Prevalence and Antibiotic Resistance of the *Escherichia coli* among Different Meat Types: A Comprehensive Study in Iran

Abstract

The widespread use of antibiotics in Iranian meat production is a serious issue that has to be addressed right away. In the livestock business, antibiotics are frequently used as a prophylactic to treat and prevent infections. Antibiotic-resistant bacteria, on the other hand, pose a serious threat to human health due to the overuse and misuse of antibiotics in animal agriculture. The main objective of the present study is determining the prevalence of antibiotic-resistant *Escherichia coli (E. coli)* in different types of meat in Iran. Totally, 5532 samples isolated from beef, chicken, and turkey (1690, 2581 and 1261, respectively) were gathered from five provinces in Iran over a period of six months (May-October 2023). Because it was impossible to visit every province in Iran for data gathering, five of the most populous provinces—which together account for over a third of the nation's total population—were chosen. Ten antibiotics along with multidrug resistance were examined to ascertain the samples' resistance. The results illustrate that our samples were more resistance to tetracycline, ampicillin, and cefoxitin. Moreover, the prevalence of *E. coli* in meat in the Razavi Khorasan province is remarkably low specially in beef and turkey meat. Because chicken meat in Iran is produced and distributed through a centralized system, there may not be much of a difference in antibiotic resistance amongst provinces. Authors suggest that public awareness initiatives are necessary to inform consumers about the dangers of antibiotic-resistant bacteria in meat. Encouraging consumers to make knowledgeable decisions and insist on meat devoid of antibiotics can push market forces in the direction of sustainable and ethical farming methods.

Keywords: Antibiotic, Resistance, *E. coli*, Meat safety

Introduction

The economics of food safety are complex because it is challenging to quantify the concept of "food safety," which is based on producers' and consumers' perceptions of safe food (Verbeke et al., 2007). Additionally, food losses and decreased food availability for populations experiencing food insecurity can result from the presence of food hazards. The discussion of policymaking, which incorporates science, politics, culture, and global consensus, goes beyond simply identifying a dangerous organism and the foodborne disease it causes (Kinsey, 2005; McCain, 2015). The leading cause of hospitalization and other deaths worldwide remains foodborne illnesses, despite notable advancements in pathogen survival and food cleaning

techniques. Although conventional antibacterial techniques such as pasteurization, pressurized preparation, radioactivity, and synthetic antiseptics have serious drawbacks such as high upfront costs, potential for malfunctions due to corrosiveness, and negative effects on the organoleptic properties and possibly nutritional significance of the foodstuffs, they have the ability to reduce bacterial activity in nutrition to varying degrees (Imran et al, 2023).

Worldwide, the poultry industry is growing. The most popular poultry in the world is chicken, and its demand is rising. But chicken also poses a risk to human health, particularly since it can spread infectious illnesses brought on by Salmonella, Listeria, and other common foodborne pathogens. Preventing pathogenic bacterial biofilm in the chicken industry is essential due to the increasing risks to food safety caused by recurring contamination, the rapid deterioration of meat, and the bacteria's resistance to cleaning and disinfection techniques frequently used in chicken processing plants (Chowdhury et al, 2023).

Poultry is the preferred protein for millions of consumers worldwide (Ashrafudoulla et al., 2021a; Merino et al., 2019). Global egg production reached 83 metric tons (Mt) in 2019—a 63% increase from 2000—and chicken meat was the most produced meat globally, making up about 40% of all meat produced (FAO, 2021). This trend is expected to continue as the world's population grows. By 2030, 95.6 Mt of eggs will be produced worldwide, and 145 Mt of chicken meat will be consumed, an increase of 15 Mt from 2022. About half of the anticipated rise in total meat output will come from this (King et al., 2017; OECD-FAO, 2021). Accordingly, one of the poultry industry's main responsibilities going forward is to satisfy consumer demand (Chowdhury et al., 2023).

