Identification and Characterization of Antimicrobial Resistance Potential Bacteria Pathogens from IFSERAR Livestock Farms and Farm Handlers

Ariom T. O.[1]* Atayese A. O.[2] Ike W. E.[2] Anyanwu P. A.[2] Ariom N. D.[2] Igwe D. O.[4] Famakinde S. A.[1] and Ekpo U. F.[3]
1. Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture Abeokuta, Ogun State, Nigeria
2. Department of Microbiology, Federal University of Agriculture Abeokuta, Ogun State, Nigeria
3. Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Nigeria
4. Department of Geology, College of Environmental Resources Management, Federal University of Agriculture, Abeokuta
* Corresponding author: ariomto@funaab.edu.ng

Abstract: The problems imposed by the emergence of antimicrobial resistance and its spread in both humans and livestock, due to excessive use of antibiotics in livestock presents serious risks to public health. The susceptibility profile of isolates from IFSERAR livestock farms was investigated. A total of forty (40) rectal, cloacae and ear swab samples of goat, poultry and handlers, were collected from livestock farms using sterile swab sticks. Specimens were sent to the laboratory for isolation, identification, characterization of the associated bacteria and antibiotic susceptibility testing were carried out using standard microbiological techniques. A total of 34 distinct species of bacteria were isolated, which included *Escherichia coli, Staphylococcus aureus, Pseudomonas* spp., *Klebsiella pneumoniae, Bacillus* spp, *Shigella* spp, *Salmonella* spp, and *Enterococcus* spp. were identified. All isolates from different livestock farms and their handlers exhibited 100% resistance to ceftazidime antibiotics. Isolates from farm handlers, Kalahar, and KalaWAD goats exhibited 85.7%, 71.4%, and 57.1% resistance to cefoxitin respectively. The least resistance (35.7%) to cefoxitin was exhibited by isolates from poultry farms. Isolates from farm handlers and the KalaWAD goat farm exhibited 71.4% resistance to vancomycin, while isolates from poultry and kalahari goat farm exhibited 57.1% and 42.5% resistance respectively. High resistance of 71.4% was exhibited by isolates from poultry farms only, while all other isolates to gentamycin exhibited 14.3% resistance. Ciprofloxacin resistance to isolates from Kalahari, KalaWAD, poultry and farm handlers was 14.3%, 28.6%, 42.9% and 28.6% respectively. The findings of this research suggest, that the bacteria isolates exhibit a variety of resistance to antibiotics used, which is a pointer to a pool of resistance genes within the livestock farm isolates.

Key word: Antimicrobial resistance, bacteria pathogens, livestock, farm handlers

INTRODUCTION

The threat and cost of antimicrobial resistance (AMR) have attracted global attention. Antibiotic-resistant pathogens have been reported to cause nearly thirty-three thousand deaths annually in the continent of Europe only and four million and ninety-five thousand global deaths, cumulating to an annual loss of €1.5 billion in medical costs and reduced productivity (Caneschi *et al.,* 2023). Similarly, the abuse and misuse of antibiotics in livestock raise serious concerns for public health due to the growth of bacteria resistant to antibiotics in livestock production, which limits the treatment options available for both human and animal pathogens (Kummerer, 2009). This trend of AMR presents a major setback in the production of safe food and animals. This is no longer news as WHO already recognized it as a major emerging global problem. (Nelson *et al.,* 2019). Livestock is often given large amounts of antimicrobials, some of which belong to the class of drugs used in treating humans and are still being used in the treatment of bacterial infections and as growth promoters. However, this excessive antimicrobial abuse gives rise to bacteria resistance, which poses a threat to both animals and humans. The economic impact of such resistance can be devastating (Scott *et al.,* 2018). Livestock such as poultry, pigs, cattle, and goats are commonly reared worldwide for food and income. The geometric increase in population and urbanization has resulted in high demand for animal products hence the intensive production of livestock experienced globally even on industrial scale for decades (Kimera *et al*., 2020). A typical example is the global increase in fish consumption, reported to be twice the population growth in recent decades. According to FOA, global fish production reached its peak at around 171 million tons in 2016 (FAO, 2018). This scenario is what brought about the abuse of antibiotics (a use that is expected to more than double by 2030), which has been linked to an increase in AMR (Scott *et al.,* 2018). The majority of the antibiotics used in livestock are for non-therapeutic purposes, like their use as growth promoter. It is alarming that sub-lethal concentrations of this substance have been detected in livestock's gastrointestinal environments, which can impede the growth of vulnerable bacterial populations (Zhu *et al.,* 2017). The growing demand for protein from animal sources has aggravated the use of antibiotics in livestock production. This practice is of serious concern since the use of antibiotics to increase yield results in a grave antimicrobial resistance threat (O'Neill, 2015). More worrisome is that this use of antibiotics in livestock is linked to pathogens that are resistant to antibiotics being found in humans (O'Neill, 2015). Antibiotics are used in livestock, for several purposes metaphylactic, therapeutic, and prophylactic use as a cheaper way of improving hygiene vis-a-vis preventing infection in livestock. A use of antibiotics in livestock that is of great concern is its administration at sub-therapeutic doses in feed or water to improve growth and production (As growth promoter) (Caneschi *et al.*, 2023). Antibiotic misuse has been linked to changes in microbial resistance traits, which frequently result in treatment failure and a rise in the cost of infection control (Ariom *et al.,* 2019). Specifically, antibiotic resistance has proven to be of significant challenge in the quest for safe food production and is now recognized as a major emerging problem of global significance (Nelson *et al.,* 2019). Livestock is a major contributor to the emergence and spread of AMR. Due to rising market demand and related financial incentives, livestock farmers in developing nations continue to use antibiotics to promote animal growth (WHO, 2019). A practice that encourages the presence of antibiotic resistance organisms in our agricultural production system and food chain which remains a potential route of exposure for all. Our main worries are the spread of livestock waste on croplands and the potential for antibiotic-resistant microbes from the farm to reach the general public through the food chain or animal handlers. The most concerning aspect of farm-raised superbugs is their ability to transmit resistant genes and genetic materials to other bacteria, including those from different genera and species that have never been exposed to antibiotics before (Awugbemi *et al.,* 2018). Therefore, it is unrealistic to think that we can effectively tackle the significant problem of antibiotic resistance in society by solely focusing on humans where only 20% of available antibiotics are used and ignoring animals where 80% of available antibiotics are used (Kimera *et al*., 2020). Hence, the aim of this study was to identify antibiotic-resistant bacteria and characterize the resistant strains in IFSERAR livestock farms.

