

Evaluation of bacterial load and Multi-drug Antibacterial resistant *Salmonella* and *Clostridium* isolates from contaminated tools used in butcheries around Ibadan

Fasanmi OG^{1,*}, Adekunle OF¹, Okuneye OJ¹, Akanbi IO¹, Adegor EC², Agbato OA¹, Lamidi BK¹, Emikpe OO¹, Owolabi OT³, Olusegun OB³, Oyedepo MO¹

¹Federal College of Animal Health & Production Technology, Moor Plantation, Ibadan, Nigeria

²Department of Public Health, National Open University of Nigeria, Niheria

³Department of Animal Health & Production Technology, Rufus Giwa Polytechnic, Owo, Nigeria

ABSTRACT

This study was designed to evaluate the bacterial load and antibiotics resistance prevalence of *Salmonella* and *Clostridium* isolates from tools in butcheries around Ibadan. The samples were aseptically collected from the tools and subjected to microbiological analysis. The food-borne bacteria isolated include *Salmonella*, *Escherichia coli*, *Clostridium* and *Campylobacter* spp, but only *Salmonella* and *Clostridium* spp were subjected to antibacterial sensitivity testing. The total bacterial count was highest ($7.020 \pm 0.160 \times 10^5$ /cfu/m) in the table scrapings at sampled butcheries, while the least ($4.550 \pm 0.200 \times 10^5$ /cfu/m) was recorded from the axe.

This study showed high antibiotics resistance prevalence, especially with the *Salmonella* sp; Chloramphenicol (57%), Enrofloxacin (57%), Trimethoprim (53%) and Oxytetracycline (51%). On the other hand, the *Clostridium* isolates were resistant to the antibiotics at varying percentages, but with highest resistance prevalence of 49% for gentamycin. Comparatively, the *Salmonella* isolates are more resistant to the common antibiotics than the *Clostridium* isolates. Multi-drug resistance has been demonstrated against common antibiotics such as enrofloxacin, oxytetracycline, gentamycin, amoxicillin and chloramphenicol, the resistance has also spread to the Fluoroquinolones (Ciprofloxacin) which are drugs of last resort.

Consequently, we recommend that butchers are trained on general hygiene, and also, antibiotic prescription and dispensing are handled by qualified personnel.

Keywords: Antibacterial resistance, Antibiotics, Butchery, Food borne bacteria, Ibadan

INTRODUCTION

The microbiological quality of meat is dependent on the physical status of the animal at slaughter, the spread of contamination during slaughter,

Vol No: 07, Issue: 06

Received Date: December 08, 2023

Published Date: December 19, 2023

*Corresponding Author

Fasanmi OG

Federal College of Animal Health & Production Technology, Moor Plantation, Ibadan, Nigeria

Email: Fasanmi.olubunmi@fcahptib.edu.ng

Citation: Fasanmi OG, et al. (2023). Evaluation of bacterial load and Multi-drug Antibacterial resistant *Salmonella* and *Clostridium* isolates from contaminated tools used in butcheries around Ibadan. Mathews J Vet Sci. 7(6) :33.

Copyright: Fasanmi OG, et al. © (2023). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

processing and sales to end users, the temperature, and other conditions of storage and distribution [1,2]. The need for adequate control and inspection during rearing of livestock and in slaughterhouses, processing plants, shops and adequate microbial assessment of fresh meats before consumption is recommended to reduce possible contamination [3,4].

The prevalence of a particular strain of bacteria depends on factors which persist during processing, transporting and storage. Over the years, there have been outbreaks of infections associated with consumption of contaminated meat, and the predominant microorganisms isolated include *Salmonella* spp., *Staphylococcus* spp., *Shigella* spp., *Escherichia* spp., *Listeria* spp., and *Clostridium* spp. [5,6]. Microbial safety of food is a significant concern of consumers and industries today. The rapid and accurate identification of food-borne pathogenic bacteria in food is important; both for quality assurance and to trace pathogens within the food chain supply [7,8].

The presence of *Salmonella* and *Clostridium* microbes and associated surfaces may contribute to the contamination of meat, unless such equipment's are thoroughly sanitized, otherwise they may continue to contaminate foodstuff also [9]. Food borne disease represents the most important public health troubles worldwide. The potential of food borne pathogens to cause illness or even cause death in consumers highlights the importance of such event and subsequent need of their monitoring and prevention. Millions of cases of illnesses, chronic complications and deaths are reported in many countries on a yearly basis [10].

