96% of pig farmers, wash their hands after handling antibiotics. Of this number, 83% wash their hands using soaps and water, 8% use water only and another 8% use disinfectants with water. It is only in Atwima Nwabiagya district, where 3 out of 19 farmers representing 16% of farmers, do not wash their hands after handling antibiotics. It is these three that forms the 4% of farmers among all respondents who do not wash their hands after antibiotic handling. Among the percentage of farmers who use soap and water only are those who bath after antibiotics handling; it is worthy of note that it is only in Ejisu-Juaben district that farmers bathed after handling antibiotics or general farm work. Throughout all the districts

however, majority of the interviewed farmers used soap and water only to wash themselves and a minority used disinfectants or water only.

Table 15 shows that none of the respondents from Atwima Nwabiagya gets contact with antibiotics during their handling. A few farmers get contact with antibiotics in Kwabre East, Bosomtwe and Atwima Kwanwoma districts. The number of farmers who get contact with antibiotics in Ejisu-Juaben district is very substantial (35%), more than half the number of those who do not get contact with the antibiotics. On the average however, majority of the farmers did not get contact with antibiotics during their use.

Table 16: Means of body washing after handling antibiotics

Means of body washing after handling antibiotics	Ejisu-Juaben (n=38)	Atwima Nwabiagya (n=16)	Bosomtwe& Atwima Kwanwoma (n=2)	Kwabre East (n=12)	Total (n=68)
Water only	2 (5%)	2 (13%)		2 (17%)	6 (8.8%)
Soap and water	28 (74%)	11 (68%)	2 (100%)	8 (66%)	49 (72.1%)
Bathing	8 (21%)	-	-		8 (11.8%)
Water and disinfectant	-	3 (19%)	-	2 (17%)	5 (7.4%)

From the interviews conducted, the farmers do not so much wash their hands because of handling the antibiotics but as a routine measure after every visit to the farm. According to most of the farmers, this is as a result of the strong scents emanating from their clothes and bodies after working on the farm; this practise helps them to become easily re-incorporated into society. The high number of farmers washing their hands after working on the farm helps prevent the transfer of antibiotics or bacteria from the farm to the house, one of the pathways of resistant bacteria transfer into the community.

Majority of the farmers in every district use some kind of protection whenever they administer antibiotics to their animals, forming 68% of all respondent farmers in the region. All respondents in Bosomtwe and Atwima Kwanwoma districts used some form of protection.

Table 17: Types of protection used by pig farmers during antibiotic use

Types of protection used	Ejisu-Juaben (N=29)	Bosomtwe & Atwima Kwanwoma (N=4)	Kwabre East (N=8)	Atwima Nwabiagya (N=12)	Total (n=53)
Rubber boots	18 (62%)	3 (75%)	5 (62.5%)	5 (41.7%)	31 (58.5%)
Gloves	15 (51.7%)	1 (25%)	6 (75%)	7 (58.3%)	29 (54.7%)
Masks	3 (10.3%)	-	4 (50%)	-	7 (13.2%)
Glasses	1 (3.4%)	1 (25%)	-	-	2 (3.8%)
Clothes	15 (51.7%)	2 (50%)	5(25%)	1 (8.3%)	23 (43.4%)
Gloves only	3 (10.3%)	-	2 (25%)	6 (50%)	11 (20.8%)
Clothes only	3 (10.3%)	-	1 (12.5%)	1 (8.3%)	5 (9.4%)
Rubber boots only	4 (13.8%)	1 (25%)	1(12.5%)	4 (33.3%)	10 (18.9%)
Rubber boots and gloves only	5 (17.2%)	1(25%)	-	1 (8.3%)	7 (13.2%)
Gloves, rubber boots, clothes and mask only	2 (6.9%)	-	4 (50%)	-	6 (11.3%)
Gloves, rubber boots and clothes only	2 (6.9%)	-	-	-	2 (3.8%)
Rubber boots and clothes only	5(17.2%)	1 (25%)	-	-	6 (11.3%)
Rubber boots, gloves and mask only	1 (3.4%)	-	-	-	1 (1.9%)
Glasses and clothes only	1 (3.4%)	1 (25%)	-	-	2 (3.8%)
Gloves and clothes	3 (10.3%)	-	-	-	3 (5.7%)

The most prominent protection adopted by farmers in all the districts during their handling of antibiotics is rubber boots, gloves and working clothes. In Ejisu-Juaben district, more than half of the farming population, that is one out of every two farmers, uses clothes, rubber boots or gloves when

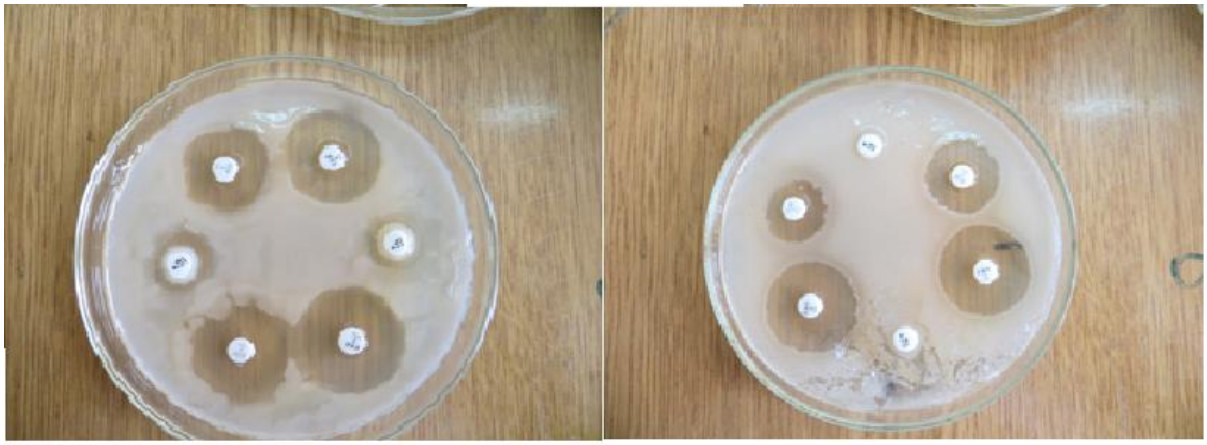
handling antibiotics; however, only 7% of all the farmers use all three items together when handling the antibiotics. Generally, nose masks and eye glasses are the least adopted among farmers in all the districts. The number of farmers who use only one item of protection or two or more protection items together is very abysmal throughout all the districts. Farmers in Atwima Nwabiagya, Kwabre East and Bosomtwe Atwima Kwanwoma do not have a very encouraging habit of wearing protective items during farming activities.

4.4 Antibiotic sensitivity test

CLSI and EUCAST have no breakpoint values for benzyl penicillin, erythromycin and enrofloxacin against members of the Enterobacteriaceae family; hence, only their zones are recorded here.

4.4.1 Antibiotic sensitivity test: Prevalence of resistance across the districts

The antibiotic susceptibilities of the enterobacteria isolates from all the districts are represented by figure 12 and table 18. Though there is a variation in resistance to the various antibiotics across the districts, the Ejisu-Juaben, Bosomtwe and Atwima Kwanwoma districts together have the highest percentages of resistance to all the antibiotics, except norfloxacin and doxycycline which resistances are highest in Kwabre east and Atwima Nwabiagya districts respectively. Gentamicin resistance is low throughout all the districts though it belong to the same class with streptomycin (aminoglycosides) which has the highest resistance throughout all the districts. This interesting observation shows that streptomycin resistance does not have a significant effect, by way of cross or co-resistance, on gentamicin resistance. Tables 5 and 6 reflect the low use of the fluoroquinolones and gentamicin in all the districts.



Antibiotic sensitivity results. The various zone sizes around the individual discs are meaasured to define resistant or susceptible bacteria using CLSI or EUCAST breakpoints. The discs overgrown with bacteria clearly shows the inability of the antibiotic to inhibit the growht of the bacteria

Figure 12: Antibiotic sensitivity results

Compared to the other districts, Bosomtwe and Atwima Kwanwoma districts use the lowest number, but not quantity, of antibiotics (tables 5 and 6): penicillin-streptomycin, tetracycline and trimethoprim. However, there is resistance to ciprofloxacin, gentamicin and sulphamethoxazole-trimethoprim. The factors leading to the high incidences of resistance within this district are difficult to comprehend especially when compared with the practices prevalent in the other districts. That the isolates from this district show more resistance to six out of eight antibiotics than all the other districts is concerning. Further studies must be carried out solely on this district, notorious during our visits for outbreaks of animal diseases, to better understand and help the farmers reduce the number of resistant species prevalent in the district.

Ejisu Juaben district had the highest prevalence of resistance (51.88%; table 18). Resistance to amoxicillin, streptomycin, tetracycline, doxycycline and sulphamethoxazole-trimethoprim is higher in this district. The least incidences of resistance are to the fluoroquinolones and gentamicin. The higher density of farms in this district plus the relatively higher numbers of antibiotics used make this data unsurprising. However, it is indicative of the fact that the district is breeding a lot of resistance genes that can be dangerous to the inhabitants.

Atwima Nwabiagya and Kwabre east districts also have very high prevalence of resistance to all the antibiotics in varying degrees; however, resistance to the fluoroquinolones and sulphamethoxazole-

trimethoprim is absent in the Atwima Nwabiagya district. In all, Atwima Nwabiagya has the lowest prevalence of resistance compared to the other districts. After Ejisu-Juaben, Kwabre east is the only district with resistance to all the antibiotics tested.

