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Research Article

Evaluation of Antibacterial Efficacy of *Alchornea cordifolia* Leaf Extracts against Multidrug-Resistant Enterobacteriaceae Isolated from Poultry Birds

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ABSTRACT
The continual monitoring of resistance in poultry isolates is of epidemiological significance. This study investigated the antibacterial potency of methanol extracts of *Alchornea cordifolia* against drug-resistant Enterobacteriaceae from healthy chickens in Umuahia, Abia State. The organisms were isolated and characterized by sequencing their 16S rRNA gene, and the antibiotic susceptibility profile was ascertained. The inhibitory activities of the extracts were tested using standard methods. Fifteen (15) microorganisms were isolated, but predominant among them were *Proteus mirabilis* (40.0%), *Salmonella* spp (26.7%), *Klebsiella pneumoniae* (20.0%), and *Enterobacter aerogenes* (13.3%). The nucleotide sequences of bacterial 16Sr RNA gene retrieved from the isolates were deposited in the GenBank database with accession numbers PQ555018 and PQ555019. The results revealed a moderate level of resistance to cephalosporins, as 40.0% of the isolates showed resistance to ceftriaxone and 46.7% to cefuroxime. Ofloxacin and levofloxacin exhibited significant activity as 73.3% and 80% of the isolates being susceptible. The predominant resistance pattern (ceftriaxone, tetracycline, and azithromycin) occurred in 93.3% of isolates. *Proteus mirabilis* exhibited the broadest resistance (as 5/6 of the strains were resistant to CRO-TET-CPR-AZN-GEN), while *Salmonella* spp and *Klebsiella pneumoniae* showed resistance to ≥5 antibiotics. The crude extracts of the leaves of *Alchornea cordifolia* exerted significant activity in a dose-dependent manner, with inhibition zones ranging between 11.0mm to 17.0mm at the 200mg/ml concentration. *Proteus mirabilis* was most susceptible to the leaf extract, as evident by its increased zone of inhibition. The potency of *Alchornea cordifolia* against the isolates (MIC between 12.5mg/mL and 50mg/mL) highlights its potential as an alternative therapy.

Keywords: *Alchornea cordifolia*; Antibiotics; Enterobacteriaceae; Poultry; Resistance

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INTRODUCTION

The Enterobacteriaceaeis a broad family of Gram-negative bacteria that comprise both free-living and indigenous flora of the lower gastrointestinal tract of various animals and humans (Denton, 2017). Enterobacterial pathogens include *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, Enterobacter *cloacae*, *Proteus* spp., *Citrobacter* spp., *Serratia marcescens*, and *Salmonella* spp. (Abebe *et al.,* 2020; Moreira de Gouveia *et al.,* 2024). These organisms are regularly found in the intestines of animals including poultry mostly as harmless, while the pathogenic forms are less affected by effective prevention and treatment interventions. This group of bacteria are significant causes of serious infections and many of the most important members of this family are becoming increasingly resistant to currently available antimicrobials (Hariharan *et al.,* 2015).

Globally, antimicrobial resistance (AMR) is increasing in several species of *Enterobacteriaceae*, particularly against beta-lactams, quinolones, fluoroquinolones, aminoglycosides, and

Ceftriaxone (Hariharan *et al.,* 2015). Antimicrobial resistance has long been a concern in livestock, leading to numerous therapeutic failures. It also represents a public health risk because antibiotics resistant bacteria (ARBs) and antibiotics resistant genes (ARGs) can be transferred to humans. Food animals and their surrounding environment are recognized hotspots for ARBs and ARGs due to the common use of antimicrobials for prophylactic purposes as well as their heavy use as growth promoters (Aijaz *et al.,* 2023). The increased burden of poultry morbidity and mortality has accentuated the high demand and the use of antimicrobial drugs (Ndukui *et al.,* 2021). However, the over and misuse of these antimicrobials agents in livestock lead to development of resistance and subsequent transfer of resistance genes among bacteria to animals, animal products and the environment (Thriemer *et al.,* 2018). This threat of AMR strains of bacteria challenges the dependable treatment for many infectious diseases of poultry as well as humans. Contact with livestock, particularly poultry, as well as illicit use of antimicrobial agents in livestock farming practice without veterinary supervision are major risk factor for the emergence and spread of antibiotics resistant food- borne pathogens such as *Escherichia coli, Salmonella,* and *Campylobacter* (Scott *et al.,* 2018). The emergence of AMR from poultry and poultry farms is recognized as a potential community health concern as it can be transmitted through food chains and direct contact with poultry and poultry products.