MATERIALS AND METHODS

Study area: This research was conducted at the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR). The research hub of the Federal University of Agriculture Abeokuta (FUNAAB), Nigeria. It operates livestock farms housing goats, cattle, poultry, fish, and rabbits within the FUNAAB. The farms are made up of Nigeria indigenous Muturu cattle; Kalahari, KalaWAD and West African dwarf goat; Fulani and Yoruba ecotype chicken; *Orechromis niloticus* and *Tilapia* cultured in earth pond and concrete tanks. It is situated about 6 km, west of the FUNAAB campus. The area is located within longitude

3023’29” E and Latitude 70 13’53” N. The major IFSERAR livestock farms are the farming system practised in IFSERAR livestock farm is a combination of both intensive and semi-intensive livestock farming systems. Antibiotics are used prophylactically, for the treatment of sick animals and as growth promoters.

Ethical approval: Approval for the research's ethics was obtained from the Veterinary Medicine ethics committee, FUNAAB (FUNAAB/COLVET/CREC/2023/01/02). The consent of the farm handlers was sorted for, and the dignity of the animal was taken into consideration during sample collection.

Sample collection: A total of forty (40) rectal and ear swab samples were aseptically collected from various IFSERAR livestock farms and farm handlers using sterile swab sticks. [(Livestock 33), Kalahari goat (8), KalaWAD goat (16), Poultry (9), (Farm handlers (7)]. This was done by carefully inserting and gently rolling the sterile swab into the rectum and ear of the livestock and handlers respectively. Each swab was sealed, labelled, and immediately taken to the laboratory for isolation, identification, and antimicrobial susceptibility testing. A global positioning system (GPS) (Magellan GPS 315) was used to georeference each farm to get the geographic coordinates (longitudes and latitudes) of the IFSERAR livestock farms.

Bacteria isolation from the swab samples collected: The method of isolation was by cultural method. The sample was inoculated on nutrient agar (TM, India) and selective diagnostic media like MacConkey agar (TM Media, India) and Mannitol salt agar (Flu, UK). The media used in this experiment were prepared appropriately based on instructions from the company. Swab samples were inoculated on the media and incubated at 37°c for 24 hours. After the incubation period, the characteristics were observed, and smears were prepared for Gram staining to examine the morphology of the cells.

Purification of bacteria isolates: Based on their morphology, single colonies of bacteria were chosen at random from different medium plates. These bacterial cultures were then subcultured on nutrient agar plates to isolate them in pure forms and then kept on nutrient slants in the refrigerator at 4^{o}C until needed for biochemical analysis and microscopic characterization.

Characterization and identification of bacterial isolates: The pure isolates were characterized and identified using morphological and biochemical characteristics like pigmentation, motility, macro and microscopic morphology, colonial morphology, indole, catalase, coagulase and citrate utilization test. These tests were carried out according to standard microbiological procedures (Cheesbrough, 2006).