Food borne diseases are common and costly preventable disease that are of public health concern, and which is treated with available antibiotics, however, the emergence of multidrug resistance bacteria has been reported worldwide, and this phenomenon limits the choice of antibiotic therapy in some bacteria-related infections or diseases [11].

Several studies have shown that the continuous use of antibiotics in animal rearing and other agricultural activities including the rearing and production of livestock as a major driving force behind the development and dissemination of antimicrobial-resistant *Salmonella* in the community [12,13]. The undue use of antimicrobial agents as growth promoting agents and prophylactic agents in the production of food producing animals such as livestock is a major contributing factor to the widespread emergence and dissemination of drug resistant bacteria around [14].

Bearing the aforementioned in mind, this study was designed to evaluate the bacterial load and antimicrobial susceptibility profile and antibiotics resistance of *Salmonella*

and *Clostridium* isolates from tools in butcheries around Ibadan southwest local government area, Ibadan.

MATERIALS AND METHODS

Study Location and Materials

The study was carried out in four selected market area under Ibadan South West Local Government Area (IBSW LGA), which is Apata, Oluyole, Mobil and Ring road. The reason for choosing these study areas was because there is more butchery stands in those markets, the GPS coordinate of IBSW LGA is 7°21'17"N, 3°52'3"E, it has an area of 40 km² and a population of 282,585 as at the 2006 census. This study ran from 12th February 2023 to 15th April 2023. This covered the periods for sample collection and all laboratory activities.

The materials used in the course of this study include well-labelled sterile swab sticks, well-labelled sterile sample bottles, ice packs, cooler, Petri dishes, autoclave, wire loop, agar, Autoclave, Oven, Fridge, measuring cylinder, Test tube, Methylated spirit, weighing scale and colony counter.

Sample Collection and Laboratory Procedures

A total number of ninety-six (96) samples were collected in all, twenty-four (24) samples per location and samples were collected from six (6) butchery outlets. The samples were collected aseptically by using sterile swab sticks to swab the tools (knives, axe and cutting wood) used in the butchery outlets and put in different well labelled sterile nylon to indicate the sample collected. Scraping of cutting table was done using sterile blade and was put in different well labelled sterile nylon and immediately put into cooler containing ice packs to prevent deterioration. It was transported immediately to the Microbiology laboratory at the University of Ibadan where laboratory analysis was carried out. The samples were analysed for microbial quality as described by FAO [15]. The samples were placed on trypticase-soya –agar (TSA) for trophic bacteria. Petri dishes were incubated at 37°C for 48 - 72 h while the cultures were observed daily under a stereoscopic microscope for the presence of bacterial colonies. The media used were weighed out and prepared according to the manufacturer's specifications with respect to the given instructions and directions. The serial dilution method was used for total microbial counts. Pure isolates of resulting growth were identified using morphological and biochemical methods described by Lennette, et al. [16] and Jolt, et al. [17]. The number of occurrence of each identified bacterium isolates were recorded. The sterility of each batch of test medium was confirmed by incubating one or two un-inoculated tubes or plates along with the inoculated tests. The un-inoculated tubes or plates were always examined to show no evidence of bacterial growth.

Determination of Antibiotic susceptibility of the *Salmonella* and *Clostridium* isolates

The standard disk diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines was applied for antimicrobial susceptibility testing. Commercially available multi-disks Rapid labsR comprising of Gentamicin (10µg), Ciprofloxacin (5µg), Amoxicillin (25µg), Tetracycline (10µg), Enrofloxacin (5µg), Chloramphenicol (30µg) and Trimethoprim (5µg). Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocol [18] on Mueller Hinton agar. Discrete colonies from a 24 h nutrient agar plates were suspended in sterile normal saline in a test tube to achieve a bacterial suspension equivalent to 0.5 McFarland Turbidity Standard. A cotton swab was dipped into the bacterial suspension and used to inoculate the entire surface of a Mueller Hinton agar plate, rotating the plate to ensure confluent growth of the bacterium. The antimicrobial susceptibility disks were placed on the surface of the inoculated plate with flame sterilized forceps. The plates were incubated in an inverted position for 16–18 h at 35–37°C. The diameter of the zone

of inhibition produced by the antibiotic disks was measured to the nearest millimeter (mm) using a transparent ruler. The criteria for categorizing the diameter of zones of inhibition into sensitive (S) or intermediate (I) or resistant (R) were based on the interpretive charts of the Clinical and Laboratory Standards Institute [18].

