Hochschule für Angewandte Wissenschaften Hamburg

Fakultät Life Science

Biotechnologie

Bachelorarbeit

Prevalence and Antibiotic Resistance of *Acinetobacter baumannii* in Agricultural Settings in Rural Ghana: Implications for Antimicrobial Stewardship

Vorgelegt von: Boubacar Diallo

Matrikelnummer: [redacted]

Email: [redacted]

Ort der Abgabe: HAW-Hamburg, FSB LS

am 17.07.2023

1.Gutachter: Herr Prof. Dr. Julien Béthune (HAW Hamburg)

2.Gutachter: Frau Dr. Denise Dekker (BNITM Hamburg)

Die Abschlussarbeit wurde betreut und erstellt im Bakteriologie Labor des Kumasi Center for Collaborative Research in Tropical Medecine, Ghana in Zusammenarbeit mit dem Bernhard Nocht Institut für Tropenmedizin, Hamburg

Abstract

Introduction: *Acinetobacter baumannii* (*A. baumannii*) is an opportunistic pathogen commonly found in healthcare settings, posing a significant concern due to its association with hospital-acquired infections and antibiotic resistance. Despite its prevalence in the hospital environment, there is limited knowledge about the occurrence and antimicrobial resistance of *A. baumannii* in poultry and livestock animals. This study aimed to analyse the antimicrobial profile and resistance patterns of *A. baumannii* in these settings and raw meat samples. The research is significant for developing strategies for the management of antimicrobial resistance in rural farming communities in Ghana and addressing the global challenge of antimicrobial resistance. The findings will inform policies and practices related to antimicrobial use in the agricultural sector, particularly in low- and middle-income countries.

Materials and Methods: This study investigated the presence and antimicrobial resistance of *A. baumannii* in poultry, livestock, and raw meat in rural Ghana. Samples were collected from various sources, including farms, markets, shops, and abattoirs. The organisms were isolated and identified by using specific laboratory procedures. Antimicrobial susceptibility testing was conducted following established guidelines. Data analysis was performed using MS Excel, and a map of the sample sites was created using QGIS software.

Results: *A. baumannii* was found in 7.77% of 1,287 stool samples, with the highest occurrence in commercial farm chickens. Antibiotic susceptibility testing showed that most isolates were susceptible to most of the antibiotics that were tested. However, a few numbers of isolates demonstrated resistance to cotrimoxazole and tetracycline. On the other hand, *A. baumannii* was confirmed in 8.46% of 260 meat samples, predominantly in chicken samples from cold stores. Antibiotic susceptibility was generally high but with a small proportion of isolates showing resistance. No multiple drug resistance (MDR) *A. baumannii* isolates were detected, but cultured unidentified organisms exhibited multiple drug resistance (MDR) and multidrug resistance.

Discussion: The study found that *A. baumannii* was prevalent in stool and meat samples, with higher rates during specific months and the rainy season, and contamination in chicken and beef, highlighting the need for continuous surveillance and judicious antibiotic use.

Conclusion: The presence of *A. baumannii* in both stool and meat samples underscores the possibility of food animals serving as reservoirs, emphasizing the importance of worldwide initiatives to monitor and tackle antibiotic resistance in the food chain.

Table of Contents

Abstract i

List of Figures iv

List of Tables v

1. Introduction 1

1.1 Occurrence of *A. baumannii* in animals and the environment 1

1.2 Occurrence of *A. baumannii* in meat 3

1.3 The interplay of poultry and livestock farming, antibiotic usage, and their implications for antibiotic resistance in food animals 4

1.4 Objectives 6

2. Materials and Methods 6

2.1 Study site 6

2.2 Sampling frame 7

2.3 Sample collection 8

2.4 Isolation of *A. baumannii* 9

2.5 Identification of *A. baumannii* 10

2.6 Antibiotic susceptibility testing (AST) 11

2.7 Data analysis 11

3. Results 11

3.1 Stool samples 11

3.1.1 Samples collection 11

3.1.2 Prevalence of *A. baumannii* in stool samples from both commercial and backyard poultry and livestock farms 12

3.1.3 Seasonal patterns of *A. baumannii* incidence 14

3.1.4 Antibiotic susceptibility testing for *A. baumannii* isolated from stool samples....... 15

3.2 Meat samples 16

3.2.1 Samples collection 16

3.2.2 Prevalence of *A. baumannii* in meat samples 18

3.2.3 Antibiotic susceptibility testing for *A. baumannii* isolated from meat samples....... 20

3.3 Prevalence of multiple drug resistance (MDR) and multidrug resistance in *A. baumannii* isolates in both stool and meat samples. 21

4. Discussion 21

5. Conclusion 26

6. Acknowledgements 26

7. References 27

List of Figures

Figure 1: Geographical location of the sample sites in Assin Fosu in the Central Region, Ghana. The figure provides a general overview of the sample sites in Assin Fosu. Additionally, it displays the geographical location of the Central Region along with all its districts…………..7

Figure 2: The sampling plan for the study site encompassed 12 months. In this figure, the "n" in the first line can have the following meanings depending on the sample sites: The number of poultry farms, the total population of free-range poultry, the number of markets, the number of retail shops, and the number of abattoirs. In the second line, the "n" refers to the total number of collected samples over 50 weeks (app. 1 year)……………………………………………8

Figure 3: Sampling of chicken faeces. The figure shows an example of a sampling of chicken faeces in a commercial chicken farm…………………………………………………...9

Figure 4: Distribution of identified organisms in stool samples among the presumptive *A. baumannii* selected. The number of times a specific organism was identified is denoted by n………………………………………………………………………………14

Figure 5: Prevalence of *A. baumannii* by season. The number of *A. baumannii* isolates for each month is denoted by n…………………………………………………………………15

Figure 6: Distribution of meat samples by type of sample……………………………………17

Figure 7: Distribution of identified organism in meat samples among the isolates selected for the VITEK® MS system. The number of times a specific organism was identified is denoted by n………………………………………………………………………………20

List of Tables

Table 1: Zone diameter interpretive standards for *A. baumannii*…………………………….....11

Table 2: Distribution of samples collected from commercial and backyard farms……………12

Table 3: Distribution of samples collected from poultry and livestock farms…………………12

Table 4: Distribution of sampled animals and their proportion in the study…………………...12

Table 5: Selection and test results of the presumptive *A. baumannii* isolates…………………13

Table 6: Biochemical test results for the presumptive *A. baumannii* isolates from stool samples………………………………………………………………………………..13

Table 7: Prevalence of *A. baumannii* in different sample types and farms……………………14

Table 8: Antibiotic susceptibility testing results and origin of resistant *A. baumannii* isolates……………………………………………………………………………….....15

Table 9: Antibiotic susceptibility testing results and origin of unidentified organisms………16

Table 10: Summary of samples collected by location category………………………………17

Table 11: Sample distribution by type of sample and location category………………………17

Table 12: Sample distribution by type of sample and country of origin………………………17

Table 13: Results of colony colour and bacterial growth on agar plates………………………19

Table 14: Biochemical test results for the presumptive *A. baumannii* isolates from meat samples………………………………………………………………………………..19

Table 15: Distribution of *A. baumannii* isolates from meat samples across sampling sites and type of samples…………………………………………………………………………..19

Table 16: Distribution of *A. baumannii* isolates from meat samples across sampling countries and type of samples……………………………………………………………………….19

Table 17: Antibiotic susceptibility profile and resistance origins of *A. baumannii* isolates from meat samples…………………………………………………………………………….20

1. Introduction

Acinetobacter baumannii (*A. baumannii*) is a Gram-negative, aerobic-, non-flagellated coccobacillus bacterium that is commonly isolated from the environment. It is an opportunistic pathogen to humans commonly associated with healthcare settings, making it a significant concern in hospitals and other healthcare facilities. In 2019, the United States' Center for Diseases Control and Prevention (CDC) released statistical data specifically about carbapenem-resistant *A. baumannii* within the country. The data highlights around 8,500 reported cases of carbapenem-resistant *A. baumannii* infections among patients who were hospitalized, along with an estimated 700 deaths occurring in the year 2017 [1].

A. baumannii is considered a recently emerged human pathogen, believed to have first appeared in military healthcare facilities during the Iraq War [2]. Once considered a bacterium with low virulence, *A. baumannii* has now become a prominent member of the ESKAPE group, which includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. This group comprises pathogens that show reduced susceptibility to antibiotics and play a significant role in the occurrence of hospital-acquired infections (HAIs) [3,4]. *A. baumannii* has gained attention due to its ability to cause a wide range of infections, including pneumonia, bloodstream infections, urinary tract infections, and wound infections. Nearly all these infections occur in patients who recently received care in a healthcare facility. The bacterium is known for its ability to survive in harsh environments and on various surfaces, making it difficult to eradicate. It can persist on medical equipment, such as ventilators, catheters, and bedrails, increasing the risk of transmission within healthcare settings. Furthermore, *A. baumannii* is notorious for its high levels of antibiotic resistance, making it a challenging pathogen to treat. In 2017, the World Health Organization (WHO) responded to this situation by designating carbapenem-resistant *A. baumannii* (CRAB) as the number one priority among a published list of 12 antibiotic-resistant bacteria, underscoring the clinical significance and global impact of CRAB infections [5]. Infections that have been caused by CRAB can only be treated with "last-line" antibiotics [6]. For instance, despite its potential kidney toxicity, colistin (polymyxin E) is regarded as the final option in treating CRAB infections. However, strains of colistin-resistant bacteria have been identified [7].

1.1 Occurrence of *A. baumannii* in animals and the environment

The distribution of *A. baumannii* in farm animal populations and their environment remains poorly understood, and there is a significant lack of information, particularly regarding

antimicrobial resistance [8]. Food animals have been recognized as a potential reservoir for *Acinetobacter* spp. in several countries around the world including Senegal [9], China [10], France [11], and Argentina [12]. *A. baumannii* has been documented as a source of mastitis, pneumonia, and sepsis in livestock such as cattle and pigs. Horses have also been known to experience wound infections, septicaemia, bronchopneumonia, neonatal encephalopathy, and eye infections. Similarly, dogs and cats have shown cases of *A. baumannii* isolated from wound infections, bloodstream infections, and urinary tract infections [13,14]. A recent study focusing on isolates obtained from cattle has revealed that these animals carry a remarkably diverse population of *A. baumannii*. Interestingly, this population of *A. baumannii* has been found to be susceptible to most antimicrobial agents [15]. Furthermore, the study finds that seasonality is a significant factor in the occurrence of *A. baumannii*, with higher temperature and humidity during certain seasons contributing to the prolonged survival and increased growth of the bacterium [15]. A study conducted in Lebanon in 2015 by Rafei et al. revealed a substantial presence of *A. baumannii* isolates in animal samples, with a notable proportion originating from cow samples. Additionally, the study reported the occurrence of carbapenem-resistant strains [16].