Antibiotic sensitivity testing of the bacterial isolates: The susceptibility profiling was done using Kirby Bauer disc diffusion method, following criteria published by the Clinical and Laboratory Standard Institute (CLSI, 2018) guidelines. Mueller-Hinton agar plates were prepared in compliance with the guidelines provided by the manufacturer. Five millilitres of nutrient broth culture were shaken and incubated at 37 ^{o}c until the visual turbidity reached the McFarland standard (0.5 or 1.5× 10.8 cfu/ml). After at least 3 minutes, the following discs were impregnated with antibiotics: Vancomycin (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), ceftazidime (30 µg), amoxicillin-clavulanate (30 µg), gentamycin (30 µg), cefoxitin (30 µg) and placed on Mueller-Hinton agar plates at equidistance using sterile forceps. For twenty-four hours, the plates were incubated at 37°c. Each antibiotic-impregnated disc had a distinct zone of inhibition surrounding it, which was measured to the nearest millimetre and interpreted as susceptible (S) or resistant (R) based on CLSI recommendations.

RESULTS

Of the 40 swab specimens collected from livestock and handlers, 35 (86%) potential pathogens were isolated. Among the isolates from IFSERAR livestock farms, *Escherichia coli* demonstrated the highest prevalence rate of 31%, followed by *Staphylococcus aureus* (20%), *Pseudomonas aeruginosa* (17%), *Klebsiella pneumoniae* (9%), *Bacillus subtilis* (9%), *Shigella flexneri* (6%), *Salmonella typhimurium* (6%) and *Enterococcus faecalis* (3%). The least number of isolates recorded was *Enterococcus faecalis* with only one isolate (3%). Considering the different livestock farms and handlers, the highest number of potential pathogens was isolated from poultry farms (31%), same number of isolates (20%) were recorded in other livestock farms and handlers (Table 1). All the organisms isolated from Kalahari goat farm exhibited multidrug resistance (MDR) except *Klebsiella pneumoniae*. In the same vein, all the organisms isolated from KalaWAD goat farm exhibited multi-drug resistance except *Pseudomonas aeruginosa*. Moreso, organisms isolated from Poultry farms also exhibited resistance to more than three different classes of antibiotics used in the study except one single isolate of *Escherichia coli.* All the potential pathogens isolated from the farm handlers exhibited multi-drug resistance. This is indicative of the farm handlers being a possible source of resistance organisms in the farm (Table 2 and Table 3).

All the bacterial isolates from Kalahari goat farm, KalaWAD goat farm, Poultry farm (livestock) and its handlers exhibited 100% resistance to ceftazidime antibiotic. On the antibiotic cefoxitin, bacteria isolated from farm handlers exhibited 85.7% resistance, while isolates from Kalahari and KalaWAD exhibited 71.4% and 57.1% resistance respectively. The least resistance to cefoxitin antibiotic was exhibited by isolates from poultry farms (35.7%). Another antibiotic the isolates exhibited high resistance to was vancomycin. The isolates from farm handlers and KalaWAD goat farm exhibited 71.4% resistance to vancomycin, while isolates from poultry and Kalahari goat farm exhibited 57.1% and 42.5% resistance respectively. Low resistance was exhibited by the isolates to gentamycin, ciprofloxacin, and tetracycline antibiotics. However, a high resistance of 71.4% was exhibited by isolates from poultry farms only, while all other isolates to gentamycin exhibited 14.3% resistance. Ciprofloxacin resistance to isolates from Kalahari goat, KalaWAD goat, poultry and farm handlers are 14.3%, 28.6%, 42.9% and 28.6% respectively. The isolates' level of resistance from different farms to tetracycline antibiotics were; isolates from Kalahari goat farm and farm handlers 42.9%, isolates from KalaWAD goat farm 28.6% and isolates from poultry 35.7%. Isolates from the Kalahari (14.3%) and KalaWAD (14.3%) and poultry (21.4%) exhibited low resistance to amoxicillin clavulanic acid except the isolates from farm handlers that exhibited 57.1% resistance to the amoxicillin clavulanic acid antibiotic (Figures 3 - 7).

Table 1: Distribution of potential pathogen isolated from IFSERAR livestock farm

Isolate	Sample				
	KAGF	KWGF	PLF	FHE	TOTAL (%)
Escherichia coli	2	1	4	0	11 (31%)
Staphylococcus aureus	2	2	1	2	7 (20%)
Pseudomonas aeruginosa	1	2	0	3	6 (17%)
Klebsiella pneumoniae	2	0	0	1	3 (9%)
Shigella flexneri	0	2	0	0	2 (6%)
Bacillus subtilis	0	0	2	0	3 (9%)
Salmonella typhimurium	0	0	2	0	2 (6%)
Enterococcus faecalis	0	0	0	1	1 (3%)
TOTAL	7	7	11	7	35 (86%)