Table 18: Summary of isolated Enterobacteria susceptibilities

Summary of isolated Enterobacteriaceae sensitivity to antibiotics		Ejisu-Juaben district	Atwima Nwabiagya district	Bosomtwe and Atwima Kwanwoma districts	Kwabre East district	Grand Total
Amoxicillin	Susceptible	5	2	-	1	8
	Intermediate	1	3	1	3	8
	Resistant	19	3	7	5	34
Ciprofloxacin	Susceptible	21	9	5	8	43
	Intermediate	2	-	-	-	2
	Resistant	3	-	3	2	8
Norfloxacin	Susceptible	23	9	8	7	47
	Intermediate	2	-	-	-	2
	Resistant	1	-	-	1	2
Gentamicin	Susceptible	24	8	7	6	45
	Intermediate	1	1	1	1	4
	Resistant	1	-	-	1	2
Streptomycin	Susceptible	3	1	1	-	5
	Intermediate	4	2	-	3	9
	Resistant	19	6	7	5	37
Tetracycline	Susceptible	9	5	4	5	23
	Intermediate	8	1	-	1	10
	Resistant	9	2	4	4	19
Doxycycline	Susceptible	12	4	2	6	24
	Intermediate	5	1	3	1	10
	Resistant	8	4	3	1	16
Sulfamethoxazole-Trimethoprim	Susceptible	16	6	4	6	32
	Intermediate	1	1	-	1	3
	Resistant	9	-	3	3	15
Total	Susceptible	113	44	31	39	227
	Intermediate	24	9	5	10	48
	Resistant	69	15	27	22	133

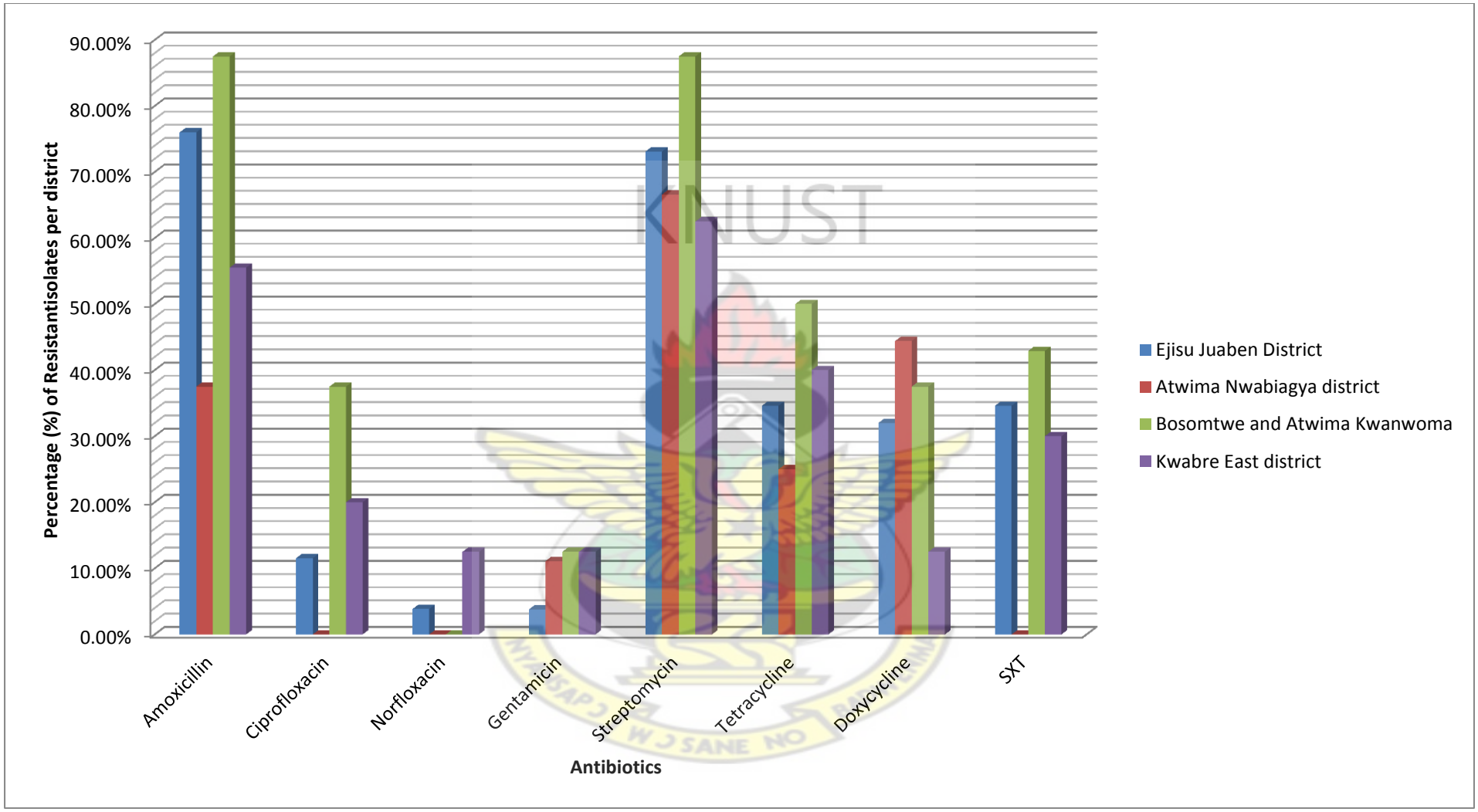


Figure 13: Antibiotic resistance per district.

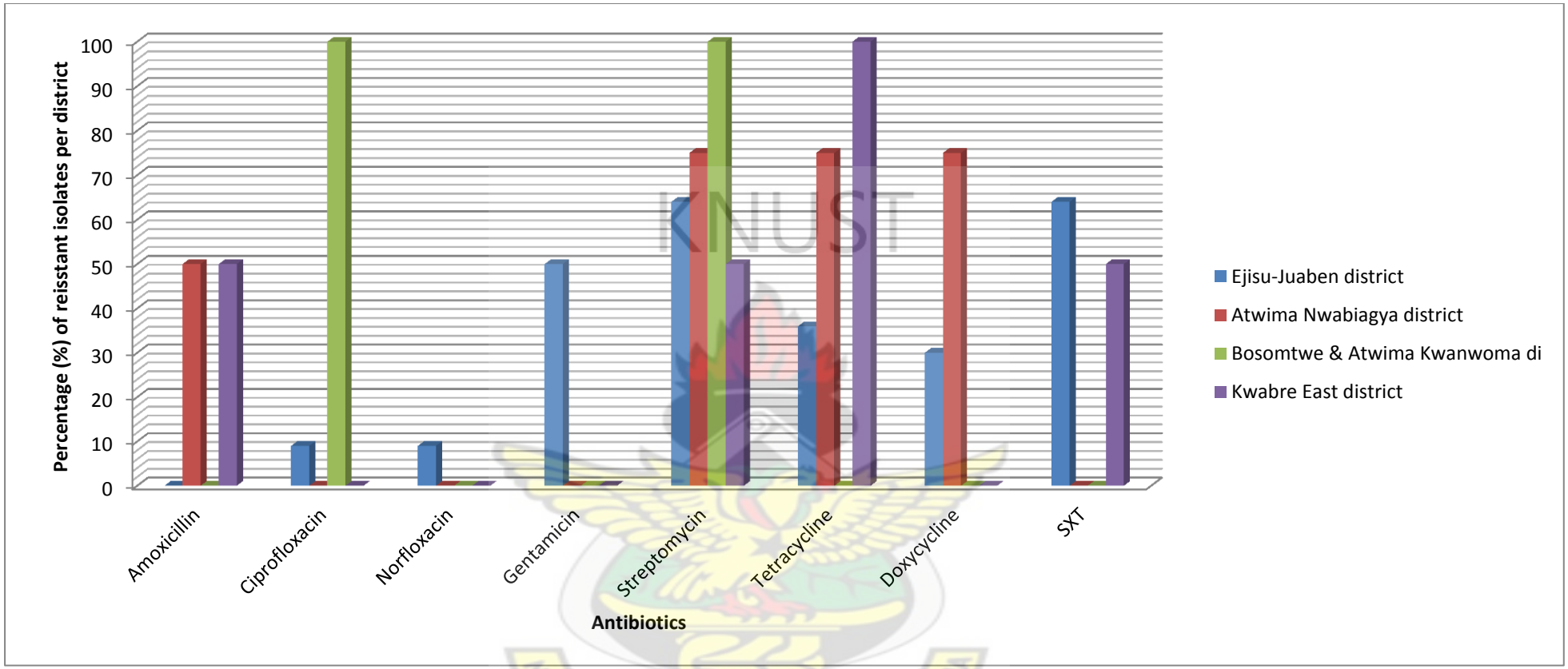


Figure 14: Resistance of *P. vulgaris* isolates from different districts

Proteus vulgaris isolates from Bosomtwe and Atwima Kwanwoma districts were all resistant to ciprofloxacin and streptomycin whiles those from Kwabre East were all resistant to tetracycline. Resistance to streptomycin and the tetracyclines from Atwima Nwabiagya isolates are also very noticeable. Clearly, the *Proteus vulgaris* isolates from Ejisu-Juaben, Atwima Nwabiagya and Kwabre East are multidrug resistant as they show resistance to almost all the antibiotics tested.

