

Original Research Article

Drug-resistant Zoonotic *Salmonella* Strains Isolated from Chicken Droppings; A Case of Ntcheu District, Mphate Area, Malawi

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Abstract: Background: *Salmonella* is one of the leading causes of foodborne illness in Malawi. The dissemination of drug-resistant strains through the food chain has important implications for the treatment failure of salmonellosis in human medicine. This study investigated the prevalence and antimicrobial resistance patterns of zoonotic *Salmonella* strains associated with poultry. **Methods:** Samples of chicken droppings from commercial poultry farms within the Mphate area of Ntcheu District in Malawi were collected using a multi-stage sampling technique followed by pooling. *Salmonella* Shigella agar and Brilliant Green agar were used to isolate the targeted zoonotic species. This was followed by the classification of isolated species using the Analytical Profile Index 20E system and an antimicrobial susceptibility test to establish their antimicrobial resistance pattern. **Results:** *Salmonella* species were found in 6 out of 12 samples, with 41.6% (5) belonging to the Non-Typhoidal *Salmonella* group. *S. typhimurium* was the most isolated serovar (60%, n=3), followed by *S. enteritidis* (20%, n=1) and *S. enterica sub-group 1* (20%, n=1). The study revealed that chicken droppings harbor *Salmonella* serovars resistant to common antibiotics, which is a public health issue. The most significant resistance was observed against Tetracycline (100%), Sulfamethoxazole (80%), Ceftazidime (60%), Gentamicin (40%), Ciprofloxacin (40%) and Ampicillin (20%). Further analysis showed that *Salmonella* var. *typhimurium* DT 104 exhibited the highest antibiotic resistance (n=6, 100%), followed by *Salmonella* var. *typhimurium* strain 2 (n=5, 83%) and *Salmonella* var. *enterica sub-group 1* (n=3, 50%). All strains that showed resistance against more than two antibiotics were found in layer chickens, with all isolates being resistant to at least one antibiotic. **Conclusion:** The findings revealed that poultry in Ntcheu District harbours several multidrug-resistant zoonotic strains of *Salmonella*. There is therefore a need to increase surveillance to monitor the emergence of resistant pathogens in food animals.

Keywords: *Salmonella*, Antimicrobial resistance, Poultry, Foodborne illness.

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1. INTRODUCTION

Poultry is a major source of low-cost and high-quality proteins, accounting for approximately one-third of the overall meat production globally [1]. However, the industry is hampered by disease outbreaks leading to losses in meat and egg production [3]. Some common pathogenic microorganisms implicated in outbreaks hampering poultry production include *Salmonella*, *Escherichia*, and *Staphylococcus* species which interestingly shape the poultry microbiota network [3-5]. The highly ubiquitous genus of *Salmonella* consists of different serovars divided into typhoidal and non-

typhoidal serovars [6]. The non-typhoidal serovars cause invasive non-typhoidal salmonellosis (iNTS) in both humans and animals. In contrast, typhoidal serovars are highly adapted to human hosts, which are responsible for causing enteric fever [7]. Currently, over 2500 different serovars have been isolated and identified within the two species of *Salmonella bongori* and *Salmonella enterica* [8]. The serovars most commonly isolated in poultry are regularly occurring in humans suggesting a probable epidemiological connection between poultry and human infections [9].

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Non-typhoidal *Salmonella* species annually cause around 93 million cases of gastroenteritis and 155,000 deaths worldwide [11, 10]. Many of these cases have been recorded in Africa, which is also, considered to be one of the continents with the highest number of foodborne diseases [12]. This could be so because, in most developing countries in Africa, such as Malawi, domestic animals live near humans in both urban and rural setups [9]. Infection of the chickens leads to faecal shedding of the pathogen into the environment and subsequent infection of humans [10]. Nevertheless, ingestion of contaminated meat and poultry products is regarded as the major mode of transmitting *Salmonella* to humans [9]. The common serotypes of *S. enteritidis* and *S. typhimurium* constitute about 40 % of all clinical gastroenteritis cases attributed to the consumption of egg and poultry products [2-10].

