ASSESSMENT OF MICROBIAL LOAD AND MULTIDRUG-RESISTANT PROFILE OF BACTERIAL FLORA FROM CATTLE IN BAUCHI, NIGERIA

Abstract

Aim: The study aimed to assess the bacterial load of in rectal swabs from cattle by isolating*Enterococcus*spp and *Escherichia coli,* and determining the multidrug-resistant pattern of the isolates.

Study design: The study is a clinical-veterinary laboratory investigation involvingthe isolationand determination ofthe multidrug-resistant (MDR) profile of *Enterococcus* sppand *E. coli* isolated from cattlerectal.

Place and Duration of study: This studywas carried out in the Yelwa and Gubi campuses Farm centersof AbubakarTafawaBalewa University (ATBU),Bauchi, Nigeria,in period extended frombetween April to June 2021.

Methodology: Fresh rectal swabsampleswere collected from the randomly selected cattle and labeled. The samples were immediately transported and processed in the Microbiology laboratory at Yelwa Campus, and the bacterial load of each sample was determined using standard techniques. *Enterococcus* sppand *E. coli were* isolated using differential culture media followed by an appropriate biochemical identification test. Theisolates were subjected tothe Kirby-Bauer disc diffusion method, to assess the antimicrobial susceptibility pattern.

Results: In Yelwa, the highest microbial load is 2.7 x 10^{12} CFU/g. while the lowest microbial load is 2.0 x 10^{12} CFU/g. In the Gubi campus, the highest microbial load is 3.4 x 10^{12} CFU/g. while the lowest microbial load is 2.7 x 10^{12} CFU/g.BothinYelwa and Gubi,the result showed that most isolates of *Enterococcus* spp and *E.coli* are multidrug-resistant. In Yalwa some of the isolates showed 100% resistance against Norfloxacin, Rifampicin, Ampicillin, and Streptomycin, while Gentamycin gave the lowest multidrug resistance (57.4%). In Gubi, the highest was to ampicillin with (90.6%) frequency, whilethe lowest resistance was found in Chloramphenicol (11.3%). In Yelwa, a high percentage resistance(92.6%)was observed in Streptomycin, and Cephalexin has the lowest (20.4%). In Gubi, all the *E. coli*isolateshad 100% resistance against sulfamethoxazole, and the lowest wasin Ofloxacin(43.4%).

Conclusion: This study found that cattle in the area are reservoirs of bacteria that are both part of the normal flora and opportunistic pathogens, and harbored resistance phenotypes. It is therefore advocated that the use of these animals' faeces as manure should be done with caution, particularly after pre-treatments.

Keywords: Multidrug-Resistance,Yelwa, Gubi,*Enterococcussp*, and *Escherichia coli*, Cattle.

1.0 INTRODUCTION

The development of large-scale concentrated animal feeding operations (CAFOs) has increased the extensive use of veterinary antimicrobials in the treatment of infections, prevention of diseases, and promotion of growth [1,2]. Similarly, antimicrobials administered to cattle provide selective advantages for antimicrobial-resistant bacteria (ARBs) to develop in cattle intestines [3]. Cattle manure could be a reservoir of bacteria

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carrying Antimicrobial Resistant Genes (ARGs) and Mobile Genetic Elements (MGEs) such as plasmids [4,5]. These antimicrobial-resistant bacteria can be transmitted to humans and cause an infection or transform the normal human flora to be pathogenic and resistant to drugs. This is increasingly becoming a threat to human health in our society. Among such prominent bacteria are *Escherichia coli* and *Enterococcus species*.

*Escherichia coli*is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded animals [6]. Most *E. coli* strains are harmless, but some serotypes (EPEC, ETEC, etc.) can cause serious food poisoning in their hosts and are occasionally responsible for food contamination incidents that prompt product recalls, The harmless strains are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K_2[7], *E. coli* and other facultative anaerobes constitute about 0.1% of gut microbiota in both humans and cattle [8], and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. It takes as little as 20 minutes to reproduce. *Colibacillosis*also known as: *E. coli* infection,enterotoxigenic*E. coli* (ETEC) or Septicaemic*Colibacillosis*. Various serotypes of enterotoxigenic*E. coli*can cause either diarrhoea or septicaemia in very youngcalves [9, 10]. Septicemic*colibacillosis*is a major cause of early calf deaths. Thecondition is often fatal or leads to post-septicaemicinfections that are often non-responsive to treatment.