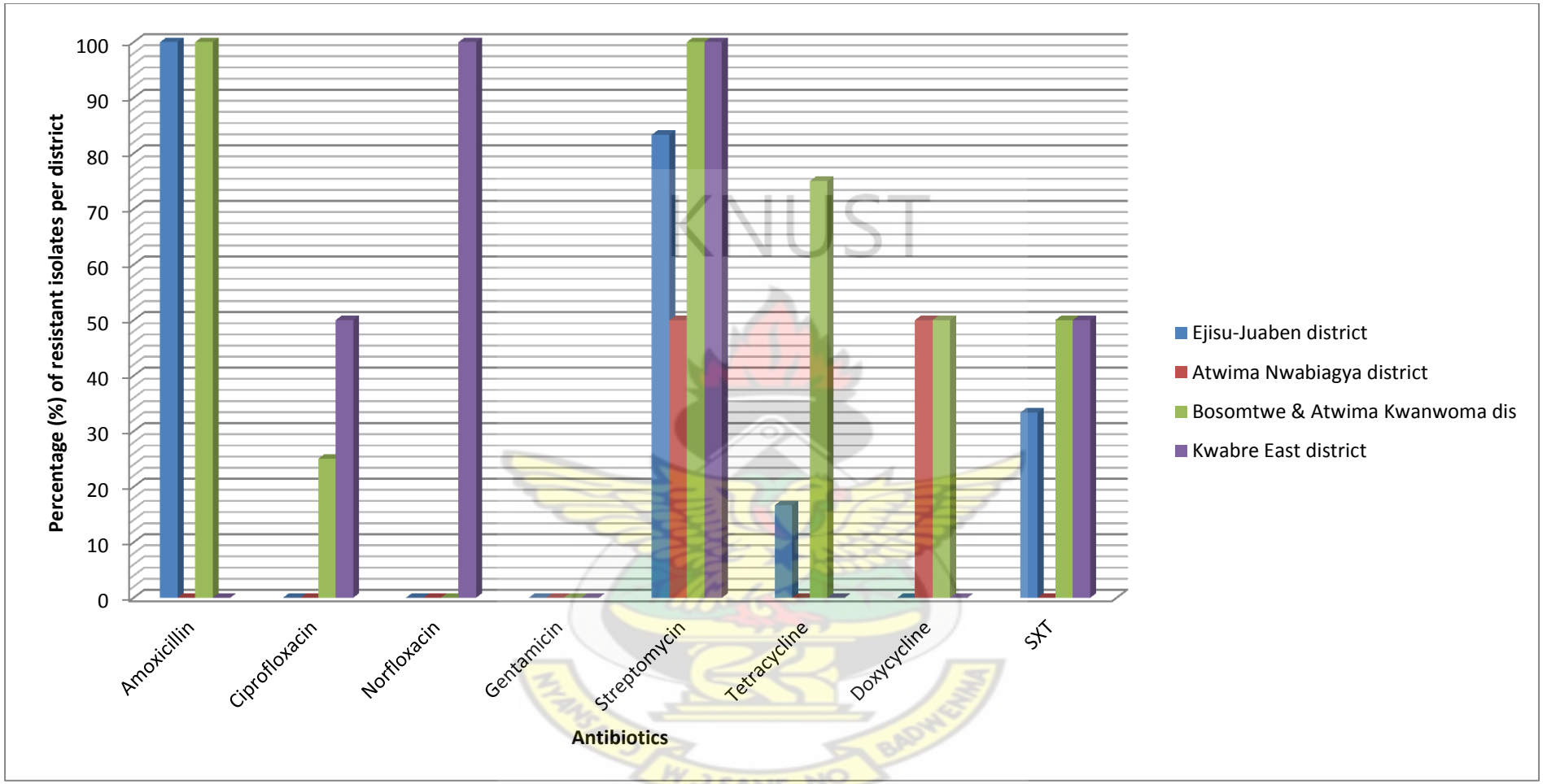


Figure 15: Resistance of *E. coli* isolates from different districts

The *E. coli* isolates from almost every district show resistance to more than one antibiotic though most farmers employ only one antibiotic at a time on the farm; a clear case of multi-drug resistance.

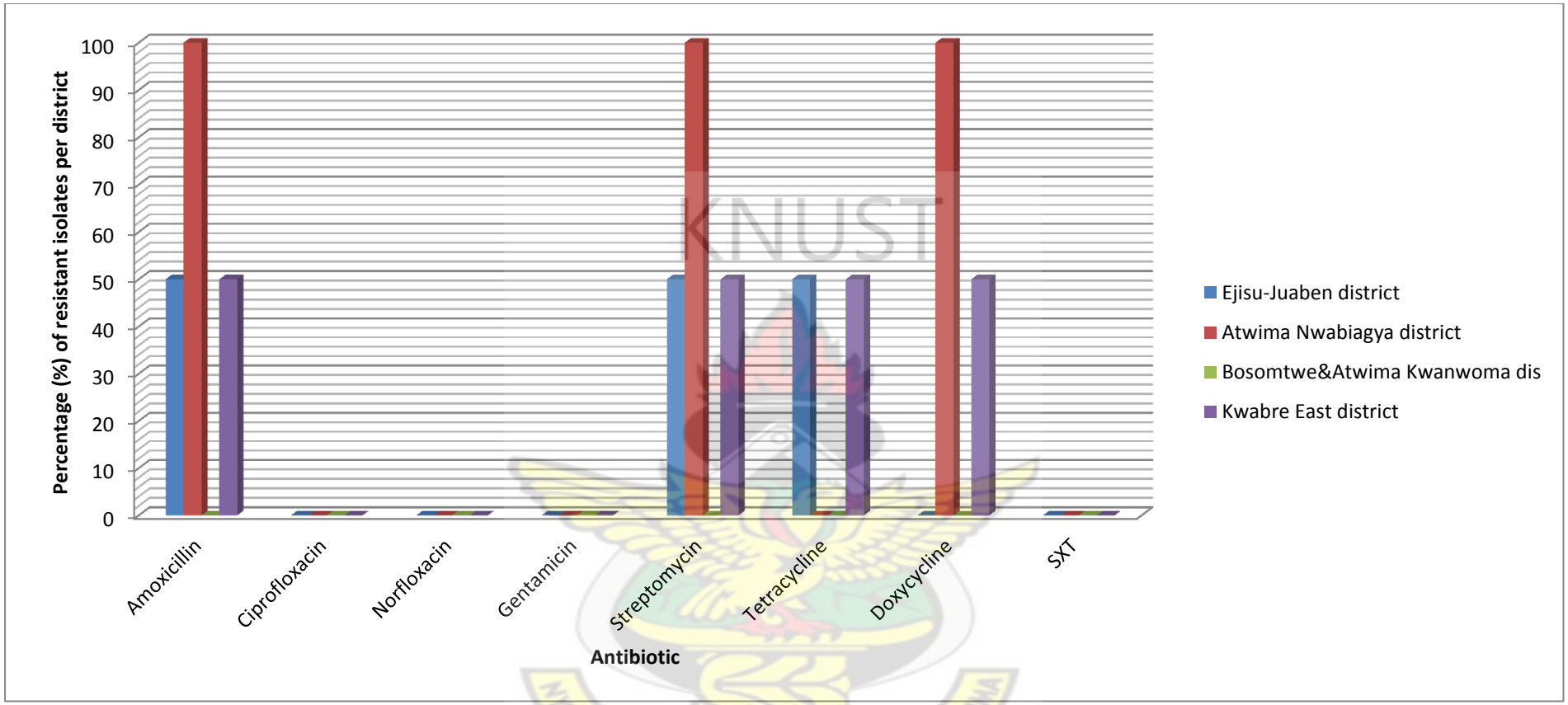


Figure 16: Resistance of *Enterobacter spp.* isolates from different districts

Enterobacter spp. from all the districts expresses multi drug resistance to amoxicillin, streptomycin and the tetracyclines whiles they are susceptible or intermediate to the fluoroquinolones, gentamicin and Sulphamethoxazole-trimethoprim. All *Enterobacter spp.* isolates from Atwima Nwabiagya are notably multidrug resistant to amoxicillin, streptomycin and doxycycline.

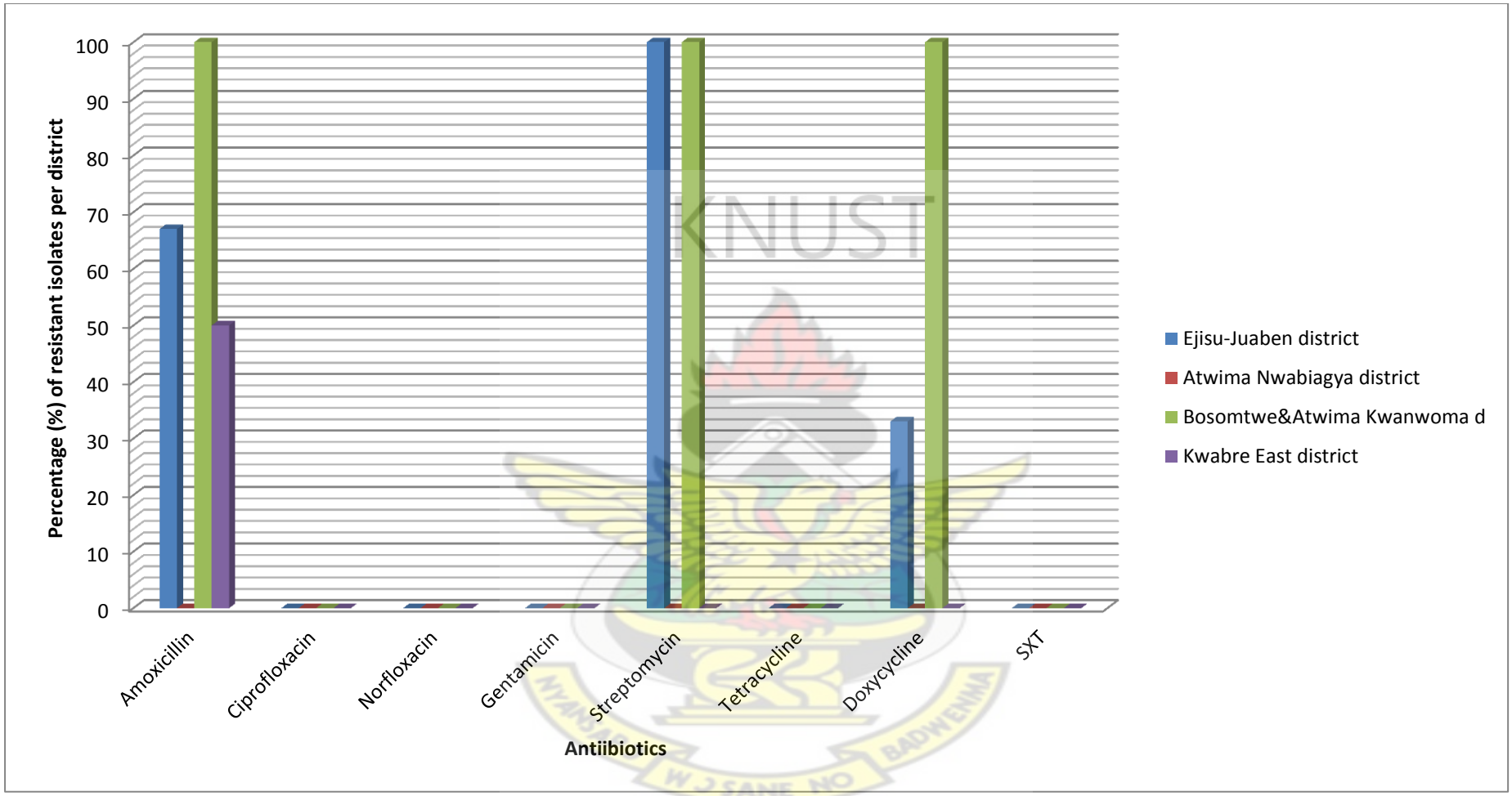


Figure 17: Resistance of *Salmonellae* isolates from different districts

Salmonella spp. isolates from Ejisu-Juaben, Bosomtwe and Atwima Kwanwoma districts are multi drug resistant; being resistant to amoxicillin, streptomycin and doxycycline.