The extensive and irrational use of antibiotics in veterinary and human medicine has seen the emergence of antimicrobial resistance (AMR) in most commensals including *Salmonella* species [13]. In many parts of the world, food-producing animals are given antibiotics daily to boost growth and prevent diseases [12]. In poultry farming antibiotics are also used as growth promoters, prophylaxes, or therapeutics [1]. For instance, a study that was conducted in Bangladesh aimed at assessing knowledge of antimicrobial use among commercial poultry farmers reported that about half of the interviewed farmers used antimicrobials as growth promoters [14]. This study concluded that one of the key drivers of growing AMR is the misuse of antibiotics associated with the knowledge gap on antimicrobials. Similarly, a study that was conducted in Ethiopia on the same topic reported that about 90% of the interviewed farmers used antibiotics without a prescription [15]. This has led some scientists to fault the practice and pronounce it as a contributor to the emergence of antimicrobial-resistant strains [16-3]. However, some researchers have also reported the contamination of chicken feed with *Salmonella* during feed preparation is another major source of antimicrobial-resistant genes [17].

AMR poses a big danger mainly as a result of failure to successfully treat patients infected with antibiotic-resistant pathogens [11]. It takes at least 10 years to develop, approve and certify antibiotics for general public use [18]. On the other hand, bacteria can evolve resistance within a few hours [11]. Gram-negative bacteria such as *Acinetobacter* species, *Escherichia coli*, *Klebsiella* species and *Salmonella* species are some of the pathogens that are extremely resistant to existing antibiotics [12-9]. Infections caused by multi-drug-resistant (MDR) organisms are associated with a high mortality rate compared to those caused by susceptible bacteria [19]. An estimated 23,000 people die annually from infections caused by MDR organisms in the United States of America alone [19].

The World Health Organization described antibiotic resistance as one of the three most critical public health threats of the 21st century [19]. Scientists anticipate that through 2050, AMR infections will probably cause the demise of 10 million humans annually if the present-day developments of antimicrobial resistance persist [1, 11]. It is also reported that MDR species contribute to a heavy financial burden with an anticipated over 20 billion dollars in loss per year in the United States alone [19]. The present study was conducted to determine the antimicrobial resistance patterns and prevalence of *Salmonella* species from poultry farms in Ntcheu District. Understanding the resistance patterns for variant strains could be key for public health interventions. Ntcheu is one of the districts that has encountered a sharp rise in the number of commercial poultry farmers with the Mphate area alone having approximately 44 poultry farmers.

2. MATERIALS AND METHODS

2.1 Study Setting

A cross-sectional study was conducted between September 2022 and October 2022. The samples analyzed in this study were collected in Mphate area in Ntcheu District, Malawi. The district is in the central region of Malawi and borders Mozambique. It has an estimated area of 3,424 km² and a total population of about 659,608 with a population density of 193/km². It is ranked 12th in terms of density, among the 28 districts of Malawi [32]. A majority of the population in the area relies on agriculture specifically vegetable production for income. Lately, it was observed that Ntcheu District has had an increase in the number of people practicing commercial poultry farming, especially within the Mphate area [14° 49' 59" S, 34° 40' 59" E]. Mphate was chosen as the study site because studies show that an increase in poultry farming is usually accompanied by an increase in the use of convectional antibiotics to protect chickens from infections, consequently contributing to the development and spread of antimicrobial resistance [20].

2.2 Selection of Poultry Farms for Sampling

The study targeted poultry farms with broilers and layers breed chickens grown for commercial use only. For layer chickens, spent layers were selected, for broiler chickens only flocks of 5 weeks old and above were selected. These stages of chickens were chosen because by this time, the chickens have been fed commercially produced feed extensively, administered a lot of antibiotics, and have been exposed to the environment, increasing the exposure to antimicrobial agents. This again gives time for the harboured pathogen to gain resistance inside the host and even already resistant pathogens to establish themselves in the host. In addition, most farmers start selling their chickens at 5 weeks, therefore, this poses a direct threat to the consumers as there are high chances of consuming the antibiotic residues and the resistant pathogens together with the products.

