

Multiple Antibiotic Resistances in *Escherichia coli* Isolated from Cattle and Poultry Faeces in Abraka, South-South Nigeria

Olivia Sochi Egbule¹ and Ibrahim Yusuf^{2*}

¹Department of Microbiology, Faculty of Science, Delta State University Abraka, P.M.B.1, Abraka, Delta, Nigeria

²Department of Microbiology, Faculty of Life Sciences, College of Natural and Pharmaceutical Sciences, Bayero University, P. M. B. 3011, Kano, Nigeria

ABSTRACT

Rise in antibiotic resistance among clinical and environmental isolates in Nigeria is becoming worrisome. The unprofessional sales and consumption of veterinary antibiotics together with lack of adequate sanitation and hygiene during slaughtering, processing and consumption of cattle and poultry products in Abraka, Delta State of Nigeria, could spread antibiotic resistant bacteria to the surrounding environments. A study was therefore conducted to isolate *Escherichia coli* from 200 poultry litters and 200 cattle faeces and screen them for beta-lactamases. Antimicrobial susceptibility of 412 *E. coli* strains isolated from 400 samples of fresh and dry faeces of cattle and poultry and 25 samples each of rectal and cloacae swabs were carried out. All the strains were screened phenotypically for extended spectrum beta-lactamase (ESBL), carbapenemase and metallo beta-lactamase (MBL). Results showed a high incidence of resistance to all the antibiotics except meropenem. Resistance to nitrofurantoin, amoxicillin and sulfamethoxazole-trimethoprim reached 80-90%, while resistance to other beta-lactams and fluoroquinolones ranges from 40-60%, and over 50% of them exhibited multidrug resistance. Strains of *E. coli* from poultry droppings are more resistant to antibiotics than their counterparts from cow dungs. ESBL, carbapenemase and MBL production was detected in 10.5, 5.26 and 7.89% of isolates from cow dungs and 27.2, 10.2 and 6.81% isolates from poultry droppings. High level of antibiotic resistance and incidence of ESBL, carbapenemase, and MBL have public health implication as poor sanitation,

ARTICLE INFO

Article history:

Received: 03 December 2018

Accepted: 04 April 2019

Published: 30 May 2019

E-mail addresses:

oliviaegbule@gmail.com (Olivia Sochi Egbule)

iyusuf.bio@buk.edu.ng (Ibrahim Yusuf)

* Corresponding author

poor water supply and lack of personal hygiene among the handlers, processors and consumers are very high in the locality.

Keywords: Antibiotic, carbapenemase, cow, ESBL, faeces, poultry, resistance

INTRODUCTION

The overuse of antibiotics in animal agriculture both in developed and developing countries has been considered as an important factor that contributes to the evolution and spread of antibiotic-resistant pathogens (ARPs) in human and environments. Wastes from human, animals, domestic and hospital sources can serve as a major reservoir of these ARPs (Diallo et al., 2013; Dierikx et al., 2010; Egea et al., 2012). In addition, during animal farming, the animals themselves and their broader environment can also serve as important reservoirs of ARP genes which have the potentiality of being exchanged within or across bacterial species. During production or processing of animals or their products, excreta and other fluids with possibly large amount of ARPs can come into contact with a human food or water, from where serious infections with potential high mortality may occur (Costa et al., 2009; Korzeniewska et al., 2013).

Scientists in the field of antibiotic resistance research globally have called for the restriction of antibiotic use in animal husbandry. However, in developed countries, there is an increase in the level of compliance, but in the developing countries, the non-therapeutic use of antibiotics is increasing, mainly for economic gain, where

growth promotion of chicken and cattles is heavily dependent on large use of antibiotics (Braykov et al., 2016). A study in 3 Nigerian states has revealed that, tetracycline is the most widely used antibiotic for livestock production, followed by flouroquinolones and betalactams (Adesokan et al., 2015).

