

Plasmid Profiling and Antibiotics Resistance in Enteric Bacteria Associated with Poultry in Rivers State

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ABSTRACT

A growing public health concern is the frequency of antibiotic resistant enteric bacteria in poultry. This study examined the patterns of antibiotic resistance and plasmid presence in enteric bacteria isolated from poultry samples obtained in Rivers State, Nigeria. Enteric bacteria were isolated, identified, and subjected to antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method. The plasmid profile of each strain was determined through gel electrophoresis. Findings showed high prevalence of antibiotic-resistant enteric bacteria in poultry meat, with resistance observed against multiple classes of antibiotics commonly used in poultry production. Plasmids were identified in a substantial proportion of these antibiotic-resistant strains, suggesting the potential role of plasmids in mediating antibiotic resistance. Also, 60% of the isolates possessed TEM genes while 50% possessed SHV genes. Isolates *Aeromonas caviae*, *Acinetobacter baumannii* and *Enterobacter hormachei* possessed both genes. This study highlighted the critical requirement for increased monitoring of antibiotic-resistant microorganisms in chicken production systems in Rivers State. These findings have significance for the preservation of public health, antimicrobial stewardship, and food safety.

Keywords: Poultry, enteric bacteria, *Enterobacter hormachei*, plasmid, extended Spectrum β -lactam genes, food safety.

Introduction

Poultry Farming is pretty straight forward; it is the rearing of domestic birds (primarily chickens, turkeys, ducks, and geese), etc., for meat, eggs, or both, depending on the type of poultry farming you are interested in (Rekwot *et al.*, 2015). In Nigeria, most poultry farmers focus on rearing Chickens and Turkeys, with chicken being the most preferred. About 85 million of Nigeria's populations, 4 in every 10 Nigerian, are into poultry production, primarily small scale to medium scale poultry farming. There is hardly any part of the country where you wouldn't see these two-legged creatures roaming around the neighborhood or being reared (Anosike *et al.*, 2018).

The food chain especially those associated with poultry could pose a great public health concern if they function as means for the development and spread of bacteria in the community which are resistant to antimicrobial agents.

Marcus *et al.* (2007) in a study on the reassessment of risk factors for sporadic *Salmonella* serotype *enteritidis* infections, reported that poultry is the most vital means for the transmission of *Salmonella* to humans and that infections from other bacterial pathogens arise from the ingestion of poultry meat contaminated by these bacteria

The extensive use of antimicrobials in the production of feed for animals has led to increased occurrence of antibiotic resistance in developing countries such as Nigeria (Gaser *et al.*, 2012). This is as a result of the use of antimicrobials such as those of the β -lactam class for treatment of illnesses caused by bacteria, prophylaxis as well as for promotion of growth (Waters *et al.*, 2011). Resistance to extended Spectrum β -lactam drugs (ESBLs) such as ceftriaxone, cefotaxime and Ceftazidime which are 3rd generation cephalosporins by enterobacteria especially, has been reported as a result of the promiscuous use of these antibiotics (Gaser *et al.*, 2011).

The secretion of Extended-Spectrum Beta-Lactamases (ESBLs) as an antibiotic resistance mechanism to third generation cephalosporins, is frequent among bacteria of the enterobacteriaceae family such as *Escherichia coli* and *Klebsiella pneumonia* (Pitout and Laupland, 2008). However, in recent time, resistance to these drugs has been reported owing to the secretion of extended spectrum beta-lactamases in enterobacteria (Garcia-Graells et al., 2012; Geser et al., 2011). Microorganisms which are ESBL resistant are often resistant to antibiotics of phenicols, aminoglycosides, potentiated sulfonamides and fluoroquinolone class (Geser et al., 2012). Subsequently, ESBL resistant bacteria have also been termed multi-drug resistant microbes. Infections caused by these ESBL-resistant bacteria thus have limited therapeutic options as the

bacteria exhibit multidrug resistance (Blaak et al., 2014). This study therefore seeks to provide information on the presence of extended Spectrum β -lactam drugs (ESBL) genes in enteric bacteria isolated from poultry.

Materials and Methods

Study Area

The study was carried out in three (3) Poultry farms located in Elioparanwo, Rivers State University campus, and Aluu. These poultry farms are located in Obio-Akpor and Port Harcourt City Local Government Areas of Rivers State. The map of the study area is presented in Fig. 1.