2.3 Sample Collection and Transportation

By the time the study was conducted, Mphate area had a total of 44 registered commercial poultry farmers. We decided to use a census approach due to the smaller population size. All farmers were identified and contacted, a majority had their chickens falling out of the inclusion criteria, with some being out of stock, and some rejecting to their farms being sampled for their own reasons. Only 10 farmers consented to enrol in the study. Chicken droppings were aseptically collected from a chicken house into a sterile zip-lock bag through a multi-stage sampling technique followed by sample pooling. From farms that kept both layers and broiler breeds of chicken, two samples were collected. Only two farms had both broilers and layers meeting the inclusion criteria, hence, a total of 12 samples were collected. The samples were kept under a cold chain until arrival at the University of Malawi, Microbiology Laboratory where the samples were analysed.

2.4 Isolation, Identification and Classification of *Salmonella* Species

About 10g of each pooled sample was firstly incubated in Buffer Peptone Water (BPW), (90ml) at 37°C for 24 hrs. Thereafter a loop-full of every sample from the pre-enrichment media was then stricken onto *Salmonella* Shigella Agar (SSA) using a sterile inoculation loop. Colonies typical of *Salmonella* from the SSA were then transferred onto Brilliant Green Agar (BGA) to isolate zoonotic species of the pathogen. Identification was done through colony characterization of the isolates on SSA and BGA, gram stain and motility test (microscopically and on motility medium). The Analytical Profile Index 20E system was used to classify the isolated species of *Salmonella* into different serovars. To ensure consistency in the results and confirmation, the samples were also sent to Mzuzu University Microbiology Laboratory where similar tests were done on the samples blindly.

2.5 Antimicrobial Susceptibility Test

Pure isolates from BGA were subjected to antimicrobial susceptibility testing using the Kirby Bauer disk diffusion method. A quality control test was done to assess the integrity of the antimicrobial disks as well as the Mueller Hinton agar media before running the test on the samples. *Escherichia coli* ATCC 25922 was used as a quality control isolate. The isolates were tested

against Ciprofloxacin (5µg), Gentamicin (10µg), Ampicillin (10µg), Sulfamethoxazole (25µg), Ceftazidime (10µg), and Tetracycline (30µg). Results were read and interpreted according to guidelines by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

2.6 Data Analysis

All results were validated and checked for missing entries and entry errors. The prevalence of *Salmonella* or MDR strains was determined by the number of samples from which the pathogen was isolated divided by the total number of tested samples. Descriptive statistics were used to calculate the proportion of resistance to different antimicrobials amongst *Salmonella* isolates tested using the disk diffusion technique. In this study, an isolate was classified as MDR if it was resistant to more than two antimicrobials used. Data analyses were performed using Microsoft Excel and Statistical Package for Social Science v.20.

3. RESULTS

3.1 Origin of Samples

All the 10 farms that were sampled were small-scale farms that had chickens ranging from 50-400. These were the farms that met the study inclusion criteria. The 10 commercial farms that were sampled in the study are shown in Figure 1.

3.2 *Salmonella* Serovars Isolated from Chicken Droppings in Ntcheu District

Salmonella was isolated from 6 samples out of the 12 samples analysed. Only 5 (41.6%) isolates belonged to the Non-Typhoidal *Salmonella* (NTS) group. In terms of the breed of chickens in which the pathogens were isolated, 60% (n=3) were from layers and 40% (n=2) were broilers. All 5 isolates belonged to the sub-species *Enterica* which was further classified into 3 serovars namely, *S. typhimurium*, *S. enteritidis*, and *S. enterica* subgroup 1. However, within the *S. typhimurium* serovar, a strain of *S. typhimurium* DT 104 was identified. This meant that *S. typhimurium* was the most isolated serovar (60%, n=3), followed by *S. enteritidis* (20%, n=1) and *S. enterica* subgroup 1 (20%, n=1) (Table 1).

Table 1: Different *Salmonella* Serovars Isolated from Chicken Droppings in Ntcheu District. (n=5).