Statistical Analysis

All data for the isolated bacterial counts were subjected to analysis of variance (ANOVA) and Duncan multiple range test was used for post-hoc comparison of means found to be statistically significant ($p < 0.05$).

Analysis was also carried out for the frequency of occurrence or prevalence for antibiotics susceptibility and specifically the resistance prevalence of *Salmonella* and *Clostridium* isolates using descriptive statistics.

RESULTS

Table 1 shows the comparison of the total bacterial count from the different tools used at the butchery, the table and cutting wood show that the cutting surfaces (table and cutting wood) have the highest number of bacteria respectively than the knife and axe with a significant difference of ($p < 0.05$).

Table 1: Comparison of total bacterial count from tools at butchery outlets around IBSW LGA.

Tool	Total count x 105/cfu/ml
Table scrapings	7.020±0.160a
Knife	5.350±0.180c
Axe	4.550±0.200d
Cutting wood	6.400±0.180b

^{abcd} mean values that are in the same column with different superscripts are significantly different $p < 0.05$

The percentage of occurrence of *salmonella* and *clostridium* isolates obtained from the tools used in butchery outlet in Ibadan south west local government are shown in table 2 below indicating the prevalence of *salmonella* isolates to be highest in the cutting table surface (30.2%), followed by the

cutting wood with 28.3% and the least (18.9%) was observed in the axe. Also, the highest prevalence of *clostridium* species was isolated from the cutting table (37.7%) and lowest from the knives (15.1%).

Table 2: Prevalence of *Salmonella* and *Clostridium* isolates from the tools used in butchery outlets around Ibadan South West LGA.

Tool	Number of isolates	
	Salmonella	Clostridium
Cutting tables	16(30.2)	20(37.7)
Knives	12(22.6)	8(15.1)
Axes	10(18.9)	9(17.0)
Cutting woods	15(28.3)	16(30.2)
Total	53	53

Table 3 shows the antimicrobial susceptibility patterns of *Salmonella* and *Clostridium* isolates to common antibiotics based on the diameter of the zone of inhibition of each antibiotic. Generally, for the *Salmonella* and *Clostridium* spp. the isolates exhibited high resistance level to common antibiotics, and different variations in intermediate and susceptible class. Considering the *Salmonella* isolates, 52.8% were susceptible to Ciprofloxacin, making it the best

antibiotics among the others, this is followed by amoxicillin that 37.7% of the isolates are susceptible to, the worst result was observed with gentamycin with just 20.8% of the isolates susceptible. While for *Clostridium* spP. Most of the isolates are susceptible to common antibiotics; Ciprofloxacin (72%), Amoxicillin (64%), Enrofloxacin (60%), and Oxytetracycline (57%), but the *Clostridium* isolates are averagely resistant to Gentamycin (49%).

Table 3: Antibacterial susceptibility of *Salmonella* and *Clostridium* isolates from tools used in butchery outlets around IBSW LGA.

Antimicrobial	<i>Salmonella</i> (53)			<i>Clostridium</i> (53)		
	S	I	R	S	I	R
<i>Oxytetracycline</i>	15	11	27	30	8	15
<i>Chloramphenicol</i>	12	11	30	23	12	18
<i>Gentamycin</i>	11	18	24	18	9	26
<i>Enrofloxacin</i>	15	8	30	32	12	11
<i>Ciprofloxacin</i>	28	10	15	38	10	5
<i>Trimethoprim</i>	12	13	28	20	17	16
<i>Amoxicillin</i>	20	14	19	34	12	7

S: Sensitive I: Intermediate R: Resistant

Table 4 shows the prevalence of antibacterial resistant *Salmonella* and *Clostridium* isolates from tools used in butchery outlets around IBSW LGA. There is high level of multi-drug resistance of the isolates (above 50%), especially with the *Salmonella* spp; Chloramphenicol (57%), Enrofloxacin (57%), Trimethoprim (53%) and Oxytetracycline (51%), while the isolates are also resistant to gentamycin (45%),

amoxicillin (36%) and the others. The *Clostridium* isolates were resistant to the antibiotics at varying percentages, but the highest prevalence (49%) of resistance was with gentamycin. Comparatively, the *Salmonella* isolates are more resistant to the common antibiotics than the *Clostridium* isolates.