The two main pathogens of concern that have prompted recalls of fresh meat products are Salmonella and *E. Coli* O157:H7. According to the Centers for Disease Control, salmonella infections were the top cause of death in the US in 2012. (CDC, 2015). Furthermore, because of the refrigerated storage environment that promotes the organism's growth, L. monocytogenes is the pathogen of concern in ready-to-eat meat and poultry products (Malley et al., 2015). Future efforts to manage pathogens linked to meat will remain a top priority. The application of antimicrobial interventions, animal identification, traceability and recall activities, animal health and welfare, and innovative processing technologies are significant factors that affect pathogen control and meat safety. Additionally, for use in lab and field settings, more advanced and quick pathogen detection techniques must be developed (McCain, 2015).

Anaerobic, rod-shaped, Gram-negative *Escherichia coli (E. coli)* is a member of the Enterobacteriaceae family of bacteria that are found in the stomachs of humans, warm-blooded

animals, cold-blooded animals, and various environments (Ramos et al, 2019; Abdelwahab et al, 2022; Ramatla et al, 2023). Since it develops antibiotic resistance more quickly than other common bacteria, *E. Coli*, a normal resident of the digestive tracts of warm-blooded animals and humans, is utilized as an indicator bacterium (Miranda et al. 2008). Therefore, its presence consistently indicates faecal contamination and suggests a potential enteric pathogen contamination (Adeyanju and Ishola, 2014).

Sadly, the prolonged use of antibiotics resulted in the development of antimicrobial resistance in pathogens and increased danger of antimicrobial resistance genes spreading throughout the environment. Antimicrobial resistance is currently increasing to dangerously high levels and posing a global threat to public health (Nguyet et al, 2022).

Analyzing *E. coli's* role in the antimicrobial resistance phenomenon should take into account two distinct but related contexts that eventually come together to address a single, shared problem: a significant influence on human health. These two viewpoints include the rise in infections globally attributed to *E. Coli* strains that are resistant to drugs and the bacterium's capacity to spread its genetic resistance to other bacteria. Because of these two characteristics, *E. Coli* has become a major player in the pandemic of antibiotic resistance. It is also easily transmitted from animals to humans through the fecal-oral route. Second, because the microbe can live in the stomachs of both humans and animals, it can interact closely with a wide variety of other bacteria. This interaction gives E. *Coli* the capacity to both act as a donor of genetic material to other bacteria and to take up resistance genes from other microbes (Galindo-Méndez, 2020). In Iran, the most popular meat sources are chicken, beef, and turkey meat. It should be mentioned, though, that due to a sharp increase in the price of beef, there has been a recent rise in the consumption of turkey meat. The finding of *E. Coli* in Iranian meat is extremely significant for food safety and public health. Meat can get contaminated by harmful *E. Coli* strains when it is slaughtered, processed, or stored improperly. Ensuring the safety and quality of meat products is crucial in Iran, as the country consumes a significant amount of meat. When *E. Coli* strains are found, trustworthy detection techniques like PCR (Polymerase Chain Reaction) and immunoassays are used to find them. This allows for quick action and intervention to stop the spread of dangerous bacteria. Enforcing stringent laws and thorough testing procedures in the meat sector can contribute to the protection of public health and guarantee that Iranian consumers can obtain safe and uncontaminated meat products. The key point is that while different Iranian provinces test and remove bacteria from meat in accordance with the same national laws, various provinces and businesses have their own requirements. Therefore, in this study, while examining the prevalence of *E. coli* in 5 densely populated

provinces of Iran, it is tried to propose the strategies of the selected province as a suitable solution to the rest of the country's provinces.