Regarding the poultry industry, there was a reported incidence in 2011 where an extremely aggressive strain of *A. baumannii* caused a widespread occurrence on a commercial chicken farm in China. This outbreak resulted in the death of over 3000 chicks that were only six days old. The study confirmed that *A. baumannii* was the main pathogen causing chicks mortality on that Chinese farm [17]. A research team conducted by Whilarm Gotfried has examined the occurrence of *A. baumannii* in choana samples obtained from white stork nestlings in Poland. The findings demonstrated that *A. baumannii* was identified in 25% of the sampled Choana specimens, marking the highest recorded prevalence rate documented thus far for any endothermic species [18]. The study also reported a single isolate of *A. baumanni* from geese [18]. A random sampling of a zoological collection in Japan led to the isolation of *A. baumannii* from the faeces of wild birds, although the presence of any pathological conditions in the birds was not mentioned [19]. Insights from Abu Dhabi Falcon Hospital, an investigation was conducted to examine the presence and transmission of *A. baumannii* in the falcon facilities of Abu Dhabi Falcon Hospital. The monthly screening of the facility from 2006 to 2008 revealed the detection of one *A. baumannii* isolates within a short period. It was observed that the falcons acquired *A. baumannii* through contaminated wild birds, possibly via faecal contamination. However, the extent of *A. baumannii* in wild birds remains unclear [20].

Concerning environmental samples associated with poultry, a study investigated the antimicrobial resistance profiles of two *A. baumannii* isolates obtained from sewage water within a poultry slaughterhouse in Germany. These isolates exhibited the same sequence type and carried numerous genetic determinants associated with resistance [21]. The presence of these bacteria has also been identified in the air of the facility where ducks are hatched. The authors hypothesized that these bacteria could potentially contribute to the development of respiratory illnesses among workers in the hatchery [22,23]. Liu et al. also highlighted the potential for transmission between humans and chickens through direct contact and handling [17].

In the natural environment, there is a lack of frequent reports regarding the presence of viable multidrug-resistant *Acinetobacter* spp. with clinical significance. Although it is indeed true that *A. baumannii* can be found in patients and hospital environments during outbreaks, this particular species does not have any recognized natural habitat beyond the hospital setting [24]. Wastewater samples from Brazilian hospitals have revealed the presence of *A. baumannii* isolates that are resistant to multiple drugs [25]. *A. baumannii* has also been isolated from hospital solid waste [26]. In a soil-related study focusing on the occurrence of *A. baumannii*, only a single soil sample out of the 49 tested contained an *Acinetobacter* spp. However, this particular isolate did not demonstrate any genetic relation to known clinical isolates [2].

1.2 Occurrence of *A. baumannii* in meat

Despite food animals being acknowledged as a potential reservoir for *Acinetobacter* spp., extensive research on the prevalence of this bacterial species in raw meat samples is limited. In Hong Kong, a considerable portion of meat samples, precisely 75% out of 36 samples of pork and beef, showed notable levels of *Acinetobacter* spp. detection [27]. In Lebanon, reports have also emerged regarding the presence of *Acinetobacter* spp. isolates in meat samples [16]. In a study conducted by Carvalheira et al. in Portugal, thirteen species of *Acinetobacter* including *A. baumannii* (n=7) were also identified using *rpoB* gene sequencing [28]. In their study, Marí-Almiral et al. examined 138 meat samples of poultry, swine, and beef in Lima, Peru. They successfully retrieved twelve isolates of the *Acinetobacter* genus from five distinct samples of calf meat. Strains were identified, by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) [29].

In contrast to other studies that focus solely on the presence of *A. baumannii*, Hamouda et al. did not find this species in any of the 27 meat samples (cow, chicken, and pork) obtained from retail supermarket chains in Edinburgh [30]. However, Lupo et al. successfully identified

A. baumannii in 25% of 248 meat samples (chicken, turkey, veal, beef, and pork) from Switzerland using MALDI-TOF MS. Among these samples, poultry meat was found to be the most commonly contaminated [31]. Besides, Tavakol et al. identified 22 isolates of *A. baumannii* through the Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) method in a total of 126 animal meat samples, including chicken, bovine, camel, turkey, and ovine [32]. In Isfahan, Iran, Askari et al. conducted a study involving 194 different types of raw meat samples, such as bovine, ovine, caprine, camel, chicken, and turkey. They successfully isolated *A. baumannii* from 20.1% of these samples, with ovine raw meat showing the highest contamination rate at 32.1% [33]. The occurrence of antibiotic-resistant strains of *Acinetobacter* spp. in meat poses an additional public health concern. The study conducted by Lupo et al. and Rafei et al. indicated that *A. baumannii* isolates identified in meat samples exhibited overall susceptibility to antibiotics commonly used in clinical settings. However, there were sporadic instances of resistance observed for colistin, ciprofloxacin, tetracycline, ceftazidime, and piperacillin-tazobactam [16,31]. According to Marí-Almiral et al., eleven isolates of the *A. baumannii* group displayed a high susceptibility profile [29]. However, Tavakol et al. reported that the *A. baumannii* isolates they recovered often exhibited resistance to tetracycline, trimethoprim, cotrimoxazole, and gentamicin. On the other hand, only a small number of isolates showed limited levels of resistance to imipenem, azithromycin, meropenem, rifampin, levofloxacin, ceftazidime, and tobramycin [32]. Additionally, Askari et al. found that *A. baumannii* strains in their study exhibited resistance to certain categories of antimicrobials commonly used to treat *A. baumannii* infections [33].

The presence of *Acintobacter* spp. in meat is of special concern since it was demonstrated that it can survive more than 60 min under thermal processing at 60 °C [34].

1.3 The interplay of poultry and livestock farming, antibiotic usage, and their implications for antibiotic resistance in food animals

Throughout the world, the surge in antibiotic resistance has reached perilously high levels, presenting a substantial and pressing risk to global health, food security, and the advancement of societies. Reports of community-acquired infections attributed to *A. baumannii* are on the rise, particularly in tropical or subtropical regions around the world [35].

The occurrence of antibiotic-resistant bacteria in food animal samples can be attributed, at least in part, to the extensive use of antimicrobial agents for treatment, prevention, growth promoters, and control of diseases in animals used for food production. This heightened use created a stronger selective pressure on bacteria, favouring the survival and proliferation of resistant

strains [13,36]. Based on the European Medicines Agency/European Surveillance of Veterinary Antimicrobial Consumption report in 2013 (EMA/ESVAC, 2013), tetracyclines and penicillins were the most commonly utilized antimicrobials for animals raised for food production across the 26 EU/EEA countries. Polymixins, aminoglycosides, and fluoroquinolones were also used, but to a lesser extent [37]. On the other hand, there is a limited level of antibiotic resistance observed in carbapenem antibiotics, as this class of antibiotic is not permitted for treating animals used for food production due to their importance in human medicine, potential contribution to antibiotic resistance, and the risk of harm to humans consuming meat from treated animals [38].

In Ghana, livestock production plays a significant role in the agricultural sector and makes a substantial contribution to fulfilling food requirements, supplying draught power, and maintaining soil fertility and structure through manure, and income. Most rural households in Ghana raise livestock as part of their agricultural activities, with livestock farming often being combined with crop farming. In the southern regions, poultry farming is the most common, whereas cattle production is primarily concentrated in the Savannah zones. Sheep and goat production, on the other hand, is widespread across the country [39]. However, there is a lack of comprehensive studies in the country that specifically investigates the usage of antimicrobials in food-animal farming [40]. Nevertheless, findings from a study conducted by Donkor et al. in 2012 indicated that approximately 41% of livestock farmers in Ghana employ antibiotics for the purpose of preventing infections on a monthly basis. Furthermore, the team concluded that antibiotic usage in animal husbandry in Ghana is driven by the interest of livestock keepers to prevent and treat animal infections rather than growth enhancement [40]. The findings from a study conducted to examine the utilization of essential antibiotics in poultry farming in Ghana revealed that tetracyclines (24.17%), aminoglycosides (17.87%), penicillins (16.51%), and fluoroquinolones were frequently administered [41]. In their study about antimicrobial usage in commercial and domestic poultry farming in the Ashanti Region of Ghana, Paintsil et al. concluded that there are high levels of antimicrobial usage in both commercial and domestic poultry farming in the Ashanti region in Ghana, which could have a potential impact on One Health. Moreover, the study related that the most common active ingredients contained in the antimicrobial for commercial poultry were oxytetracycline, tylosin, streptomycin, neomycin, and colistin. For domestic poultry, almost all farmers who employed antibiotics used only amoxicillin [42].

1.4 Objectives

Antimicrobial resistance (AMR) is recognized as a global issue that impacts countries regardless of their income or level of development [43]. However, its consequences are particularly pronounced in low- and middle-income countries (LMICs). A comprehensive analysis of AMR data in Africa revealed that 42% of countries lacked available data, there was a significant level of resistance to commonly prescribed antibiotics, and the quality of microbiological data was poor [44]. The use of antibiotics has risen considerably in LMICs for both human and animal health, driven by economic improvements and dietary changes [45]. Currently, LMICs often lack comprehensive antibiotic policies for manufacturing, dispensing, and prescription, which are further affected by low socio-economic status, large population size, and challenged healthcare systems [46]. The irrational use of antimicrobials in developing countries is a complex and multifaceted problem, necessitating a comprehensive understanding for the development of effective control policies. Even with the advent of new medicines, the persistence of antibiotic resistance as a major threat requires behavioural changes in antimicrobial usage.

The objective of this investigation is to assess the antimicrobial profile and determine the level of antimicrobial resistance exhibited by *A. baumannii* in poultry and livestock farming, as well as in raw meat consumed within rural Ghana. By analysing the prevalence and resistance patterns of this bacterium, the study aims to provide valuable insights into the potential risks associated with antimicrobial use in the poultry and livestock sectors, contributing to the development of effective strategies for antimicrobial resistance management in Ghana's rural farming communities.