Generally in Nigeria, and specifically in Abraka, Delta State, poultry farming and cattle rearing are promoted for economic development and nutrition supplementation strategy by all levels of government. As a result of this sustained increase in poultry production and concurrent use of veterinary antibiotics in Nigeria, rapid rise in antibiotic consumption in animals was observed in some states from 2010 to 2013, with the majority of the antibiotics acquired without professional prescriptions and were wrongly used (Ojo et al., 2016). This practice of indiscriminate antibiotic use, may impose selection pressure on bacteria which can lead to emergence of multi-drug resistant bacteria in the animal and subsequent passage to human, since the antibiotics, their metabolites, and resistant bacteria can be excreted with urine and faeces to the surrounding environment especially where poor sanitation and lack of effective infection control management are in place (Diallo et al., 2013; Yusuf & Adam, 2014).

Extended-spectrum β -lactamase (ESBL) and carbapenemase-producing enteric bacteria such as *Escherichia coli* and *Salmonella* sp. are globally recognised ARPs, with high spreading rate and mortality (Dierikx et al., 2010). Several infections caused by ARP producing

ESBLs and carbapenemase such as urinary tract infections, bacteraemia, peritonitis, pneumonia, intra-abdominal infections, and meningitis have a record of resisting treatment with many antibiotics such as ceftazidime, cefotaxime, aztreonam, fluoroquinolones and even antibiotics that are rarely used in many hospitals (Motayo & Akinduti, 2014; Yusuf et al., 2014).

Given the sustained use of antibiotics indiscriminately in Abraka for cattle and poultry production, there is need for studies that will determine the antibiotic susceptibility pattern of *E. coli* isolated from cow rectum, poultry cloacae and faeces (dungs and droppings) obtained from farms and surrounding environment and also to examine the *E. coli* for ESBL and carbapenemase production.

MATERIALS AND METHODS

Study Site

This study was carried out in Abraka, a town in Delta State located in South-South geo-political zone of Nigeria. The town was selected due to their relatively high poultry and cattle production in the region. Fertile land and a long period of rainfall provide evergreen grass for Fulani's owned cattle to graze. Poultry and livestock activities in the area provide a source of income to a large number of both registered and unregistered pharmaceutical outlets that provide drugs including antibiotics to both poultry and cattle producers. While poultry farms are often reared in a cage system, cattle in the region are reared mainly in semi-intensive systems where the animals are taken out to

graze and then returned to their pens later in the evening. Both poultry farmers and cattle rearers have unregulated access to veterinary drugs in the region.

Sample Collection, Processing, Isolation, and Characterization of *Escherichia coli*

A total of 200 poultry litter samples was collected from Delta State University, Abraka poultry farms and 200 faecal samples of cattle from cattle farms located in Abraka and environs. For each, four sampling sites were identified and coded as P1, P2, P3, P4 and C1, C2, C3, C4 for poultry and cattle farms respectively. The cattle and poultry farms were independent of each other epidemiologically and to the best of our ability, the animals in the farms were healthy and not suspected of having disease or infection. At each sampling site, 50 samples were collected during the months of April to June 2017 in triplicates in sterile bottles. Samples P1, P2, C1, and C2 were taken fresh while P3, P4, C3 C4 were taken from dry faeces of the respective animals. To collect fresh poultry droppings, a large sterile autoclavable polythene was spread on clean and disinfected cage at 7pm. The feet of 6 week old broiler chickens were immersed in mild disinfectant solution before putting them back to the cage. The chickens were fed overnight and samples of fresh droppings were collected in the morning (7am) while dried ones were collected in the evening (7pm). For cow dungs, middle portion of fresh and dry dungs that is not in contact with soil were

collected with the aid of sterile spatula. Rectal and cloacal swabs from 25 randomly selected cattle and poultry were collected aseptically with sterile swab sticks. Swabs were collected equally from all the sampling sites. All the samples were transported to the laboratory within 1 h for bacterial isolation. About 1 g of faecal samples were homogenized in 9 mL of buffered peptone water and 1 ml were rediluted in another 9 mL of buffered peptone water to make a dilution of 1:10. Cloacal swabs were soaked in 10 mL of buffered peptone water.