<i>Salmonella</i> serovars	Prevalence % (n)
<i>S. enterica</i> sub-group 1	20 (1)
<i>S. enteritidis</i>	20 (1)
<i>S. typhimurium</i>	60 (3)
Typhimurium further classification	
<i>typhimurium</i> DT 104	33 (1)
<i>typhimurium</i> other strains	67 (2)

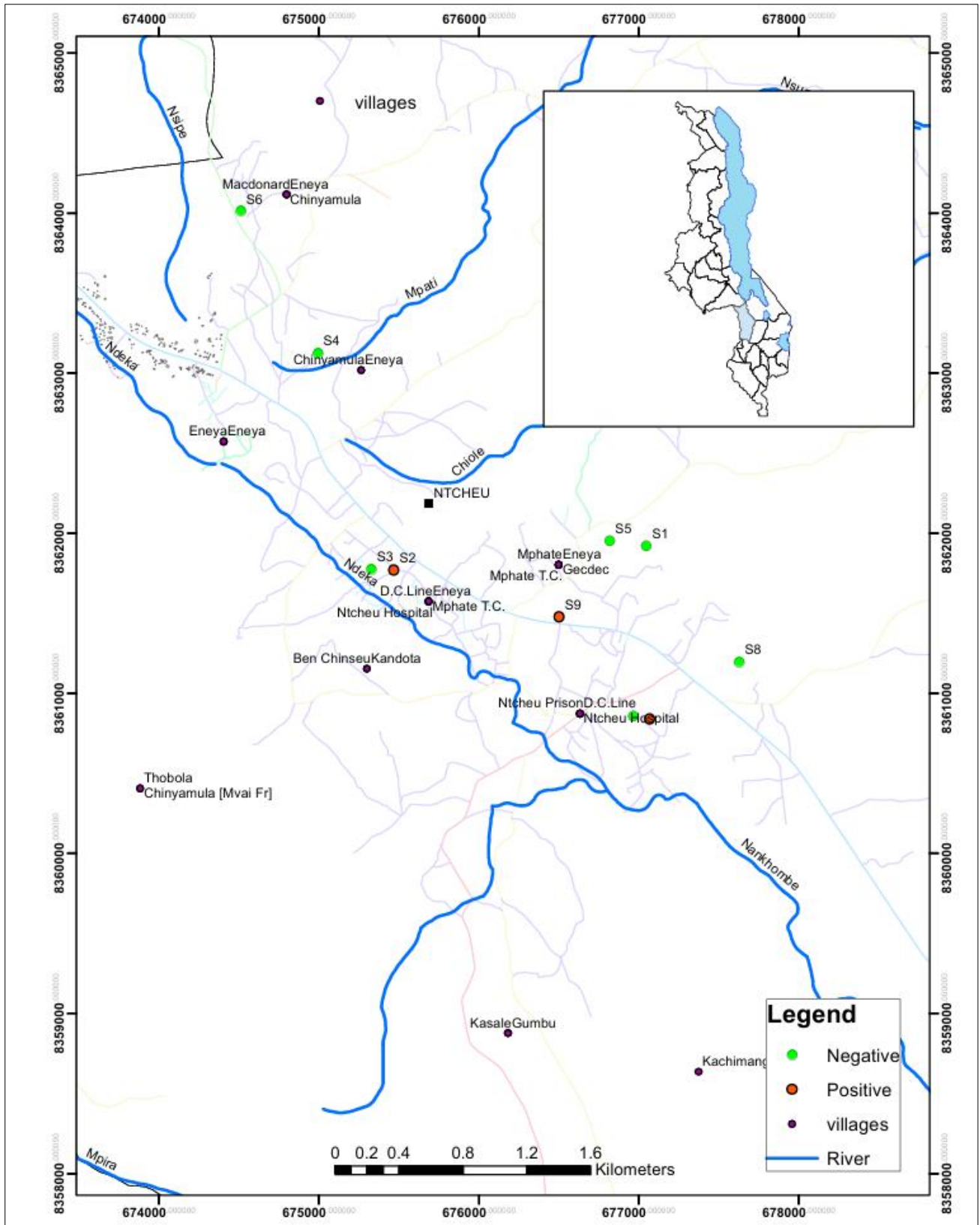


Figure 1: Map of Ntcheu District showing Mphate area/Boma where chicken dropping samples were collected. The spots in green indicate the site where individual samples were collected from individual farms. The spots in red indicate the sites where MDR *Salmonella* strains were isolated

3.3 Antimicrobial Susceptibility Profile of Isolated *Salmonella* Species

As shown in Figure 2 and Table 2 the isolated *Salmonella* serovars in the current study were resistant to some common antibiotics suggesting a public health problem. The isolated pathogens demonstrated a similar

rate of antimicrobial resistance against the antibiotics with the most resistance shown against Tetracycline (100%), Sulfamethoxazole (80%), Ceftazidime (60%), Gentamicin (40%), Ciprofloxacin (40%), Ampicillin (20%).