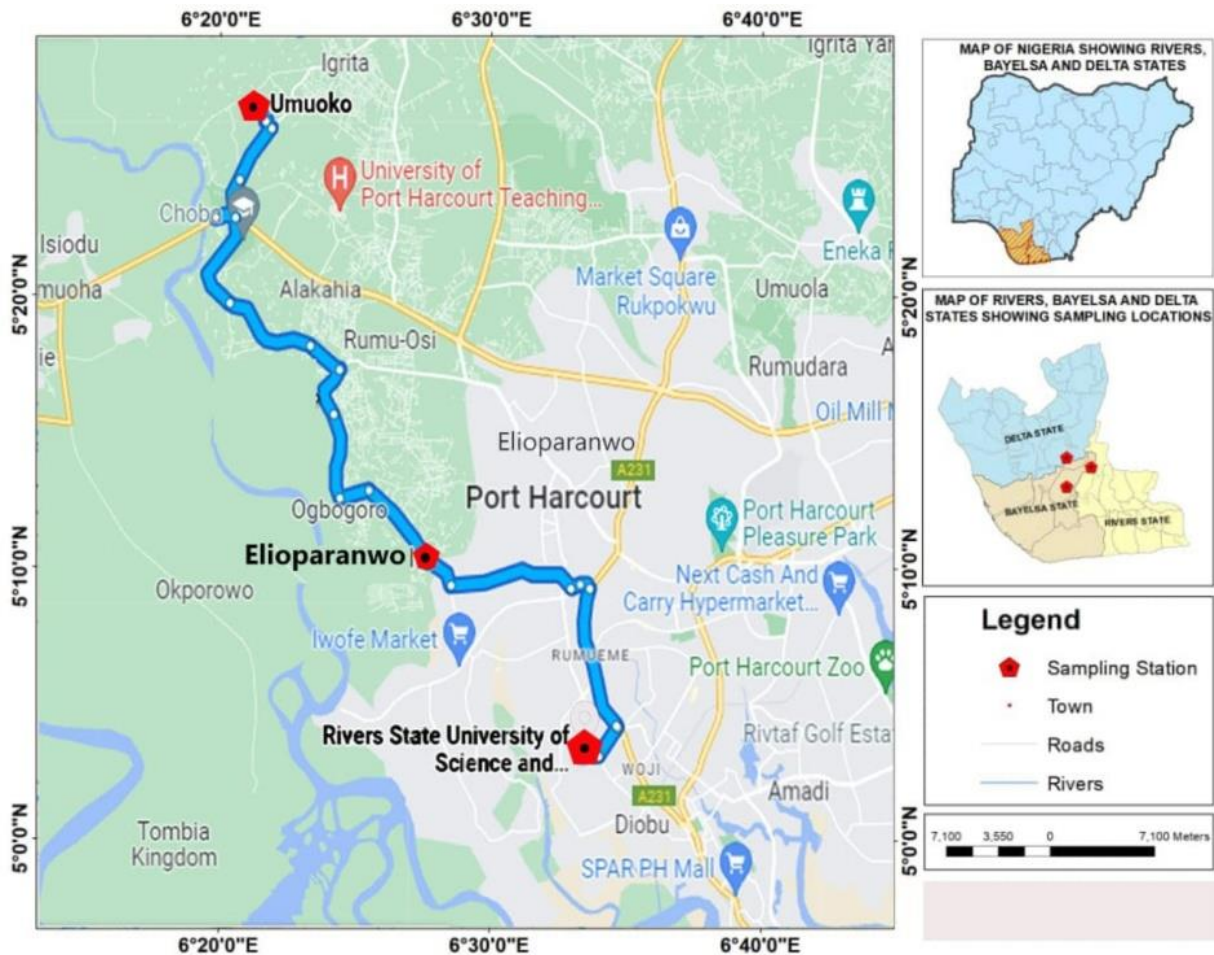


Fig. 1: Map of the Study area where the farms are situated

Microbiological Analysis

Isolation and Identification of Bacteria

Swab samples from Drinkers trays and Door handles were streaked directly on respective agar plates while other samples of soil, water, poultry dropping, muscle, wings, and intestine were analysed using the spread plate method after conducting a ten-fold serial dilution to obtain dilutions of 1:10⁶. Aliquots (0.1mL) of an appropriate dilution were inoculated on freshly prepared nutrient agar, McConkey agar, and Eosin methylene blue agar and incubated at 37°C for 24 hours for the isolation of total heterotrophic bacteria, total coliform and faecal coliform bacteria, respectively. Isolates were identified based on their colonial/morphological characteristics (Cheesebrough, 2006).

Preservation of Isolates

Discrete bacterial colonies that grew on respective media were subcultured using streak plate method onto freshly prepared nutrient agar and incubated at 37°C for 24 hours in order to obtain pure culture. The pure bacteria cultures were then maintained according to the method adopted by Amadi *et al* 2014 using 10% (v/v) glycerol suspension at - 4°C

Antimicrobial Susceptibility Testing

The susceptibility pattern of the isolates to antibiotics such as Cefuroxime (30µg), Cefotaxime (30µg), Cefixime (5µg), Ofloxacin (5µg), Gentamicin (30µg), Nalidixic acid (30µg), Amoxicillin/Clavulanic acid (25/10µg), Levofloxacin (5µg), Imipenem (10µg), Nitrofurantoin (30µg), Ampicillin/Cloxacillin (30µg) was carried out using the disc diffusion method (CLSI, 2019). Muller-Hinton agar (Difco Laboratory, Michigan, USA) was used for the susceptibility test. The organisms were standardized using the 0.5 McFarland standard. This was done by transferring the test isolates into test tubes containing 4ml normal saline which has been sterilized by autoclaving at 121°C for 15 minutes. The turbidity of the test isolate was compared with the turbidity of the 0.5 McFarland standard. Swab sticks were dipped into tubes containing the standardized isolates and were inoculated horizontally and vertically on the prepared Mueller-Hinton agar plates by spreading gently. This was allowed for 5 minutes to dry before discs containing the antibiotics were placed on the surface

of the seeded plates and incubated at 37°C for 24 hours. After incubation, zone diameter was read and interpreted as susceptible, resistant or intermediate (CLSI, 2019).

Amplification of Resistant genes

SHV and TEM gene are members of extended spectrum beta-lactamases which are often mediated by plasmid (Behrooz *et al.*, 2010; Ramazanzadeh *et al.*, 2010). They are often identified in Enterobacteriaceae family. Polymerase chain reaction method of detection was performed as described by Fatemeh *et al.* (2012). SHV genes from the isolates were amplified using the SHV F: 5' CGCCTGTGTATTATCTCCCT-3' and SHV R: 5'-CGAGTAGTCCACCAGATCCT-3' primers while TEM genes from the isolates were amplified using the TEMF: 5'-ATGAGTATTCAACATTTCCG TG-3' and TEMR: 5'-TTACCAATGCTTAATCAGTGA G-3' primers. All on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 56°C for 40 seconds; extension, 72°C for 50 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 300bp product size.

Results

The results of the percentage susceptibility of isolates of *Acinetobacter* isolates from the various farms is presented in Fig. 1. Results showed that all forty (40) isolates of *Acinetobacter* sp isolated from the Aluu farm, were susceptible to Ofloxacin, Nalidixic acid, and Nitrofurantoin. In Elioparanwo farm, the susceptibility of the isolate was very low and the highest susceptibility of 76% was recorded for ofloxacin showing 76% sensitivity to the isolates while 50% sensitivity was recorded for Nalidixic acid. Also, in RSU farm, the isolates were 100% susceptible to ofloxacin 48% was susceptible to nalidixic acid. The isolates were highly resistant to gentamycin and other beta-lactam antibiotics.