As alternatives to antibiotics, several studies have emphasized the importance of good management practices, biosecurity, and use of medicinal plants in poultry feeding system to limit the dissemination of these AMR bacteria (Onunkwo *et al.,* 2021). Several countries have relied on plants for the supply major pharmaceuticals and healthcare products (Jamshidi-Kia *et al.*, 2018). This is because plants have been demonstrated to possess a vast reservoir of compounds that have numerous biological activities including antimicrobial properties (Ruban and Gajalaksmi, 2016). One of such plants is *Alchornea cordifolia* which has been routinely utilized as a local remedy for cold (Ebenyi *et al.,* 2017). Also, according to Siwe *et al.* (2016), it is utilized to treat illnesses caused by several bacterial species. The leaves and stem bark are the portions that are most commonly used in medicine, however the leaf has greater potency (Ebenyi *et al.*, 2017).

Due to their potential to spread to humans as foodborne pathogens, antibiotics resistant bacteria in animals poses a growing threat and because Enterobacterial species are among the most significant and abundant animal/human pathogens, their antibiotic susceptibility must be routinely monitored. Several studies including those of Benklaouz *et al*. (2020), Benameur *et al*. (2021), Barour *et al*. (2022) and Belmahdi *et al*. (2022) have reported high rates of AMR in *E. coli* isolates of avian origin in Algeria. However, data on the AMR of Enterobacteriaceae other than *E. coli* are scarce in poultry farms. Therefore, in this study, we aimed to investigate AMR in Enterobacterial isolates from healthy broiler chickens and assess the antibacterial potency of *Alchornea cordifolia* leaf extracts against the isolates.

MATERIALS AND METHODS

Collection of Samples and Inoculation

Cloacal samples were collected from poultry birds at different farms locations in Umuahia metropolis, the Capital of Abia State. The specimens were inoculated within 24 h of collection into Selenite-F broth (Titan Biotech Ltd, India) for enrichment and incubated at 37°C for 18 - 24 h and subsequently plated on *Salmonella-Shigella* agar (Titan Biotech Ltd, India) for primary isolation. The culture plates were incubated aerobically at 37°C for 24 h and observed for growth through the formation of colonies. Colonies that grew in the plates were purified by streak plate method on nutrient agar and subsequently maintained on Nutrient agar slants.

Biochemical characterization of isolates

Standard microbiological techniques were performed for biochemical characterization of the isolates. Biochemical tests were carried out according to standard procedures as described in Cheesbrough (2006), which included Indole, Citrate utilization, Urease, Methyl Red, Voges Proskauer and Triple Sugar Iron tests.

Molecular Characterization

Genomic DNA Extraction and Electrophoresis

Genomic DNA was extracted from the isolates using the Zymo Research Bacterial/Fungal DNA Miniprep kit (Zymo Research Inc., California, USA) according to the manufacturer's instructions. Ten (10) µL of extracted DNA mixed with 2µL of loading dye was electrophoresed on 0.8% agarose gel in 1X TBE buffer containing 0.5µg/mL ethidium bromide along with 1kb ladder (Fermentas) at 150V for 45min. The gel was visualized using a UV transilluminator (Biobase, China) and photographed.

PCR Reaction: amplification of DNA

The PCR reaction was performed on the extracted DNA samples using universal degenerate primers 27F Forward 5'AGRGTTTGATCMTGGCTCAG 3 and 1492R reverse 5'GGTTACCTTGTTACGACTT 3' (De Santis *et al.,* 2007) that amplifies the entire 16s

variable region at annealing temperature of 58°C in a final reaction volume of 25µL.

DNA Sequencing

DNA sequencing was performed by Sanger (dideoxy) sequencing technique to determine the nucleotide sequence of the specific microorganism isolated using automated PCR cycle- Sanger Sequencer™ 3730/3730XL DNA Analyzers from Applied Biosystems (Russell, 2002). The nucleotide sequences obtained were analysed by BLAST analysis on the American database (http://blast.ncbi.nlm.nih.gov). For every set of isolate, a read was BLASTED and the resultant top hits with minimum E-score for every BLAST result showing species name was used to name the specific organism.