Key: KAGF = Kalahari Goat Farm. KWGF = KalaWAD Goat Farm. PLF = Poultry Farm. FHE = Farm Handlers

Table 2: Antibiotics sensitivity profile of bacteria isolated from IFSERAR livestock farms

FARM	ISOLATES	CIP	TE	FOX	CN	AMC	VA	CAZ
KAGF	*Escherichia coli*	S	R	R	R	S	S	R
	Staphylococcus aureus	R	S	R	S	**S**	R	R
	Pseudomonas aeruginosa	S	S	R	S	R	R	R
	Staphylococcus aureus	S	S	R	S	S	R	R
	Escherichia coli	S	S	R	S	S	S	R
	Klebsiella pneumonia	S	S	S	S	S	S	R
	Klebsiella pneumonia	S	R	S	S	S	S	R
KWGF	*Staphylococcus aureus*	S	R	R	R	S	R	R
	Pseudomonas aeruginosa	S	S	R	S	S	R	R
	Staphylococcus aureus	S	S	R	S	S	R	R
	Escherichia coli	S	S	R	S	S	S	R
	Shigella flexneri	R	R	S	S	R	R	R
	Shigella flexneri	R	R	S	S	S	S	R
	Pseudomonas aeruginosa	S	S	S	S	S	R	R
PLF	*Escherichia coli*	R	R	S	R	**S**	S	R
	Escherichia coli	S	S	R	R	R	R	R
	Staphylococcus aureus	**S**	**S**	**S**	R	**S**	S	R
	Salmonella typhimurium	**S**	**S**	**S**	R	R	S	R
	Bacillus subtilis	**S**	**S**	**S**	R	S	R	R
	Escherichia coli	R	R	S	R	S	R	R
	Bacillus subtilis	R	R	**S**	R	S	R	R
	Escherichia coli	S	S	R	R	S	R	R
	Bacillus subtilis	R	**S**	R	R	R	R	R
	Salmonella typhimurium	R	S	R	R	S	R	R
	Escherichia coli	S	R	S	S	S	S	R
	Escherichia coli	S	S	S	S	S	S	R
	Escherichia coli	S	S	R	S	S	R	R
	Escherichia coli	R	R	S	S	S	S	R

Key: KAGF = Kalahari Goat Farm. KWGF = KalaWAD Goat Farm. PLF = Poultry Farm. FHI = Farm handlers. CIP = Ciprofloxacin TE = Tetracycline FOX = cefoxitin CN = Gentamycin AMC = Amoxicillin/Clavulonic acid CAZ = Ceftazidime. VA = Vancomycin

Table 3: Antibiotics sensitivity profile of bacteria isolated from IFSERAR farm handlers

Isolates	CIP	TE	FOX	CN	AMC	VA	CAZ
Staphylococcus aureus	S	R	R	S	R	R	R
Pseudomonas aeruginosa	S	S	R	S	R	R	R
Pseudomonas aeruginosa	S	R	R	S	S	S	R
Enterococcus faecalis	S	S	R	R	R	R	R
Pseudomonas aeruginosa	R	R	S	S	S	S	R
Klebsiella pneumonae	R	S	R	S	R	R	R
Staphylococcus aureus	S	S	R	S	S	R	R