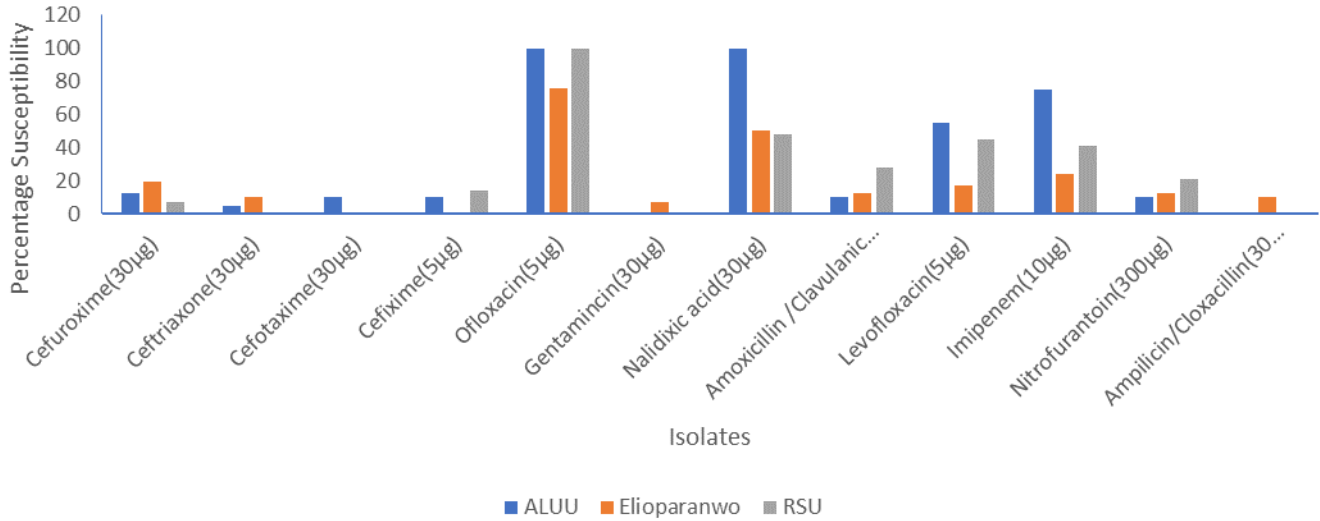


Fig. 1: Percentage susceptibility of *Acinetobacter* sp in the various farms

Results of the percentage susceptibility of isolates of *Aeromonas* in the various farms are presented in Fig. 2. Results showed that the percentage susceptibility of 41 isolates of *Aeromonas* from Aluu farm to Cefuroxime, Ceftriaxone, Cefotaxime, Cefixime, Ofloxacin, Gentamycin, Nalidixic acid, Amoxicillin /Clavulanic Acid, Levofloxacin, Imipenem, Nitrofurantoin and Ampicillin/Cloxacillin were 41.4, 34, 46, 49, 100, 44, 100, 44, 0, 39, 46 and 34%, respectively while for isolates in Elioparanwo, the susceptibility to same antibiotics were 42, 43, 49, 100,

100, 100, 51, 38, 100, 45, 43.4 and 42%. The response of the isolates from RSU farm was recorded as 0, 34, 100, 100, 48, 100, 100, 41, 48, 38, 41 and 38%, respectively for same antibiotics. Results showed that ofloxacin and gentamycin was the most effective antibiotics in Aluu farm as the isolates displayed 100% susceptibility. In Elioparanwo farm, Cefixime, Ofloxacin, Gentamycin and Levofloxacin were the most effective antibiotics while in RSU, Cefotaxime, Cefixime, Gentamycin and Nalidixic acid were the most effective antibiotic.

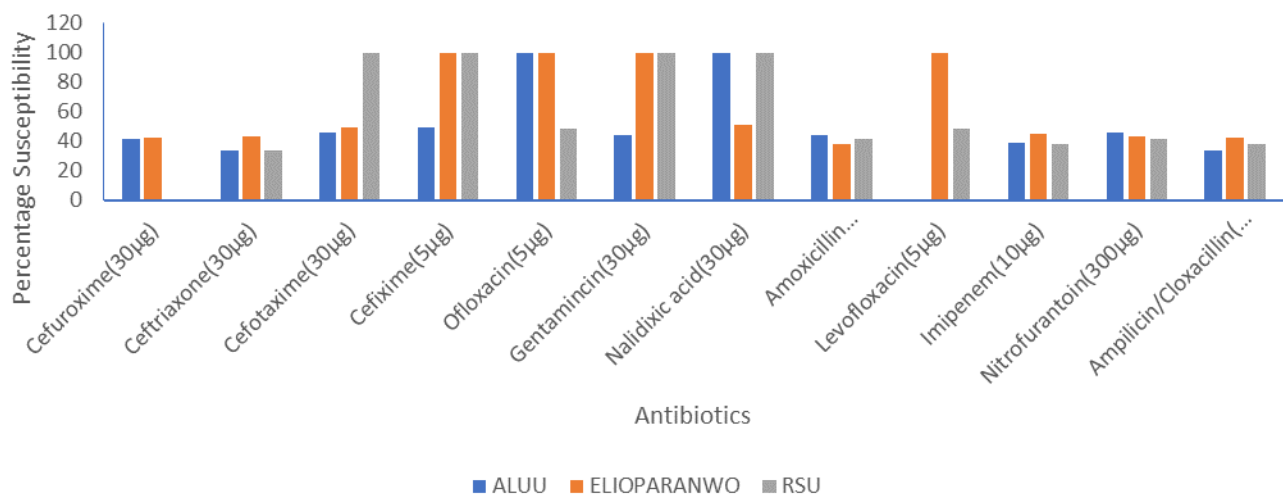


Fig. 2: Percentage susceptibility of *Aeromonas* isolates in the various farms

The results of the percentage susceptibility of *Citrobacter* isolates in the various farms are presented in Fig. 3. Results showed that the susceptibility of *Citrobacter* sp in Aluu farm to cefotaxime, ofloxacin, Amoxicillin /Clavulanic acid, imipenem and nitrofurantoin were 13.3, 66.7, 53.3, 66.7 and 66.7% while susceptibility pattern of the isolates in Elioparanwo farm is given as 25, 28.6, 10.7, 21.4, 14.3, 100 and 75 for cefotaxime, Ofloxacin, Gentamycin, Nalidixic acid, Amoxicillin /Clavulanic acid, imipenem and nitrofurantoin. The susceptibility recorded for this isolate to Cefuroxime, Ceftriaxone, Cefotaxime, ofloxacin, Amoxicillin /Clavulanic acid, imipenem, nitrofurantoin and Ampicillin/ Cloxacillin antibiotics were 23.5, 11.7, 23.5, 100, 58.8, 17.6, 47, 100 and 11.8%. Ofloxacin, imipenem and nitrofurantoin had similar antibiotic effect on the isolates in Aluu farm and was the most effective in the farm while imipenem followed by Nitrofurantoin were the best antibiotics against the isolates in Elioparanwo farm. Ofloxacin and nitrofurantoin were the best antibiotics in the RSU farm against the isolate.

The results of the percentage susceptibility of *Citrobacter* isolates in the various farms are presented in Fig. 4. Results showed that the percentage susceptibility of the twenty-two isolates of *C.* were 18.2, 0, 27.3, 0, 59.1, 0, 0, 45.5, 18.1, 100, 45.5 and 0% for Cefuroxime, Ceftriaxone, Cefotaxime, Cefixime, Ofloxacin, Gentamycin, Nalidixic acid, Amoxicillin /Clavulanic acid, Levofloxacin, Imipenem, Nitrofurantoin and Ampicillin/Cloxacillin, respectively. The percentage susceptibility of the isolate from Elioparanwo and RSU farm were 0, 30.3, 0, 18.2, 100, 15.1, 6.1, 100, 0, 30.3, 100 and 15.2%; and 0, 0, 21.7, 8.7, 100, 8.7, 0, 100, 0, 100, 100 and 13% for Cefuroxime, Ceftriaxone, Cefotaxime, Cefixime, Ofloxacin, Gentamycin, Nalidixic acid, Amoxicillin /Clavulanic acid, Levofloxacin, Imipenem, Nitrofurantoin and Ampicillin/Cloxacillin, respectively. Results further showed that Imipenem was the only effective antibiotics in the Aluu farm as it was able to completely inhibit the growth of the isolates while in Elioparanwo and RSU farms, ofloxacin, Nitrofurantoin and Ampicillin/Cloxacillin were the most effective antibiotics.