2. Materials and Methods

2.1 Study site

The investigation was carried out in Assin Fosu, which serves as the administrative centre of the Assin Central Municipal District located in the Central Region of Ghana (Fig. 1). Boasting a population of over 2.8 million residents, the Central Region ranks as the second most densely populated region in Ghana, only surpassed by the Greater Accra region [47]. The Central Region is located in the southern part of the country, along the Gulf of Guinea. It is bordered by the Greater Accra Region to the east, the Western Region to the west, the Ashanti Region to the north, and the Eastern Region to the northeast. Agricultural activity and animal farming are significant components of the Central Region of Ghana's economy. The results of a recent study

about the Entrepreneurial Ecosystem conducted in the Central Region have demonstrated a significant proportion of the population involved in the Agricultural sector. Additionally, the study has shown that farming is the primary agricultural sector activity [48]. The Central region occupies a prominent position within Ghana's tourism sector, renowned for its tourist attractions. Based on data from the Ghanaian Ministry of Tourism, Arts, and Culture, it has been observed that the Central Region of Ghana draws the highest number of visitors among all the regions in the country[49]. However, the surge in tourism brings forth the potential risk of spreading infections. With a considerable proportion of tourists arriving from international destinations, notably European cities like Hamburg, the likelihood of contagious diseases being transmitted increases.

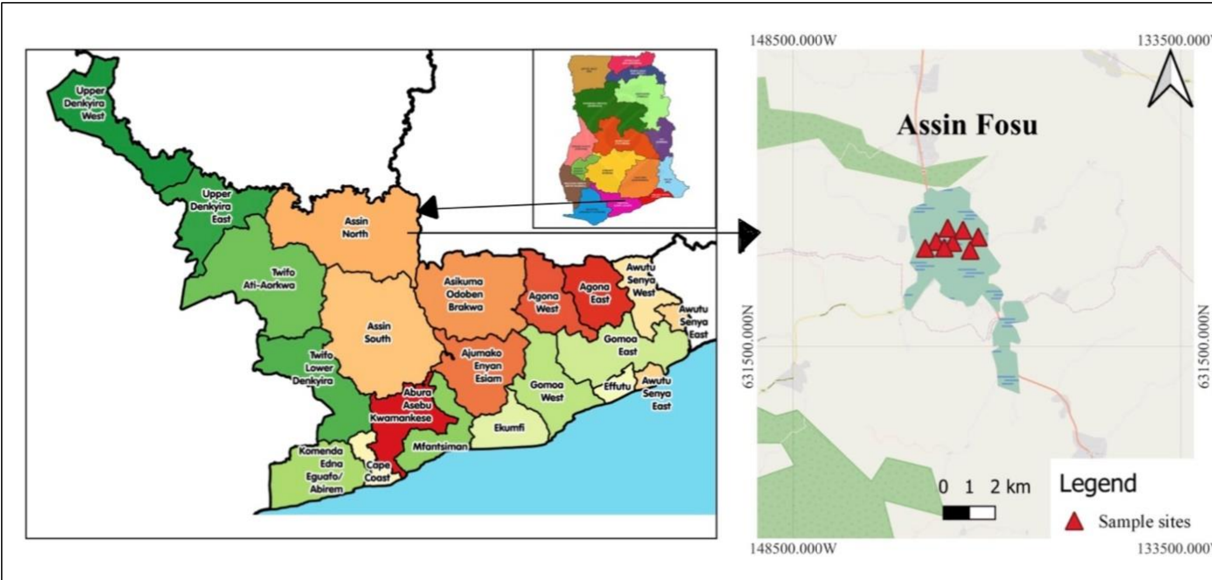

Figure 1: Geographical location of the sample sites in Assin Fosu in the Central Region, Ghana. The figure provides a general overview of the sample sites in Assin Fosu. Additionally, it displays the geographical location of the Central Region along with all its districts.

2.2 Sampling frame

In collaboration with the Animal Research Institute in Accra (Ghana), three large poultry/cattle farms in the study district were visited and registered. Only the farms that dispatched poultry or cattle to the nearby slaughterhouse and had their meat sold locally in the sampled markets and retail shops were selected. Likewise, contact was made, and registration was done for all significant markets, retail shops, and abattoirs within the study area. The sampling site was visited every week, and samples were gathered according to Figure 2.

Furthermore, the study evaluated the occurrence of AMR in free-range farms, commonly known as backyard farms, where it is expected that antibiotic usage is minimal due to lower

disease risk. The meat sourced from these free-range farms significantly contributed to the total supply of poultry products available in local markets in the study area.

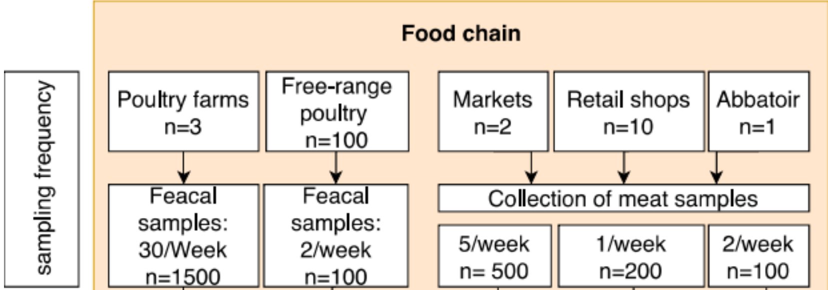

Figure 2: The sampling plan for the study site encompassed 12 months.
In this figure, the "n" in the first line can have the following meanings depending on the sample sites: The number of poultry farms, the total population of free-range poultry, the number of markets, the number of retail shops, and the number of abattoirs. In the second line, the "n" refers to the total number of collected samples over 50 weeks (app. 1 year)

2.3 Sample collection

Sampling took place weekly from April 2022 to March 2023. During each sampling visit, a number of 30 single faecal droplets from poultry and/or livestock on farms were gathered. Poultry included chicken, turkey, and duck, while livestock included cows, pigs, goats, and sheep. A farm was considered commercial if it possessed a minimum of 500 enclosed poultry with an intensive housing system. On the other hand, smallholder farms, which engaged in small-scale agriculture, were households that kept free-roaming poultry and/or an enclosed livestock sheltered by basic or temporary roofing. From each farm, the total samples collected were approximately 10% of the population of farm animals kept. The collection of samples involved using a sterile spatula to collect around 2g of a fresh individual faecal droplet. The collected sample was put into a sterile plastic container without the need for any preservatives to be added (Figure 3).

In markets, retail stores, and the slaughterhouse, 10 single meat samples weighing approximately 15g were weekly collected and placed in sterile homogenizer bags. The collected meat samples were either local meat or exported from other countries. These samples were then refrigerated in a cool box during transportation to the laboratory.

The collected samples, obtained from a place (farm and/or market) were carefully transported in a refrigerated container. To ensure accurate documentation, the GPS coordinates of the collection sites were determined using the eTrex 10 device from Garmin Ltd. After transportation, the samples were promptly processed within 2-4 hours at the bacteriology

laboratory of the Kumasi Center for Collaborative Research in Tropical Medicine (KCCR) in Ghana.

Figure 3: Sampling of chicken faeces. The figure shows an example of a sampling of chicken faeces on a commercial chicken farm.

2.4 Isolation of *A. baumannii*

The organisms were isolated through a weekly procedure, ensuring the isolation of new organisms each week. A detailed explanation of this weekly procedure is provided below.

Day 1: To enhance their growth, the meat samples were first cut in small portions and placed into an enrichment broth (Brain Heart Infusion broth, Oxoid, United Kingdom) before further analysis. In contrast, pre-enrichment was not performed for the stool samples as it was possible to directly streak them onto an agar plate. The stool samples were spread onto Chrom Extended Spectrum Beta-Lactamase (Chrom-ESBL) plates specifically designed to identify ESBL-producing organisms. Both the pre-enriched meat samples and the Chrom-ESBL plates were then placed in an incubator at a temperature of 35-37 °C for 18-24 hours in the normal atmosphere.

Day 2: On the second day, the meat samples were inoculated onto Chrom-ESBL plates cultured in BHI broth. In the case of the stool samples, colonies exhibiting a white colour on the Chrom-ESBL plates were chosen, as it is expected for *A. baumannii* to display this colour on Chrom-ESBL plates [50]. Additionally, the white colonies were streaked onto Blood Agar (BA) plates to generate plates that ensure purity. Subsequently, all plates were incubated at a temperature of 35-37 °C for 18-24 hours in the normal atmosphere.

Day 3: Regarding the meat samples, colonies exhibiting a white colour on the Chrom-ESBL plate were transferred onto BA plates to generate a plat that ensures purity. These plates were then placed in an incubator at a temperature of 35-37 °C for 18-24 hours in the normal atmosphere. As for the stool samples, biochemical tests were carried out on this day. The indole and oxidase tests were performed consecutively. It is expected that *A. baumannii* would yield negative results for both the indole and oxidase tests. The organisms that were successfully isolated were preserved at - 80 °C using the Microbank™ system for subsequent investigations.

Day 4: Identical biochemical tests were conducted for the meat samples on that day. The isolated organisms were stored at - 80 °C using the Microbank™ system for subsequent analyses.

2.5 Identification of *A. baumannii*

The Identification process began after obtaining a significant number of isolates. 130 isolates were selected, including 100 isolates from the stool samples and 30 isolates from the meat samples. All isolates were streaked again on Chrom agar plates without the ESBL supplement. This step was performed to ensure that they were not contaminated with other organisms during storage. The plates were then incubated at a temperature of 35-37 °C for 18-24 hours in the normal atmosphere. The next day, purity plates were prepared by selecting white colonies and streaking them on MacConkey plates. Biochemical tests, including indole, oxidase, and citrate tests, were conducted. This additional step was performed to improve the chances of isolating *A. baumannii*. Since *A. baumannii* is a Gram-negative bacterium, it was expected to grow on the MacConkey plate, and any organisms that did not grow were eliminated. Indole and oxidase tests were expected to yield negative results, while the citrate test was expected to be positive.

Species identification was conducted using the VITEK® MS system (biomerieux, Marcy-l'Étoile, France). Initially, a sterile glass tube was filled with 3 mL of 0.45% Sodium Chloride Inhalation Solution, USP (CareFusion, USA). Then, a few colonies from the overnight culture were transferred into the glass tube using a sterile loop. The contents were vortexed, and the density was measured as 0.5 McFarland units using the DensiCheck Plus (biomerieux, Marcy-l'Étoile, France). Finally, the Vitek Cards were placed in the glass tubes, and species confirmation was carried out using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) with the VITEK® MS system. The results were then saved in a Folder on the desktop.

2.6 Antibiotic susceptibility testing (AST)

All confirmed *A. baumannii* isolates underwent antimicrobial susceptibility testing using the disk diffusion method (Kirby Bauer). The results were then interpreted based on the 2022 European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [51]. *A. baumannii* was inoculated onto Mueller Hinton agar supplemented with 5% sheep blood, and antibiotic disks (Oxoid, United Kingdom) were placed on the agar. The susceptibility plates were then incubated at a temperature of 35-37 °C for 18-24 hours in the normal atmosphere. Following incubation, the diameter of the growth inhibition zone was measured and compared with the interpretation table (table 1) provided by EUCAST for each antibiotic/organism group. The results were categorized as resistant (R), susceptible with increased exposure (I), or susceptible (S).