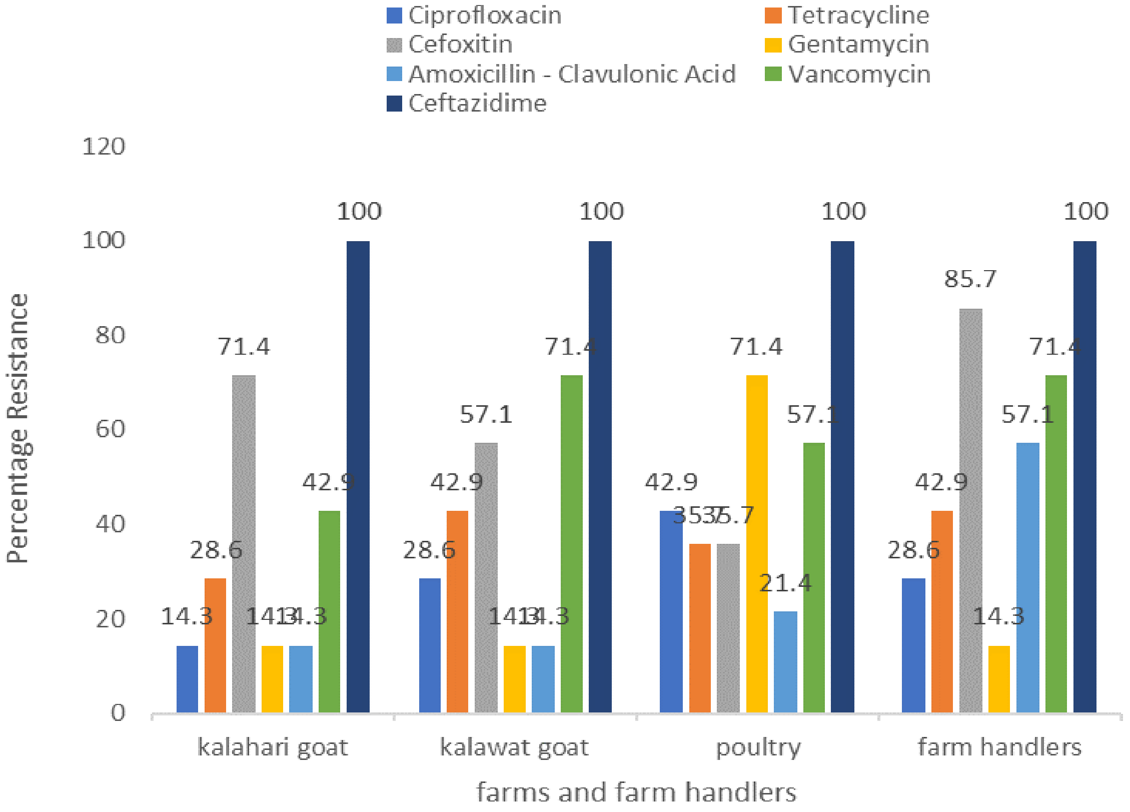

Figure 3: Percentage resistance of bacteria isolated from IFSERAR livestock farms and farm handlers

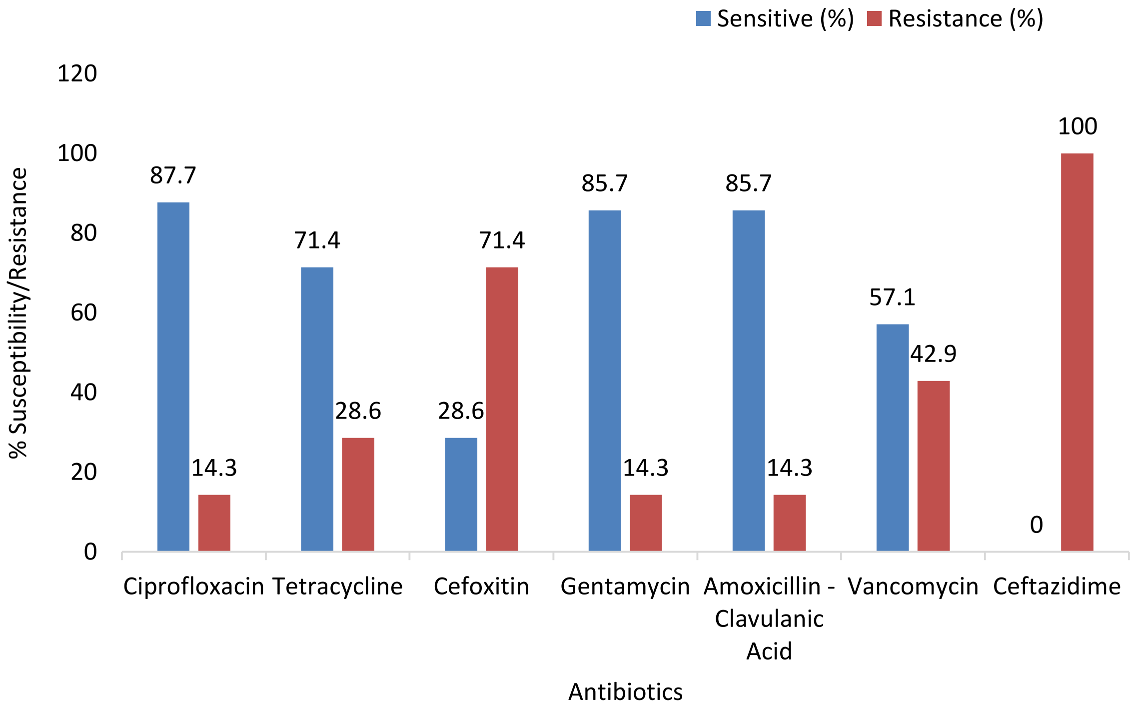

Figure 4: Percentage susceptibility and resistance of bacteria isolated from IFSERAR kalahari goat farm