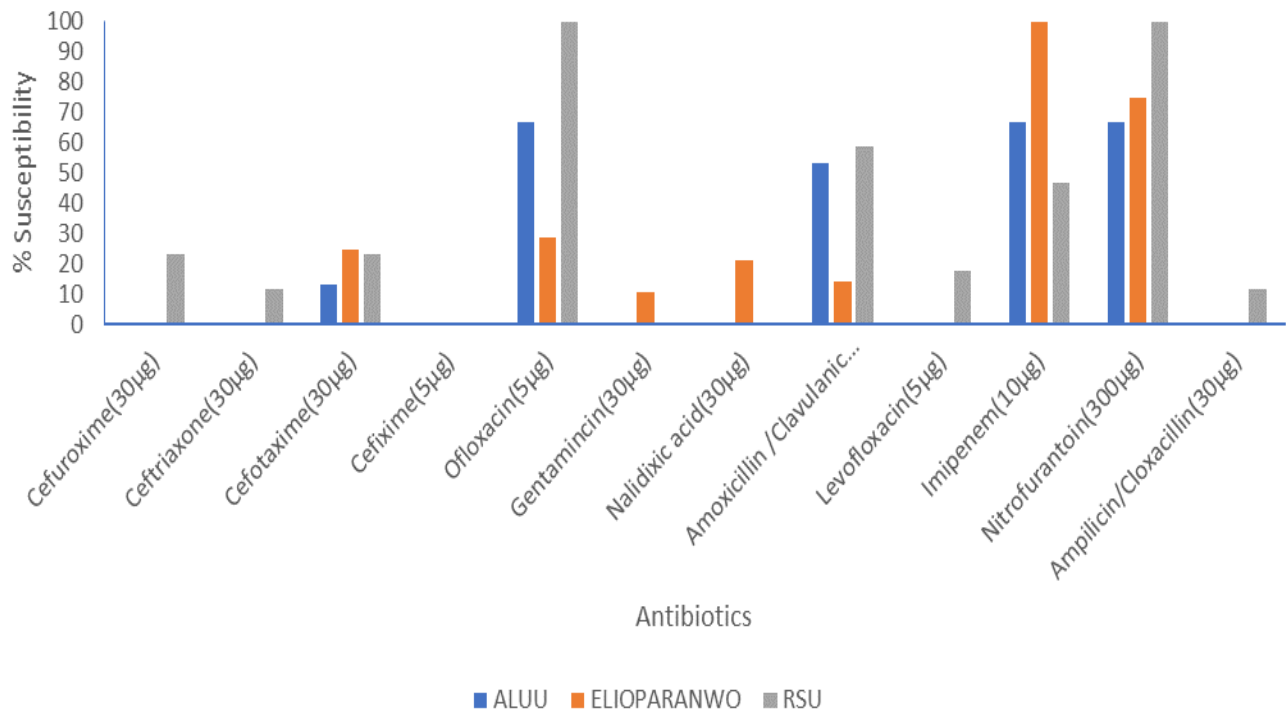


Fig. 3: Percentage susceptibility of *Citrobacter* isolates in the various farms

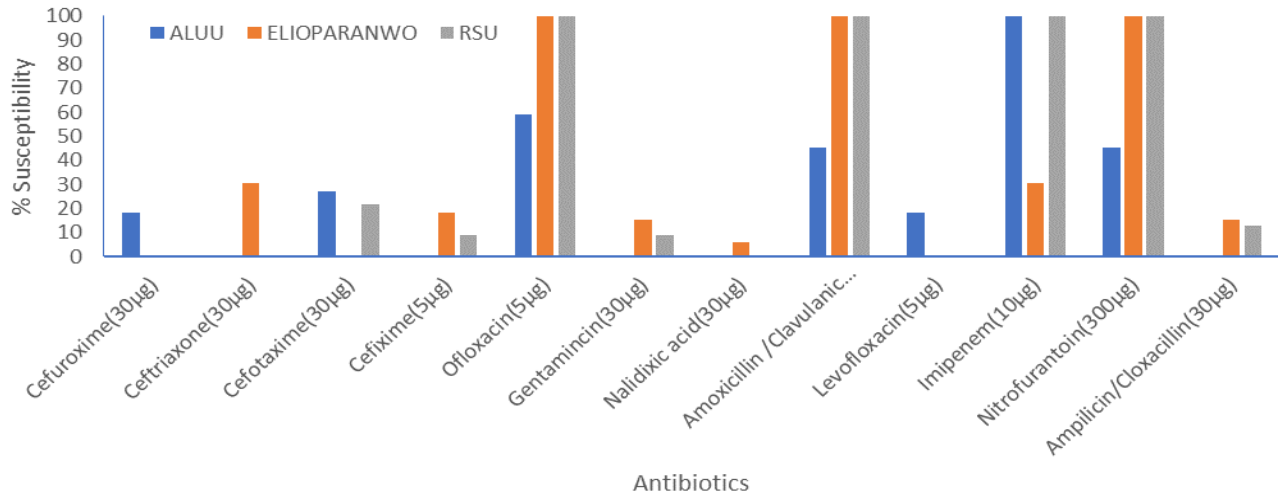


Fig. 4: Percentage Susceptibility of Citrobacter isolates in the Various Farms

Results of the percentage susceptibility of *Enterobacter* isolates in the various farms are presented in Fig. 5. Results showed that the susceptibility pattern of the isolates from Aluu farm were 40.9, 36.4, 45.5, 40.9, 45.5, 100, 45.5, 36.4, 100, 45.5, 43.2 and 36.4% for Cefuroxime, Ceftriaxone, Cefotaxime, Cefixime, Ofloxacin, Gentamycin, Nalidixic acid, Amoxicillin /Clavulanic Acid, Levofloxacin, Imipenem, Nitrofurantoin and Ampicillin/Cloxacillin, respectively. While the percentage susceptibility of these isolates in Elioparanwo farm to similar antibiotics agents 45.1, 46.5, 46.5, 43.7, 47.9, 49.3, 46.5, 43.7, 49.3, 49.3, 49.3 and 45.1%. In RSU farm, the percentage

susceptibility of these isolates to similar antibiotics agents was 42.9, 40.5, 100, 40.5, 47.6, 100, 100, 42.9, 45.2, 100, 40.5 and 35.7%. Results further showed that disparity in degree of activity of the antibiotics against the bacterial agents for instance same antibiotics having a different activity against a particular isolate in different regions/ farms. Gentamycin, Nalidixic acid, and Imipenem were the most effective antibiotics against *Enterobacter* isolates in the RSU farm while Gentamycin and Levofloxacin were the most effective in Aluu farm. All the antibiotics demonstrated some level of effectiveness but were not completely effective against the isolates of *Enterobacter* sp in Elioparanwo farm.