Enterococcus species represent a subgroup of the group D faecal *Streptococcus* and as a coccus, they are spherical and occur either singly, in pairs, or as short chains. They are Gram-positive, facultatively anaerobic, lactic-acid-producing bacteria that live as commensal bacteria in the gastrointestinal tract of humans and animals. Members of this group include *Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum*, or *Enterococcus casseliflavus, Enterococcus mundtii,* and *Enterococcus avium*, which exhibit significant differences in the incidence of virulence factors, antibiotic resistance genes and distribution in fresh and dry cattle manure [11]. *Mundtii* is the species most commonly reported in cattle manure [12]. Generally, the enterococci are considered avirulent and harmless, however, *E. faecalis* and *E. faecium* are notable opportunistic pathogens causing nosocomial infection in humans. Important clinical infections caused by *Enterococcus*includes Urinary tract infections, bacteremia, endocarditis, diverticulitis, meningitis and spontaneous bacterial peritonitis. They are also one of the environmental causative agents of mastitis.

Antimicrobial agents are the major drugs of choice of physician's desk to treat pathogenic infections. It has been observed that some clinicians prescribe the medicine based on the symptoms instead of performing diagnostic tests. This prescribing pattern may be one of the reasons for the development of resistant bacteria to antibiotics [13], Therefore, Antimicrobial susceptibility testing (AST) plays an important role to check the effectiveness of a drug against a bacterium and select the best drug that acts against the bacterium.Livestock practices vary from one individual to another and from one geographical location to the other, but eventually, influence the microbial structure of manure released by the animals. Manure provides a different biological and physicochemical environment to microorganisms [14] Viruses represent another group of pathogens that exist in cattle manure. Originally, these pathogens inhabit the intestinal

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tracts of animals and are typically shed in this habitat asymptomatically. Seemingly, both animals and humans on and off farms are exposed to the potential health risks allied to inadequate management of manure. Consequently, the fate of these pathogens in manure to pollute, contaminate and infect the environment and humans, respectively, is based on the pathogen's ability to survive in manure following excretion [14]. The aim of this study is to therefore determine the microbial loadantimicrobial susceptibility patterns of bacteria (*E. coli* and *Enterococcus sp*) from cattle in Yelwa and Gubi campus of AbubakarTafawaBalewa University Bauchi, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Study Area

This study was carried out at the Yelwa and Gubi campuses of AbubakarTafawaBalewa University (ATBU) ,Bauchi, North-Eastern Nigeria. Yelwa covered a total land mass of 15km^2. The Gubi campus is located in Gubi village of Ganjuwa local Government of Bauchi state, covering 48 km^2. The area is dominated by tropical Sudan Savannah, characterized by agricultural activities both on a large and small scale which include crop production and cattle rearing.

2.2 Samples Collection

A total of 107rectal samples wererandomly collected from randomly selected white Fulani cattle breed from the two campuses, between April to June 2021. A 10g each of the fresh faecaland rectal samples were collected aseptically into a sterile plain container using a Scoop and labeledappropriately. The samples were transported using an icebox with ice packs to the Microbiology laboratory at Yelwa Campus of AbubakarTafawaBalewa University Bauchifor immediate processing.

2.3 Sample Processing

The bacterial load of each sample was determined by serial dilution of each sample. A 1gram of the sample was diluted in 10 ml of distilled water. A seven-fold serial dilution was carried out and 0.5ml of 10^{-7} diluentwas inoculated on every 3 plates of Nutrient agar media (Oxoid, UK) and incubated at 37^{o}C for 24 hours, and the average colonies counted were identified. *Enterococcus sp* was isolated using Brain Heart infusion broth (BHI) (LAP M) containing 5% glycerol. A 1ml of the homogenized mixture of the medium with the sample was added to 3ml Bile aesculin broth (BEB) (LAP M) and incubated under aerobic conditions at 37^{o}C for 24 hrs. A loopful from BEB was streaked on a Bile aesculin agar (BEA) (LAP M) and incubated under aerobic conditions at 35^o C for 48 hours. The plates were observed for white or grey typical colonies surrounded by black zones [15].