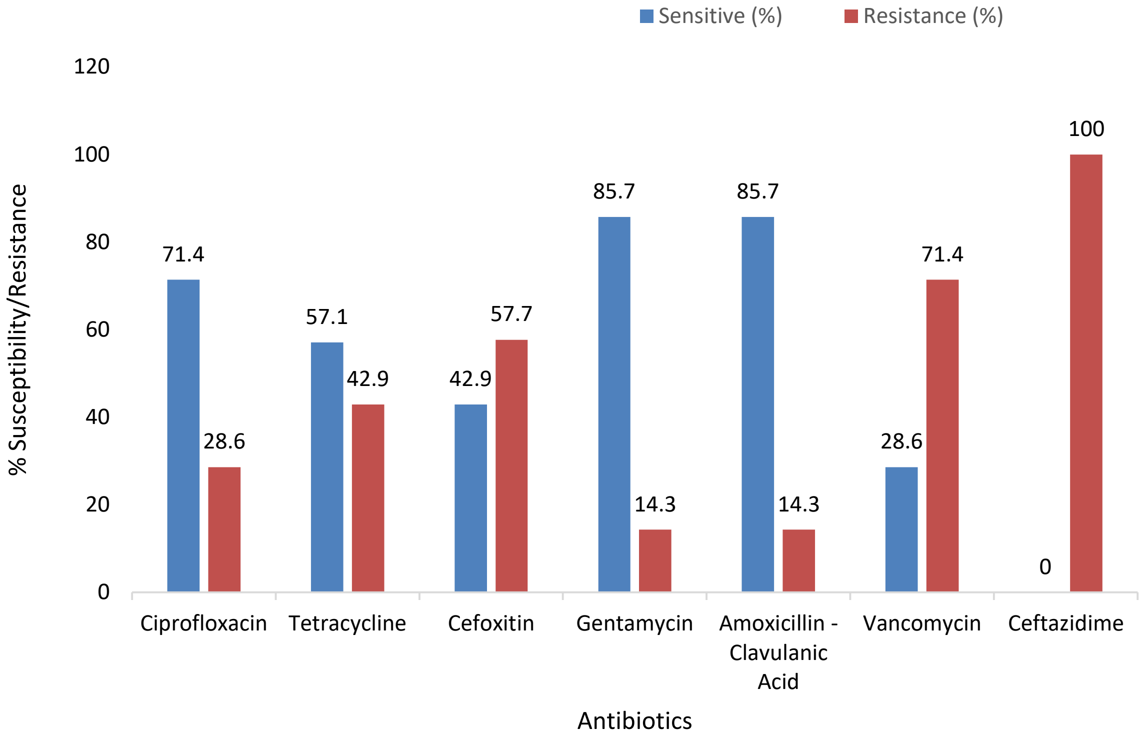

Figure 5: Percentage susceptibility and resistance of bacteria isolated from IFSERAR Kalawad goat farm

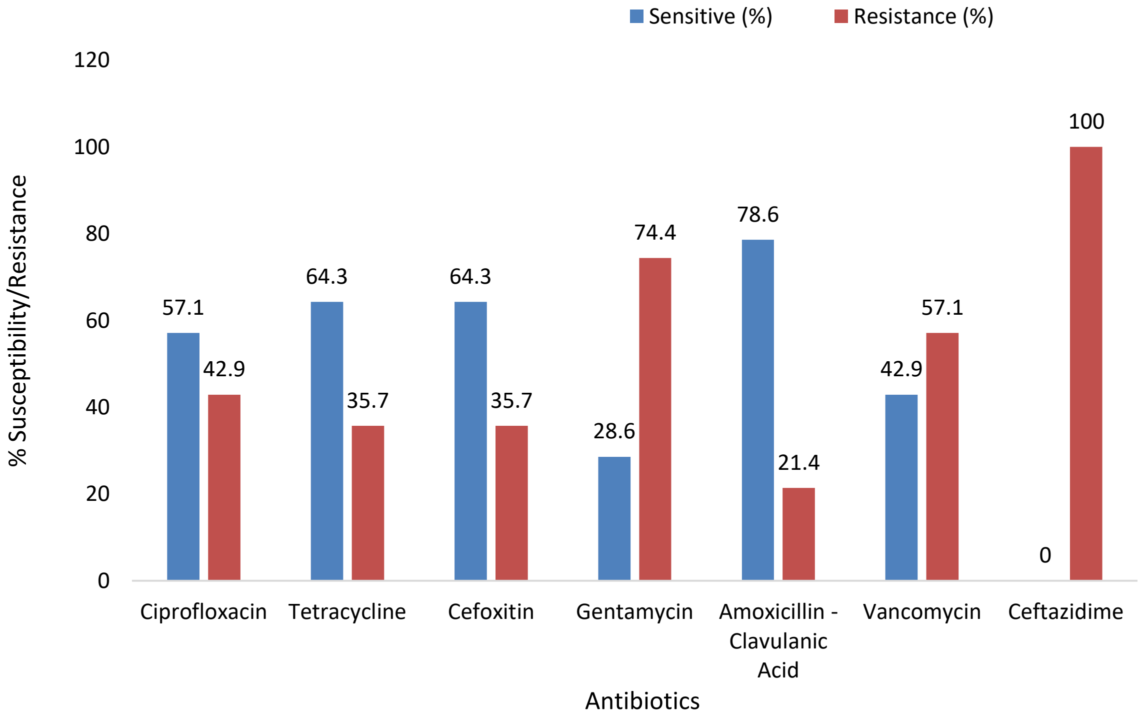

Figure 6: Percentage susceptibility and resistance of bacteria isolated from IFSERAR poultry farm