Table 1: Zone diameter interpretive standards for *A. baumannii*

Antibiotics	**Disc content (μg)**	**Zone diameter breakpoint (mm)**	
		S≥	**R<**
Cotrimoxazole	5	16	11
Tetracycline	30	15	12
Gentamicin	10	17	17
Ciprofloxacin	5	21	21
Meropenem	10	21	15
Imipenem	10	24	21

2.7 Data analysis

Data were transferred to a Microsoft Excel spreadsheet (Version 2305; Microsoft Corp., Redmond, WA, USA) for analysis. QGIS software, version 3.28.7 (QGIS Development Team, Zurich, Switzerland) was used to draw a map showing the location of the sample sites.

3. Results

3.1 Stool samples

3.1.1 Samples collection

A combined number of 79 farms were included in the study, consisting of 25 commercial farms and 54 backyard farms. A total of 1,287 samples were gathered, with 750 (58.28%) originating from commercial farms and 537 (41.72%) obtained from backyard farms (Table 2). A large portion of the collected faecal samples (77.62%, totalling 999 samples) was obtained from

poultry, including chickens, turkeys, and ducks. The remaining samples (22.38%, amounting to 288 samples) were collected from other types of livestock, such as goats, sheep, cows, and pigs (Table 3). Out of the 1,287 samples collected, chickens were the most frequently sampled animal, comprising 63.79% of the total number of samples. Turkeys and pigs were the subsequent most sampled animals, accounting for 8.55% and 8.00% respectively. On the other hand, ducks and sheep had the lowest number of samples, representing 5.13% and 2.56% of the total samples respectively (Table 4).

Table 2: Distribution of samples collected from commercial and backyard farms.

Type of farm	Total number	% of total samples collected
Commercial farms	25	58.28% (750)
Backyard farms	54	41.72% (537)
Grand Total	79	100.00% (1,287)

Table 3: Distribution of samples collected from poultry and livestock farms.

Type of farm animals	Total number	Total samples collected
Poultry farms	52	77.62% (999)
Livestock farms	27	22.38% (288)
Grand Total	79	100.00% (1,287)

Table 4: Distribution of sampled animals and their proportion in the study.

Type of animal	Sum of number of samples	% of total number of samples by type of animal
Chicken	821	63.79%
Turkey	110	8.55%
Pig	103	8.00%
Cow	85	6.60%
Goat	69	5.36%
Duck	66	5.13%
Sheep	33	2.56%
Grand Total	1,287	100.00%

3.1.2 Prevalence of *A. baumannii* in stool samples from both commercial and backyard poultry and livestock farms

A total of 100 (7.77%) were identified as presumptive *A. baumannii* isolates out of the 1,287 stool samples collected. Among the 100 presumptive *A. baumannii* isolates initially selected, four were excluded because they exhibited a colour other than white on the Chrom agar plate. Among the remaining 96 isolates, all of them (100%) grew on the MacConkey agar plate and

tested negative for the indole test. However, a small percentage (4.17%, 4/96) of the presumptive *A. baumannii* isolates tested positive for oxidase and were subsequently eliminated. Additionally, 10.87% (10/92) of the remaining presumptive *A. baumannii* isolates were excluded from the identification process due to their negative result in the citrate test. In Table 5 and Table 6, all the results of the purity plate (PP) on Chrom and MacConkey agar plates, as well as the results of the biochemical tests, are presented.

Finally, a total of 82 (6.37%) were tested for identification out of the 1,287 stool samples collected. Among the 82 presumptive *A. baumannii* isolates analysed with the VITEK® MS system, 31 isolates (37.80%) were confirmed to be positive for *A. baumannii*. However, a significant portion (29.27%, 24/82) of the isolates could not be identified with the VITEK® MS system. The remaining organisms identified were attributed to other organisms (Figure 4).

Among the 54 backyard farms and 25 commercial farms, 3.46% (n=26) and 0.93% (n=5) respectively, tested positive for *A. baumannii*. Concerning the analysed sample types, the prevalence of *A. baumannii* isolates in chicken samples from commercial farms was found to be 3.46% (26/750). None of the chicken samples collected from backyard farms tested positive for *A. baumannii*. The remaining sample types were exclusively obtained from backyard farms, and within this subgroup, the prevalence of *A. baumannii* was relatively low. The highest incidence was observed in pigs, with 2.91% (3/103) of samples testing positive for *A. baumannii*. Ducks and sheep exhibited the lowest prevalence, with only one positive *A. baumannii* detected in the total sample collected from each animal type. Turkey, cow, and goat samples showed no incidence of *A. baumannii* (Table 7).

Table 5: Selection and test results of the presumptive *A. baumannii* isolates.

Initial selection	Exclusion	Exclusion reason
100	4	Colour other than white on chrome agar plate
Remaining isolates	**Test results**	
96	All grew on MacConkey agar plate	

Table 6: Biochemical test for the presumptive *A. baumannii* isolates from stool samples.

	Biochemical tests		
Variable	**Indole, % (n/N)**	**Oxidase, % (n/N)**	**Citrate, % (n/N)**
Positive	0.00% (0/96)	4.17% (4/96)	89.13% (82/92)
Negative	100.00% (96/96)	95.83% (92/96)	10.87% (10/92)
Grand Total	100.00% (96/96)	100.00% (96/96)	100.00% (92/92)

n, number of positive or negative; N, total presumptive *A. baumannii* isolates selected.

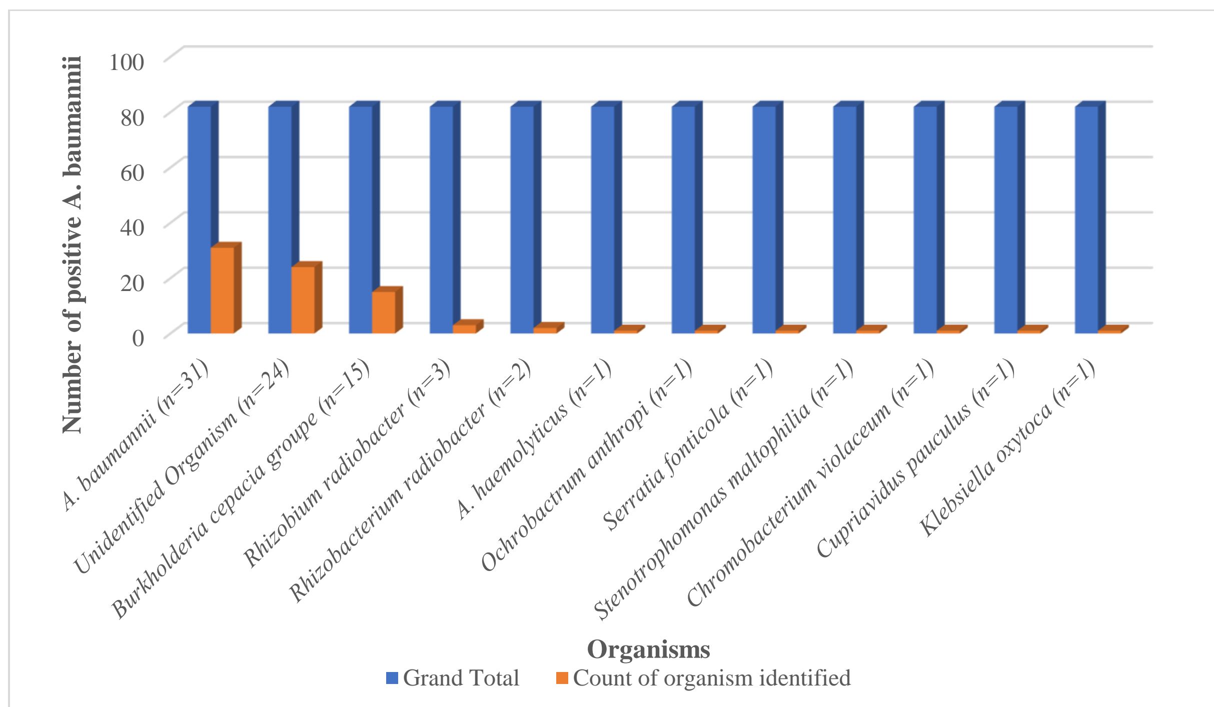

Figure 4: Distribution of identified organisms in stool samples among the presumptive *A. bauamannii* selected. The number of times a specific organism was identified is denoted by n.

Table 7: Prevalence of *A. baumannii* in different sample types and farms.

Type of sample	Type of farm	Total positive *A. baumannii*, % (n/N)
Chicken	commercial	3.46% (26/750)
Chicken	backyard	0.00% (0/71)
Turkey	backyard	0.00% (0/110)
Pig	backyard	2.91% (3/103)
Cow	backyard	0.00% (0/85)
Goat	backyard	0.00% (0/69)
Duck	backyard	1.51% (1/66)
Sheep	backyard	3.03% (1/33)
Grand Total	-	2.41% (31/1,287)

n, number of positive *A. baumannii*; N, total samples collected.

3.1.3 Seasonal patterns of *A. baumannii* incidence

The results reveal that isolates of *A. baumannii* were not detected during the months from December to February and from July to September. However, throughout the rest of the year, *A. baumannii* exhibited varying prevalence rates. The highest prevalence of *A. baumannii* was observed in the months from April to June with respective rates of 10.00% (12/120), 9.17% (11/120), and 3.33% (4/120). On the other hand, the months of March, October, and November showed the lowest prevalence, ranging from 0.68% (1/148) to 2.22% (2/90). *A. baumannii* isolation rate during the rainy season (April to October) was 3.12% (28/898), which exceeded

the 0.77% (3/389) recorded during the dry season (November to March). The seasonal prevalence by month of *A. baumannii* is shown in Figure 5.