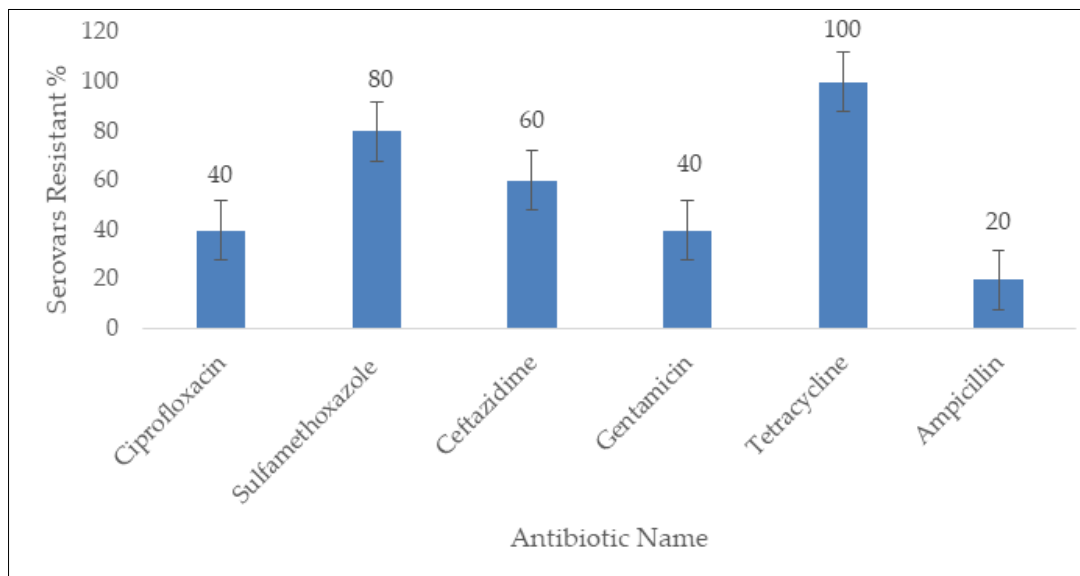


Figure 2: Serovars resistant percent against individual antibiotics tested in the study. (n=5)

Table 2.0 Shows resistance in individual serovars against tested antibiotics. The highest resistance against the antibiotics was observed in *Salmonella var. typhimurium DT 104* (n=6, 100%). *Salmonella var. typhimurium strain number 2* had the second most

resistance (n=4, 67%). *Salmonella var. enteritidis* had the third most resistance (n=3, 50%). *Salmonella var. typhimurium strain number 1* and *Salmonella var. enterica sub-group 1* showed lower resistance with (n=2, 33%) and (n=1, 17%) respectively.

Salmonella Serovars	Antibiotic Name						Number	% Resistance
	AM	TET	GM	SXT	CIP	CAZ		
S. entrc-1	SEN	RES	SEN	RES	SEN	RES	3	50
S. entrtdts	SEN	RES	SEN	SEN	SEN	SEN	1	16.6
S. typhm.1	SEN	RES	SEN	RES	SEN	SEN	2	33.3
S. typhm.2	SEN	RES	RES	RES	RES	RES	5	83.3
S. DT104	RES	RES	RES	RES	RES	RES	6	100

Key: Names of antibiotics: AM= Ampicillin; TET= Tetracycline; GM= Gentamicin; SXT= Sulfamethoxazole; CIP= Ciprofloxacin; CAZ= Ceftazidime. **Results:** RES= Resistant; SEN= Sensitive. **Serovars:** S. entrc-1= *Salmonella var. enterica sub-group 1*; S. entrtdts= *Salmonella var. enteritidis*; S. typhm-1= *Salmonella var. typhimurium strain 1*; S. typhm-2= *Salmonella var. typhimurium strain 2*; S. DT104= *Salmonella var. typhimurium definitive type 104*