Phylogenetic analysis

For construction of a phylogenetic tree, the sequences were aligned with known bacterial 16s RNAs obtained from the GenBank database by using MEGA5.2 software, Neighbour Joining method.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocol (CLSI, 2015) on Mŭeller-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 Mŭeller-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 diameters of the zone of inhibition produced by the antibiotic disks were 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 (CLSI, 2015)

Extraction of plant materials

Leaves of *Alchornea cordifolia* were collected across three locations in Abia state namely Ozuitem, Ahiaeke and Umudike, dried and ground into fine powder. Five hundred grams (500 g) of each of the powdered plant materials was soaked in 1,500 mL of methanol for 24 h at room temperature. The extracts were filtered using non-adsorbent muslin cloth into a clean beaker. The filtrate was dried by evaporating off the solvent at 50 °C in a hot air oven over a period of two days

Screening of the extracts for antibacterial activity

Exactly 0.4 g of each crude extract was reconstituted in 2 mL of dimethyl sulphoxide (DMSO) to obtain extract concentration of 200 mg/mL. This was serially diluted in 2-folds to obtain the following lower extract concentrations: (100, 50, and 25) mg/mL

Screening of extracts and fractions for antibacterial activity

The antibacterial activities of the extracts were assessed by the agar well diffusion assay as previously described by Allotey-Babington *et al.* (2014) with slight modifications. Briefly, a stock solution of 200 mg/mL of the plant extracts was made in dimethyl sulfoxide (DMSO). Further dilutions were made to obtain concentrations of 50 mg/ml and 25 mg/ml. The test organisms were reactivated by streaking out on a freshly prepared Nutrient agar plate. An aliquot of 50 µL of suspension of each isolate standardized to 0.5 MacFarland standard was aseptically inoculated unto Mŭeller-Hinton agar plate using a cotton swab to create a lawn of the organisms. Wells were created on the agar surface using a flame-sterilized cork-borer of 6 mm in diameter. An aliquot of 50µL of each of the plant extracts was loaded into each well.

The minimum inhibitory concentration (MIC) was determined by microbroth dilution technique. Serial dilutions of the plant extracts were made in test tubes containing sterile Mŭeller-Hinton broth, to obtain concentrations of 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/mL). The test tubes were inoculated with 50 µL of suspension of the test bacterium standardized to McFarland standard tube No. 0.5. The inoculated tubes were incubated aerobically at 37°C for 18-24 h. After incubation, the tubes were examined for turbidity. The tube with the lowest concentration of extracts which showed no turbidity was recorded as the MIC value for the tested extract. The minimum bactericidal concentration (MBC) was determined by streaking the contents of the tubes with no visible turbidity, separately, on freshly prepared Nutrient agar plates. The MBC is the concentration in the tube from which no growth was observed after 18-24 h of incubation (Ekundayo *et al.,* 2020).

RESULTS

A total of 15 isolates belonging to the Enterobacteriaceae family were isolated from cloacal samples of poultry birds, predominated by *Proteus mirabilis* (40.0%) followed by *Salmonella* spp (26.7%), *K. pneumoniae* (20.0%) and

Enterobacter aerogenes (13.3%) as shown in Figure 1). DNA sequencing of the 16s rRNA gene of the isolates confirmed that the presumptive isolates were *Proteus mirabilis, Klebsiella pneumoniae* each with 98% similarity (Figure 3). The nucleotide sequences of bacterial 16s rRNA gene fragments retrieved from bacterial isolates in this study were deposited in the GenBank nucleotide sequence database under accession numbers PQ555018 and PQ555019 respectively.

The results of our study revealed a moderate level of resistance to cephalosporins as 40.0% (6/15) of the isolates showed resistance to ceftriaxone and 46.7% (7/15) of the isolates were resistant to cefuroxime. Amongst the fluoroquinolones, ofloxacin and levofloxacin exhibited significant activity against the isolates with 73.3% and 80% being susceptible respectively (Figure 2). The most predominant resistance pattern (resistance to ceftriaxone, tetracycline, and azithromycin) occurred in 93.3% (14/15) of isolates, suggesting an almost complete resistance to these vital drugs. *Proteus mirabilis* exhibited the broadest resistance (e.g., 5/6 strains resistant to CRO-TET-CPR-AZN-GEN), while *Salmonella* spp. and *Klebsiella pneumoniae* showed high resistance to ≥5 antibiotics. Despite high MDR pattern (Table 1) amongst the isolates, the *A. cordifolia* extract showed bactericidal effects against all isolates (Table 2), highlighting its potential as an alternative therapy. The methanol extract *A. cordifolia* demonstrated activity in a concentration-dependent manner.