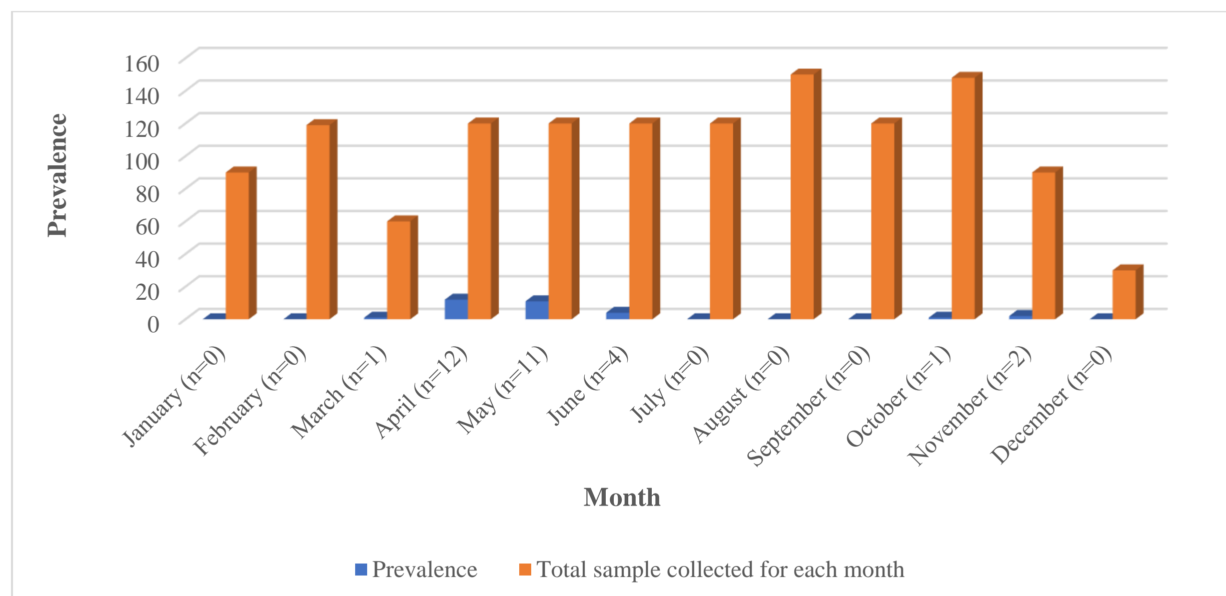

Figure 5: Prevalence of *A. baumannii* by season. The number of *A. baumannii* isolates for each month is denoted by n.

3.1.4 Antibiotic susceptibility testing for *A. baumannii* isolated from stool samples.

The majority (90.32%, 28/31) of the *A. baumannii* isolates that were identified, exhibited a susceptible response to most of the antibiotics that were tested. However, there were three isolates that showed a resistance response to three of the tested antibiotics: Cotrimoxazole, Tetracycline, and Ciprofloxacin. Two out of the three resistant isolates were from chicken samples originating from commercial farms and the remaining resistant isolate was from sheep samples originating from a backyard farm (Table 8). None of the 31 isolates showed a susceptibility at increased exposure (I) response to any of the antibiotics tested. Furthermore, none of the isolates displayed resistance to the antibiotics of the carbapenem class that were tested. However, three (12.50%, 3/24) of the unidentified organisms were found to be resistant to carbapenems. Specifically, two of these isolates showed resistance to Meropenem, while one isolate exhibited resistance to Imipenem. All three carbapenem-resistant isolates were isolated from chickens originating from commercial farms (Table 9).

Table 8: Antibiotic susceptibility testing results and origin of resistant *A. baumannii* isolates.

Antibiotics	AST results for *A. baumannii*		Origin of the resistant isolate(s)	
	Number of (S)	Number of (R)	Type of farm	Sample type
Cotrimoxazole	30	1	Commercial	Chicken
Tetracycline	30	1	Commercial	Chicken
Gentamicin	31	0	NA	NA

Antibiotics	AST results for *A. baumannii*		Origin of the resistant isolate(s)	
	Number of (S)	Number of (R)	Type of farm	Sample type
Ciprofloxacin	30	1	Backyard	Sheep
Meropenem	31	0	NA	NA
Imipenem	31	0	NA	NA

S, susceptible; R, resistant; NA, not applicable (No resistant isolates in that category)

Table 9: Antibiotic susceptibility testing results and origin of resistant unidentified organisms isolates.

Antibiotics	AST results for unidentified organisms		Origin of the resistant isolate(s)	
	Number of (S)	Number of (R)	Type of farm	Sample type
Cotrimoxazole	23	1	Commercial	Chicken
Tetracycline	21	3	Commercial	Chicken
Gentamicin	24	0	NA	NA
Ciprofloxacin	23	1	Commercial	Chicken
Meropenem	22	2	Commercial	Chicken
Imipenem	23	1	Commercial	Chicken

S, susceptible; R, resistant; NA, not applicable (No resistant isolates in that category)

3.2 Meat samples

3.2.1 Samples collection

The meat samples were gathered from 30 different locations, including 21 cold stores, 7 retail shops from the local market, and 2 abattoirs. A collection of 260 meat samples were gathered, with 170 (65.38%) originating from cold stores, 70 (26.92%) originating from the market, and 20 (7.69%) obtained from abattoirs (Table 10). The collected samples consisted of three different types. Among these, chicken accounted for the highest percentage of samples collected, representing 57.69% (150/260). Beef followed with 23.08% (60/260), while cow samples 19.23% (50/260) were the least collected (Figure 6). Furthermore, the chicken samples were exclusively collected from cold stores, whereas the cow samples were gathered from both markets and abattoirs. On the other hand, the beef samples were collected from a combination of cold stores, markets, and abattoirs (Table 11). Regarding their country of origin, the majority of the collected samples consisted of local meat (34.62%, 90/260), obtained either from retail shops in the market or abattoirs. However, a significant portion of the samples were imported from various countries. Within this subgroup, France (11.54%, 30/260) represented the primary source of imported meat samples, followed by Argentina, Brazil, and the USA (United States of America) with a percentage of 9.62% (25/260) for each country. However, the country of origin for a notable portion of the sampled meat remained unknown (Table 12).

Table 10: Summary of samples collected by location category.

Location category	Count of Location category	Sum of samples collected, % (n/N)
Cold store	21	65.38% (170/260)
Market	7	26.92% (70/260)
Abattoir	2	7.69% (20/260)
Grand Total	30	100.00% (260/260)

n, number of samples collected for each location category; N, total sample collected.

Table 11: Sample distribution by type of sample and location category.

Type of samples	Location category	Sum of number of samples collected, % (n/N)	Grand Total, % (n/N)
Chicken	Cold store	57.69% (150/260)	57.69% (150/260)
Beef	Market	11.54% (30/260)	23.08% (60/260)
	Cold store	7.69% (20/260)	
	Abattoir	3.85% (10/260)	
Cow	Market	15.38% (40/260)	19.23% (50/260)
	Abattoir	3.85% (10/260)	
Grand Total		100.00% (260/260)	100.00% (260/260)

n, number of samples collected for each location category; N, total sample collected.

Table 12: Sample distribution by type of sample and country of origin.

Country of Origin	Type of samples			
	Chicken	Beef	Cow	Grand Total, % (n/N)
Local	NA	40	50	34.62% (90/260)
Unknown	40	5	NA	17.31% (45/260)
France	30	NA	NA	11.54% (30/260)
USA	25	NA	NA	9.62% (25/260)
Argentina	20	5	NA	9.62% (25/260)
Brazil	20	5	NA	9.62% (25/260)
Netherland	15	NA	NA	5.77% (15/260)
Ireland	NA	5	NA	1.92% (5/260)
Grand Total	150	60	50	100.00% (260/260)

n, number of samples collected for each location category; N, total sample collected; NA, not applicable (No samples collected).

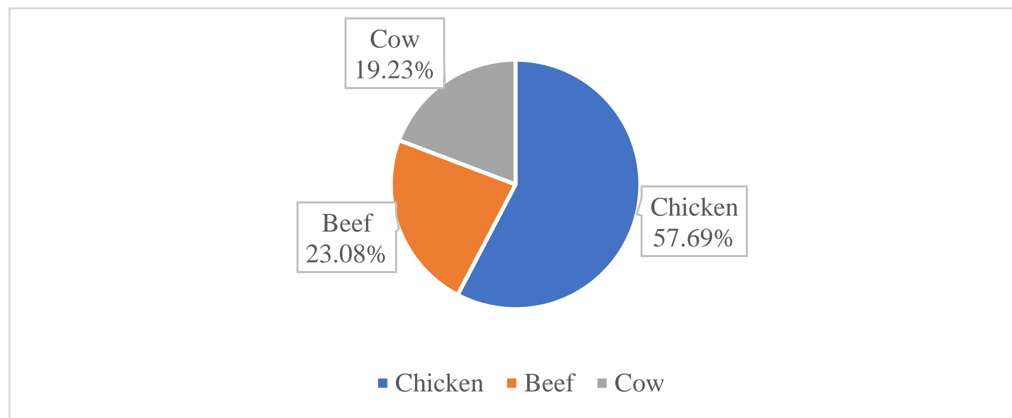

Figure 6: Distribution of meat samples by type of samples

3.2.2 Prevalence of *A. baumannii* in meat samples

Among the 260 meat samples collected, a total of 30 isolates (11.54%) were identified as presumptive *A. baumannii*. One of the initial 30 presumptive *A. baumannii* isolates was excluded due to its colour deviation from the expected appearance on the Chrom agar plate. Among the remaining 29 isolates, all of them (100.00%, 29/29) grew on the MacConkey agar plates and tested negative for the Indole test. However, a small portion (6.90%, 2/29) of the presumptive *A. baumannii* isolates tested positive for oxidase and were subsequently removed from consideration. Furthermore, 7.41% (2/27) of the remaining presumptive *A. baumannii* isolates were excluded from the identification process due to their negative result in the Citrate test. Tables 13 and 14 show the PP (Purity Plates) results on both Chrom- and MacConkey agar plates, as well as the results of the biochemical tests.

Ultimately, from the 260 collected meat samples, a total of 25 samples (9.62%) underwent the identification testing. Among these 25 *A. baumannii* presumptive isolates that underwent analysis through the VITEK® MS system, 22 isolates (88.00%) were successfully confirmed as *A. baumannii*. Nevertheless, a small fraction of the isolates (12.00%, 3/25) exhibited identification results corresponding to organisms distinct from *A. baumannii* (Figure 7).

Out of the locations included in the study, *A. baumannii* was detected in 7.65% (n=13) from cold stores, 11.43% (n=8) from the market, and 5.00% (n=1) from abattoirs. Among the chicken samples, 6.00% (9/150) tested positive, with the positive results exclusively found in the cold store as chicken samples were not gathered from retail shops (markets) and abattoirs. Among beef samples, 11.66% (7/60) tested positive. The highest prevalence among cow samples was observed in the cold stores (20.00%, 4/20), followed by 10.00% (3/30) in the market. No positive *A. baumannii* were found in the abattoir samples. The cow samples had a positivity

rate of 12.00% (6/50), with positive results observed in both markets (12.0%, 5/40) and abattoir (10.00%, 1/10). Overall, the grand total indicates an 8.46% (22/260) positivity rate across all samples and locations (Table 15). Concerning their country of origin, there is variability in the prevalence of positive *A. baumannii* isolates. Among local samples, 10.00% (9/90) tested positive for chicken, with 7.50% (3/40) for beef and 12.00% (6/50) for cow. For unknown origin samples, 2.22% (1/45) tested positive, with 2.50% (1/40) for chicken and no positive samples reported for beef. France had a 10.00% (3/30) positivity rate for chicken, while the USA had an 8.00% (2/25) positivity rate for chicken. Argentina and the Netherlands reported no positive samples for chicken. Brazil showed a 20.00% (5/25) positivity rate for chicken, with 10.00% (2/20) for beef and 60.00% (3/5) for cow. Ireland had a 20.00% (1/5) positivity rate for beef (Table 16).