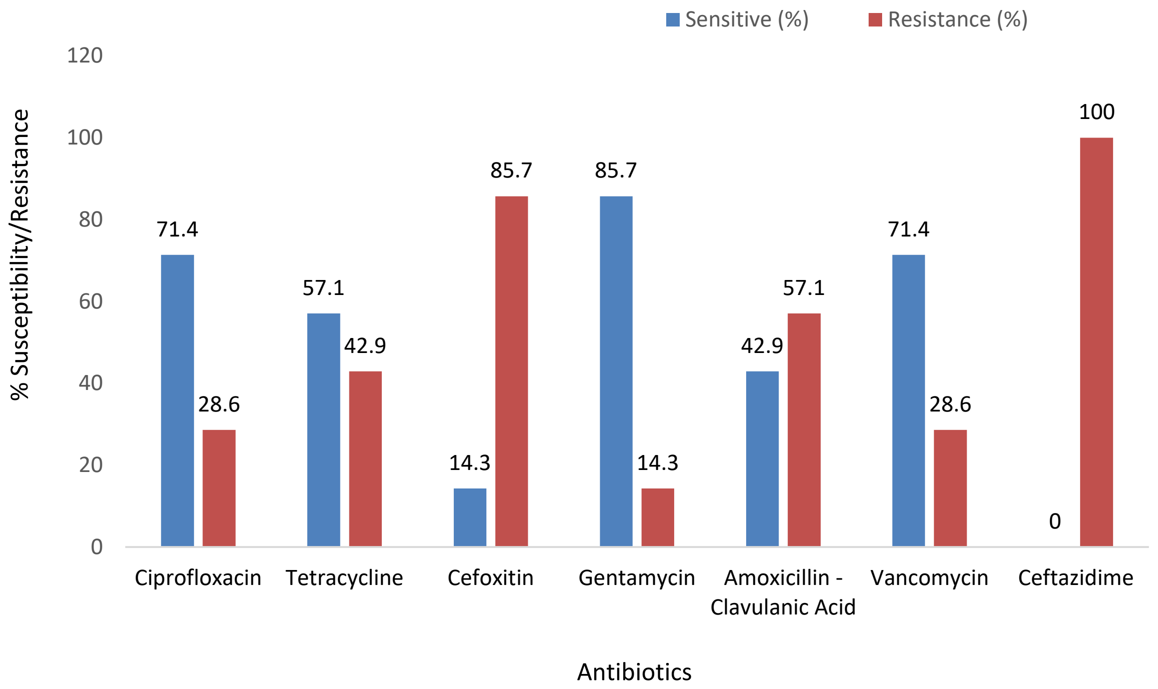

Figure 7: Percentage susceptibility and resistance of bacteria isolated from IFSERAR farm handlers