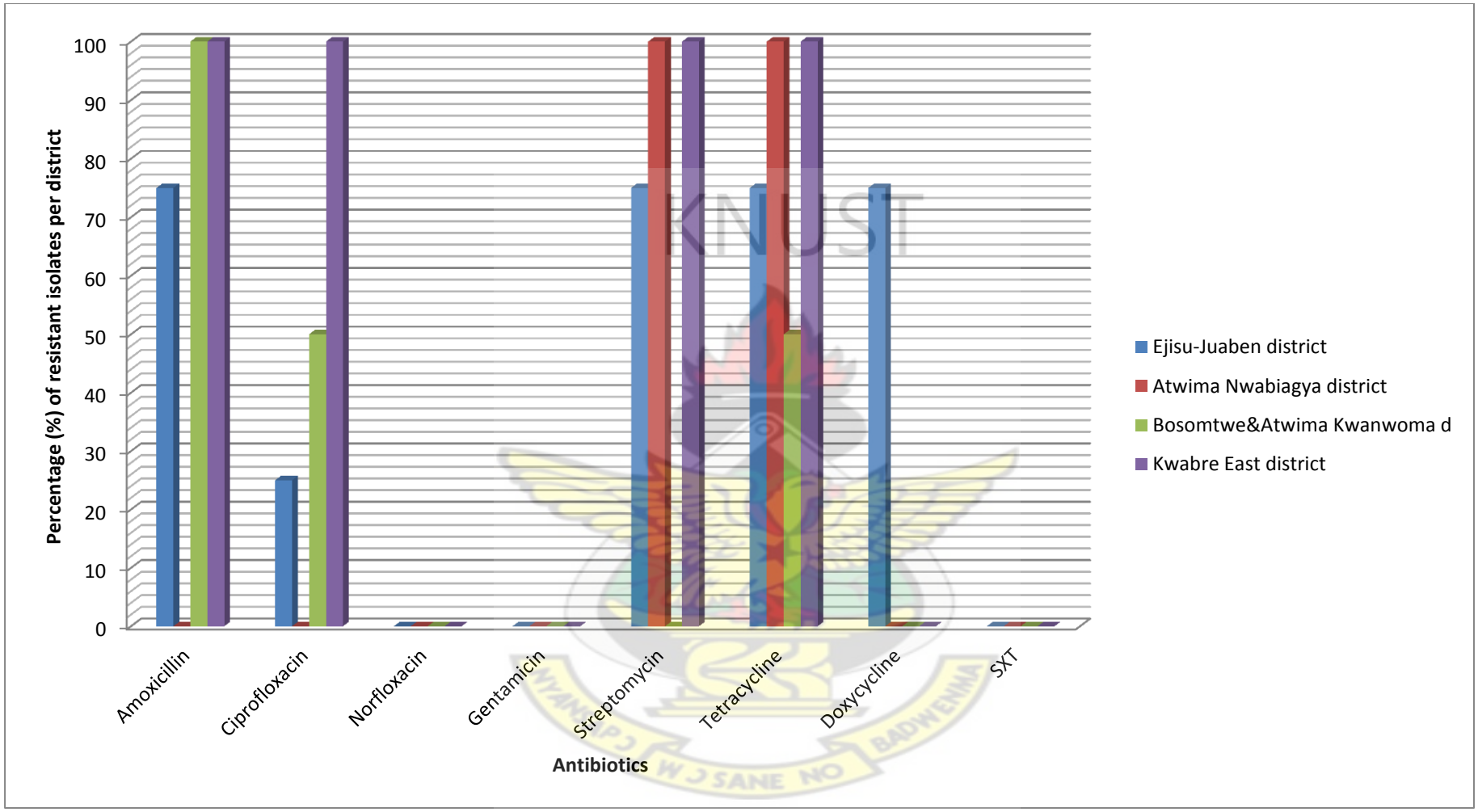


Figure 18: Resistance of *S. typhi* isolates from different districts

The *S. typhi isolates* show multi drug resistance to amoxicillin, ciprofloxacin, streptomycin and tetracycline; antibiotics of importance in both clinical and veterinary medicine.

CHAPTER FIVE

5.0 DISCUSSION

Global concerns about the increasing incidences of antibiotic resistance has turned the focus of microbiological research towards finding the causes, means of spread and ways of preventing resistant bacterial species from enlarging their borders.

5.1 Farm practices

The practices of the farmers in terms of antibiotic storage, waste disposal and body washing after handling antibiotics are not according to those accepted and recommended by veterinarians. The practice of disposing antibiotics and farm waste through drains into the backyard contaminates the surrounding soils with antibiotics and bacteria exposed to them. Extensive reviews by Thiele-Bruhn (2003) and Chee-Sanford and colleagues (2009) showed that poor animal farm waste management practices are making soils, ground water and surface water reservoirs of resistance genes and resistant bacteria.

Due to the hot temperature conditions of the tropics (an average of 25ºC), most antibiotics tend to breakdown, making them ineffective at the recommended doses (Okeke *et al.*, 1999). Consequently the storage sites of the farmers are not conducive for the continual effectiveness of the antibiotics.

The washing methods of the farmers (83% use water and soap; section 4.3.16) cannot totally eradicate bacteria should there be an outbreak on the farm. This practice cannot prevent the transfer of resistance from farms to humans.

Increased education of farmers and supervision of farm activities by veterinarians is necessary to correct the wrong practices of pig farmers.

5.2 GPS map and siting of pig farms

The siting of pig farms is a risk factor for the acquisition of resistant bacteria and resistance genes from such farms (Hamscher *et al*., 2003; The Lancet Infectious Diseases Commission, 2013). Several studies have reported the presence of antibiotics, resistant bacteria and resistance genes in wells/boreholes found on farms, water bodies near farms and in the surrounding atmosphere of farms; resistance genes have been found as far as 250m downstream of lagoons and ground water (Teuber, 2001; The Lancet Infectious Diseases Commission, 2013). The farms surveyed for this study (figures 5 to 8) were in most cases situated far away from homes and human settlements except in a few cases in Ejisu and Edwenase (in the Ejisu-Juaben districts) and Akropong (in Atwima Nwabiagya district) where the farmer lived with his family near the farm. Such closeness to pig farms as already shown in documented literature is hazardous in transmitting resistant bacteria from farms to humans through the farmers(Price *et al.*, 2007; The Lancet Infectious Diseases Commission, 2013).

Furthermore, high levels of resistant bacteria found on the farms (figures 13 to 18) and the nearness of most pig farms to surface water (section 4.3.7), coupled with the poor sewage discharge practices can contaminate ground water as shown in previous studies with groundwater located near farms (Thiele-Bruhn, 2003). This can be hazardous to animals and humans in farms that use wells/borehole. This threat is especially alarming in cases where the farms are situated so close to human settlements in which ground and surface water is depended upon for domestic purposes. A molecular typing study of the faecal bacterial isolates of the farmers, people living around the farms and of the pigs will be necessary to establish a concrete evidence of resistance gene and resistant bacteria transfer via the soil, ground and surface water as shown in other studies (Thiele-Bruhn, 2003; The Lancet Infectious Diseases Commission, 2013).

The GPS maps (figures 5-8) shows the towns located near pig farms. These towns are at risk of any disease breakout from the farms. Consequently, it should be the focus of veterinary and medical attention to continually screen human populations living in close proximity to pig farms to pre-empt any zoonosis epidemic of resistant bacteria. The farms represented on the maps are not exhaustive as farms which are not in the pig farmers unions are scarcely represented. Farmers with difficult-to-

access farms or farmers who were absent at the times of visitation are mostly unrepresented. However the GPS maps give a clear idea of the distribution of farms within the districts.

5.1.1 Incidences of resistant isolates per district

The results obtained in this study reflects those of other studies in that there was resistance to antibiotics not used in those districts as well as resistance to those that were used (Glad *et* al., 2010; Donkor *et al*., 2012; The Lancet Infectious Diseases Commission, 2013) showing that antibiotics usage increases resistance among pigs. The presence of resistance to antibiotics not used among the farms is not new as documented evidence supports the presence of resistance phenotypes among pig farms with no antibiotic usage (Zhu *et al*., 2012). There is no available data on the resistance prevalence of bacteria to antibiotics in the districts studied. However, the high prevalence of resistance among the districts to amoxicillin (34 isolates; table 18), streptomycin (37 isolates; table 18) and the tetracyclines (35 isolates; table 18) agrees with a study by Donkor and colleagues (2012) among *E. coli* isolates from livestock (including pigs) in Accra.

The summary of resistant isolates per district (table 18) shows the relatively higher resistance prevalence (51.88%) in Ejisu Juaben district followed by Bosomtwe and Atwima Kwanwoma districts (20.30%) to all the antibiotics. It is therefore important that more studies be carried out to determine the extent of bacterial resistance in these districts. Contingency measures to contain the spread of the resistant clones will be in the interest of public health. Further studies to ascertain the mechanisms responsible for resistance to antibiotics not used in the farms (ciprofloxacin) will be helpful in understanding the epidemiological situation on pig farms.

All the districts had a higher percentage of susceptible strains more than resistant ones (table 18). The percentage of resistant strains to one or more antibiotic from each of the districts is substantial; this is especially obvious in the Bosomtwe and Atwima Kwanwoma districts where percentages of strains showing resistance to one or more antibiotic were very close. This table (18) however, establishes that there are resistant strains present in all the districts in substantial numbers; there was at least resistance to every antibiotic used on isolates from Ejisu-Juaben district (table 18).

Figures 13 to 18 show that *Proteus vulgaris, E. coli, Enterobacter, Salmonella spp.* and *S. typhi* isolates from all the districts are multidrug resistant. Resistance to antibiotics not used on the farms, as seen in the results, can only be as a result of horizontal transfer of resistant genetic elements like plasmids, transposons and integrons from the immediate environment of these enterobacteria isolates.

In the Ejisu-Juaben district and also in the other districts (table 18), resistance to amoxicillin and streptomycin was very high (76% and 73.08% respectively) whereas resistance to the fluoroquinolones (ciprofloxacin and norfloxacin) and gentamicin was very low (11.54% and 3.85% respectively). This may be influenced by the higher percentage of farmers using penicillin-streptomycin antibiotic formulations (tables 5 and 6) vis-à-vis the percentage usage of ciprofloxacin, norfloxacin and gentamicin. Gentamicin is in the same antibiotic class with streptomycin (aminoglycosides). However, the percentage of streptomycin usage far outweighs that of gentamicin, suggesting that the increased incidence of resistance to streptomycin may be due to the higher use of streptomycin. This pattern also rules out the possibility of cross resistance. Amoxicillin usage is very low throughout all the districts. The high case of resistance to amoxicillin is possibly due to cross resistance from the high use of penicillin. The use of penicillin can trigger the selection of beta-lactamase producers which can in turn transfer these genes to other Enterobacteriaceae, leading to increased resistance to amoxicillin which is not resistant to these enzymes.