For*Escherichia coli,*the broths were inoculated to eosin methylene blue (EMB) agar (Oxoid, UK) and incubated under appropriate growth conditions. The plates were then examined for typical and presumptive round colonies of *E. coli* with a metallic sheen. Biochemical tests were performed for further identification of the isolates as described by Evans *et al.* [15].

Antimicrobial susceptibility analysiswas carried out using the Kirby-Bauer disc diffusion method, according the clinical and laboratory standards Institute (CLSI) guidelines [16]. For this study, isolates were identified as antimicrobial-resistant (AMR) based on the

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criteria of resistance to at least one antimicrobial agent and as multidrug-resistant (MDR) isolate by the criteria of resistance to at least two different antibiotics[16].

3.0 RESULTS AND DISCUSSION

The analysis of microbial load of cow dung from Yalwa and Gubi campuses revealedthat, in Yelwa, the highest microbial load was recorded in Week4, with 2.7 x 10^{12} CFU/gand, the lowest microbial load was recorded in Week5, with 2.0 x 10^{12} CFU/g. In Gubi campus the highest microbial load was observed in Week1, with 3.4 x10^{12} CFU/g. While the lowest microbial load is clearly observed in Week4 and Week5 with 2.7 x 10^{12} CFU/g.

Multidrug resistance pattern of *Enterococcus sp*isolated from cattle in Yelwa and Gubi. The result shows that all the isolates are multidrug-resistant, as indicated by resistance to more than one drug. In Yalwa, some of the isolates showed 100% resistance against; Norfloxacin, Rifampicin, Ampicillin, andStreptomycin antibiotics while Gentamycin, shows the lowest multidrug resistance of 57.4%. In Gubi, the isolates showed multidrug resistance against Norfloxacin, Rifampicin, Erythromycin, Chloramphenicol, Gentamycin, Ciprofloxacin, and Ampicillin. The highest is ampicillin with a 90.6%, level of resistance. The lowest resistance is observed in Chloramphenicol (11.3%).

Table1: Microbial load of cow dung isolated from Yelwa and Gubi Campus

Location/Week	Number of Samples Collected (n = 156)	Mean number of Colonies	Mean Microbial load (cfu/g)	Percentage (%)
Yelwa				
Week 1	18	113	2.25 x 10^{12}	19.5
Week 2	15	122	2.4 x 10^{12}	20.6
Week 3	15	113	2.3 x 10^{12}	19.7
Week 4	15	132	2.7 x 10^{12}	31.5
Week 5	15	112	2.0 x 10^{12}	17.2
Gubi				
Week 1	18	169	3.4 x 10^{12}	23.2
Week 2	15	139	2.8 x 10^{12}	19.2
Week 3	15	148	3.0 x 10^{12}	20.5
Week 4	15	133	2.7 x 10^{12}	18.5
Week 5	15	136	2.7 x 10^{12}	18.5

Multidrug resistance pattern of *Escherichia coli* isolated from cow dug in Yelwa and Gubi can also be observed. In Yelwa, a high percentage of (92.6%) multidrug resistance is observed in Streptomycin, and Cephalexin have the lowest multidrug resistance of 20.4%. In Gubi, all the isolates of *E. coli* revealed 100% resistance against sulfonamides while the lowest multidrug resistance was found in Ofloxacin (43.4%).