Table 4: Prevalence of antibacterial resistant *Salmonella* and *Clostridium* isolates from tools used in butchery outlets around IBSW LGA.

Antimicrobial	<i>Salmonella</i> Resistant isolates (%)	<i>Clostridium</i> Resistant isolates (%)
Oxytetracycline	27(51)	15(28)
Chloramphenicol	30(57)	18(34)
Gentamycin	24(45)	26(49)
Enrofloxacin	30(57)	11(21)
Ciprofloxacin	15(28)	5(9)
Trimethoprim	28(53)	16(30)
Amoxicillin	19(36)	7(13)

There is high level of multi-resistance of *Salmonella* and *Clostridium* isolates to each of these antibacterial agents compared to the level of sensitivity and frequency of the

isolates that were intermediate to the antibacterial (Figure 1).

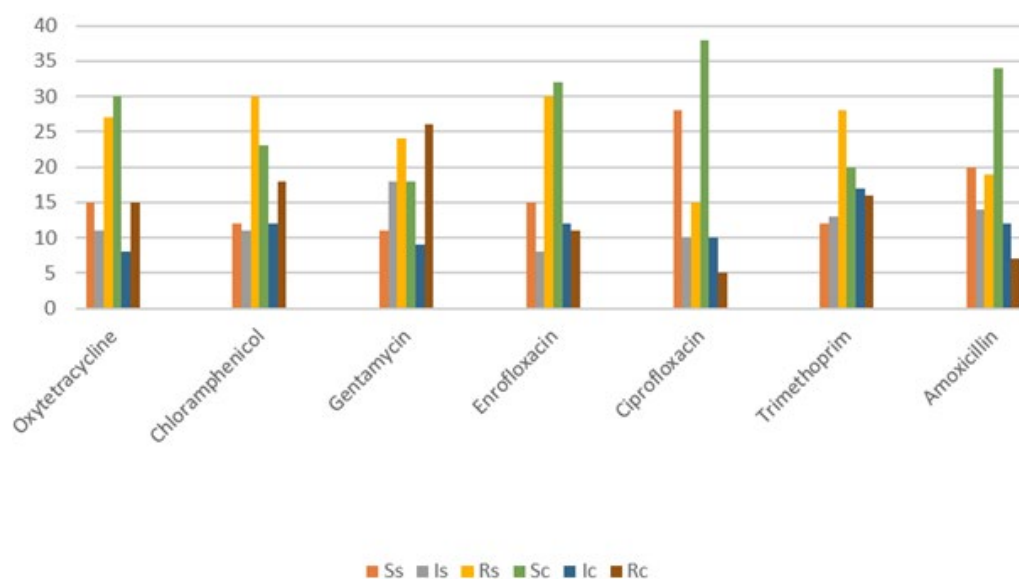


Figure 1: Antimicrobial susceptibility of *Salmonella* and *Clostridium* isolates from tools used in butcheries around IBSW LGA Ibadan.

DISCUSSION

This study enabled us to identify different types of meat-borne bacteria, among which are *Salmonella* and *Clostridium* sp., bacteria that are of public health importance on the tools sampled at the selected butcheries around IBSW LGA, which is an indication of different levels of contamination. This finding further corroborates earlier submissions by Aslama, et al. [19] and Deliephan, et al. [20] that *Salmonella* and *Clostridium* can cause varying degrees of contamination due to poor hygiene on surfaces and hence leading to food-borne infections. The relatively high prevalence rate of *Salmonella* and *Clostridium* isolated in this study should be of concern to health authorities, especially for the fact that the local government area is highly populated, as such an outbreak could have dire consequences. The high prevalence could be traced to low sanitary practices observed in most of the butchery outlets. This prevalence rate agree with the submission of Enabulele, et al. [21] who worked on resistance pattern of *salmonella* isolates from food, animal and human sources. It also confirms the report of Anyanwu, et al. [22] who observed a pattern of *salmonella* infection that appears to be spreading in Nigeria in the form of epizootics.