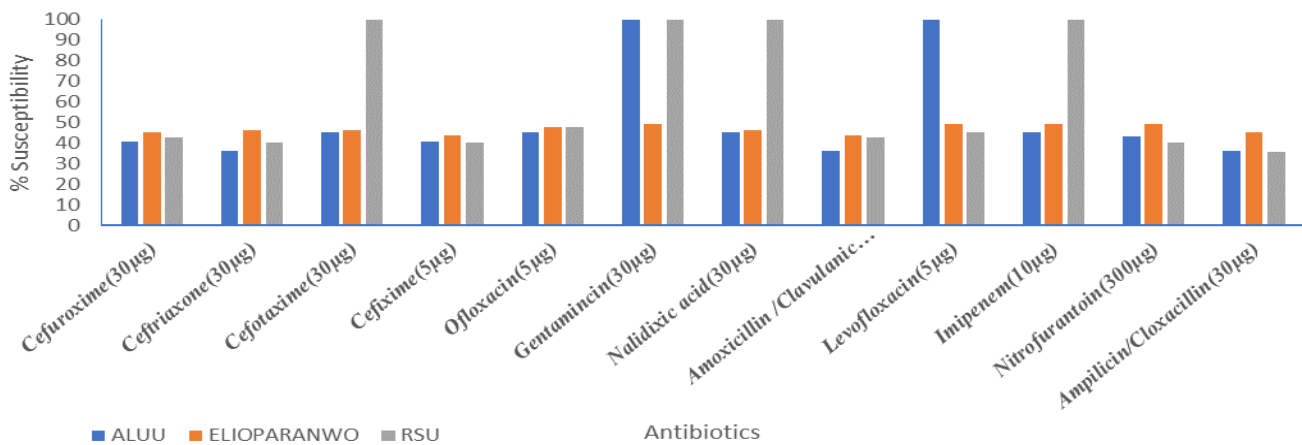


Fig. 5: Percentage Susceptibility of Enterobacter isolates in the Various Farms

Results of the percentage susceptibility of *Myroides* isolates in the various farms are presented in Fig. 6.

Results showed that the susceptibility pattern of the isolates from Aluu farm to Cefuroxime, Ceftriaxone,

Cefotaxime, Cefixime, Ofloxacin, Gentamycin, Nalidixic acid, Amoxicillin /Clavulanic Acid, Levofloxacin, Imipenem, Nitrofurantoin and Ampicillin/Cloxacillin were 9.5, 0, 28.6, 23.8, 100, 28.6, 42.9, 23.8, 100, 90.5, 42.9 and 9.5%, respectively.

While in Elioparanwo farm, the percentage susceptibility was 15, 30, 0, 0, 50, 20, 35, 10, 50, 45,

100 and 15, respectively. The response of the isolates from RSU farm to the antibiotics was 20.7, 41.4, 13.8, 37.9, 44.8, 37.9, 100, 6.9, 48.3, 41.4, 100 and 20.7%. Ofloxacin was the most effective antibiotics against the isolates of *Myroides* in Aluu farm while Nitrofurantoin was one of the most effective in Elioparanwo and RSU. Nalidixic acid was also very effective against the isolates in RSU farm.

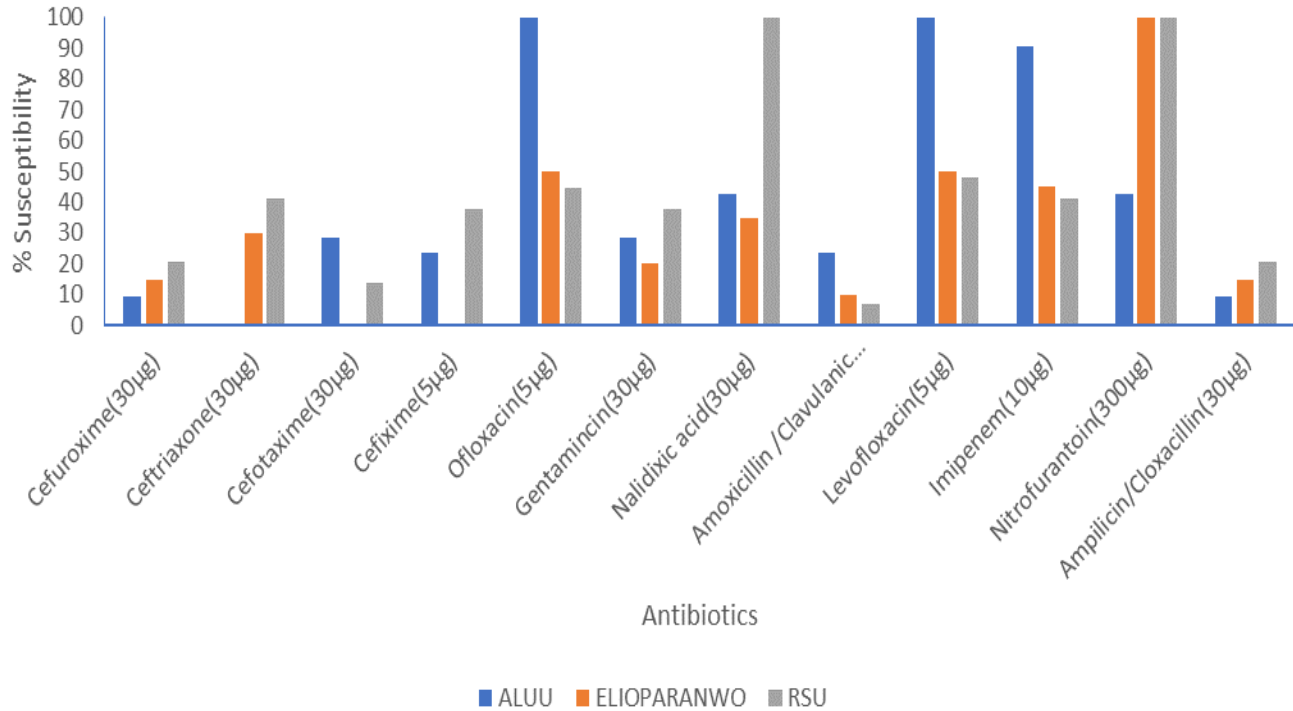


Fig. 6: Percentage Susceptibility of *Myroides* isolates in the Various Farms

Results of the SHV gene is presented in plate 1. Results showed that the SHV genes were detected/visualized having 300 base pair in lane 1, 5, 6, 7 and 9 for *Aeromonas caviae*, *Acinetobacter baumannii* and *Enterobacterhormaechei* while *Citobacter* and *Myriodes odoratiminus* isolates lacked the SHV genes.

Results of the TEM gene are presented in Plate 2. Results showed that TEM genes were

detected/visualized having 400base pair in lane 1,2,3,5,7 and 10 for *Aeromonas caviae*, *Myroides odoratimimus*, *Acinetobacter baumannii*, *Enterobacter hormaechei* and *Citrobacter braakii*.

Table 1 shows the results of the Molecular Identification of Isolates with their Accession Number. It also shows the corresponding extended spectrum beta-lactamases (ESBL) genes (SHV and TEM) that were detected.