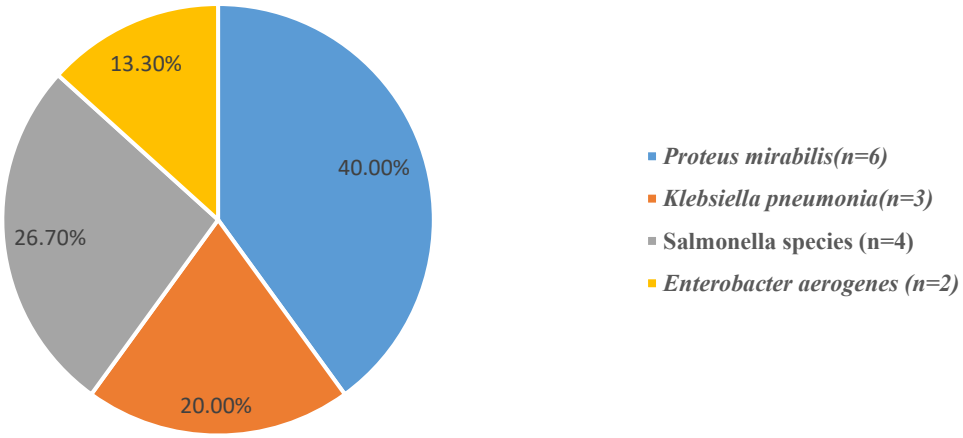

Figure 1: Frequency of Occurrence of the Enterobacteriaceae Isolates in Poultry

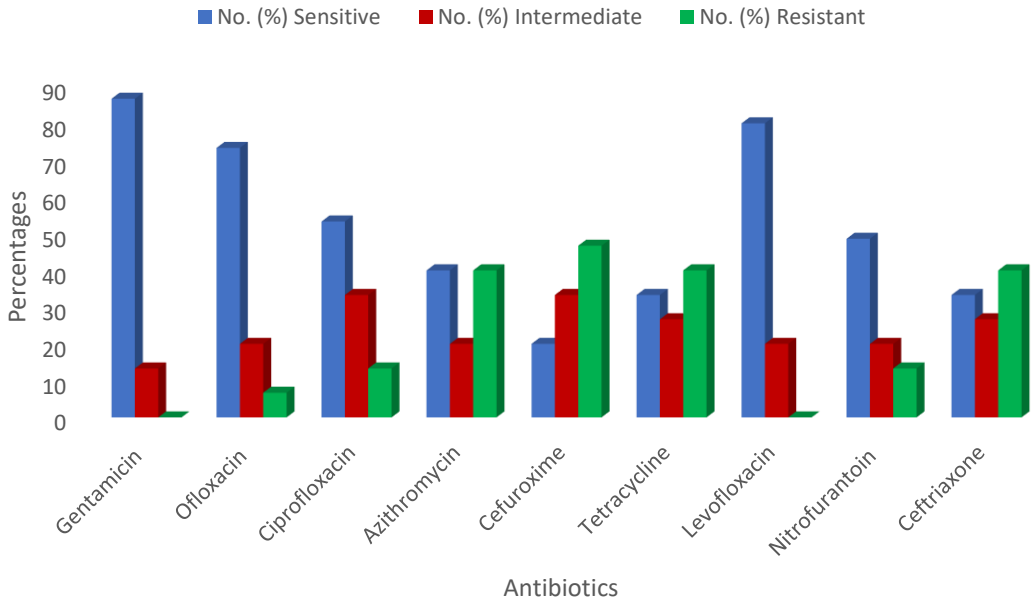

Figure 2: Antimicrobial Susceptibility Pattern of the enterobacterial isolates

Table 1: Multidrug Resistant Patterns of the Isolates (n=15)

Resistance pattern	MDR bacterial strains No (%)				
	Enterobacter aerogenes(n=2)	*Klebsiella pneumonia* (n=3)	*Salmonella* sp. (n=4)	*Proteus mirabilis*(n=6)	Total MDR
CRO-TET-CPR-GEN-NIT-AZN	2	2	1	3	8(53.3)
CRO-TET-CPR-GEN-NIT-OFL	2	2	2	3	9(60.0)
CRO-TET-CPR-AZN-GEN	1	2	2	5	10(66.7)
CRO-TET-OFL-GEN-NIT	0	1	1	4	6(40.0)
CRO-TET-LBC-CPR-GEN	0	2	2	5	9(60.0)
CRO-TET-CPR-AZN	1	3	4	4	12(80)
CRO-TET-OFL-GEN	0	0	3	3	6(40.0)
CRO-TET-AZN	2	3	4	5	14(93.3)
CRO-TET-GEN	0	2	3	4	9(60.0)