Food safety refers to the conditions and practices that preserve the quality of food to prevent contamination and food-borne illnesses, which mean the food or meat, must be wholesome

[23]. Whenever there are biological contaminants in meat, they render the food/meat unwholesome and are therefore not fit for human consumption [24]. The contamination of food has become a global public health issue affecting developed as well as developing nations. The contamination can be caused by physical, chemical, and biological agents. Among the biological agents, bacteria are most frequently encountered as a cause of contamination throughout the world [25]. Access to sufficient amounts of safe and nutritious food is key to sustaining life and promoting good health. It also creates a vicious cycle of disease and malnutrition, particularly affecting infants, young children, elderly and the immuno-compromised persons [26].

Also, there are evidences of multi-drug resistant *Salmonella* and *Clostridium* isolates from these same locations to the antibiotics tested in this study. Basically, in livestock production, antibiotics are used for a number of reasons which include among others, therapeutic treatment, disease prophylaxis and as growth promoters. The successful use of antibiotics in veterinary medicine notwithstanding its use has become particularly worrisome, especially for the fact of the potential to extend such drug into the human food chain or the possibility of reduced efficacy of such drugs which has been observed in some reports to be administered by non-qualified personnel [27].

Antimicrobial drugs are key in the treatment of diseases, and their use is essential to protect both human and animal health. However, antimicrobial misuse in the livestock sector, aquaculture and crop production is a major concern as a risk for the emergence and spread of antimicrobial resistant micro-organisms [28]. This is a major global threat of increasing concern to human and animal health. It also has implications for both food safety and food security and the livelihoods of millions of farmers. It has been established that majority of microbes which cause infection in animals can also cause outbreaks in human beings [29].

However, the extent to which the use of antimicrobial agents in food animals or humans contributes to antimicrobial-resistant microorganisms in humans varies between the different microorganisms and region of occurrence [30].

This study observed multi-drug resistant *Salmonella* sp. that has the following prevalence of resistance to Oxytetracycline (51%), Chloramphenicol (57%), Gentamicin (45%), Enrofloxacin (57%), Ciprofloxacin (28%), Trimethoprim (53%) and Amoxicillin (36%). The cause of this high prevalence could be attributed to the irrational use of antibiotics [31]. The high prevalence of resistant isolates to enrofloxacin (57%) and trimethoprim (53%) observed in this study agrees with the submissions of Shang, et al [32]. The *Clostridium* isolates were resistant to the antibiotics at varying percentages, but the highest prevalence (49%) of resistance was with gentamycin. Comparatively, the *Salmonella* isolates are more resistant to the common antibiotics than the *Clostridium* isolates. Unlike the high resistance of the *Salmonella* isolates recorded in this study, the *Clostridium* isolates have good sensitivity to fluoroquinolone which is contrary to the submission of Sholeh, et al [33] that the high-risk antimicrobials for *C. difficile* isolates development showed a high level of resistance, the highest was seen in fluoroquinolones and clindamycin. But this study showed that the low resistance of *Clostridium* to amoxicillin/clavulanate is in line with the submission of Sholeh, et al [33] that it showed almost no resistance, while Tetracycline resistance was present in 20% of human clinical *C. difficile* isolates, which is further supported by this study

Most worrisome however is the finding that there is some significant degree of resistance to the Amoxicillin and Ciprofloxacin. These antibiotics are comparatively new and expensive as such the resistance to them in this study give a cause for concern as they would have been the antibiotics of choice should there be an outbreak of any gastrointestinal tract infection that may arise from *salmonella* and *Clostridium* [34,35].

When humans ingest antimicrobial-resistant microbes in food, some species of microbes may cause severe illness, these

and other species may also serve as a source of transferable resistance determinants for other microorganisms, including human pathogens [36].

It has been established that there is an emergent clone of *Salmonella*, especially the *enterica* variant called *Salmonella infantis* first discovered in Israel, but it is all over the world as at today [37,38]. The emergence of the clone of *Salmonella Infantis* is a cause for concern, as its sudden rise in retail meats and contributions to human illnesses is combined with a propensity for multidrug-resistance [39].

CONCLUSION

Due to the confirmation of excessive presence and prevalence of food borne bacteria on tools used at the sampled butcherries, food safety around IBSW LGA, Ibadan has been called to question. *Salmonella* and *Clostridium* species according to the results of these studies have demonstrated multi-drug resistant characteristic to common antibiotics such as enrofloxacin, oxytetracycline, gentamycin, amoxicillin and chloramphenicol, the resistance has also spread to the Fluoroquinolones (Ciprofloxacin) which is supposed to be the drug of last resort. Hence the prevalence of multi-drug resistant *Salmonella* and *Clostridium* species revealed limitation of effective treatment of *Salmonella* and *Clostridium* infections with common antibiotics.