Methods

To study antibiotic-resistant *E. coli*, a range of materials and methods can be employed. Firstly, bacterial strains of *E.* coli with known antibiotic resistance profiles should be obtained. These strains can be acquired from culture collections or isolated from clinical samples. Once the strains are collected, they can be cultured in appropriate growth media under controlled laboratory conditions. To test the antibiotic susceptibility of these strains, the Kirby-Bauer disk diffusion method or the broth microdilution method can be used. These methods involve exposing the bacteria to different antibiotics and measuring their ability to grow in the presence of the drugs. Additionally, molecular techniques, such as polymerase chain reaction (PCR), can be employed to identify specific resistance genes present in the *E. coli* strains. This allows for a deeper understanding of the mechanisms behind their resistance. Furthermore, whole-genome sequencing can be utilized to analyze the entire genetic makeup of the bacteria, providing insights into their evolutionary history and potential resistance mechanisms. These techniques, along with proper controls and statistical analysis, enable researchers to investigate and better comprehend the prevalence and characteristics of antibiotic-resistant *E. coli*.

Sampling and susceptibility Testing

The sampling of *E-coli* in beef, chicken, and turkey from five provinces in Iran over a six-month period is a crucial step in ensuring the safety and quality of these food products. In this study, due to the impossibility of access to all the provinces, 5 provinces were selected which have the largest population in Iran and in total account for more than a third of the country's population, which can be a very good sample for the whole country (including Tehran, Razavi Khorasan Isfahan, Fars, Khuzestan). By conducting regular sampling from May 2023 to October 2023, experts can identify any potential sources of contamination and implement necessary measures to prevent the spread of *E-coli*. This process involves collecting samples from various stages of the production chain, including farms, slaughterhouses, and processing facilities. These samples are then analyzed in specialized laboratories to detect the presence of *E-coli* and determine its strain. The data collected from this comprehensive sampling can provide valuable insights into the prevalence and distribution of *E-coli*, allowing for targeted interventions and improved food safety practices. It is an essential endeavor to safeguard public health and ensure that consumers can enjoy their favorite meat products without any worry.

First, samples were cultivated for 24 hours at 37 °C in McConkey agar medium (HiMedia Laboratories, Mumbai, India). To obtain pure culture, the lactosepositive colonies were then divided and subcultured on McConkey agar. According to the guidelines provided by the Clinical Laboratory Standards Institute (CLSI) 2018, the qualitative disc diffusion method by Kirby Bauer procedure was utilized on Mueller-Hinton agar (Merck, Germany) to ascertain the isolates' resistance to antibiotics against drugs that are frequently used in the veterinary and medical fields.

Nalidixic acid, ampicillin, tetracycline, ampicillin-sulbactam, cefoxitin, cefazolin, ceftriaxone, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole susceptibilities were tested for each isolate.

Results

Fig 1 as well as tables 1-3 summarizes the finding of data analysis.