Table 2: Mean Diameter Zones of Inhibition (mm) produced by Methanol Extracts of *Alchornea cordifolia* against the isolates

Test Organisms	Extract concentration (mg/ml)			Control Gentamicin
	200	100	50	
Proteus mirabilis	15.0 ± 0.70	11.0 ± 0.00	10.0 ± 0.00	27.0 ± 1.41
Enterobacter aerogenes	11.0 ± 1.41	0.0 ± 0.00	0.0 ± 0.00	21.5 ± 0.70
Salmonella species	12.0 ± 0.70	10.0 ± 0.70	8.0 ± 0.00	24.0 ± 0.00
Proteus mirabilis	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	19.5 ± 0.70
Enterobacter aerogenes	15.0 ± 0.70	11.0 ± 0.00	10.0 ± 0.00	27.0 ± 1.41
Proteus mirabilis	11.0 ± 1.41	0.0 ± 0.00	0.0 ± 0.00	21.5 ± 0.70
Proteus mirabilis	12.0 ± 0.70	10.0 ± 0.70	8.0 ± 0.00	24.0 ± 0.00
Salmonella species	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	19.5 ± 0.70
Salmonella species	15.0 ± 0.70	11.0 ± 0.00	10.0 ± 0.00	27.0 ± 1.41
Klebsiella pneumonia	11.0 ± 1.41	0.0 ± 0.00	0.0 ± 0.00	21.5 ± 0.70
Klebsiella pneumoniae	12.0 ± 0.70	10.0 ± 0.70	8.0 ± 0.00	24.0 ± 0.00
Klebsiella pneumoniae	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	19.5 ± 0.70
Salmonella species	11.0 ± 1.41	0.0 ± 0.00	0.0 ± 0.00	21.5 ± 0.70
Proteus mirabilis	12.0 ± 0.70	10.0 ± 0.70	8.0 ± 0.00	24.0 ± 0.00
Proteus mirabilis	17.0 ± 0.70	12.0 ± 0.70	0.0 ± 0.00	24.0 ± 0.00

Table 3: MIC and MBC values (mg/ml) of extract of Methanol Extracts of *Alchornea cordifolia* against the Isolates

Organisms	Ozuitem		Ahiaeke		Umudike	
	MIC	MBC	MIC	MBC	MIC	MBC
Proteus mirabilis	50	100	50	100	50	100
Enterobacter aerogenes	12.5	25	12.5	25	12.5	25
***Salmonella* species**	50	100	50	100	50	100
Proteus mirabilis	25	50	25	50	25	50
Enterobacter aerogenes	25	50	25	50	25	50
Proteus mirabilis	25	50	25	50	25	50
Proteus mirabilis	12.5	25	12.5	25	12.5	25
***Salmonella* species**	50	100	50	100	50	100
***Salmonella* species**	25	50	25	50	25	50
Klebsiella pneumonia	25	50	25	50	25	50
Klebsiella pneumoniae	25	50	25	50	25	50
Klebsiella pneumoniae	12.5	25	12.5	25	12.5	25
***Salmonella* species**	25	50	25	50	25	50
Proteus mirabilis	50	100	50	100	50	100
Proteus mirabilis	12.5	25	12.5	25	12.5	25