RECOMMENDATION

1. Considering the high level of contamination across board, we hereby recommend that the butchers should be enlightened on general hygiene and the need to sterilize their tools before and after slaughtering.
2. Antibiotic prescription and dispensing should be handled by qualified personnel trained to do such, and avoid the indiscriminate use of antibiotics in livestock farms.
3. In order to eradicate or minimize AMR there is the need for a multidisciplinary approach to deal with AMR.

REFERENCES

1. Ilboudo J, Tapsoba F, Savadogo A, Seydi M, Traore AS. (2012). Improvement of the hygienic quality of farmhouse meat pies produced in Burkina Faso. *Adv Environ Biol.* 6(10):2627–2635.
2. Bradeeba K, Sivakumaar PK. (2013). Assessment of microbiological quality of beef, mutton and pork and its environment in retail shops in Chidambaram, Tamil Nadu. *Int J Plant Anim Environ Sci.* 3:91–97.
3. Ukut IOE, Okonko IO, Ikpoh IS. (2010). Assessment of bacteriological quality of fresh meats sold in Calabar Metropolis, Nigeria. *Electronic J Environ Agri Food Chem.* 9(1):89–100.

4. Ahouandjou HF, Baba-Moussa J, Bonou V, Dougnon Z, Adéoti R, Yedji R, et al. (2015). Evaluation of the microbiological quality of cattle carcasses in some slaughterhouses at Benin, West Africa. *Int J Sci Rep.* 1(5):228.
5. Nychas GJE, Skandamis PN, Tassou CC, Koutsoumanis KP. Meat spoilage during distribution. *Meat Sci*, 2008; 78: 77 – 89.
6. Bintsis T. (2017). Foodborne pathogens. *AIMS Microbiol.* 3(3):529-563.
7. Martinović T, Andjelković U, Gajdošik MŠ, Rešetar D, Josić D. (2016). Foodborne pathogens and their toxins. *J. Proteom.* 147:226–235.
8. Priyanka B, Patil RK, Dwarakanath S. (2016). A review on detection methods used for foodborne pathogens. *Indian J Med Res.* 144(3):327-338.
9. Atlabachew T, Mamo J. (2021). Microbiological Quality of Meat and Swabs from Contact Surface in Butcher Shops in Debre Berhan, Ethiopia. *J. Food Qual:*11.
10. WHO. (2015). Food safety.
11. Hashempour-Baltork F, Hosseini H, Shojaee-Aliabadi S, Torbati M, Alizadeh AM, Alizadeh M. (2019). Drug Resistance and the Prevention Strategies in Food Borne Bacteria: An Update Review. *Adv Pharm Bull.* 9(3):335-347.
12. Mathew AG, Jackson F, Saxton AM. (2002). Effects of antibiotic regimens on resistance of *Escherichia coli* and *Salmonella* serovar Typhimurium in swine. *J Swine Hlth Prod.* 10: 7-13.
13. Economou V, Gousia P. (2015). Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infect Drug Resist.* 8:49–61.
14. Rossolini GM, Arena F, Pecile P, Pollini S. (2014). Update on the antibiotic resistance crisis. *Clin Opin Pharmacol.* 18: 56–60.
15. FAO. (2007). FAO guidance to governments on the application of HACCP in small and/or less-developed food businesses.
16. Lennette EH, Ballows A, Hausler WJ Jr, Shadomy HJ. (1985). *Manual of Clinical Microbiology*. American Society for Microbiology Washington DC, USA.
17. Jolt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. (1994). *Bergey's manual of systematic bacteriology*. 786. 9th edn. Williams & Wilkins Co. Baltimore, Maryland,
18. CLSI. (2015). M100-S24: Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. M100-S24.
19. Aslama M, Checkley S, Avery B, Chalmers G, Bohaychuk V, Gensler G et al. (2012). Phenotypic and genetic characterization of antimicrobial resistance in *Salmonella* serovars isolated from retail meats in Alberta Canada. *Food Microbiol.* 32:110–117.
20. Deliephan A, Dhakal J, Subramanyam B, Aldrich CG. (2023). Mitigation of *Salmonella* on Food Contact Surfaces by Using Organic Acid Mixtures Containing 2-Hydroxy-4-(methylthio) Butanoic Acid (HMTBa). *Foods.* 12(4):874.
21. Enabulele OI, Ehwrieme DA, Aluyi HSA. (2008). Resistance pattern of *Salmonella* Isolates from Food, Animal and Human sources to common antimicrobial agents. *Glob J of Pure and Appl Sci.* 4(2): 179-182.
22. Anyanwu AI, Fasina PO, Ajayi OT, Rapu I, Fasina MM. (2020). Antimicrobial Resistant *Salmonella* and *Escherichia coli* Isolated from Day-old Chicks, Vom, Nigeria. *African J Clinic Exp Microbiol.* 11(1): 129-136.
23. USDA. (2023). What does food safety mean? <https://ask.usda.gov/s/article/What-does-food-safety-mean>
24. Fasanmi OG, Sansi, JAA. (2008). *Essential of meat and milk inspection and hygiene*. 1st edition, published by Tunmid prentronic, Ibadan.
25. Mahendra P, Martin H. (2021). Food Contamination Poses a Threat to Food Safety. *American Res J Food Nutri.* 3(1) 1-4
26. WHO. (2022). Food Safety Fact sheet. <https://www.who.int/news-room/fact-sheets/detail/food-safety>
27. Oluwasile BB, Agbaje M, Ojo OE, Dipeolu MA. (2014). Antibiotic usage pattern in selected poultry farms in Ogun state. *Sokoto J Vet Sci.* 12 (1).
28. FAO. (2021). Antimicrobial resistance. <http://www.fao.org/antimicrobial-resistance/en/>
29. Ghasemzadeh I, Namazi SH. (2015). Review of bacterial and viral zoonotic infections transmitted by dogs. *J Med Life.* 8(4): 1 - 5.
30. Fagbohun AF, Odunsi OO, Nwufoh OC, Agbato OA, Fasanmi OG. (2023). *Essentials of Livestock Biosecurity*. ISBN: 978-978-997-669-0. Palmarius Publishers, Ibadan.
31. Horumpende PG, Said SH, Mazuguni FS, Antony ML, Kumburu HH, Sonda TB, et al. (2018). Prevalence, determinants and knowledge of antibacterial self-medication: a cross sectional study in North-eastern Tanzania Singer AC, editor. *PLoS One.* e0206623.