Table 13: Results of colony colour and bacterial growth on agar plate.

Agar plates	**PP results**			
	Colonies colour		**Bacterial growth status**	
	White, % (n/N)	**Other Colour, % (n/N)**	**BG, % (n/N)**	**NBG, % (n/N)**
Chrom agar	96.67% (29/30)	3.33% (1/30)	NA	NA
MacConkey agar	NA	NA	100.00% (29/29)	0.00% (0/29)
Grand Total	100.00% (30/30)		100.00% (29/29)	

n, number of positive isolates; N, total isolates tested; NA, not applicable (No isolate tested).

Table 14: Biochemical test for the presumptive *A. baumannii* isolates from meat samples.

Biochemical test	**Test results**		
	Positive, %(n/N)	**Negative, % (n/N)**	**Grand Total, % (n/N)**
Indole	0.00% (0/29)	100.00% (29/29)	100.00% (29/29)
Oxidase	6.90% (2/29)	93.10% (27/29)	100.00% (29/29)
Citrate	95.59% (25/27)	7.41% (2/27)	100.00% (27/27)

n, number of positive isolates; N, total isolates tested.

Table 15: Distribution of *A. baumannii* isolates from meat samples across sampling sites and type of sample.

Type of sample	**Sampling sites**			**Grand Total, % (n/N)**
	Cold store, % (n/N)	**Market, % (n/N)**	**Abattoir, % (n/N)**	
Chicken	6.00% (9/150)	NA	NA	6.00% (9/150)
Beef	20.00% (4/20)	10.00% (3/30)	0.00% (0/10)	11.66% (7/60)
Cow	NA	12.50% (5/40)	10.00% (1/10)	12.00% (6/50)
Grand Total, % (n/N)	7.65% (13/170)	11.43% (8/70)	5.00% (1/20)	8.46% (22/260)

n, number of positive isolates; N, total samples collected; NA, not applicable (No sample collected).

Table 16: Distribution of *A. baumannii* isolates from meat samples across sampling countries and type of sample.

Country of Origin	Type of samples			Grand Total, % (n/N)
	Chicken, % (n/N)	Beef, % (n/N)	Cow, % (n/N)	
Local	NA	7.50% (3/40)	12.00% (6/50)	10.00% (9/90)
Unknown	2.50% (1/40)	0.00% (0/5)	NA	2.22% (1/45)
France	10.00% (3/30)	NA	NA	10.00% (3/30)
USA	8.00% (2/25)	NA	NA	8.00% (2/25)
Argentina	0.00% (0/20)	0.00% (0/5)	NA	0.00% (0/25)
Brazil	10.00% (2/20)	60.00% (3/5)	NA	20.00% (5/25)
Netherland	6.66% (1/15)	NA	NA	6.66% (1/15)
Ireland	NA	20.00% (1/5)	NA	20.00% (1/5)
Grand Total, % (n/N)	6.00% (9/150)	11.66% (7/60)	12.00% (6/50)	8.46% (22/260)

n, number of positive isolates; N, total samples collected; NA, not applicable (No sample collected).

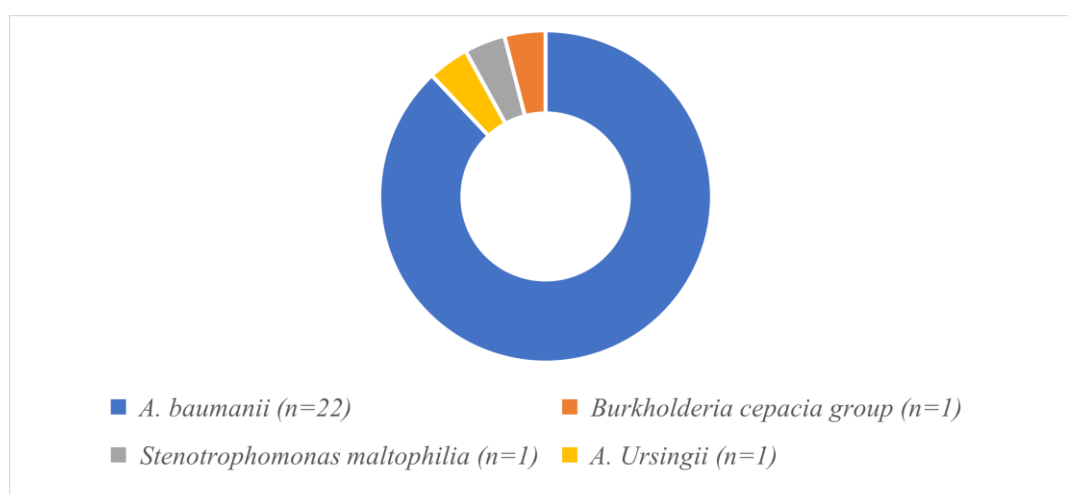

Figure 7: Distribution of identified organisms in meat samples among the isolates selected for the VITEK® MS system. The number of times a specific organism was identified is denoted by n.

3.2.3 Antibiotic susceptibility testing for *A. baumannii* isolated from meat samples.

In general, *A. baumannii* isolates from meat samples showed a favourable susceptibility profile for most of the antibiotics tested. Cotrimoxazole demonstrated high susceptibility (S), with 95.45% of the isolates being susceptible. However, a small percentage (4.54%) showed resistance (R), indicating the presence of some resistance strains. Tetracycline also showed a similar pattern, with 90.90% susceptibility (S) and 4.54% resistance (R). Additionally, 4.54% of the isolates displayed susceptibility at increased exposure (I) to Tetracycline. On the other hand, Gentamicin, Ciprofloxacin, Meropenem, and Imipenem exhibited excellent results, with 100.00% susceptibility (S) and no instances of resistance (R) or susceptibility at increased exposure (I) among the tested isolates. The two resistant strains, as well as the isolate that displayed susceptibility at increased exposure (I) to Tetracycline, were both sampled from a cold store. The resistance isolate to Cotrimoxazole was found in a sample obtained from beef

originating from Brazil, while the resistance strain for Tetracycline was identified in a sample obtained from chicken originating from France. On the other hand, the isolate that displayed susceptibility at increased exposure (I) to Tetracycline was obtained from chicken samples originating from the USA. Table 17 highlights the results of the Antibiotic susceptibility testing.

Table 17: Antibiotic susceptibility profile and resistance origins of *A. baumannii* isolate from meat samples.

Antibiotics	**AST results for *A. baumannii***			**Origin of the resistant isolate(s)**		
	Susceptible, % (n/N)	**Resistant, % (n/N)**	**Intermediate, % (n/N)**	**Type of sample**	**Sampling site**	**Country of origin**
Cotrimoxazole	95.45% (21/22)	4.54% (1/22)	0.00% (0/22)	Beef	Cold store	Brazil
Tetracycline	90.90% (20/20)	4.54% (1/22)	4.54% (1/22)	Chicken	Cold store	France
Gentamicin	100.00% (22/22)	0.00% (0/22)	0.00% (0/22)	NA	NA	NA
Ciprofloxacin	100.00% (22/22)	0.00% (0/22)	0.00% (0/22)	NA	NA	NA
Meropenem	100.00% (22/22)	0.00% (0/22)	0.00% (0/22)	NA	NA	NA
Imipenem	100.00% (22/22)	0.00% (0/22)	0.00% (0/22)	NA	NA	NA

n, number of susceptible, resistant, or intermediate isolates; N, total isolate tested; NA, not applicable (No resistant isolate in that category).

3.3 Prevalence of multiple drug resistance (MDR) and multidrug resistance in *A. baumannii* isolates in both stool and meat samples.

Multiple drug resistance, MDR (i.e., resistance to three or more antibiotics), as well as Multidrug resistance (i.e., resistance to the antibiotics typically used for the treatment of infections caused by *A. baumannii*: Meropenem, Imipenem, and Gentamicin) was not observed for any of the confirmed *A. baumannii* isolates (n=31) from the stool samples. Nonetheless, 8.33% (n/N = 2/24) of the unidentified organisms showed multiple drug resistance (MDR). Both isolates were isolated from chicken samples originating from commercial farms. Additionally, these two isolates were respectively isolated in May and August. Regarding multidrug resistance among the unidentified organisms, 12.50% (n/N = 3/24) of them showed multidrug resistance against Meropenem and Imipenem (Table 9).

Concerning the meat samples, they showed neither multiple drug resistance nor multidrug resistance (Table 17).

4. Discussion

The current study provided valuable insights into the prevalence and antibiotic-resistant profile of *A. baumannii* isolated along the food chain within a rural region of Ghana. Despite the low percentage of *A. baumannii* isolates in the current study, it provides confirmation of the presence of *A. baumannii* beyond the hospital environment. Furthermore, it highlights the role

of food animals as potential reservoirs for *A. baumannii*. Similar results regarding the prevalence of *A. baumannii* in stool samples of food animals were reported by Rafei et al. in a previous study in Lebanon where they examined 379 faecal samples gathered from cow. Among these samples, a total of 4.48% (n/N = 17/379) isolates of *A. baumannii* were reported[16]. However, in contrast to the study by Rafei et al., the current study differed in several key aspects. Firstly, the current study involved collecting stool samples from a wide range of animals, including poultry and livestock, whereas Rafei et al. specifically examined faecal samples from cows. Additionally, while Rafei et al. analysed 379 faecal samples, a larger number of stool samples specifically 1,287 were gathered in the current study. Another notable difference lies in the sample collection process. Unlike Rafei et al., who promptly collected faecal samples after defecation or directly from the animal anus to minimize potential contamination, in the present study the collected stool samples were already on the soil. This variation in sample collection methodology could have implications for the overall microbial composition and potential sources of contamination. Furthermore, in contrast to Rafei et al., who performed a pre-enrichment step by suspending the faecal samples in a 10% water solution, the current study did not include any pre-enrichment step for the stool samples. This difference in methodology could explain the high number of both unidentified bacterial species (n=24 isolates) and the identification of 27 isolates of species other than *A. baumannii*. Without a pre-enrichment step for the stool samples in the current study, the number of unidentified organisms could be attributed to the limited growth and isolation of specific bacterial species. Certain bacteria, including *A. baumannii*, might have been present in the samples but could not be accurately identified due to the lack of an enriched environment for their growth and detection. Furthermore, the identification of 27 isolates as different organisms than *A. baumannii* could be explained by the possibility that other bacterial species present in the stool samples outcompeted or overgrew *A. baumannii* during the culture process. The absence of a pre-enrichment step might have limited the ability to specifically isolate and identify *A. baumannii*, leading to the identification of alternative organisms instead.