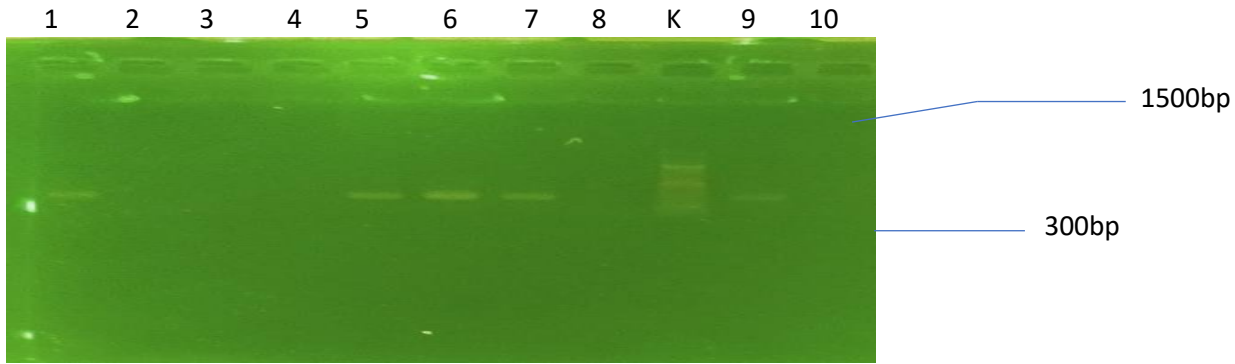


Plate 1: Showing Agarose gel electrophoresis of SHV gene of some selected bacteria isolates. Lanes 1, 5, 6, 7 and 9 represent the SHV gene bands (300bp). Lane K represents the 100bp Molecular ladder of 1500bp. (Lane 1=*Aeromonas caviae*, Lane 5=*Acinetobacter baumannii*, Lane 6=*Enterobacter hormachei*, Lane 7=*Enterobacter hormachei*, and Lane 9=*Enterobacter hormachei*)

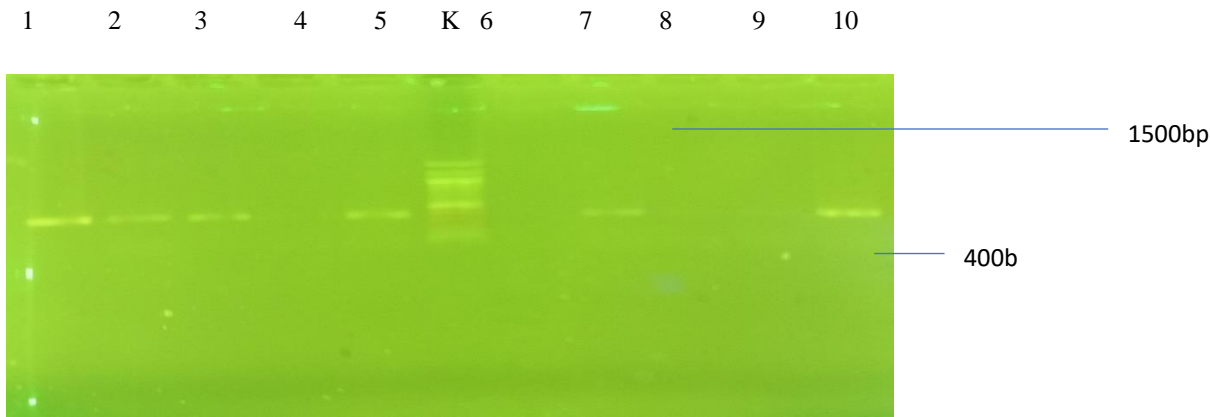


Plate 2: Agarose Gel Electrophoresis of *Bla TEM* gene of some selected bacterial isolates. Lanes 1, 2,3,5,7 and 10 represent *BlaTEM* gene band (400bp). Lane K represents the 100bp Molecular ladder of 1500bp. (lane1=*Aeromonas caviae*, Lane 2=*Aeromonas caviae*, Lane 3 =*Myriodes odoratiminus*, Lane 5=*Acinetobacter baumannii*, Lane 7 = *Enterobacter hormachei*, Lane 10 = *Citrobacter braakii*).

Table 1: Molecular Identification of Isolates with their Accession Number

Isolate Code	Molecular identity of isolate	Resistance genes (ESBL)	
		SHV	TEM
K1	<i>Aeromonas caviae</i> (CP072326)	+	+
K2	<i>Aeromonas caviae</i> (OL455994)	-	+
K3	<i>Myroides odoratiminus</i> (MN733229)	-	+
K4	<i>Enterobacter hormachei</i> (ON834331)	-	-
K5	<i>Acinetobacter baumannii</i> (ON60630)	+	+
K6	<i>Enterobacter hormachei</i> (ON834331)	+	-
K7	<i>Enterobacter hormachei</i> (ON834331)	+	+
K8	<i>Citrobacter freundii</i> (JQ231164)	-	-
K9	<i>Enterobacter hormachei</i> (ON834332)	+	-
K10	<i>Citrobacter braakii</i> (ON557391)	-	+
% Positive		50%	60%

Keys: + = present; - = absent

Discussion

Resistance to antibiotics by bacterial isolates has become a problem for public health and has potential economic ramifications as bacterial resistance to antibiotics has been intensively explored. Studies have revealed an expanding significance for free-living birds as hosts for bacteria carrying mechanisms for antibiotic resistance (Smith *et al.*, 2014; Carroll *et al.*, 2015). The findings in this present study showed that the response of the bacterial isolates to the different antibiotics varied across the farms under study. For instance, the susceptibility of *Acinetobacter baumannii* to ofloxacin and nalidixic acid in Aluu farm was 100% while 76 and 50% susceptibility was recorded for similar isolates in Elioparanwo farm. In the Rivers State University poultry farm, the isolates while being 100% susceptible to ofloxacin were 48% susceptible to nalidixic acid.

This disparity in effectiveness of antibiotics was observed in all the isolates across the farms. The trend of variation in antibiotic effectiveness or response of a particular bacterial isolate to an antibiotic from different geographical locations could be influenced by rate of antibiotics use or abuse in this location. This agreed with Robinson (2018) who had similar observations.

Horizontal gene transfer has been reported as one method bacterium obtain resistant genes (Prescott *et al.*, 2011). Liu *et al.*, (2017) reported the variability in the prevalence of Extended spectrum β -lactamases (ESBLs) producing *Citrobacter* spp among countries with reports of 4.9–20.6%, 0.2–4.6%, and 0.9% of *C. freundii* isolates from Korea, Japan and USA, respectively; and 3.5 and 60.0% of *C. koseri* isolates from USA and Japan, respectively which agreed with the present study. Thus, if an antibiotic resistant gene for gentamycin is available in one environment and absent in another environment, there is a high probability that bacterial isolates in that environment could pick up such plasmid thereby becoming resistant to gentamycin. Furthermore, the indiscriminate use of antibiotics for poultry farming and other factors influence resistance to antibiotics.

According to Saud *et al.*, (2019), the widespread use of antimicrobials in the chicken industry to prevent disease and enhance growth further activates the processes that result in the formation of bacterial strains that are resistant to common antibiotics.