32. Shang K, Kim JH, Park JY, Choi YR, Kim SW, Cha SY et al. (2023). Comparative Studies of Antimicrobial Resistance in *Escherichia coli*, *Salmonella*, and *Campylobacter* Isolates from Broiler Chickens with and without Use of Enrofloxacin. *Foods*. 12(11):2239.
33. Sholeh M, Krutova M, Forouzesh M, Mironov S, Sadeghifard N, Molaeipour L, et al. (2020). Antimicrobial resistance in *Clostridioides (Clostridium) difficile* derived from humans: a systematic review and meta-analysis. *Antimicrob Resist Infect Control*. 9:158.
34. Karaman R. (2015). From conventional prodrugs to prodrugs designed by molecular orbital methods. *Frontiers Comp Chem*. 2: 187–249.
35. Thai T, Salisbury BH, Zito PM. (2023). Ciprofloxacin continuing education activity. StartPearls Publishing LLC.
36. Wegener HC. (2012). Improving food safety through a one health approach. Washington: National Academy of Sciences. Antibiotic resistance - Linking human and animal health. 331-349.
37. Yokoyama EN, Ando T, Ohta A, Kanada Y, Shiwa T, Ishige et al. (2015). A novel subpopulation of *Salmonella enterica* serovar *Infantis* strains isolated from broiler chicken organs other than the gastrointestinal tract. *Vet Microbiol* 175:312–318.
38. Bogomazova AN, Gordeeva VD, Krylova EV, Soltynskaya IV, Davydova EE, Ivanova OE et al. (2020). Mega-plasmid found worldwide confers multiple antimicrobial resistance in *Salmonella Infantis* of broiler origin in Russia. *Int J Food Microbiol*. 319:108497.
39. Tyson GH, Li C, Harrison LB, Martin G, Hsu CH, Tate H, et al. (2021). Multidrug-Resistant *Salmonella Infantis* Clone is Spreading and Recombining in the United States. *Microb Drug Resist*. 27(6):792-799.