Besides that, there are differences in the methodology employed for isolation between the study conducted by Rafei et al. and the current study. As for the study by Rafei et al., the pre-enrichment step was followed by streaking the cultures onto MacConkey agar plates. The agar plates were supplemented with Cephradine, Amoxicillin, Fosfomycin, and Cycloheximide, and then incubated at 37 °C for 48 hours. In addition, there is another study that used a different approach for isolating bacterial cultures of *A. baumannii*. In this other study, conducted by Klotz et al., the samples were cultured on blood agar, Water-blue Metachrome-yellow Lactose agar,

and MacConkey agar containing 1 mg/L cefotaxime [15]. In contrast, the present study employed a different approach for isolating bacterial cultures of *A. baumannii*. The samples were streaked directly onto Chrom agar plates supplemented with CHROMagar^{TM} ESBL supplement (CHROMagar, Paris, France). The selection of this agar medium for this study indicates a specific interest in identifying ESBL-producing bacteria. However, during the identification process, the isolates were streaked on MacConkey agar plates to ensure the selection of only Gram-negative bacteria. This step was taken to differentiate and select the target bacteria from other types of organisms for identification. Furthermore, the incubation period in the current study was shorter (18-24 hours at 35-37 °C in normal atmosphere) compared to Rafei et al.'s study. The choice of agar medium and the differences in incubation time reflect variations in the objectives and methodologies employed. Rafei et al. aimed to isolate and identify *A. baumannii* beyond the hospital environment, while the present study focused on detecting exclusively ESBL-producing *A. baumannii*. These differences in isolation techniques and incubation conditions highlight the diversity of approaches in microbial identification and characterisation.

In the same way, the current study reported a seasonal occurrence of *A. baumannii* suggesting that the prevalence of *A. baumannii* varies throughout the year, with higher rates observed during certain months (from April to June) and during the rainy season (April to October). Furthermore, the study reported a small percentage of antimicrobial resistance (AMR) during the rainy season (n=2), whereas only one resistant pattern was reported during the dry season. The observed seasonal and monthly variations in the prevalence of *A. baumannii* have important implications for infection control strategies. The absence of *A. baumannii* during certain months suggests the possibility of seasonal fluctuations in environmental reservoirs or colonization patterns. Several factors could contribute to the reduced detection of *A. baumannii* during specific months. For instance, environmental conditions such as temperature and humidity may play a role in bacterial survival and persistence. Higher temperatures during the dry season might hinder the growth and dissemination of *A. baumannii*, resulting in decreased detection rates. Similarly, decreased humidity levels during certain months may limit the survival of *A. baumannii* in the environment or on surfaces, reducing its transmission potential. Conversely, the higher prevalence rates observed during the months from April to June indicate a period of increased *A. baumannii* activity. These findings may suggest that favourable environmental conditions during these months contribute to enhanced bacterial growth and dissemination. Warmer temperatures and increased humidity could create a more suitable environment for the survival and proliferation of *A. baumannii*, leading to higher detection

rates. Furthermore, seasonal changes in the food animals might also contribute to the observed variations. It is possible that animals sampled during certain months have a higher risk of *A. baumannii* colonisation or infection due to specific seasonal factors or activities. Moreover, the increased isolation rate of *A. baumannii* during the rainy season (April to October) suggests a potential link between environmental conditions and the transmission dynamics of the bacterium. Rainfall can impact the environmental reservoirs and routes of transmission for *A. baumannii*. Heavy precipitation may facilitate the dissemination of *A. baumannii* by creating more conducive conditions for bacterial survival and dispersion. The higher prevalence of *A. baumannii* during the rainy season, accompanied by a slightly higher number of resistance patterns, raises concerns about the potential impact of environmental factors on the development and spread of antibiotic resistance. The presence of resistant patterns during this period might be attributed to the increased use of antibiotic or the circulation strains with a higher propensity for resistance acquisition. Similar results have been reported in a previous study conducted in Germany by Klotz et al. on seasonal occurrence and carbapenem susceptibility of bovine *A. baumannii* in Germany. The study found that the prevalence of *A. baumannii* in cattle was higher during the summer months, with a peak between May and August. The authors suggest that this could be due to the increased use of antimicrobial agents during this time, as well as the warmer temperatures, which may favour the growth and survival of the bacterium [15]. This convergence of findings suggests that environmental factors, such as temperature and antimicrobial use, play a significant role in shaping the prevalence and transmission dynamics of *A. baumannii* across different contexts. The similarities in seasonal patterns between *A. baumannii* in food animals in Ghana and Germany highlight the need for global implications for infection control strategies and antimicrobial stewardship in the One Health field.

The present study also highlighted the prevalence of *A. baumannii* in meat commonly consumed in the study area. Overall, the study reported a prevalence of 8.46% positivity rate across all samples and locations. Among the specific meat samples, 6.00% of chicken samples tested positive for *A. baumannii*, exclusively in cold stores. This finding suggests that *A. baumannii* may contaminate chicken products during storage, potentially due to improper handling, storage conditions, or the introduction of contaminated poultry. Regarding beef samples, 11.66% tested positive for *A. baumannii* with the highest prevalence being observed in cold stores (20.00%) followed by 10.00% in the markets. The absence of positive *A. baumannii* among the abattoir samples suggests that the abattoir's stringent control measure may effectively minimize the presence and transmission of *A. baumannii* in beef products. Samples collected from cows

exhibited an overall *A. baumannii* positivity rate of 12.00%, with *A. baumannii* identified in both market and abattoir samples. This suggests that *A. baumannii* can be present in cow samples across the food chain, from the abattoir to the market. The detection of *A. baumannii* in cow samples further highlights the potential contamination risk during livestock production and supply.

The results of the current study provided also valuable information regarding the antibiotic susceptibility of *A. baumannii* isolates in meat samples. The study reported that *A. baumannii* isolates showed high susceptibility to most antibiotics tested, with Cotrimoxazole and Tetracycline having 95.45% and 90.90% susceptibility, respectively. Testing with Gentamicin, Ciprofloxacin, Meropenem, and Imipenem exhibited reassuring results with 100% susceptibility. The favourable susceptibility profile of *A. baumannii* isolates from the meat samples indicates that several antibiotics, such as Cotrimoxazole, Tetracycline, Gentamicin, Ciprofloxacin, Meropenem, and Imipenem, may still be effective treatment options. However, the presence of resistance and susceptibility at increased exposure to certain antibiotics (Cotrimoxazole and Tetracycline) highlights the importance of ongoing surveillance and judicious use of antibiotics to mitigate the emergence and spread of resistance. The geographical variation observed in antibiotic-resistant profiles in this study emphasizes the need for global efforts to monitor and address antibiotic resistance in *A. baumannii* and other pathogens associated with food products.

The findings of *A. baumannii* in meat samples in the present study are in concordance with previous studies such as the study conducted by Marí-Almirall et al., where 12 *Acinetobacter* isolates were found in calf samples by analysing meat samples from traditional markets in Lima, Peru. They found a low prevalence of *A. baumannii* in the meat samples, with only one strain identified out of the 12 *Acinetobacter* isolates recovered. Additionally, they found all isolates well susceptible to antibiotics [29].

The present study also revealed a lack of multiple drug resistance (MDR), as well as multidrug resistance among confirmed *A. baumannii* isolates from both stool and meat samples. However, the presence of multiple drug resistance and multidrug resistance among unidentified organisms, particularly in chicken samples, raises concerns about the potential transmission of antibiotic-resistant organisms in the food chain. Further studies are needed to identify and characterize these organisms and to evaluate their implications for public health. Continued surveillance is crucial to assess the emergence and spread of antibiotic resistance in both confirmed and unidentified organisms associated with food products.

5. Conclusion

In conclusion, this study provides valuable insights into the prevalence, antibiotic susceptibility, and seasonal variations of *A. baumannii* in the food chain in Assin-Fosu, Ghana. The detection of *A. baumannii* in food samples highlights the role of food animals as potential reservoirs. The prevalence of *A. baumannii* in stool samples was relatively low compared to previous studies, possibly influenced by differences in sample collection and isolation methods. The study also reported seasonal and monthly variations in *A. baumannii* prevalence, with higher rates observed during the months of April to June and the rainy season (April to October). These findings suggest a potential influence of environmental factors, such as temperature and rainfall, on the growth and transmission dynamics of *A. baumannii*. Regarding meat samples, *A. baumannii* was detected at varying prevalence rates, with higher prevalence observed in meat of cow, and beef samples obtained from specific locations, such as cold stores and markets. Importantly, the majority of *A. baumannii* isolates showed high susceptibility to the antibiotics tested, indicating effective treatment options. However, a small percentage of isolates exhibited resistance (R) or susceptibility at increased exposure (I) to specific antibiotics. The geographical variation in resistance patterns highlights the need for global efforts to monitor and address antibiotic resistance in *A. baumannii* associated with food products. The absence of multiple drug resistance (MDR) and multidrug resistance among confirmed *A. baumannii* isolates from stool and meat samples is encouraging. However, the presence of multiple drug resistance (MDR) and multidrug resistance among unidentified organisms, particularly in chicken samples, raises concerns about the potential transmission of antibiotic-resistant organisms in the food chain. Further studies are needed to identify and characterize these organisms and assess their implications for public health.

Overall, this study contributes to our understanding of *A. baumannii* in the food chain, emphasizing the need for continued surveillance, adherence to hygiene practices, and judicious use of antibiotics. The findings underscore the importance of a One Health approach to address the complex dynamics of *A. baumannii* transmission and antibiotic resistance in both healthcare and food production settings. Further research is warranted to elucidate the mechanisms underlying the observed resistance patterns and to develop effective strategies for prevention and control.