Isolation of *E. coli* was carried out by inoculation of 0.1 mL of appropriate dilutions of each sample on MacConkey (Oxoid, UK) plates in triplicates and incubated at 37°C for 24 h (Egea et al., 2012). Distinct colonies of *E. coli* on the plates (pink color) were identified and purified by repeated culturing. Pure cultures of presumptive *E. coli* from all the samples were characterised and identified biochemically according to standard protocol and stored in glycerol stock at -20°C until use.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the standard disk diffusion method on Mueller–Hinton agar medium (Oxoid, UK) according to the criteria established by the Clinical and Laboratory Standards Institute [CLSI], (2016). The antibiotics used were ceftazidime (CAZ-30 µg), cefuroxime (CRX-30 µg), gentamycin (GEN-10 µg), cefixime (CXM-5.0 µg), cefotaxime (CTX-30 µg), ofloxacin (OFL-5.0 µg), amoxicillin-clavulanic acid

(AUG-30 µg), nitrofurantoin (300 µg), ciprofloxacin (CPR-5 µg, sulfamethoxazole-trimethoprim (SXT-23.75/1.25 µg), meropenems (MEM-10 µg) (Oxoid UK). Isolates resistant to beta-lactams were selected and tested for ESBL production. The susceptibility breakpoints for all antimicrobials were those recommended by CLSI (2016). *Escherichia coli* that are resistant to three or more classes of antibiotics were classified as multi-drug resistant strain.

Detection of Extended-Spectrum Beta-Lactamases (ESBL) and Carbapenemase

ESBL production was detected phenotypically by the double disc synergy test (DDST) method according to the criteria established by the CLSI using CAZ and CXT discs alone and in combination with AUG (CLSI, 2011, 2016).

Screening for the production carbapenemase by the animal isolates was performed with the modified Hodge test using *E. coli* ATCC 25922 (a carbapenem susceptible strain) as the indicator strain and ertapenem (ETP) (10 µg) disc (Birgy et al., 2012).

Isolates that showed reduced susceptibility to ETP (< 24mm in diameter) were suspected as carbapenemase producers. However, all the isolates (both suspected and non-suspected) were subjected to carbapenemase confirmatory test by the modified Hodge test. Carbapenemase producers were identified by the formation of a clover leaf type indentation at the

intersection of the test organism and *E. coli* ATCC 25922 within the zone of inhibition around the carbapenem

Phenotypic Differentiation of MBLs and KPC Carbapenemase Production

MBL production was detected according to modified combined-disc tests of ETP alone and with EDTA (Kim et al., 2015). Briefly, a 0.5 McFarland standard suspension of the test isolates were inoculated on MHA plates (Oxoid, UK). Two discs separated by a distance of 20-30mm were placed on the surface of the inoculated MHA using a sterile forceps. One of the discs was ETP and the other was a sterile cut filter paper disc impregnated with 292 µg EDTA. The plates were incubated at 37°C for 24 h. The diameter of a clear zone around the ETP+EDTA was compared with a clear zone around the plain ETP-EDTA disc. Enhancement of zone around plain ETP by at least 2mm was taken as positive for MBL production.

RESULTS AND DISCUSSION

Livestock and poultry production in developing countries like Nigeria has enormous environmental, economic and health implications. At one end they provide a source of living, protein, and manure for the immediate community, but at the other end serves as reservoirs for untreatable bacteria. Cross-transmission to human is very much easier since the level of environmental sanitation and personal hygiene among the handlers and consumers is very low.