In this study, bacteria, *Enterococcus faecalis*and*Enterococcus fascism,*non-fastidious bacteria specifically *Escherichia coli,* were isolated infrom107 rectal swabs from cattle. Cattle dung is composed of about 80% water and supports a matrix ofundigested plant material that is rich in nutrients, microorganisms, and their byproducts [17]. Similar bacterial isolates (*Staphylococcus aureus, Bacillus* spp, *Pseudomonas aeruginosa*, *Escherichia coli,*and *Salmonella* spp) were isolated from cattle dung in Kampar, Malaysia, and Ekiti state, Nigeria [18,19]. However, the lower parts of the gut of cattle contain

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various microorganisms including*Enterococcus* spp, *B. subtilis*, and *Lactobacillus* spp[20].

Table 2: Multidrug-resistant pattern of *Enterococcus spp*isolated from cow dung in Yelwa and Gubi

Location	Antimicrobial Agents (mcg)	Number (%) of isolates (*E. coli* = 54) (*E. spp.* = 53)and Susceptibility pattern	
Yelwa		**Sensitive**	**Resistant**
	Amoxicillin (20)	11(22.4)	43(79.6)
	Ampicillin (20)	00(0.0)	54(100)
	Chloramphenicol (30)	21(38.9)	33(61.1)
	Ciprofloxacin (10)	20(37.0)	34(63.0)
	Erythromycin (30)	22(40.7)	32(59.3)
	Gentamycin (10)	23(42.6)	31(57.4)
	Levofloxacin (20)	10(18.5)	44(81.5)
	Norfloxacin (10)	00(0.0)	54(100)
	Rifampicin (20)	00(0.0)	54(100)
	Streptomycin (30)	00(0.0)	54(100)
Gubi			
	Amoxicillin (20)	34(64.2)	19(35.8)
	Ampicillin (20)	05(9.4)	48(90.6)
	Chloramphenicol (30)	47(88.7)	06(11.3)
	Ciprofloxacin (10)	46(86.8)	07(13.2)
	Erythromycin (30)	44(83.0)	09(17.0)
	Gentamycin (10)	45(84.9)	08(15.1)
	Levofloxacin (20)	31(58.5)	22(41.5)
	Norfloxacin (10)	43(81.1)	10(18.9)
	Rifampicin (20)	40(75.5)	13(24.5)
	Streptomycin (30)	27(50.9)	26(49.1)

The microbial load of each sample was determined in Yalwa Campus the highest microbial load was found to be 3.56 x 10^{12}CFU/g and 5.78 x 10^{12}CFU/g in Gubi Campus. This result is in line with bacterial density in mammal's faeces, ranging from 10^{11} to 10^{12} CFU/g as reported byNikolina*et al.* [21]. The microbial load was high because the sample was freshly collected and analyzed immediately.

The presence of a high level of resistance to the tested antibiotics was observed in this study. The *Enterococcus* sppand *Escherichia coli* isolated were 100 % resistant to Norfloxacin, Rifampicin, Streptomycin, Ampicillin, and Sulfamethoxazole. However, Multidrug resistance is observed in all the isolates. This showed that the majority of Gram-positive isolated bacteria were resistant to Beta-lactams antibiotic while Gram-negative was more resistant to Fluoroquinolones antibiotics. Studies have shown that

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Escherichia coli and *Enterococcus spp*are sources of resistance genes for strains isolated from cattle dung even though they inhabit different ecosystems [22].

Resistance gene profiles are believed to play a very important role in mediating and transferring resistance to antibacterial drugs in the bacteria population. They can be localized in discrete transposable elements of DNA called transposons and plasmids, which are mobile, and can move from one DNA molecule to another [5]. This can lead to the rapid spread of antibiotic resistance in a bacteriapopulation and explains the emergence of multi-resistant strains [23].

Bacteria use three main strategies to get protected against β-lactams: alteration in Penicillin Binding Proteins (PBPs) which reduces the affinity of β- lactams, efflux pumps which remove the antibiotic from the bacterial periplasmic space, and production of β-lactamases which hydrolyze the ring of β-lactams [24, 25]. However, resistance to quinolones has been a problem ever since Nalidixic acid was introduced into clinical medicine more than 40 years ago [25]. Generally, three mechanisms of resistance to quinolones are currently recognized: mutations that alter the drug targets, mutations that reduce drug accumulation, and plasmids that protect cells from the lethal effects of quinolones [26].