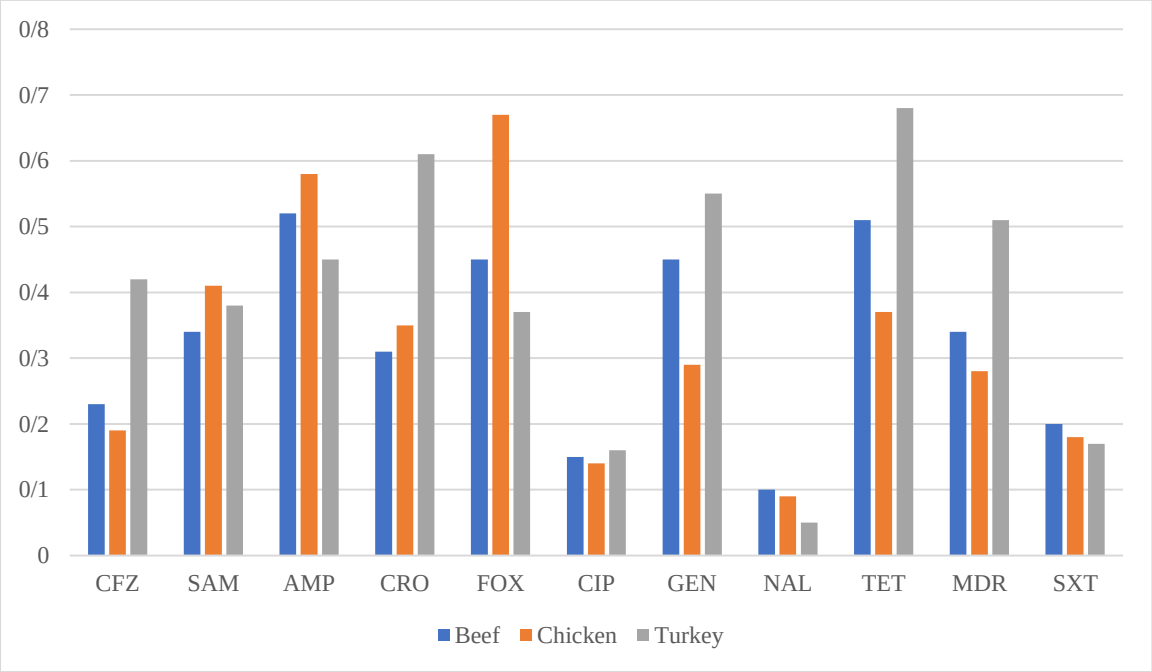

Figure 1: Prevalence of antibiotic resistance among *E. coli* isolates (beef, chicken and turkey samples)

According to figure 1, the emergence of antibiotic-resistant *E. coli* in meat in Iran is a concerning issue. Several factors contribute to the prevalence of resistance to antibiotics such as tetracycline (TET), ampicillin (AMP), and cefoxitin (FOX) in *E. coli* strains found in meat. One key factor is the overuse and misuse of antibiotics in livestock farming. Antibiotics are often used to promote growth and prevent infections in animals, but their excessive and improper use leads to the development of antibiotic resistance. Additionally, inadequate regulation and control measures in the meat production industry may contribute to the spread of antibiotic-resistant *E. coli*. Poor hygiene practices, unsanitary conditions, and improper handling of meat products can all facilitate the transmission of resistant bacteria. Furthermore, the global nature of the meat trade may also play a role, as international movement of meat can introduce and spread antibiotic-resistant strains. Addressing these issues requires a multifaceted approach, including stricter regulation of antibiotic use in livestock, improved hygiene practices in meat production, and increased awareness and education on the proper use of antibiotics to mitigate the rise of antibiotic-resistant *E. coli* in meat.

Table 1. Prevalence of *E. coli* in beef, chicken and turkey meat collected from 5 selected provinces of Iran

Province	Meat	No. of Sample	No. of Positive Sample	Prevalence (%)
Tehran	Beef	366	182	0.497
	Chicken	541	172	0.318
	Turkey	281	104	0.370
Razavi Khorasan	Beef	381	56	0.147
	Chicken	518	198	0.382
	Turkey	259	27	0.104
Isfahan	Beef	298	135	0.453
	Chicken	520	208	0.400
	Turkey	219	102	0.466
Fars	Beef	352	141	0.401
	Chicken	521	281	0.539
	Turkey	274	93	0.339
Khuzestan	Beef	293	98	0.334
	Chicken	481	193	0.401
	Turkey	228	67	0.294

The prevalence of *E. coli* in meat in the Razavi Khorasan province is remarkably low due to a combination of strict regulations, meticulous farming practices, and a focus on food safety. The province's agricultural sector ensures that all livestock undergo regular health screenings and are raised in clean and hygienic environments. Farmers prioritize the use of safe and high-quality animal feed, minimizing the risk of contamination. Additionally, the local meat processing facilities adhere to stringent hygiene standards, implementing rigorous sanitation practices and conducting regular inspections. Razavi Khorasan province places great emphasis on educating farmers and workers about proper handling and storage techniques to prevent bacterial growth. Furthermore, the implementation of advanced testing methods helps to detect and address any potential contamination issues promptly. This collective commitment to food safety has led to the low prevalence of *E. coli* in meat, ensuring consumers can have confidence in the quality and safety of the meat products in Khorasan province.