With the higher patronage of the tetracyclines among the farmers (tables 5 and 6), it is interesting that resistance to tetracycline and doxycycline is not as high as that of amoxicillin and streptomycin in all districts. However, there is a close margin between the incidences of resistance and susceptibility such that in Ejisu-Juaben, Bosomtwe and Atwima Kwanwoma districts, the resistance and susceptibility incidences for tetracycline have the same percentages (34.62%; table 18). The percentages for doxycycline and tetracycline with respect to the incidences of resistance and susceptibility describe a clear case of cross-resistance due to the higher use of Oxytetracycline among the farmers; this is the main type of the tetracycline class used in veterinary medicine. The number of organisms with intermediate resistance to tetracycline and doxycycline is substantially higher than that of all other antibiotics for all the districts. This is descriptive of the shifting trend from susceptibility to resistance

to the tetracyclines. With the continued use of the tetracyclines, all the intermediate strains shall develop resistance to the organisms. The percentage of resistance to the tetracyclines (34.31%) is thus not surprising as they are widely used among farmers in all districts.

The resistance incidences among all the districts to Sulphamethoxazole-trimethoprim are almost equal to that of the tetracyclines though Sulphamethoxazole alone is not used by the pig farmers. Moreover, trimethoprim is one of the least patronised antibiotics by the pig farmers (tables 5 and 6). Subsequently the high percentage of resistance incidences (30%) to Sulphamethoxazole-trimethoprim in all the districts is possibly due to cross resistance from the use of sulphadimidine which is one of the most patronised of the antibiotics among the farmers (table 6).

The prevalence of resistance to antibiotics not used in the districts is indicative of the presence of multidrug resistant species. Further molecular studies will be necessary to determine the resistance mechanisms involved in these resistant bacteria. The presence of resistant species in our farms is not safe for our food chain as these can easily end up on our plates (Gilchrist *et al.*, 2007; WHO, 2011) to cause fatal clinical infections. The need for a concerted effort by veterinarians, policy makers, health care stakeholders and scientists to nip this menace in the bud is very imperative.

5.2 Types of antibiotics used and motivation for use

The antibiotics used by the pig farmers agree with those used by livestock farmers in Accra (Donkor *et al*. 2012) and in Kenya where two separate studies reported that the tetracyclines, sulphonamides, fluoroquinolones and aminoglycosides were commonly used among livestock farmers (Mitema *et al.* 2001; Irungu *et al.* 2011). This study corroborates with those from other studies throughout the world (Lee *et al*., 2001; Page and Gautier, 2012) where the dominant veterinary antibiotic classes are the tetracyclines, penicillins, macrolides, sulphonamides, pleuromutilins, lincosamides, fluoroquinolones, aminoglycosides and polymixins are used in different orders of importance depending on the particular countries. There are however differences in that the pleuromutilins, lincosamides and polymyxins are not used in Ghanaian piggery. Moreover, the macrolides do not have so much

prominence as seen in other countries. However, like all other countries, all these antibiotics fall into classes used in clinical medicine.

Like other regions in Ghana and other African countries the use of antibiotics for growth promotion in piggery and other livestock is unknown or little practised (Mitema *et al.,* 2001; Irungu *et al.,* 2011; Donkor *et al.*, 2012) as was observed in this study. In poultry, especially, there are antibiotics found in manufactured feed which farmers might use without intentionally having growth promotion in mind (Donkor *et al*. 2012). Though other reports state that farmers used antibiotics for prevention and treatment of disease, especially in poultry, (Donkor *et al*,, 2012) this was not the case with the pig farms studied. Antibiotics were used mainly for treatment and little or none for prevention and the percentages of antibiotics used for treatment in other studies (>90%) are in agreement (Mitema *et al*., 2001).

To the best of our knowledge, there is a dearth of publications reporting the use of probiotics among pigs and other livestock in Africa and Ghana. And the result of this study is not an exception. Probiotics are now emerging on the Ghanaian market basically among poultry and not pig farmers. Their promotion and adoption among the farmers shall greatly reduce the use of antibiotics and reduce the burden of resistance in the country.

5.2.1 Sources of antibiotics, dosage forms and routes of administration

Antibiotic resistance through off-label use, misuse, abuse, in food animal production can only be curbed through veterinary oversight and prescription (WHO, 2011; Page and Gautier, 2012). Consequently, the practice of buying antibiotics without prescriptions from non-veterinarians or their use without veterinary supervision as seen in this study and mirrored in studies undertaken in other countries (Wageh *et al*., 2013; The Lancet Infectious Diseases Commission, 2013), is inimical to the fight against inappropriate antibiotic use in veterinary medicine.

The routes of administration and dosage forms of the antibiotics used by the farmers is the same as reported by studies throughout the world (Page and Gautier, 2012); feeding solid antibiotics (in the form of powders and granules) through water and feed is done in advanced countries where large

populations of pigs are kept. In such conditions, critical care is taken to ensure accurate dosing per animal and this requires skill and expertise.

5.3 Antibiotic resistance

5.3.1 Resistance per organism

The current reports of multi drug and pan drug resistance among *Enterobacteriaceae*, noticeably, *E. coli*, *Enterobacter* and *Salmonellae* to most β-lactams, including oxy-iminocephalosporins, monobactams and carbapenems with concomitant resistance to all clinically important antibiotics like the aminoglycosides, fluoroquinolones, tetracyclines, macrolides, sulphonamides and Trimethoprim were not observed in these isolates (Bush, 2013; The Lancet Infectious Diseases Commission, 2013). Nevertheless, the resistance of the *E. coli*, *Enterobacter* and *Salmonellae spp.* isolates, is reflective of the common antibiotics used among the pig farms and corroborates with already available data on resistance among *E. coli* to the penicillins, aminoglycosides and tetracyclines reported by Donkor and colleagues (2012). These results show the waning effect of the tetracyclines, penicillins, aminoglycosides and sulphonamides among clinical and veterinary settings. Hence, great care should be taken to control antibiotic use in veterinary medicine to control the spread of resistant bacteria from animals to humans, as reported previously (Levy *et al.*, 1976), to protect the efficacy of our reserved antibiotic arsenals from possible inefficacy. Multi drug resistance in *E. coli, Enterobacter spp.* and *Salmonellae* will be disastrous for public health as they are associated with fatal zoonotic and clinical infections. Diarrhoea resulting from *E. coli* and *Salmonellae spp.* infections will exacerbate the situation via the increased shedding of resistant clones in the environment.

Most of the organisms (more than 80%) are susceptible to the fluoroquinolones and gentamicin in all the districts, showing that the commensals in the GIT might not have or have very little pool of genes that are resistant to the fluoroquinolones and gentamicin (table 18). Due to the little exposure of the GIT flora to the fluoroquinolones and gentamicin, there has been little or no selection of resistant strains. Moreover, the very minor percentage of organisms resistant to the fluoroquinolones and gentamicin has not been able to transfer their resistance to the other commensals.

Clearly, the *Proteus vulgaris* isolates (figure 14) from Ejisu-Juaben, Atwima Nwabiagya and Kwabre East are multidrug resistant as they show resistance to almost all the antibiotics tested. The farmers do not use all these antibiotics concomitantly on a given farm; consequently, the multidrug resistance observed is possibly due to cross and co resistance via horizontal gene transfer of resistance genetic elements between bacteria. *Proteus vulgaris* is a common commensal in the intestinal flora of humans and animals. The resistance levels shown indicate that it has been exposed to antibiotics or received resistance from other intestinal bacteria. These resistances can be easily transferred to pathogens or in conditions of suppressed immunity, cause untreatable urinary tract infections (Talaro and Talaro, 2002; Chee-Sanford *et al.,* 2009).

The *E. coli* isolates (figure 15) from almost every district show resistance to more than one antibiotic though most farmers employ only one antibiotic at a time on the farm; a clear case of multi-drug resistance. All the *E. coli* isolates from Kwabre east are resistant to norfloxacin and streptomycin. Those from Bosomtwe and Atwima Kwanwoma districts are also very resistant, especially to amoxicillin, streptomycin and tetracycline. Such resistant species can easily transfer their resistant genes to other pathogens either in the pigs or in the pork consumer. The presence of resistance in *E. coli* suggests that the pigs have been exposed to antibiotics and or resistance genes.

Enterobacter spp. from all the districts (figure 16) save Bosomtwe and Atwima Kwanwoma express multi drug resistance to amoxicillin, streptomycin, tetracycline (except Atwima Nwabiagya) and doxycycline (save Ejisu Juaben) whiles they are not resistant to the fluoroquinolones, gentamicin and Sulphamethoxazole-trimethoprim. All *Enterobacter spp.* isolates from Atwima Nwabiagya are notably multidrug resistant to amoxicillin, streptomycin and doxycycline. It is interesting to note that there is no *Enterobacter spp.* resistance to the fluoroquinolones, sulphamethoxazole-trimethoprim and gentamicin in all the districts, indicating that resistance to these antibiotics in the other organisms from the same districts and farms have not been able to transfer their resistance genes to *Enterobacter spp*. *Enterobacter spp.* also shows that commensals harbour resistance genes which they can transfer to pathogens, serving as a continual resistance genes reserve. Appropriate antibiotic use and the resort

to probiotics are necessary to reduce the exposure of commensals to antibiotics, thus reducing the selection of resistant genes and the reservoir of resistant genes.

Salmonella spp. isolates (fig. 17) from Ejisu-Juaben, Bosomtwe and Atwima Kwanwoma districts are multi drug resistant; being resistant to amoxicillin, streptomycin and doxycycline. Infact, all Bosomtwe and Atwima Kwanwoma isolates are resistant to amoxicillin, streptomycin and doxycycline. *Salmonellae* have been known to cause fatal diseases the world over and several studies have noted their increasing resistance to several antibiotics (WHO, 2011). Fortunately, their resistance is limited to a few antibiotics and barely to two districts. *Salmonellae* are not common intestinal denizens, being zoonotic pathogens. There is subsequently the possibility that the isolates have had a shorter stay and exposure to antibiotics in the intestines of their host.