6. Acknowledgements

I would like to express my sincere gratitude to the One Health Bacteriology team at the Bernhard Nocht Institute für Tropen Medizin (BNITM), particularly Dr. Denise Dekker and

Dr. Stefan Berg, for their valuable support and guidance throughout this research project. Their expertise and contributions have been instrumental in the success of this study. I would also like to extend my appreciation to the Bacteriology team at the Kumasi Center for Collaborative Research (KCCR), headed by Dr. Charity Wiafe Akenten. Their collaboration and assistance in sample collection and laboratory work were indispensable in obtaining the data for this study. Furthermore, I would like to acknowledge the support and supervision provided by my esteemed supervisor, Prof. Dr. Julien Béthune. His expertise, guidance, and valuable insights have greatly contributed to shaping the structure and direction of this research paper. I am especially also grateful to Cynthia Kyerewaa and Charlotte Adu for their supervision and assistance during the laboratory work. Their expertise and dedication were essential in the successful execution of the experiments. Lastly, I would like to extend my heartfelt thanks to my family and all those who have supported me throughout this research endeavour. Your encouragement and belief in my ability have been a constant source of motivation. This research would not have been possible without the collaboration, support, and encouragement of all the individuals mentioned above. I am deeply appreciative of their contributions to this study.

7. References

1. CARBAPENEM-RESISTANT ACINETOBACTER. .

2. Scott P, Deye G, Srinivasan A *et al.* An outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus complex infection in the US military health care system associated with military operations in Iraq. Clinical Infectious Diseases 2007; 44: 1577–1584.

3. Boucher HW, Talbot GH, Bradley JS *et al.* Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. Clinical Infectious Diseases 2009; 48: 1–12.

4. Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther 2013; 11: 297–308.

5. WHO publishes list of bacteria for which new antibiotics are urgently needed. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (19 May 2023, date last accessed).

6. Hamidian M, Nigro SJ. Emergence, molecular mechanisms and global spread of carbapenem-resistant acinetobacter baumannii. Microbial Genomics 5 2019.

7. Lesho E, Yoon E-J, McGann P *et al.* Emergence of Colistin-Resistance in Extremely Drug-Resistant Acinetobacter baumannii Containing a Novel pmrCAB Operon During Colistin Therapy of Wound Infections. J Infect Dis 2013; 208: 1142–1151.

8. Wareth G, Neubauer H, Sprague LD. Acinetobacter baumannii – a neglected pathogen in veterinary and environmental health in Germany. Veterinary Research Communications 43 2019.

9. Kempf M, Rolain J-M. Emergence of resistance to carbapenems in Acinetobacter baumannii in Europe: clinical impact and therapeutic options. Int J Antimicrob Agents 2012; 39: 105–114.

10. Wang Y, Wu C, Zhang Q *et al.* Identification of new delhi metallo-β-lactamase 1 in acinetobacter lwoffii of food animal origin. PLoS One 2012; 7.

11. Lupo J. Carbapenemase-producing Acinetobacter spp. in Cattle, France. .

12. Ledesma MM, Díaz AM, Barberis C *et al.* Identification of Lama glama as reservoirs for Acinetobacter lwoffii. Front Microbiol 2017; 8.

13. Müller S, Janssen T, Wieler LH. Multidrug resistant Acinetobacter baumannii in veterinary medicine--emergence of an underestimated pathogen? Berl Munch Tierarztl Wochenschr 2014; 127: 435–46.

14. van der Kolk JH, Endimiani A, Graubner C, Gerber V, Perreten V. Acinetobacter in veterinary medicine, with an emphasis on Acinetobacter baumannii. J Glob Antimicrob Resist 2019; 16: 59–71.

15. Klotz P, Higgins PG, Schaubmar AR *et al.* Seasonal occurrence and carbapenem susceptibility of bovine Acinetobacter baumannii in Germany. Front Microbiol 2019; 10.

16. Rafei R, Hamze M, Pailhoriès H *et al.* Extrahuman Epidemiology of Acinetobacter baumannii in Lebanon. Appl Environ Microbiol 2015; 81: 2359–2367.

17. Liu D, Liu ZS, Hu P *et al.* Characterization of a highly virulent and antimicrobial-resistant Acinetobacter baumannii strain isolated from diseased chicks in China. Microbiol Immunol 2016; 60: 533–539.

18. Wilharm G, Skiebe E, Higgins PG *et al.* Relatedness of wildlife and livestock avian isolates of the nosocomial pathogen Acinetobacter baumannii to lineages spread in hospitals worldwide. Environ Microbiol 2017; 19: 4349–4364.

19. Ahmed AM, Motoi Y, Sato M *et al.* Zoo animals as reservoirs of gram-negative bacteria harboring integrons and antimicrobial resistance genes. Appl Environ Microbiol 2007; 73: 6686–6690.

20. Muller MG, George AR, Walochnik J. Acinetobacter baumannii in localised cutaneous mycobacteriosis in falcons. Vet Med Int 2010; 2010.

21. Savin M, Parcina M, Schmoger S, Kreyenschmidt J, Käsbohrer A, Hammerl JA. Draft Genome Sequences of Acinetobacter baumannii Isolates Recovered from Sewage Water from a Poultry Slaughterhouse in Germany. Microbiol Resour Announc 2019; 8.

22. Martin E, Ernst S, Lotz G, Linsel G, Jäckel U. Microbial exposure and respiratory dysfunction in poultry hatchery workers. Environ Sci Process Impacts 2013; 15: 478–84.

23. Martin E, Jäckel U. Characterization of bacterial contaminants in the air of a duck hatchery by cultivation based and molecular methods. J Environ Monit 2011; 13: 464–70.

24. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii* : Emergence of a Successful Pathogen. Clin Microbiol Rev 2008; 21: 538–582.

25. Ferreira AE, Marchetti DP, De Oliveira LM, Gusatti CS, Fuentefria DB, Corção G. Presence of OXA-23-Producing Isolates of *Acinetobacter baumannii* in Wastewater from Hospitals in Southern Brazil. Microbial Drug Resistance 2011; 17: 221–227.

26. Hossain Md, Rahman N, Balakrishnan V, Puvanesuaran V, Sarker Md, Kadir M. Infectious Risk Assessment of Unsafe Handling Practices and Management of Clinical Solid Waste. Int J Environ Res Public Health 2013; 10: 556–567.

27. Houang ETS, Chu YW, Leung CM *et al.* Epidemiology and infection control implications of Acinetobacter spp. in Hong Kong. J Clin Microbiol 2001; 39: 228–234.

28. Carvalheira A, Casquete R, Silva J, Teixeira P. Prevalence and antimicrobial susceptibility of Acinetobacter spp. isolated from meat. Int J Food Microbiol 2017; 243: 58–63.

29. Marí-Almirall M, Cosgaya C, Pons MJ *et al.* Pathogenic Acinetobacter species including the novel Acinetobacter dijkshoorniae recovered from market meat in Peru. .

30. Hamouda A, Vali L, Amyes SGB. Gram-Negative Non-Fermenting Bacteria from Food-Producing Animals are Low Risk for Hospital-Acquired Infections. Journal of Chemotherapy 2008; 20: 702–708.

31. Lupo A, Vogt D, Seiffert SN, Endimiani A, Perreten V. Antibiotic resistance and phylogenetic characterization of acinetobacter baumannii strains isolated from commercial raw meat in Switzerland. J Food Prot 2014; 77: 1976–1981.

32. Tavakol M, Momtaz H, Mohajeri P, Shokoohizadeh L, Tajbakhsh E. Genotyping and distribution of putative virulence factors and antibiotic resistance genes of Acinetobacter baumannii strains isolated from raw meat. Antimicrob Resist Infect Control 2018; 7.

33. Askari N, Momtaz H, Tajbakhsh E. Prevalence and phenotypic pattern of antibiotic resistance of Acinetobacter baumannii isolated from different types of raw meat samples in Isfahan, Iran. Vet Med Sci 2020; 6: 147–153.

34. Survival of clinical and food Acinetobacter spp. isolates exposed to different stress. .

35. Meumann EM, Anstey NM, Currie BJ *et al.* Genomic epidemiology of severe community-onset Acinetobacter baumannii infection. Microb Genom 2019; 5: 1–13.

36. Marshall BM, Levy SB. Food animals and antimicrobials: Impacts on human health. Clinical Microbiology Reviews 24 2011 718–733.

37. European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). https://www.ema.europa.eu/en/veterinary-regulatory/overview/antimicrobial-resistance/european-surveillance-veterinary-antimicrobial-consumption-esvac (26 June 2023, date last accessed).

38. WHO GUIDELINES ON USE OF MEDICALLY IMPORTANT ANTIMICROBIALS IN FOOD-PRODUCING ANIMALS. .

39. FAO in Ghana. Ghana at a glance https://www.fao.org/ghana/fao-in-ghana/ghana-at-a-glance/en/ (28 May 2023, date last accessed).

40. Donkor ES, Newman MJ, Yeboah-Manu D. Epidemiological aspects of non-human antibiotic usage and resistance: Implications for the control of antibiotic resistance in Ghana. Tropical Medicine and International Health 2012; 17: 462–468.

41. VE B, C A. Antibiotic Practices and Factors Influencing the Use of Antibiotics in Selected Poultry Farms in Ghana. J Antimicrob Agents 2016; 2.

42. Paintsil EK, Ofori LA, Akenten CW *et al.* Antimicrobial usage in commercial and domestic poultry farming in two communities in the ashanti region of ghana. Antibiotics 2021; 10.

43. TACKLING DRUG-RESISTANT INFECTIONS GLOBALLY: FINAL REPORT AND RECOMMENDATIONS THE REVIEW ON ANTIMICROBIAL RESISTANCE CHAIRED BY JIM O'NEILL. 2016.

44. Tadesse BT, Ashley EA, Ongarello S *et al.* Antimicrobial resistance in Africa: A systematic review. BMC Infect Dis 2017; 17.

45. Vikesland P, Garner E, Gupta S, Kang S, Maile-Moskowitz A, Zhu N. Differential Drivers of Antimicrobial Resistance across the World. Acc Chem Res 2019; 52: 916–924.

46. Kakkar M, Sharma A, Vong S. Developing a situation analysis tool to assess containment of antimicrobial resistance in South East Asia. BMJ (Online) 2017; 358: 14–19.

47. Ghana statistical Service, 2021. .

48. Mensah MSB, Amarteifio ENA. Exploratory Study of the Entrepreneurial Ecosystem in Central Region, Ghana. 2019;

49. Ministry of Touris A and culture, republic of G. Kakum National Park . https://www.motac.gov.gh/tourist-sites/#:~:text=Ghana's%20most%20visited%20attraction%20is,Attandanso%20in%20the%20Central%20Region. (18 June 2023, date last accessed).

50. Colonies Appearance. https://www.chromagar.com/en/product/chromagar-esbl/ (15 July 2023, date last accessed).

51. The European Committee on Antimicrobial Susceptibility Testing - EUCAST. https://www.eucast.org/ (20 June 2023, date last accessed).