3.4 Prevalence of Multidrug-Resistant *Salmonella* Isolated from Chicken Droppings in Ntcheu District

The isolates were regarded as multi-drug resistant (MDR) when they exhibited resistance against more than 2 antibiotics and extensive drug-resistant (XDR) if they were resistant to all 6 antibiotics used in

the study. All 5 isolates were resistant to at least 1 antibiotic. It was observed that 40% (2) of the isolates showed resistance to more than 2 antibiotics while 20% (1) was an XDR. Notably, the MDR and XDR strains were isolated from layer chickens.

Table 3: Prevalence of MDR *Salmonella* Species Isolated from Chicken Droppings in Ntcheu District

Type	Source	Prevalence % (n)
Resistant to 1 antibiotic	Layer chickens	20 (1)
Resistant to 2 antibiotics	Layer chickens	20 (1)
Multi-drug resistant (MDR)	Layer chickens	40 (2)
Extensive drug-resistant (XDR)	Layer chickens	20 (1)

4. DISCUSSION

Salmonellosis is a major public health concern and continues to have serious economic consequences in the poultry industry worldwide. The global increase in multidrug resistance (MDR) *Salmonella* species is worrying. This is the first study describing antimicrobial resistance patterns in *Salmonella* species originating from chickens in Ntcheu District, Malawi. *Salmonella spp.* isolates from chicken droppings showed higher resistance levels to Tetracycline, Sulfamethoxazole, and Ceftazidime as compared to most other classes. Tetracycline and sulfamethoxazole were the most commonly available over-the-counter medications locally and the two most commonly used in treatment of chickens.

Data presented in Table 1 demonstrates that the most prevalent *Salmonella* species in Ntcheu District were *Salmonella typhimurium* (60%), followed by *Salmonella enteritica sub-group 1* (20%) and *Salmonella enteritidis* (20%). These findings align with a study conducted in South Korea, where *S. typhimurium* was the most frequently isolated serotype (23.4%), followed by *S. enteritidis* (21.9%) and other serotypes [21]. Similarly, a study conducted in Bangladesh also reported that *S. typhimurium* and *S. enteritidis* were the most commonly isolated serotypes [22]. In contrast, different findings were reported in Ghana, where the most isolated serovars were *S. kentucky* (26%), followed by *S. virchow* (11.3%) and *S. enteritidis* (10%) [23]. In Zambia, *S. enteritidis* had the highest isolation rate (47.1%), followed by *S. sendai* (9%) and *S. ruanda* (6%) [24]. In Brazil, the highest isolation rate was observed for *S. minnesota* (35.7%), followed by *S. heidelberg* (32.1%) and *S. enteritidis* (26.8%) [25].

The variation in serotype isolations could be attributed to geographical location, as certain serovars have been reported to be endemic and isolated only in specific regions of the world [26]. However, these current findings are consistent with several studies, including a meta-analysis that evaluated the prevalence and diversity of *Salmonella enterica* serovars in animal-based foods across different continents (Africa, the Americas, Asia, Europe, and Oceania). The results of this analysis revealed that *S. typhimurium* and *S. enteritidis* have a global distribution and are commonly isolated in African communities [26]. Poultry, in particular, continues to play a significant role in the transmission of these serovars to humans [26].