The *S. typhi isolates* show multi drug resistance to amoxicillin, ciprofloxacin, streptomycin and tetracycline; antibiotics of importance in both clinical and veterinary medicine (fig. 18). *S. typhi* shows similarity in resistance to sulphamethoxazole-trimethoprim, norfloxacin and gentamicin with *Salmonella spp.* The absolute resistance shown by Kwabre east isolates to tetracycline, streptomycin, ciprofloxacin and amoxicillin requires attention—especially as ciprofloxacin is a drug of choice in typhoid fever cases. Atwima Nwabiagya isolates are only resistant to two antibiotics, but in this the resistance is absolute. The isolates from Ejisu-Juaben (almost 80%) also show marked resistance to five antibiotics: amoxicillin, ciprofloxacin, streptomycin, tetracycline and doxycycline. One interesting observation is the high level of resistance to ciprofloxacin vis-à-vis the absence of resistance in norfloxacin, and that between streptomycin and gentamicin, tetracycline and doxycycline (save in the Ejisu-Juaben isolates)—antibiotics belonging to the same class. One would expect that resistance in one antibiotic would affect resistance in another antibiotic of the same class (cross resistance) (WHO, 2011). In this case however, such possibilities are very minimal, raising questions about the means by which the *S. typhi* acquired its resistance: the only sound reason being their exposure to antibiotics in the intestines.

CHAPTER SIX

6.0 CONCLUSION

The prevalent conditions on pig farms in Ashanti regions, especially antibiotic storage conditions, waste management practices and body washing methods are not the most appropriate as recommended by veterinarians. washing methods. Prevalence of resistance among enterobacteria isolates to the selected antibiotics (except to the fluoroquinolones and gentamicin) was observed in all the districts but significantly higher in Ejisu-Juaben, Bosomtwe and Atwima Kwanwoma districts. *E. coli* and *Salmonellae* resistance to more than one antibiotic was observed in farms from various districts. Resistance to more than two antibiotics (multi drug resistance) was observed in more than 78% of the resistant isolates.

6.1 Recommendations

- During the study, it was observed that there were no strict regulations and a well-functioning regulatory body governing the sales of veterinary antibiotics. Under such circumstances, it would be difficult to supervise the type of antibiotics sold to farmers or to keep all sales of antibiotics strictly under prescription. Consequently, it is very important that such institutions be established to regulate the sales of veterinary antibiotics as a means of reducing resistance through the sales of antibiotics of clinical importance for farm animals' production.
- Further surveillance studies must be carried out in the Ejisu-Juaben, Bosomtwe and Atwima Kwanwoma districts to ascertain the causes for the development and spread of resistance, the levels and trends in resistance among various bacteria to help veterinary authorities make informed decisions about the best means to contain the high prevalence of resistance found in these districts.
- The use of veterinary antibiotics requires stricter legislations by government to limit its sales to only veterinarians. This is important to ensure their prudent use and prevent off-label use.

By such legislations, veterinarians can better educate farmers on better usage of the antibiotics as they come to buy the antibiotics from these professionals.

- Probiotics are not common on the Ghanaian market and no farmer was seen using one throughout the study. Probiotics can significantly reduce the need for antibiotics by preventing bacterial infections. A promotion of their use by veterinarians and legislation is imperative to reduce the amount of antibiotics used on pigs.
- Finally, there is an urgent need to organize seminars, symposia and workshops for pig farmers on a regular basis to teach them better management practices that shall make the use of antibiotics limited and unnecessary.

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APPENDIX

APPENDIX A: Questionnaire

A. RESEARCH INSTRUMENT

DEPARTMENT OF PHARMACEUTICS

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

COLLEGE OF HEALTH SCIENCES

Kwame Nkrumah University of Science and Technology, Kumasi

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*This Research Instrument is designed to collect Data for a Study on the Topic "**Impacts of types and handling practices of antibiotics by pig farmers in Ashanti Region, Ghana on antibiotic resistance**"*

Date: ……. Interview No…… Farm Code……..

GPS coordinates: ……

A. GENERAL INFORMATION

PART I – RESPONDENT/MANAGER/OWNER DETAILS

The questionnaire must be filled when possible in the presence of the worker/operator who is in charge of applying the chemicals in the farm. However, some basic details on the farm owner must also be recorded.

Respondent:

General education level	Type of education in pig production 1=workshop 2=training course 3=university degree 4=in-service training/on the job training 5=none 6=other	Organizing institution 1=extension service, 2=university courses 3=pig feed company 4= pig drug company 5=other	Frequency (times/year)	Brief description of the content of the educational course

Farm owner (if it is not the respondent):

General education level	Type of education in pig production 1=workshop 2=training course, 3=university degree 4= in-service training 5=none 6=other	Organizing institution 1=extension service 2=university 3=pig feed company 4= pig drug company 5=other	Frequency (times/year)	Brief description of the content of the educational course

B. INFORMATION ON THE USE OF ANTIBIOTICS

1. Which antibiotics are used and how?

A Product name	B Active ingredient	C % of active ingredient (1)	D Form (2)	E Reason for use (3)	F Mode of use (4)	G Dose	H Dose Units (5)	I Number of applications per day (-)	m. Treatment length (days)	n. Average number of times the treatment is done per production cycle (-)
Oxybac	Oxytetracycline-HCl	50%	A B C	A B C D E	A B C	50	g	1	7	2
Form A	Formaldehyde	90%	A B C	A B C D E	A B C	20	mg	1	1	1
			A B C	A B C D E	A B C					

(1) In some cases the active ingredient content is not displayed as a %. Instead, some products just mention e.g. oxytetracycline-HCl 500mg. In this case one must calculate the % of active ingredient based on the whole weight or volume of the package.

Example1: if the package contains 1kg of formulated product and the label says oxytetracycline-HCl 500g, then the % of active ingredient is calculated as (500g/1000g)x100 = 50%.

Example2: if the package contains 1L of formulated product and the label says oxytetracycline-HCl 200g, then the % of active ingredient is calculated as (200g/1000g)x100 = 20% (assuming density of 1, so 1L = 1kg = 1000g)

(2) A=pellet, B=solid (powder), C=liquid, D=other (please specify)

(3) A= water treatment, B= nutritional supply, C = diseases prevention, D = disease treatment, E=other (please specify)

(4) A=directly to water B=mixed with feed C=used to clean equipment, D= other (please specify)

(5) mg, ml, L, g, kg (Please if the farmer reports a different unit, e.g. 2 table spoons, 3 cups, keep record of the weight or volume and convert into a standard unit).

(6) m^2, ha

C: Antibiotic knowledge

2. Are drugs/chemicals administered according to safety instructions described

i. On the package? (Y/N) Which ones: ……..

ii. By veterinarian/technicians? (Y/N)

iii. Extension officer? (Y/N)

iv. Experiences? (Y/N)

v. Friends/others? (Y/N)

3. How are the drugs (antibiotics) stored?

Commercial name	**Type of container** 1. glass solid container 2. metal solid container 3. plastic container 4. plastic bag 5. paper bag 6. other	**Status of container** 1. Closed 2. Opened	**Site of storage** 1. shelf 2. cupboard 3. others: ….
	1 2 3 4 5 6	1 2	1 2 3
	1 2 3 4 5 6	1 2	1 2 3

4. Others (state): ……

5. Do you use the same tools for all the antibiotics or different tools for each type of antibiotic?

Yes/ No

6. Are the tools used for handling the following chemicals washed after their use?

Antibiotics (Y/N)

Disinfectants (Y/N)

Pesticides (Y/N)

Probiotics (Y/N)

7. If yes, where is the waste water discharged after washing?

i. In to the sewage system

ii. Court yard

iii. Soil

iv. Drainage canal/water system

v. other

8. Is there any direct contact between the skin of the workers and the antibiotics?

i. Antibiotics (Y/N)

ii. Disinfectants (Y/N)

iii. Pesticides (Y/N)

iv. Probiotics (Y/N)

9. Do farm workers use any protection during handling of antibiotics? **Yes / No**

10. If **yes**, which types of protection do workers use during antibiotics' handling?

Commercial name	**Protective measure** (1=gloves, 2=rubber boots, 3=mask, 4=glasses 5 = clothes)	**Type of handling** (1=mixing into feed, 2=loading and applying into water, 3=disinfection)	**Appropriate use (according to manufacturer or veterinarian's instructions)** 1 Yes 0 No
	1 2 3 4 5	1 2 3	1 2
	1 2 3 4 5	1 2 3	1 2

11. Do workers clean their hands/take a shower each time after handling of antibiotics or contact with water/feed containing antibiotics? **Yes/No**

13. If **Yes**, how are the hands/body cleaned?

1. with water

2. with water and soap
3. Other (please state): ……..

C. WATER DISCHARGE

1. What is the source of the water used on the farm? ……
2. Is the water at the farm used for any other purpose than for pig farming? **Yes / No**
3. If **yes**, which purpose? .....
4. How is the farm's waste water treated /processed? …….
5. Is there any water body (stream, river, lagoon, Lake Etc.) found around the farm? **Yes/ No**
6. If **yes,** then please state: …….
7. Is the water from the effluent recipient used by the local population? **Yes / No**
8. If **Yes**, for which purpose? .....
9. GPS coordinates effluent discharge point:……E……..N

APPENDIX B: Culture Media

1. Bismuth sulphite agar (modified): Oxoid [CM 0201]

Ingredients	Amount (g/l)
Peptone	5.0
'Lab-Lemco' powder	5.0
Glucose	5.0
di-sodium phosphate	4.0
Ferrous sulphate	0.3
Bismuth sulphite indicator	8.0
Brilliant green	0.016
Agar	12.7

40g of bismuth sulphite was weighed into a one litre conical flask and made to volume with distilled water. The mixture was distributed into test tubes in 20mL portions, closed with cotton wool and dissolved by heating in a microwave oven. They were then stabilised in a water bath prior to being poured unto petri dishes

2. MacConkey Agar (Scharlau-01-118)

Ingredients	Amount (g/l)
Peptone	20.0
Lactose	10
Bile salts #3	15.0
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0

51.5g of MacConkey agar powder was weighed into a one litre conical flask and made to volume with distilled water. The mixture was dissolved by heating in a microwave oven. The mixture was distributed into test tubes in 20mL portions, closed with cotton wool and sterilised by autoclaving at 115°C for 30min (Arnold and sons Ltd., Basildon). They were then stabilised in a water bath prior to being poured unto petri dishes

3. Mueller-Hinton Agar (Oxoid-CM 0337)

Ingredients	Amount (g/l)
Beef, dehydrated infusion from	300
Casein hydrolysate	17.5
Starch	1.5
Agar	15.0

[Meets CLSI standard M6-A2 for dehydrated culture media]

38g of Mueller-Hinton agar was weighed into a one litre conical flask and made to volume with distilled water. The mixture was dissolved by heating in a microwave oven. The mixture was distributed into test tubes in 20mL portions, closed with cotton wool and sterilised by autoclaving

at 121°C for 15min (Arnold and sons Ltd., Basildon). They were then stabilised in a water bath prior to being poured unto petri dishes

4. X.L.D. Agar (LAB-LAB032)

Ingredients	Amount (g/l)
Xylose	3.75
L-lysine	5.0
Lactose	7.5
Sucrose	7.5
Sodium chloride	5.0
Yeast extract	3.0
Agar No. 2	13.0
Phenol red	0.08
Sodium Desoxycholate	1.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8

53.5g of X.L.D. agar was weighed into a one litre conical flask and made to volume with distilled water. The mixture was distributed into test tubes in 20mL portions, and dissolved by heating in a microwave oven. They were then stabilised in a water bath prior to being poured unto petri dishes

5. Triple sugars, Iron (TSI) Agar (Oxoid CM0277)

Ingredients	Amount (g/l)
'Lab-Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0

Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0

65g of T.S.I. agar was weighed into a one litre conical flask and made to volume with distilled water. The mixture was dissolved by heating in a microwave oven. The mixture was distributed into test tubes in 20mL portions, closed with cotton wool and sterilised by autoclaving at 115°C for 30min (Arnold and sons Ltd., Basildon). They were stabilised in a water bath prior to being poured aseptically into petri dishes. They were allowed to set in a sloped form with a butt about 1 in. deep.