DISCUSSION

Antimicrobial resistance (AMR) poses a significant threat to global health due to the growth in mortality and economic burden brought by resistant organisms in Human and livestock animals (Aworh *et al.*, 2019). The indiscriminate use of antibiotics has been identified as one of the most prevalent risk factors that can lead to the establishment, selection, and spread of AMR bacteria in livestock (Joshua *et al.*, 2018; Adebowale *et al.*, 2020). In Nigeria, antibiotics are still widely used for this purpose even though the practice are not allowed in most other countries as growth promoters. This is evident in research that found that by the year 2030, Africa would have the highest expected increase of antibiotic use of 37% in food-producing animals (Caneschi *et al.*, 2023). The percentage prevalence of organisms isolated (*E. coli, (*31%), *Staph. aureus* (20%), *P. aeruginosa* (17%), *K. pneumoniae* (9%), *Shigella flexneri* (6%), *S. typhimurium* (6%) and *Enterococcus faecalis* (3%)) agrees with findings reported by Cantas *et al.*, Marshall and Levy, and Adebowale *et al.* who isolated *Escherichia coli, Shigella* spp. *Staphylococcus aureus, Enterococcus* spp. and *Salmonella* spp. (Marshall and Levy, 2011, Cantas *et al.*, 2013, Adebowale *et al.*, 2022). Arbab *et al.*'s research conducted in 2021 also revealed that *E. coli* and *S. aureus* were the most frequently found infection agents, accounting for 58% and 54%, respectively, followed by *Salmonella* spp. 21%, and *Shigella* spp. 29% (Arbab *et al.*, 2021). This shows that *E. coli* and *Staphylococcus aureus* are the most common pathogen in livestock. Although the presence of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis, Shigella flexneri, Salmonella typhimurium,* and *Enterococcus faecalis* indicates a high burden that may pose a risk to both human and animal health. Therefore, that of *Escherichia coli* and *Staphylococcus aureus* could be attributed to be among the normal flora of the livestock. This study reported MDR isolates from both humans and animals, which are resistant to commonly used antibiotics such as ceftazidime (100%), vancomycin (57%), gentamycin (71%), cefoxitin (71%), ciprofloxacin (43%) tetracycline (43%), amoxicillin clavulanic acid (14%) as major findings. All the organisms isolated from Kalahari, KalaWAD goat farm and poultry farm exhibited multidrug resistance (MDR) except *Klebsiella pneumoaniae, Pseudomonas aeruginosa* and *Escherichia coli* respectively. All the potential pathogens isolated from farm handlers exhibited multi-drug resistance. The findings of this research agree with that of Chambers *et al.* (2009) where *Staphylococcus aureus* and *Escherichia coli* isolate showed resistance to cefoxitin, vancomycin, ceftazidime, tetracycline and gentamycin which indicates its multidrug resistance. Multidrug resistance (MDR) observed in this study may be due to antibiotic abuse, overuse or maybe that the resistance is chromosomally or plasmid-mediated bearing in mind the none static nature of antimicrobial susceptibility to bacteria (Dick *et al.*, 2015). More so, the possibility of vertical and horizontal transfer of resistant genes could be possible because of the reported MDR. For instance, tetracycline has been linked with MDR bacteria co-selecting resistant genes to critically important antibiotics in humans (Herrick *et al*., 2014). All the bacterial isolates from Kalahari goat farm, KalaWAD goat farm, poultry farm (livestock) and its handlers exhibited 100% resistance to ceftazidime antibiotic. The findings of this research agrees with that of Ariom *et al.* (2019) on both hospital and community-acquired MRSA, in which the authors reported a 100% resistance to both community and hospital isolates of *Staph. aureus* on ceftazidime antibiotics. This is also in agreement with that of Adebowale *et al*. (2022) that reported 100% resistance to ceftazidime antibiotic in research conducted on life poultry birds. This high level of resistance exhibited by isolates to ceftazidime indicates that this beta-lactam antibiotic cannot be used as a treatment option in the case of a disease outbreak. The least resistance to cefoxitin antibiotic was exhibited by isolates from poultry farms, while highest resistance to vancomycin was exhibited by isolates from Kalawad goat farm handlers. The increased resistance (isolates from farm handlers on cefoxitin (85.7%), Kalahari (71.4%), KalaWAD (57.1%)) observed among these antibiotics is not strange as they have been reported to be among the most used antibiotic classes in food-producing animals. (Caneschi *et al.*, 2023). Thus, suggesting the high chance of its misuse in livestock farms. This finding is also in agreement with a study conducted by Alonso *et al*. (2017) where *Escherichia coli* isolate demonstrated a high increase in resistance (60.6%) to quinolones and cephalosporins which are among the critically important antibiotics (CIAs). In this study, low resistance was exhibited by the isolates to gentamycin, ciprofloxacin, and tetracycline antibiotics, while a high resistance of 71.4% was shown by isolates from poultry farms only. The low resistance recorded against gentamycin, ciprofloxacin, tetracycline and amoxicillin clavulanic acid antibiotics corroborated the findings of Adesokan *et al.* (2015), where they reported tetracycline, ciprofloxacin, gentamycin and amoxicillin clavulanic acid as among the most effective antimicrobials used by livestock farmers in Southwest Nigeria. More so, the high resistance (57.1%) observed among the farm handlers in this study shows a possible transmission of antibiotic resistance, as demonstrated by Founou *et al.* (2016).

CONCLUSION

Antibiotic resistance as observed in this research has public health implications because multi-drug resistance is extremely common. A similar pattern of resistance was observed among the livestock animals and their handlers. Most worrisome, is the fact that some of the resistant isolates are opportunistic pathogens and infection caused by these organisms may be difficult to treat and can spread among human populations.

This calls for caution, in the indiscriminate and inappropriate use of antibiotics in food-producing animals and humans. Though gentamycin, ciprofloxacin, tetracycline and amoxiclav were relatively effective against the isolates in this study, there is still an urgent need for periodic antimicrobial resistance surveillance to detect any changes in resistance patterns over time. Concerning the high antimicrobial resistant profile observed on the potential pathogens isolated from the farm handlers, it is important to routinely educate the farm handlers on the danger of AMR and proper hygienic practices that would decrease the need for therapeutic use of antibiotics in their routine farming activities.

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