Of the total 400 faecal samples, comprising cow dungs and poultry droppings, and 50 swabs, a total of 412 *E. coli* were isolated, 188 from poultry samples and 224 from cow samples. Sixty eight percent (68.0%) and 73.6% of the isolates were from fresh poultry and cattle faeces respectively, while the remaining comes from dry samples. Specifically, 56% and 76% of the total rectal and cloacal swabs of cow and poultry respectively, yielded positive culture of *E. coli*. Of the total *E. coli* isolates, 94 and 88 from poultry and cattle samples respectively were resistant to at least two antibiotics while 61 and 51 were multidrug resistance respectively (Table 1). Further, the *E. coli* isolated from rectal and cloacal swabs were 42.8% and 63.1% resistant to at least 2 commonly used veterinary antibiotics in the area. The result indicates that highly resistant *E. coli* was present in faecal samples from poultry and cattle reared in the study area, possibly due to a general increase in the unprofessional use of antibiotics in animal production in the entire region. This is serious; especially in such community where awareness level of effect and sources of ARPs even among the health care workers is very limited, so for members of the community where literacy level is very low (Yusuf et al., 2015). The presence of 28.5% and 10.5% multi drug resistant *E. coli* in the cloacal and rectal swabs of the apparently healthy cattle and poultry is also worrisome, and may be due to prolonged exposure of *E. coli* in the gastro intestinal tracts of the animals to antibiotics. These increased population of resistant

Table 1
 Screening of isolates from various sampling sites of cattle and poultry farms for susceptibility to selected common antibiotics

Sample code	N° of <i>E. coli</i> isolated	N° of <i>E. coli</i> resistant to at least 2 antibiotics (%)	N° of <i>E. coli</i> resistant to multiple antibiotics (%)	N° of <i>E. coli</i> susceptible to all antibiotics (%)
P1	67	34 (50.7)	15 (22.3)	18 (26.8)
P2	58	33 (56.8)	20 (34.4)	5 (8.6)
P3	32	10 (31.2)	13 (40.6)	9 (28.1)
P4	28	11 (39.2)	9 (32.1)	7 (25.0)
C1	71	31 (43.6)	15 (21.1)	11 (15.4)
C2	64	19 (29.6)	14 (21.8)	2 (3.12)
C3	28	17 (60.7)	8 (28.5)	0 (0.00)
C4	31	9 (29.0)	12 (38.7)	10 (32.2)
PC	14	6 (42.8)	4 (28.5)	4 (28.5)
CR	19	12 (63.1)	2 (10.5)	5 (26.3)
Total	412	182(44.1)	112(27.1)	71(17.2)

Note: PC=poultry cloacal swab, CR=Cow rectal swab

strains of *E. coli* will remained in the environment and may later cause infection in the animals or human that consume them.

Of specific interest, 44.1% of the total isolates were resistant to at least two antibiotics commonly used for human treatment in hospitals while 27.1% were resistant to multiple antibiotics. Surprisingly, only 71 (17.2%) of the isolates were susceptible to tested antibiotics. Possibility of community acquirement of these bacteria in the study area is very easy and will be rapid, since cattle are allowed to move about freely in the environment, with their faeces scattered all over the environment. Even though, poultry in the study area are often caged in farms meant for commercial production, but the poultry manures are heaped with no appropriate disposal means. Studies in developed countries where cattle and poultry are caged or ranched and infection control system is

functional, showed that certain amount of *E. coli* producing ESBL were found in meats of broilers, turkeys and other livestock (Dierikx et al., 2010; Egea et al., 2012; Horton et al., 2011).