6. Soya Peptone broth (Oxoid-Code N. L44)

Ingredients	Amount (g/l)
Soya peptone	4.5
Sodium chloride	7.2
Potassium dihydrogen phosphate	1.26
Dipotassium hydrogen phosphate	0.18
Magnesium chloride (anhydrous)	13.58
Malachite green	0.036

26.75g of Soya peptone broth powder was weighed into a one litre conical flask and made to volume with distilled water. The mixture was dissolved by heating in a microwave oven. The mixture was distributed into test tubes in 20mL portions, closed with cotton wool and sterilised by autoclaving at 115°C for 30min (Arnold and sons Ltd., Basildon).

APPENDIX C: Sensitivity test results

Table 19: Percentages of resistance to antibiotics per district

Districts	Total number of tests producing susceptible results	Total number of tests producing intermediate results	Total number of tests producing resistant results	Total number of sensitivity tests
Ejisu-Juaben	113 (54.85%)	24 (11.65%)	69 (33.5%)	206 (100%)
Atwima Nwabiagya	44 (64.71%)	9 (13.24%)	15 (22.06%)	68 (100%)
Bosomtwe & Atwima Kwanwoma	31 (49.21%)	5 (7.94%)	27 (42.86%)	63 (100%)
Kwabre East	39 (54.93%)	10 (14.08%)	22 (30.99%)	71 (100%)
Grand total	227 (55.64%)	48 (11.76%)	133 (32.60%)	408 (100%)

Table 20: Summary of inhibition zones produced by erythromycin, penicillin and enrofloxacin to Enterobacteria isolates

District	Ranges of zones of inhibitions: Erythromycin				Ranges of zones of inhibitions: Benzyl Penicillin				Ranges of zones of inhibitions: Enrofloxacin			
	0-9.9mm	10-15mm	16-20mm	>20mm	0-9.9mm	10-15mm	16-20mm	>20mm	0-9.9mm	10-15mm	16-20mm	>20mm
Ejisu-Juaben	8	11	1	6	17	4	3	2	2	2	4	19
Atwima Nwabiagya	2	5	-	-	3	5	2	-	-	1	1	7
Bosomtwe & Atwima Kwanwoma	1	4	-	2	7	1	-	-	2	-	1	5
Kwabre East	6	2	1	1	8	2	-	-	-	2	2	7
Total	17	22	2	9	35	12	5	2	4	4	8	38

Table 21: Summation of inhibition zones produced by erythromycin, penicillin and enrofloxacin to Enterobacteria

Districts	Summation of inhibition zones ranges produced by erythromycin, penicillin and enrofloxacin				Total
	0-9.9mm	10-15mm	16-20mm	>20mm	
Ejisu-Juaben	27 (34.18%)	17 (21.52%)	8 (10.13%)	27 (34.18%)	79 (100%)
Atwima Nwabiagya	5 (19.24%)	11 (42.31%)	3 (11.54%)	7 (26.92%)	26 (100%)
Bosomtwe & Atwima Nwabiagya	10 (43.48%)	5 (21.74%)	1 (4.35%)	7 (30.43%)	23 (100%)
Kwabre East	14 (45.16%)	6 (19.35%)	3 (9.68%)	8 (25.81%)	31 (100%)
Grand total	56 (35.22%)	39 (24.53%)	15 (9.43%)	49 (30.82%)	159 (100%)

Table 22: Summary of susceptibility tests

District	Organism	A			B			C			D			E			F			G			H		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ejisu-Juaben	*1*	3	-	7	9	1	1	8	2	1	10	-	1	3	1	7	4	3	4	5	2	3	4	-	7
	2	-	-	6	5	-	-	6	-	-	6	-	-	-	1	5	2	3	1	3	2	-	4	-	2
	3	1	-	1	1	1	-	2	-	-	2	-	-	-	1	1	1	-	1	2	-	-	1	1	-
	4	-	1	2	3	-	-	3	-	-	3	-	-	-	-	3	2	1	-	1	1	1	3	-	-
	5	1	-	3	3	-	1	4	-	-	3	1	-	-	1	3	-	1	3	1	-	3	4	-	-
Atwima Nwabiagya	*1*	1	1	2	4	-	-	4	-	-	3	-	-	-	1	3	-	1	3	1	-	3	4	-	-
	2	1	-	-	2	-	-	2	-	-	2	-	-	1	-	1	2	-	-	1	-	1	2	-	-
	3	-	-	1	1	-	-	1	-	-	1	-	-	-	-	1	1	-	-	-	-	1	1	-	-
	5	-	2	-	2	-	-	2	-	-	2	-	-	-	-	2	-	-	1	1	1	-	2	-	-
Bosomtwe &	*1*	-	2	-	-	-	1	1	-	-	1	-	-	-	-	1	1	-	-	-	1	-	1	-	-
	2	-	-	4	3	-	1	4	-	-	3	1	-	-	-	4	1	-	3	1	1	2	2	-	2

Atwima	*4*	-	-	1	1	-	-	1	-	-	1	-	-	-	-	1	1	-	-	-	-	1	1	-	-
Kwanwoma	*5*	-	-	2	1	-	1	2	-	-	2	-	-	1	-	-	1	-	1	1	1	-	-	-	1
Kwabre	*1*	-	1	1	2	-	-	2	-	-	2	-	-	-	1	1	-	-	2	1	-	-	1	-	1
East	*2*	-	1	-	1	-	1	-	-	1	1	-	-	-	-	1	1	1	-	-	1	-	1	-	1
	3	-	1	1	2	-	-	2	-	-	1	1	-	-	1	1	1	-	1	1	-	1	2	-	-
	4	1	-	1	2	-	-	1	-	-	1	-	-	-	1	-	2	-	-	2	-	-	1	-	-
	5	-	-	1	-	-	1	1	-	-	1	-	-	-	-	1	-	-	1	1	-	-	-	1	-

Key: *Proteus vulgaris (1), E. coli (2), Enterobacter spp. (3), Salmonella spp.(4)* and *S. typhi (5)* from different districts that are susceptible (S), intermediate (I) and resistant (R) to Amoxicillin (A), Ciprofloxacin (B), Norfloxacin (C), Gentamicin (D), Streptomicin (E), Tetracycline (F), Doxycycline (G) and Sulphamethoxazole-Trimethoprim (H).

Table 23: Summary of inhibition zones produced by erythromycin, enrofloxacin and penicillin

District	Organism	Sum of inhibition zones ranges: Erythromycin				Ranges of zones of inhibitions: Benzyl Penicillin				Ranges of zones of inhibitions: Enrofloxacin			
		0-9.9mm	10-15mm	16-20mm	>20mm	0-9.9mm	10-15mm	16-20mm	>20mm	0-9.9mm	10-15mm	16-20mm	>20mm
Ejisu-Juaben	*1*	5	4	1	1	9	1	1	-	-	-	3	8
	2	1	3	-	2	3	2	-	1	-	-	-	6
	3	-	1	-	1	-	-	2	-	-	1	1	-
	4	1	1	-	1	1	1	-	1	-	-	1	2
	5	1	2	-	1	4	-	--	-	-	1	-	3
Atwima Nwabiagya	*1*	-	2	-	-	2	2	1	-	-	1	1	2
	2	1	1	-	-	-	1	1	-	-	1	-	2
	3	-	1	-	-	-	1	-	-	-	-	-	1
	5	1	1	-	-	1	1	-	-	-	-	-	1
Bosomtwe & Atwima Kwanwoma	*1*	-	-	1	-	-	1	-	-	-	-	-	1
	2	-	3	-	1	4	-	-	-	-	-	-	2
	4	-	1	-	-	1	-	-	-	-	-	-	1
	5	1	-	-	1	2	-	-	-	1	-	-	1
Kwabre	*1*	1	-	-	-	1	-	-	-	-	-	1	1

East	*2*	1	1	-	-	2	-	-	-	-	-	1	1
	3	1	-	-	-	1	1	-	-	-	-	-	2
	4	2	-	-	-	1	1	-	-	-	1	-	1
	5	1	-	-	-	1	-	-	-	-	1	-	-

Key: erythromycin (A), penicillin (B) and enrofloxacin (C) in the presence of *Proteus vulgaris (1), E. coli (2), Enterobacter spp. (3), Salmonella spp.* (4) and *S. typhi* (5)

APPENDIX D: GPS Coordinates

Table 24: Kwabre East district pig farms GPS table showing only farms that were visited showing the latitude (LAT), longitudes (LONG) and the towns the farms are located.