Table 2. ANOVA test for antibiotic resistance *E. coli* in beef, chicken and turkey in 5 different provinces

		Mean Square	F	Sig.
Beef	Between Groups	2.243	4.318	.007
	Within Groups	0.513		
	Total			
Chicken	Between Groups	0.381	1.034	.355
	Within Groups	0.375		
	Total			
Turkey	Between Groups	1.418	4.087	.005
	Within Groups	0.307		
	Total			

The difference in antibiotic resistance in chicken meat between Iran's provinces may not be significant due to several factors. The government's efforts in enforcing proper antibiotic usage and promoting responsible farming practices could minimize the variation in resistance levels. Additionally, the centralized production and distribution system in Iran may also play a role. This system ensures that chickens are sourced from reputable farms and undergo standardized processes, reducing the likelihood of variations in antibiotic usage and subsequent resistance. Due to the shortage of chicken in some provinces, there is always a flow of chicken distribution between different provinces of Iran so that the shortages of each province can be supplied from other provinces. For this reason, the chicken consumed in several provinces may be sourced from a specific source.

Table 3. ANOVA for Antibiotic resistance in 5 different provinces based on antibiotics

		Mean Square	F	Sig.
CFZ	Between Groups	0.298	1.091	.198
	Within Groups	0.243		
	Total			
SAM	Between Groups	0.385	1.039	.418
	Within Groups	0.313		
	Total			
AMP	Between Groups	1.397	4.124	.001
	Within Groups	0.418		
	Total			
CRO	Between Groups	0.409	1.881	.352
	Within Groups	0.381		
	Total			
FOX	Between Groups	0.277	1.209	.391
	Within Groups	0.251		
	Total			
CIP	Between Groups	0.213	1.009	.171
	Within Groups	0.207		
	Total			
GEN	Between Groups	0.391	1.299	.218
	Within Groups	0.298		
	Total			
NAL	Between Groups	2.786	4.008	.008
	Within Groups	0.495		
	Total			
TET	Between Groups	0.448	1.151	.213
	Within Groups	0.315		
	Total			
MDR	Between Groups	1.388	3.267	.002
	Within Groups	0.546		
	Total			
SXT	Between Groups	0.508	1.413	.408
	Within Groups	0.345		
	Total			

According to Table 3, ampicillin (AMP), multidrug resistance (MDR) and nalidixic acid (NAL) differences among the examined provinces are significant. These significant differences among provinces have profound implications for public health and healthcare systems. Ampicillin resistance, which is the ability of bacteria to withstand the effects of the commonly used

antibiotic ampicillin, is a growing concern as it limits treatment options for bacterial infections. The variability in ampicillin resistance among provinces suggests differences in the prevalence of antibiotic-resistant bacteria and the effectiveness of antibiotic stewardship programs. Similarly, multidrug resistance, defined as the resistance to multiple classes of antibiotics, poses a major challenge in the treatment of bacterial infections. The variations in multidrug resistance across provinces highlight the need for coordinated efforts in surveillance, prevention, and control of antibiotic resistance. Nalidixic acid resistance, specifically in the context of fluoroquinolones, is significant as this class of antibiotics is commonly used to treat a range of infections. The differences observed in nalidixic acid resistance among provinces reflect the varying patterns of antibiotic usage, the presence of specific resistance mechanisms, and potentially the quality of healthcare practices. Understanding these differences and their underlying factors is essential for designing targeted interventions, implementing appropriate antibiotic prescribing guidelines, and promoting antimicrobial stewardship to combat the rising threat of antibiotic resistance on a regional and national level.

Discussion and Conclusion

Based on the results of the research, the general situation of antibiotic resistance in the investigated meats is not at a suitable level. Many commercially available chemical agents are used in the poultry industry to prevent microbial contamination, but their efficacy in eliminating pathogens from the surrounding environment of the poultry processing plant has been reported to be lacking (Merino et al., 2019). Although many common organic acids have a limited efficacy range or require too high of a concentration for practical use in food applications, they are naturally occurring constituents of numerous foods (Ricke et al., 2005).