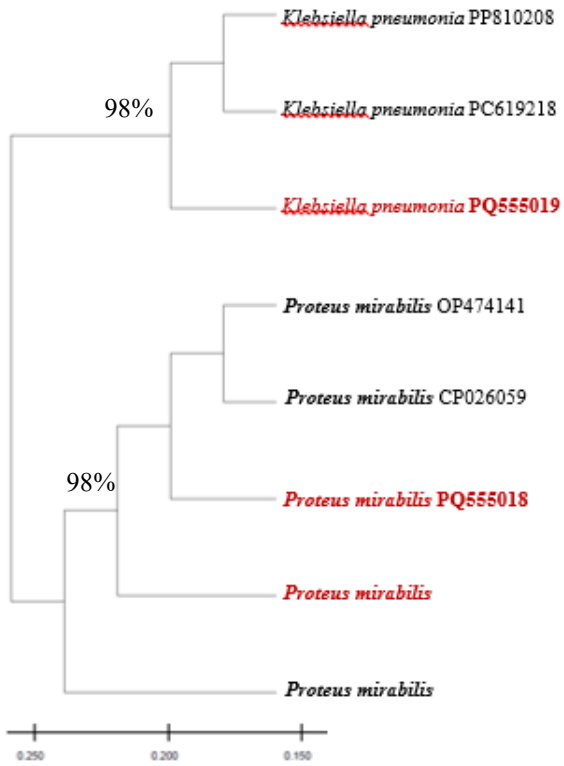

Figure 3: Phylogenetic Tree of the isolated Enterobacteriaceae against other related species

DISCUSSION

In this study, the most predominant isolate was *Proteus mirabilis* (40.0%) followed by *Salmonella* spp (26.7%), *K. pneumoniae* (20.0%) and *Enterobacter aerogenes* (13.3%). Previous studies by Ngai *et al.* (2021) and Langata *et al.* (2019) have also reported isolating *P. mirabilis* and *Salmonella* spp. from chicken droppings which supports the findings of this present study. In the study of Ojja *et al.* (2024), the overall prevalence of the chicken colonization by *Enterobacteriaceae* was 95.0% (190/200) with isolation of three members of this family; *E. coli* in 60.5% (121/200), *Enterobacter* spp in 27.9% (46/200) and *Klebsiella pneumoniae* in 11.5% (23/200) from commercial laying chickens in the 10 LGAs studied. Again in the study of Kamel *et al.* (2024), *E. coli* was the most isolated bacterium (n = 194, 80.50%), followed by *Proteus mirabilis* (n=21, 8.71%), *Escherichia fergusonii* (n = 8, 3.32%), *Salmonella* spp. (n = 7, 2.90%), *Enterobacter*

cloacae (n = 4, 1.66%), *K. pneumoniae* (n = 3, 1.25%). The occurrence of some of these species has been previously reported in avian farms in Algerian by Benklaouz (2023), where 315 *Enterobacteriaceae* strains were isolated with a predominance of *E. coli* (55.23%) followed by *Proteus* spp. (16.8%) isolates; in addition, a few isolates were identified as *Salmonella* spp. (1.90%). In another study conducted by Ojja *et al.* (2024) in Nigeria involving 287 *Enterobacteriaceae* isolates collected from laying chicken, *E. coli* remained the most frequent (59.6%), but significant rates were also detected for *Enterobacter* spp. (27.9%) and *K. pneumoniae* (12.5%). A similar set of isolates were also reported in the study of Benklaouz *et al.* (2020). These reports are in agreement with the isolates obtained from this study.

Regrettably, these poultry farms could contribute to environmental contamination with antibiotic-resistant bacteria, which are transmitted to chickens, livestock, and humans through direct contact or through contaminated food products (Moawad *et al.,* 2018**).** Similar studies carried out in Nigeria in 2011 and 2019 showed comparatively similar results (Johnson *et al.,* 2019**).** However, most of the previous studies demonstrated that the frequency of *E. coli* from chicken faeces could vary greatly with the time of sample collection, chicken age, and the diet (Nhung *et al.,* 2017).

The results of our study revealed a relatively moderate level of resistance to third-generation cephalosporins (40% for ceftriaxone and 46.7% for cefuroxime), which can be attributed to the rare use of these molecules in poultry farms. This finding are in agreement with those reported by Kamboh *et al*. (2018), who reported rates of (41.13%) for ceftriaxone and (33.33%) for ceftazidime. However, Faife *et al*. (2020) observed higher resistance rates for ceftriaxone (74%) and ceftazidime (67%). Amongst the fluoroquinolones, ofloxacin and levofloxacin exhibited significant activity against the isolates with 73.3% and 80% being susceptible respectively. The findings from this study showed that 8/15 (53.3%) of the isolates were susceptible to ciprofloxacin, 5/15 (13.3%) were of intermediate susceptibility and only two (13.3%) were susceptible to the antibiotic. The study of Benameur *et al*. (2018) in western Algeria reported a very high level of resistance to ciprofloxacin. However, these values are higher than those found in the previous studies, such as in China (He *et al.,* 2020), Mozambique (Faife *et al.,* 2020), and Ethiopia (Mitkie *et al.,* 2023). An elevated level of resistance was also noted for tetracycline (40%). This finding is however, lower than that reported in Pakistan (Kamboh *et al.,* 2018), where the resistance rate was 84.42%. Globally, tetracyclines have become the most frequently used antibiotics in livestock and poultry farming owing to their numerous advantages, including low cost, high efficiency, and wide range of action. This study also revealed that isolates from birds showed high resistance toward Azithromycin (40.0%). Significant proportion of resistance against similar antibiotics as used in this study has also been reported from Nigeria (Omoya and Ajayi, 2016), Ethiopia (Ali *et al.,* 2020), Vietnam (Nguyen *et al.,* 2015), and China (Yasin *et al.,* 2017).