FARM_ID	LAT	LONG	X_UTM	Y_UTM	LOCATION
KED01	6.77273	-1.58569	656301.887	748852.612	Fawoade
KED02	6.84937	-1.46873	669203.701	757366.994	Kasaaam
KED03	6.76102	-1.54133	661209.220	747572.215	Nwamase
KED04	6.7656	-1.54111	661232.020	748078.747	Nwamase
KED05	6.76523	-1.54201	661132.657	748037.534	Saaman
KED06	6.76583	-1.54361	660955.593	748103.352	Saaman
KED07	6.75971	-1.54735	660544.195	747425.364	Bosore
KED08	6.75818	-1.55609	659578.567	747253.302	Bosore
KED09	6.75324	-1.57924	657021.143	746699.514	Truba
KED10	6.7436	-1.57803	657158.008	745633.925	Duase (Part of K)
KED11	6.82163	-1.55166	660047.292	754271.066	Hemang
KED12	6.81624	-1.55622	659545.073	753673.529	Hemang
KED13	6.81343	-1.55718	659439.895	753362.482	Hemang
KED14	6.79518	-1.53835	661527.258	751350.641	Wadie Adwumakase
KED15	6.79254	-1.53887	661470.661	751058.535	Wadie Adwumakase
KED16	6.79264	-1.5387	661489.418	751069.649	Wadie Adwumakase
KED17	6.79656	-1.53687	661690.386	751503.737	Wadie Adwumakase
KED18	6.79888	-1.53519	661875.306	751760.847	Wadie Adwumakase
KED19	6.81558	-1.54058	661273.957	753605.745	Aboaso
KED20	6.81036	-1.52789	662678.333	753032.769	Aboaso
KED21	6.80951	-1.52737	662736.095	752938.950	Aboaso

Table 25: GPS table of Ejisu-Juaben pig farms that were visited showing the latitude (LAT), longitudes (LONG) and the towns the farms are located

FARM ID	LAT	LONG	X_UTM	Y_UTM	Farm location
FL01	6.72471	-1.46566	669586.651	743582.632	Ejisu
FL02	6.71801	-1.47074	669027.356	742839.961	Ejisu
FL03	6.72477	-1.46537	669618.690	743589.367	Ejisu
FL04	6.72465	-1.46593	669556.822	743575.903	Ejisu
FL05	6.7248	-1.46586	669564.509	743592.515	Ejisu
FL06	6.72486	-1.46498	669661.775	743599.455	Ejisu
FL07	6.72469	-1.46575	669576.708	743580.389	Ejisu
FL08	6.72464	-1.46588	669562.353	743574.814	Ejisu
FL09	6.7247	-1.4654	669615.398	743581.616	Ejisu
FL10	6.72472	-1.46541	669614.286	743583.824	Ejisu
FL11	6.72472	-1.4658	669571.170	743583.689	Ejisu
FL12	6.67919	-1.44588	671789.344	738555.705	Ejisu
FL13	6.73155	-1.44981	671336.529	744344.557	Besease-Gyamaase
FL14	6.72797	-1.44951	671370.950	743948.768	Besease-Gyamaase
FL15	6.72835	-1.44923	671401.772	743990.888	Besease-Gyamaase
FL16	6.73371	-1.44919	671404.313	744583.638	Besease-Gyamaase
FL17	6.73321	-1.45093	671212.128	744527.735	Besease-Gyamaase
FL18	6.72473	-1.46563	669589.960	743584.854	Besease-Gyamaase
FL19	6.69279	-1.43312	673195.362	740064.140	Edwenase
FL20	6.69078	-1.43188	673333.168	739842.300	Edwenase
FL21	6.68738	-1.43435	673061.276	739465.439	Edwenase
FL22	6.7373	-1.48163	667816.795	744969.366	Abankro
FL23	6.73816	-1.48092	667894.990	745064.712	Abankro
FL24	6.73832	-1.47824	668191.207	745083.328	Abankro
FL25	6.73853	-1.47792	668226.511	745106.661	Abankro
FL26	6.74792	-1.45806	670418.753	746151.940	Asotwe
FL27	6.74894	-1.44926	671391.222	746267.823	Asotwe
FL28	6.75035	-1.44867	671455.949	746423.955	Asotwe
FL29	6.65466	-1.46817	669333.294	735835.366	Achinakrom
FL30	6.65537	-1.46957	669178.254	735913.400	Achinakrom
FL31	6.6868	-1.4455	671828.700	739397.389	Onwe
FL32	6.67625	-1.4437	672031.402	738231.347	Onwe
FL33	6.6707	-1.4419	672232.360	737618.228	Onwe

FARM ID	LAT	LONG	X_UTM	Y_UTM	Farm location
FL01	6.72471	-1.46566	669586.651	743582.632	Ejisu
FL02	6.71801	-1.47074	669027.356	742839.961	Ejisu
FL03	6.72477	-1.46537	669618.690	743589.367	Ejisu
FL04	6.72465	-1.46593	669556.822	743575.903	Ejisu
FL05	6.7248	-1.46586	669564.509	743592.515	Ejisu
FL06	6.72486	-1.46498	669661.775	743599.455	Ejisu
FL07	6.72469	-1.46575	669576.708	743580.389	Ejisu
FL08	6.72464	-1.46588	669562.353	743574.814	Ejisu
FL34	6.67615	-1.44282	672128.734	738220.596	Onwe
FL35	6.68124	-1.4482	671532.120	738781.595	Onwe
FL36	6.80478	-1.42338	674232.182	752452.150	Juaben
FL37	6.82071	-1.41652	674984.691	754216.279	Juaben
FL38	6.82047	-1.41652	674984.778	754189.738	Juaben
FL39	6.67105	-1.50564	665184.825	737635.120	Apeadu-Kokoben
FL40	6.66944	-1.50011	665796.782	737458.939	Apromase
FL41	6.6672	-1.5203	663565.248	737204.494	Apromase
FL42	6.75966	-1.57144	657881.284	747411.953	Kenkanse (Adwumam Manhyia)
FL43	6.7572	-1.56106	659029.498	747143.307	Adwumam

Table 26: GPS table of Bosomtwe and Atwima Kwanwoma districts pig farms showing the latitude (LAT), longitudes (LONG) and the towns the farms are located

FARM_ID	LAT	LONG	X_UTM	Y_UTM	Farm Location
BAK_01	6.56312	-1.55292	659992.2	725684.7	Behinase
BAK_02	6.56332	-1.55279	660006.5	725706.9	Behinase
BAK_03	6.56462	-1.5522	660071.3	725850.8	Behinase
BAK_04	6.56294	-1.5527	660016.6	725664.9	Behinase
BAK_05	6.56295	-1.55268	660018.8	725666	Behinase
BAK_06	6.5626	-1.55207	660086.3	725627.5	Behinase
BAK_07	6.56205	-1.55142	660158.4	725566.9	Behinase
BAK_08	6.56269	-1.54998	660317.4	725638.1	Behinase
BAK_09	6.56236	-1.55018	660295.4	725601.5	Behinase
BAK_10	6.561086	-1.55116	660187.5	725460.3	Behinase
BAK_11	6.56073	-1.5494	660382.2	725421.5	Behinase
BAK_12	6.56755	-1.54797	660538.2	726176.1	Behinase

FARM_ID	LAT	LONG	X_UTM	Y_UTM	Farm Location
BAK_01	6.56312	-1.55292	659992.2	725684.7	Behinase
BAK_02	6.56332	-1.55279	660006.5	725706.9	Behinase
BAK_03	6.56462	-1.5522	660071.3	725850.8	Behinase
BAK_04	6.56294	-1.5527	660016.6	725664.9	Behinase
BAK_05	6.56295	-1.55268	660018.8	725666	Behinase
BAK_13	6.56274	-1.54981	660336.2	725643.7	Behinase
BAK_14	6.58707	-1.56248	658927.4	728330	Abourntem
BAK_15	6.58709	-1.5625	658925.2	728332.2	Abourntem
BAK_16	6.587	-1.56237	658939.6	728322.3	Abourntem
BAK_17	6.58703	-1.56252	658923	728325.6	Abourntem
BAK_18	6.5864	-1.56754	658368.1	728254.3	Abourntem
BAK_19	6.58649	-1.5675	658372.5	728264.3	Abourntem
BAK_20	6.5585	-1.49865	665995.2	725191.5	Swedru-Abrankese
BAK_21	6.5584	-1.49869	665990.9	725180.4	Swedru-Abrankese
BAK_22	6.55745	-1.49871	665989	725075.3	Swedru-Abrankese
BAK_23	6.63262	-1.55405	659844.9	733369.6	Esereso
BAK_24	6.74496	-1.74236	638992.6	745734.4	Gyaakye-Pramso

Table 27: GPS table of Atwima Nwabiagya pig farms showing the latitude (LAT), longitudes (LONG) and the towns the farms are located

FARM_ID	LAT	LONG	X_UTM	Y_UTM	FARM LOCATION
ANFL_01	6.73347	-1.73774	639506.6	744465.3	Koforidua
ANFL_02	6.75275	-1.72798	640579.9	746599.9	Akropong
ANFL_03	6.75172	-1.727	640688.5	746486.3	Akropong
ANFL_04	6.75157	-1.72698	640690.7	746469.7	Akropong
ANFL_05	6.74978	-1.72865	640506.7	746271.3	Akropong
ANFL_06	6.74745	-1.73022	640333.8	746013.2	Akropong
ANFL_07	6.73817	-1.74066	639182.5	744984.1	Akropong
ANFL_08	6.74447	-1.74043	639206.1	745680.8	Akropong
ANFL_09	6.74496	-1.74236	638992.6	745734.4	Akropong
ANFL_10	6.7715	-1.79052	633662	748655.5	Akropong
ANFL_11	6.73955	-1.71741	641752.1	745143.4	Esaase

FARM_ID	LAT	LONG	X_UTM	Y_UTM	FARM LOCATION
ANFL_01	6.73347	-1.73774	639506.6	744465.3	Koforidua
ANFL_02	6.75275	-1.72798	640579.9	746599.9	Akropong
ANFL_03	6.75172	-1.727	640688.5	746486.3	Akropong
ANFL_04	6.75157	-1.72698	640690.7	746469.7	Akropong
ANFL_05	6.74978	-1.72865	640506.7	746271.3	Akropong
ANFL_06	6.74745	-1.73022	640333.8	746013.2	Akropong
ANFL_12	6.6581	-1.90036	621549.9	736088.5	Anyinamso No. 1
ANFL_13	6.64929	-1.90255	621310	735113.9	Anyinamso No. 2
ANFL_14	6.76057	-1.78874	633861.7	747447.4	Adankwame-Mfensi
ANFL_15	6.77231	-1.7912	633586.6	748744.8	Mfensi
ANFL_16	6.73813	-1.74066	639182.5	744979.7	Entensere
ANFL_18	6.65834	-1.90015	621573.1	736115.1	Gyakobaa
ANFL_19	6.67569	-1.79853	632802.6	738059.6	Nkawie
ANFL_20	6.63632	-1.93179	618080.6	733672.8	Kantenkyire