According to Kim et al. (2020), the most common antimicrobial resistance in Korea was to the quinolone subclass (NAL), which was followed by the penicillin subclass (AMP) and the tetracycline subclass (TET). These findings are consistent with our own research; although NAL is less prevalent in the current study. Furthermore, Davis et al. (2018) noted that the high prevalence of resistance among E. Coli isolates from conventionally-raised turkey meat suggests that conventional turkey production uses more antimicrobials than does raising without antibiotics and in organic systems. However, resistance prevalence among *E. Coli* from chicken meat was more closely associated with brand than with production category. This finding suggests that differences in antimicrobial use and other brand-level variations occurred during production and/or processing.

Antibiotic resistance is a growing concern in the world of medicine and agriculture. The overuse and misuse of antibiotics in livestock animals is one of the leading causes of antibiotic resistance, which can result in the spread of dangerous infections that are difficult to treat. Iran is one of the countries where the use of antibiotics in meat production is a significant problem. The practice of using antibiotics in animal feed is widespread in the country, and this has led to a rise in antibiotic-resistant bacteria in meat products. The prevalence of antibiotic use in Iranian meat production is a pressing concern that requires immediate attention. In Iran, the use of antibiotics in meat production is widespread due to various factors. Farmers often administer antibiotics to livestock as a growth promoter, aiming to enhance productivity and maximize profits. The consequences of this rampant antibiotic use are alarming. The presence of antibiotic residues in meat products is a serious public health concern, as it can lead to the development and spread of antibiotic-resistant bacteria. These resistant strains can make infections difficult to treat, potentially resulting in prolonged illnesses, increased healthcare costs, and even death. Public awareness campaigns are essential to educate consumers about the risks associated with antibiotic-resistant bacteria in meat. Empowering consumers to make informed choices and demand antibiotic-free meat can drive market forces towards sustainable and responsible farming practices. Collaboration between government agencies, farmers, veterinarians, and researchers is crucial to develop and implement alternative strategies to reduce the reliance on antibiotics in meat production. This can include promoting good animal husbandry practices, improving hygiene and sanitation in farms, and exploring natural alternatives to antibiotics, such as probiotics and vaccines. One effective approach is the adoption of alternative practices such as probiotics and prebiotics. Probiotics are live, beneficial bacteria that can be added to animal feed to strengthen the immune system and support a healthy gut microbiome. Conversely, prebiotics are indigestible fibers that promote the growth of good bacteria in the digestive system. The use of antibiotics for illness prevention and growth promotion can be greatly decreased by implementing these substitutes.