In this study, *Citrobacter braakii* and *C. freundii* isolates were highly resistant to gentamycin, nalidixic acid, Ampicillin/Cloxacillin and cefixime antibiotics. Although they exhibited some level of susceptibility to ofloxacin and imipenem antibiotics, they displayed multi-drug resistance. *Citrobacter* spp., particularly *C. freundii*, are known to cause a variety of infections, including UTIs, wound infections, gastrointestinal infections, septicemia, and meningitis, particularly in immunocompromised patients and in hospital settings (Ranjan and Ranjan, 2013; Leski *et al.*, 2016a). Since this development and the discovery that *C. freundii* frequently demonstrates resistance to various classes of antibiotics coincide, it is possible that both clinical strains and environmental strains serve as a reservoir for antimicrobial resistance determinants (Liu *et al.*, 2017). In a previous study of outpatients in Bo, Sierra Leone, it was discovered that an unexpectedly large proportion of *C. freundii* isolates from UTIs were highly MDR (Leski *et al.*, 2016b). According to reports, *Citrobacter* spp. ESBL prevalence ranged from 0.5 to 36% globally (Fernandes *et al.*, 2014; Praharaj *et al.*, 2016). About 80.9% of the *Citrobacter* isolates from hospitalised patients in India produced ESBLs (Praharaj *et al.*, 2016). From this study, we screened for the blaTEM and blaSHV genes by PCR instead of testing for the ESBL phenotype and discovered that only 8.3% of the isolates were blaTEM positive, and none had the blaSHV gene. This agreed with Liu *et al.*, (2017) who reported that only 3.2% of *Citrobacter* sp possessed the blaTEM gene with no blaSHV gene. Although they posited that the low ESBL genes in their study could be because their isolates were from food source. This could also imply that the low percentage of blaTEM genes in the present study could be because the isolates are from a food source. The present study did not however, agree with a previous study which showed high susceptibility of *Citrobacter* sp to gentamycin and ceftriaxone antibiotics (Gautam *et al.*, 2019).

Acinetobacter sp like *Citrobacter* sp also exhibited MDR to many of the antibiotics. The antibiotics that displayed great inhibitory action against the isolates was ofloxacin. Although imipenem which is a carbapenem antibiotics also displayed high level of inhibitory action. This is consistent with a previous study that reported that all *Citrobacter* isolates were susceptible to the carbapenem meropenem (Aravena-Román *et al.*, 2012).

More so, the multi-drug resistance demonstrated by *Acinetobacter* sp could be due to the possession of blaTEM and blaSHV genes which are known ESBL genes that aid in the resistance to beta-lactam antibiotics (Robinson et al., 2023). The β -lactamases produced by Gram-negative and Gram-positive bacteria play vital role in resistance against β -lactam antibiotics (Dharne et al., 2008).

Furthermore, *Myroides* isolates were highly resistant to antibiotics especially the penicillin and aminoglycosides despite being susceptible to ofloxacin (a quinolone). Thus, multi-drug resistance was also observed in the *Myroides* isolates. This is consistent with a previous work by Dharne et al. (2008) who reported high resistance of *Myroides* isolates to gentamycin and other antibiotics. Mammeri et al. (2002) proposed that *Myroides* spp. possess metallo β -lactamases, and this is consistent with the present study. Very high resistance *E. coli* isolates were also observed in this study.

The present study has also showed that the MDR observed could be hinged on the presence of antibiotic resistance genes which is present in the plasmids of these isolates.

Ofloxacin is one of the fluoroquinolones antibiotics that possess bactericidal effects on both Gram negative and Gram-positive bacteria. According to The American Society of Health-System Pharmacists (2015), ofloxacin like ciprofloxacin is used for the treatment of different forms of infections such as endocarditis, respiratory infections, urinary tract infections, cellulitis, gastroenteritis, and lots more.

Thus, this statement agreed with the findings in this current study as the ofloxacin antibiotic was the most sensitive antibiotics against all the bacterial isolates in this study. Ofloxacin like other fluoroquinolones function by inhibiting the DNA gyrase and type II topoisomerase and topoisomerase IV which is needed to unwind the DNA of the bacteria (Pommier et al., 2010).

Gentamycin was also very effective against *Enterobacter*, *Aeromonas* and other gram-negative bacteria isolates even though some level of resistance was observed. Gentamycin belongs to the aminoglycoside antibiotics that function by binding to the 30S ribosomal subunit thereby disrupting the proof-reading function which leads to the synthesis of

toxic proteins caused by wrong interpretation of the mRNA (Tom et al., 2011).

In conclusion, the study on plasmid profiling and antibiotic resistance in enteric bacteria associated with poultry meat in Rivers State highlighted several critical findings: high prevalence of antibiotic resistant isolates, possession of divers ESBL genes (SHV and TEM genes).

Thus, monitoring antibiotic resistance is crucial, especially in the context of poultry production, as it relates to the food supply chain. To protect public health and maintain the potency of antibiotics for both human and veterinary treatment, efforts must be made to limit the use of antibiotics in poultry, improve hygiene standards, and strengthen surveillance.

To address the intricate problem of antibiotic resistance in the chicken sector in Rivers State and elsewhere, additional study and intervention strategies are required.

References

- Anosike, F. U., Rekwot, G. Z., Owoshagba, O. B., Ahmed, S. and Atiku, J.A. (2018). Challenges of poultry production in Nigeria. *Nigerian Journal of Animal Production*, 45(1), 252-258
- Aravena-Román, M., Inglis, T. J. J., Henderson, B., Riley, T. V., and Chang, B. J. (2012). Antimicrobial susceptibilities of *Aeromonas* strains isolated from clinical and environmental sources to 26 antimicrobial agents. *Antimicrobial Agents and Chemotherapy*. 56(2), 1110–1112.
- Behroozzi, A., Rahbar, M.V. and Yousefi, J. (2010). Frequency of extended spectrum beta-lactamase (ESBLs) producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from urine in an Iranian 1000-bed tertiary care hospital. *African Journal of Microbiology Research*. 4,881-884.
- Blaak, H., De Kruijf, P., Hamidjaja, A. R., Van Hock., H. A., Husman, D, A. and Schets, M. F. (2014). Prevalence and characteristics of ESBL producing *E. coli* in Dutch recreational waters influenced by waste water treatment plants. *Journal of Veterinary Microbiology*. 171 (3-4), 48-59.
- Carroll, D., Wang, J., Fanning, S. and McMahan, B. J. (2015). Antimicrobial resistance in wildlife:

- implications for public health. *Zoonoses and Public Health*. 62(7), 534-542.
- Cheesbrough, M. (2006). District laboratory practice in tropical countries part 2. Cambridge University Press, New York; ISBN-13 978-0-511-34842-6.
- Clinical and Laboratory Standard Institute (CLSI). (2019). 28th ed. Wayne: Clinical and Laboratory Standard Institute; 2019. Performance standard for antimicrobial disk susceptibility tests.
- Dharne, M. S., Gupta, A. K., Rangrez, A. Y., Ghate, H. V., Patole, M. S., and Shouche, Y. S. (2008). Antibacterial activities of multi drug resistant *Myroides odoratimimus* bacteria isolated from adult flesh flies (Diptera: Sarcophagidae) are independent of metallo beta-lactamase gene. *Brazilian Journal of Microbiology*. 39(2), 397–404.
- Dharne, M. S., Misra, S. P., Misra, V., Dwivedi, M., Patole, M. S., and Shouche, Y. S. (2008). Isolation of urease-positive *Ochrobactrum intermedium* in the stomach of a non-ulcer dyspeptic patient from north India. *Journal of Microbiology, Immunology, and Infection= Wei Mian yu gan ran za zhi*. 41(2), 183-186.
- Fatemeh, R. Z., Zahra, M., Mahboubeh, N.N., Mehrangiz, K. K., Kiarash, G., Abdollahim, R., Habibollah, E., Maryam, S. N. and Mahboubeh, D.H. (2012). The prevalence of TEM and SHV genes among extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae*, *Iran Journal of Basic Medical Sciences*. 15(1),654-660.
- Fernandes Azevedo, B., Barros Furieri, L., Peçanha, F. M., Wiggers, G. A., Frizera Vassallo, P. and Ronacher Simões, M. (2012). Toxic effects of mercury on the cardiovascular and central nervous systems, *International Journal of Biomedical Research*. 20, 94-98.
- Garcia-Graells, C., Antoine, J., Larsen, J., Catry, B., Skov, R. and Denis, O. (2012). Livestock veterinarians at high risk of acquiring methicillin-resistant *Staphylococcus aureus* ST398. *Epidemiol Infect*. 140:383–389.
- Gautam, N., Poudel, R., Lekhak, B., and Upreti, M. K. (2019). Antimicrobial Susceptibility Pattern of Gram-Negative Bacterial Isolates from Raw Chicken Meat Samples. *Tribhuvan University Journal of Microbiology*. 6(1), 89–95.
- Leski, T.A., Taitt, C. R., Bangura, U., Ansumana, R., Stenger, D. A. and Wang, Z.(2016b). Finished genome sequence of the highly multidrug-resistant human urine isolate *Citrobacter freundii* strain SL151 genome. *American Society of Microbiology*. 4(6),12-16.
- Liu, L., Lan, R., Liu, L., Wang, Y., Zhang, Y., Wang, Y. and Xu. J. (2017). Antimicrobial resistance and cytotoxicity of *Citrobacter* spp. in Maanshan Anhui Province, China. *Frontiers in Microbiology*. 8, 13-57.
- Liu, L., Lan, R., Liu, L., Wang, Y., Zhang, Y., Wang, Y., and Xu, J. (2017). Antimicrobial resistance and cytotoxicity of *Citrobacter* spp. in Maanshan Anhui Province, China. *Frontiers in Microbiology*. 8(JUL), 1–12
- Mammeri, H., Bellais, S., and Nordmann, P. (2002). Chromosome- encoded beta-lactamases TUS-1 and MUS-1 from *Myroides odoratus* and *Myroides odoratimimus* (formerly *Flavobacterium odoratum*) new members of the lineage of molecular subclass B1 metalloenzymes. *Antimicrob. Agents Chemother*. 46 (11):3561–3567.
- Marcus, R., Varma, J. K., Medus, C., Boothe, E, Anderson, B.J., Crume, T.L Fullerton, K.E, Moore, M. R., White, P,L, Lyszkowicz, E, Voetsch, A.C and Angulo, F.J.(2007). Reassessment of risk factors for sporadic *Salmonella* serotype enteritidis infections; a case-control study in five food net sites, 2002-2003. *Epidemiology and Infection*. 135 (1), 84-92.
- Pitout, J. D. D. and Kupland, K. B. (2008). Extended spectrum β lactamase producing enterobacteria case; An emerging public health concern *Lancet Infectious Disease*. 8, 159-166.
- Pommier, Y., Leo, E., Zhang, H and Marchand, C. (2010). "DNA topoisomerases and their poisoning by anticancer and antibacterial drugs". *Chemistry & Biology*. 17 (5): 421–33.
- Praharaj, A. K., Khajuria, A., Kumar, M. and Groves, N. (2016). Phenotypic detection and molecular characterization of beta-lactamase genes among *Citrobacter* species in a tertiary care hospital. *Avicenna Journal of Medicine*. 6,17-27.

- Prescott, C., Sherwood, L. M., Woolverton, C. J., Williey, J. M. and Harley, F. K. (2011) Microbiology. 8th Ed. New York: Macgraw-Hill Higher Education. 158-165.
- Ramazanzadeh, R., Farhadifar, F. and Mansouri, M. (2010). Etiology and antibiotic resistance pattern of community-acquired extended-spectrum beta-lactamase-producing gram negative isolates in Sanandaj. *Research Journal of Medical Science*, 4,243-247.
- Ranjan, K .P. and Ranjan, N. (2013). *Citrobacter*: An emerging health care associated urinary pathogen. *Annals of Urological Research*. 5, 313-314.
- Rekwot, G. Z., Ahmed S. and Dawang, N. C. (2015). Technical efficiency of poultry egg production in Kaduna State, Nigeria, proceedings of the 20th annual conference of the animal science association of Nigeria, 6-10th September 2015.University of Ibadan, Oyo State. 324-329
- Robinson, V. K., Aleruchi, O., Okafor, A. C., Ahuokpo, H. I., and Ipalibo, C. H. (2023). Antibiogram and Resistant Gene (Gentamicin and Estended Beta-lactam) Profile of *Escherichia coli* Isolated from Yoghurt. *Asian Journal of Biochemistry, Genetics and Molecular Biology*. 14(2), 1–11.
- Saud, B., Paudel, G., Khichaju, S., Bajracharya, D., Dhungana, G., et al. (2019). Multidrug-resistant bacteria from raw meat of buffalo and chicken, Nepal. *Veterinary Medicine International*. 1–7.
- Smith, S., Wang, J., Fanning, S. and McMahon, B. J. (2014). Antimicrobial resistant bacteria in wild mammals and birds: a coincidence or cause of concern? *Irish Veterinary Journal*.67(8),1-3.
- The American Society of Health-System Pharmacists. (2015). Ciprofloxacin Archived from the original on 23 September 2015. Retrieved June 5, 2019.
- Tom, E., Anna, C., Peter, L. and Jonathan, S. (2011). *Medical Microbiology and Infection (5th Edn)*. 147.
- Waters, A. E., Contente-Cuomo, T., Buchhagen, J., Liu, C. M., et al. (2011). Multidrug-resistant *Staphylococcus aureus* in US Meat and Poultry. *Clin Infect Dis*. 52, 1227–1230
- Wemedo, S. A. and Robinson, V. K. (2018). Evaluation of Indoor Air for Bacteria Organisms and their Antimicrobial Susceptibility Profiles in a Government Health Institution. *Journal of Advances in Microbiology*. 11(3), 1-7.