The findings presented in Figure 2 and Table 2 indicate that *Salmonella* species recovered from chicken droppings in Ntcheu District exhibited the highest resistance against Tetracycline (100%) and the least resistance against Ampicillin (20%). These results are in close agreement with a study conducted in Ghana [23], where the highest resistance was observed to nalixidic acid (89.5%), followed by tetracycline (80.7%), sulfamethoxazole (79.1%), ciprofloxacin (64.9%) and

ampicillin (26.3%). Similarly, a study in Nigeria [27], reported the highest resistance to tetracycline (90.0%), followed by nalixidic acid (76.0%), sulfamethoxazole (71.0%), ampicillin (67.0%), Gentamicin (13.0%) and ciprofloxacin (9.5%). Likewise, the results are consistent with those from Bangladesh [22], where the highest resistance was observed to ampicillin (98.8%), followed by tetracycline (94.19%) and sulfamethoxazole (67.44%). Furthermore, similar patterns were observed in Ethiopia [9], with the highest resistance reported to tetracycline (82.0%), followed by ampicillin (70.0%) and sulfamethoxazole-trimethoprim (56.0%). Despite using different concentrations and guidelines in these studies, higher resistance to antibiotics such as tetracycline, was still observed. For example, in a study conducted in Ghana, higher resistance was observed even when using 15µg of tetracycline [23]. Similarly, a higher concentration of 25µg was used in the Nigerian study, but the isolates still demonstrated higher resistance to the antibiotic [27]. In Bangladesh, a concentration of 30µg was used, yet the isolates exhibited even greater resistance. These findings suggest that certain *Salmonella* species may be nearing complete resistance to B-lactams and Sulphonamide antibiotics. This may be the reason why resistance pattern of the isolates in the reviewed studies and the present study are comparable. However, the 100% resistance to tetracycline in the current study may also be attributed to a smaller sample size.

Studies support the finding that B-lactams and Sulphonamide are the most commonly used antibiotics in poultry farming [1-29]. This present study also demonstrated that B-lactams and Sulphonamide were commonly used by farmers. This can potentially be connected to the rise in antibiotic resistance due to pathogen adaptation as a result of repeated antibiotic administration. Similar findings have been explained by a researcher in the United States where the emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA) was highly linked to the uncontrolled availability and misuse of antimicrobials [30]. Therefore, there is a need for strict control and monitoring of medications sold by vendors.

The current investigation has demonstrated that in the Ntcheu District, 40% of zoonotic *Salmonella* species associated with poultry are MDR strains. Similar findings were recorded in Ghana in 2016, where 56.3% of *Salmonella* species showed MDR patterns [23]. However, Ethiopia and Bangladesh have reported even higher prevalence rates of MDR *Salmonella* at 76% and 94% respectively [9, 10, 28]. We suspect that the rates of MDR *Salmonella* in Ntcheu could have been comparable to those in studies conducted in Ethiopia and Bangladesh if our sample size of chicken flocks was large. Larger flock sizes have been associated with a higher prevalence of Salmonellosis and MDR [9-31]. The extensive use of antimicrobials as growth promoters in large-scale poultry farming is a major contributing factor to the emergence

of MDR strains. In the present study, most small-scale farmers did not use antimicrobials as growth promoters, possibly explaining the observed resistance to antibiotics that have already lost their efficacy. Notably, all MDR serotypes isolated in this study were found in layer chickens, which have a longer lifespan compared to broilers. This suggests that the longer the period of keeping the population of layer chickens, the greater the potential for more MDR strains of the pathogen. These findings emphasize the urgent need for effective strategies to combat antimicrobial resistance in poultry farming.

5. CONCLUSION

Relative to other studies, the current investigation reported a lower prevalence of MDR *Salmonella* species however, an extensively drug-resistant strain of *S. typhimurium* DT 104 was identified. Moreover, the study spot map (Figure 3) revealed a wide distribution of the MDR strains in the area, potentially facilitating their spread and negatively impacting intervention efforts. It is worrying to note that significant resistance was registered against Ceftazidime (60%), Ciprofloxacin (40%) and Gentamicin (40%) since these are classified as critically important antibiotics for human medicine by the WHO. Therefore, urgent and strict intervention measures to control widespread use of these antibiotics is necessary to protect the community.

Ethical Approval: All research ethical considerations were followed throughout this study. An ethical clearance was obtained from Mzuzu University; Faculty of Health Science, Research Committee (Ethical Clearance Number: FOHS/REC/21/201)

Conflicts of Interest: The authors declare no conflicts of interest.

Consent: A signed informed consent was obtained from each participant. Participation was voluntary, they had the freedom to join without being influenced by material benefits or money and they were free to stop participating in the study anytime and at any stage they desired.

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