References

1. Abdelwahab, G. E., Ishag, H. Z. A., Al Hammadi, Z. M., Al Yammahi, S. M. S., Mohd Yusof, M. F. B., Al Yassi, M. S. Y., ... & Mohammed Al Muhairi, S. S. (2022). Antibiotics resistance in Escherichia coli isolated from livestock in the emirate of abu dhabi, UAE, 2014–2019. *International Journal of Microbiology*, *2022*.
2. Adeyanju, G. T., & Ishola, O. (2014). Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springerplus*, *3*, 1-9.
3. Ashrafudoulla, M., Na, K. W., Byun, K.-H., Kim, D. H., Yoon, J. W., Mizan, M. F. R., Kang, I., & Ha, S.-D. (2021). Isolation and charac- terization of *Salmonella* spp. from food and food contact surfaces in a chicken processing factory. *Poultry Science*, *100*(8), 101234.
4. CDC. 2015. Cause of death. Natl. Vital Stat. Reports. Available from: http://www.cdc.gov/nchs/data/nvsr/nvsr64/nvsr64_10.pdf
5. Chowdhury, M. A. H., Ashrafudoulla, M., Mevo, S. I. U., Mizan, M. F. R., Park, S. H., & Ha, S. D. (2023). Current and future interventions for improving poultry health and poultry food safety and security: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, *22*(3), 1555-1596.
6. Davis, G. S., Waits, K., Nordstrom, L., Grande, H., Weaver, B., Papp, K., Horwinski, J., Koch, B., Hungate, B. A., Liu, C. M., & Price, L. B. (2018). Antibiotic-resistant Escherichia coli from retail poultry meat with different antibiotic use claims. *BMC microbiology*, *18*(1), 174.
7. FAO. (2021). *Statistical yearbook 2021*. https://www.fao.org/3/cb1329en/online/cb1329en.html
8. Galindo-Méndez, M. (2020). Antimicrobial Resistance in Escherichia coli. IntechOpen. doi: 10.5772/intechopen.93115
9. Imran, A., Shehzadi, U., Islam, F., Afzaal, M., Ali, R., Ali, Y. A., ... & Rasool, A. (2023). Bacteriophages and food safety: An updated overview. *Food Science & Nutrition*.
10. Kim, S., Kim, H., Kim, Y., Kim, M., Kwak, H., & Ryu, S. (2020). Antimicrobial resistance of Escherichia coli from retail poultry meats in Korea. *Journal of Food Protection*, *83*(10), 1673-1678.
11. King, T., Cole, M., Farber, J. M., Eisenbrand, G., Zabaras, D., Fox, E. M., & Hill, J. P. (2017). Food safety for food security: Relation- ship between global megatrends and developments in food safety. *Trends in Food Science & Technology*, *68*, 160–175.
12. Malley, T. J., Butts, J., & Wiedmann, M. (2015). Seek and destroy process: Listeria monocytogenes process controls in the ready-to-eat meat and poultry industry. *Journal of Food Protection*, *78*(2), 436-445.
13. McCain, A. K. (2015). *Influence of market setting and time of purchase on counts of aerobic bacteria, Escherichia coli, and coliform and prevalence of Salmonella and Listeria in beef, pork, and chicken in Vietnam*. Mississippi State University.
14. Merino, L., Procura, F., Trejo, F. M., Bueno, D. J., & Golowczyc, M. A. (2019). Biofilm formation by *Salmonella* sp. in the poultry indus- try: Detection, control and eradication strategies. *Food Research International*, *119*, 530–540.
15. Miranda JM, Vázquez BI, Fente CA, Barros-Velázquez J, Cepeda A, Franco CM (2008) Evolution of resistance in poultry intestinal Escherichia coli during three commonly used antimicrobial therapeutic treatments in poultry. Poult Sci 87:1643–1648.
16. Nguyet, L. T. Y., Keeratikunakorn, K., Kaeoket, K., & Ngamwongsatit, N. (2022). Antibiotic resistant Escherichia coli from diarrheic piglets from pig farms in Thailand that harbor colistin-resistant mcr genes. *Scientific Reports*, *12*(1), 9083.
17. OECD/FAO (2021), *OECD-FAO Agricultural Outlook 2021-2030*, OECD Publishing, Paris.
18. Ramatla, T., Ramaili, T., Lekota, K. E., Ndou, R., Mphuti, N., Bezuidenhout, C., & Thekisoe, O. (2023). A systematic review and meta-analysis on prevalence and antimicrobial resistance profile of Escherichia coli isolated from water in africa (2000–2021). *Heliyon*.
19. Ramos, C. P., Santana, J. A., Morcatti Coura, F., Xavier, R. G. C., Leal, C. A. G., Oliveira Junior, C. A., ... & Silva, R. O. S. (2019). Identification and characterization of Escherichia

coli, Salmonella spp., Clostridium perfringens, and C. difficile isolates from reptiles in Brazil. *BioMed research international, 2019.*

20. Ricke, S. C., Kundinger, M. M., Miller, D. R., & Keeton, J. T. (2005). Alternatives to antibiotics: Chemical and physical antimicrobial interventions and foodborne pathogen response. *Poultry Science*, *84*(4), 667–675.
21. Verbeke, W., Frewer, L. J., Scholderer, J., & De Brabander, H. F. (2007). Why consumers behave as they do with respect to food safety and risk information. *Analytica chimica acta*, *586*(1-2), 2-7.