Bacteria such as *Enterococcus* sppand *E. coli,* among othersisolated from cattle dung were reported to be resistant to several antibiotics including Penicillin, Amoxicillin, Ofloxacin, Pefloxacin, Ciprofloxacin, and Tetracycline [27]. A similar resistant pattern was also reported by [28] in *Escherichia coli*, *Aeromonashydrophila*, *Salmonella typhi*, *Staphylococcus aureus,* and *Shigelladysenteriae*from cattle dung with isolates showing high resistance to ampicillin, amoxicillin, gentamicin chloramphenicol, and erythromycin. Utilization of antibiotics for other purposes other than therapy can enrich the population of resistant bacteria in the environment capable of infecting humans [29].

Nonetheless, low resistances were seen in some isolated *E. coli* against Ceporex and Ciprofloxacinwhich were exclusively isolated from cattle dung as well as some *Enterococcus spp*isolates that were also susceptible to Levofloxacin. This is in agreement with [30], who reported antibiotics susceptibility of *Nocardia*spp. ComparablyAbu *et al.* [31] reported the sensitivity of bacterial isolates to similar antibiotics such as Ciprofloxacin and Ofloxacin and opined on their value as empiric antibiotic therapy for enteric infections. It has been shown that resistance to ciprofloxacin is usually associated with resistance to other macrolides, lincosamides, and type B streptogramin, and is referred to as MLS resistance [32].

Multiple drug resistance was seen in pathogenic *Enterococcus spp*and *Escherichia coli* in this study. This may be attributed to the presence of resistance determinants on plasmids with similar selective markers or as a result of the independent, simultaneous development of resistance to different agents [31]. These suggest that bacteria have the unique characteristics of being able to transfer resistance genes from one bacterium to another in different populations and habitats [33].

Table 3: Antimicrobial susceptibility pattern of *Escherichia coli* isolated from cattle in Yelwa and Gubi

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Location	Antimicrobial Agents (mcg)	Number (%) of isolates (*E. coli* = 54) (*E. spp.* = 53)and Susceptibility pattern	
Yelwa		**Sensitive**	**Resistant**
	Ampicillin (30)	21(38.9)	33(61.1)
	Augmentin (30)	20(37.0)	34(63.0)
	Cephalexin (10)	43(79.6)	11(20.4)
	Ciprofloxacin (10)	41(75.9)	13(24.1)
	Ofloxacin (10)	12(22.2)	42(77.8)
	Gentamycin (10)	19(35.2)	35(64.8)
	Nalidixic acid (30)	33(61.1)	21(38.9)
	Reflacine (10)	30(55.6)	24(44.4)
	Streptomycin (30)	04(7.4)	50(92.6)
	Sulfamethoxazole (30)	10(18.5)	44(81.5)
Gubi			
	Ampicillin (30)	10(18.9)	43(81.1)
	Augmentin (30)	20(37.7)	33(62.3)
	Cephalexin (10)	21(39.6)	32(60.4)
	Ciprofloxacin (10)	19(35.8)	34(64.2)
	Ofloxacin (10)	30(56.6)	23(43.4)
	Gentamycin (10)	09(17.0)	44(83.0)
	Nalidixic acid (30)	22(41.5)	31(58.5)
	Reflacine (10)	21(39.4)	32(60.4)
	Streptomycin (30)	13(24.5)	40(75.5)
	Sulfamethoxazole (30)	00(0.0)	53(100)

Multi-drug resistance profiles have also been reported in enteric bacteria from both human and animal sources [34,35]. Persistent multiple drug resistance of most isolates to appropriate drugs of choice is of great public health concern and calls for periodic monitoring of antibiograms to detect possible changing patterns [36].

4.0 CONCLUSION

This study provides novel data on the status and distribution of antimicrobial resistance in food-producinganimals. The gastrointestinal microbiota of cattle harbour commensal bacterial species with various antibiotic resistance profileof public health concerns, particularly among the *Enterococci* species. Antimicrobial stewardship in veterinary medicine is very important to overcome the critical consequences MDR on human health, further investigations and monitoring studies are therefore required.

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