The analysis of the AMR patterns of all isolates showed that all isolates were MDR and exhibited resistance to at least three or more antimicrobial agents. This finding is similar to those reported in previous studies by Benklaouz (2023) and Benameur *et al*. (2018). This increase in multidrug resistance rates can be explained by the widespread use of antimicrobials for therapeutic purposes or growth promotion in poultry farms (Barour *et al.,* 2022). The most predominant resistance pattern (resistance to ceftriaxone, tetracycline, and azithromycin) occurred in 93.3% (14/15) of isolates, suggesting an almost complete resistance to these vital drugs. *Proteus mirabilis* exhibited the broadest resistance (e.g., 5/6 strains resistant to CRO-TET-CPR-AZN-GEN), while *Salmonella* spp. and *Klebsiella pneumoniae* showed high resistance to ≥5 antibiotics. This points to widespread antibiotic abuse in poultry farming, which could lead to the zoonotic spread of untreatable infections to humans (Founou *et al.,* 2021; Robinson *et al.,* 2022). The high resistance to ceftriaxone (CRO), tetracycline (TET), and azithromycin (AZN) (93.3%) aligns with global trends of escalating antimicrobial resistance (AMR) in poultry. *Proteus mirabilis* and *Salmonella* spp. exhibited particularly extensive resistance, consistent with reports identifying these organisms as major MDR pathogens in poultry farming (Ogunleye *et al.,* 2023).

Despite high MDR prevalence amongst the isolates, the *A. cordifolia* extract showed bactericidal effects against all isolates, highlighting its potential as an alternative therapy. The methanol extract *A. cordifolia* demonstrated concentration-dependent bactericidal effects, supporting traditional use of *A. cordifolia* for infections. Its potency against *Enterobacter aerogenes* (MIC = 12.5 mg/mL) is in agreement with recent findings on the efficacy of this plant against Gram-negative bacteria (Agyare *et al.,* 2023). However, the reduced activity against *P. mirabilis* and *Salmonella* spp. (MIC = 50 mg/mL) suggest strain-specific variability, possibly due to differences in resistance mechanisms (Kuete,

2022). The inhibition zones at 200 mg/mL correlate with MIC data and aligns with studies reporting flavonoids and terpenoids in *A. cordifolia* as key bioactive disruptors of bacterial cell membranes (Mbosso Teinkela *et al.,* 2021). The findings of this study suggests that secondary metabolites in *A. cordifolia* may possess novel mechanisms of action against MDR (Adedapo *et al.,* 2022). Recent work confirms that phytochemicals like alchorneine (an alkaloid in *A. cordifolia*) disrupt proton motive forces in bacteria, sensitizing them to antibiotics (Ezekwesili *et al.,* 2024). These findings are similar with those of Mbouna *et al.* (2023) as well as Ekhuemelo *et al.* (2024), who tested methanol/water extracts against ESBL-producing *E. coli, Klebsiella, Proteus* and *Salmonella* from poultry/livestock. The antimicrobial resistance rates obtained in our study are in agreement with those described by these authors, and also provide evidence supporting the fact that antimicrobial resistance of enterobacterial isolates are increasing progressively.

CONCLUSION

This study highlighted high AMR rates and MDR phenotypes in Enterobacteriaceae isolated from healthy poultry birds in Abia state Nigeria. The spread of such strains to terrestrial and aquatic environments, as well as to other food-producing animals and humans through various pathways is a cause of concern to public health. *Alchornea cordifolia* methanol extract exhibited promising bactericidal activity against poultry MDR pathogens. This study highlights the plant's potential as a complementary antimicrobial agent in the face of rising antibiotic resistance.

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