In this study, almost all the isolates were about 50% resistant to commonly used beta-lactam antibiotics such as ceftazidime, cefuroxime, cefixime and cefotaxime (Figure 1). Similarly, high resistance to fluoroquinolones was also observed. However, a higher resistance of strains of *E. coli* from both samples was observed with amoxicillin, nitrofurantoin, and sulfamethoxazole-trimethoprim. Overall resistance to meropenem was less but slightly higher in isolates from poultry wastes. This clearly reflected a high level of antibiotic abuse in poultry than in cattle in Nigeria, since most of the commercial poultry productions in the region are often carried out in the more sanitized environment due

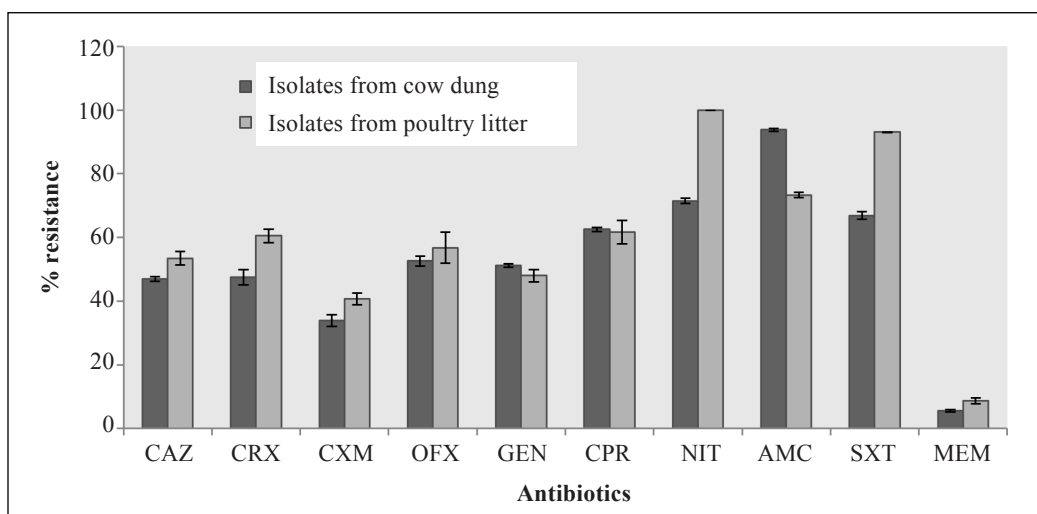


Figure 1. Antimicrobial resistance pattern of overall *E. coli* isolates from poultry litter and cow dung. Data are the means \pm standard deviations of three independent replicates

to the fragile nature of birds, hence the birds received more attention and large volume of antibiotics than cattle which are often treated with antibiotics only when sick. The result obtained in this study is in consonant with previous reports where a high level of resistance in isolates from poultry litters was reported (Sayah et al., 2005; Smith et al., 2007). A remarkable observation is the high level of resistance to the β -lactam antibiotic, particularly the 3rd generation cephalosporins, ceftazidime, and cefotaxime

even though they are rarely used in poultry and cattle treatment in the study area. Of high interest, is a low level of resistance to the meropenem, an antibiotic often reserved for treatment of life-threatening infections in human.

Screening the strains of *E. coli* isolates phenotypically for ESBLs showed that 10.5% and 27.27% of the isolates from cow dung and poultry litters respectively produces ESBL (Table 2). The prevalence of ESBL in this study, especially in poultry droppings is

Table 2
Prevalence of ESBLs, carbapenemase and MBL among *E. coli* from cow dung and poultry litter

Source	N ^o of <i>E. coli</i> screened	N ^o of ESBL producers	N ^o of carbapenemase producers	N ^o of MBL producers
Cow dung	76	8(10.5)	4(5.26)	6(7.89)
Cow rectal swab	12	2 (16.6)	2(16.6)	0(0.0)
Poultry litter	88	24(27.27)	9(10.2)	6(6.81)
Poultry cloacal swab	6	3(50.0)	2(33.3)	1(16.6)
Total	182	37 (20.3)	17 (9.34)	13 (7.14)

Note: Values in parenthesis are percentages

high when compared with the prevalence of ESBL in *E. coli* isolated from hospitalised and non-patients in neighbouring states in Nigeria as earlier reported (Aibinu et al., 2003; Iroha et al., 2009; Olowe et al., 2010; Yusuf et al., 2014). Further, carbapenemase production including MBL was also higher in *E. coli* strains from poultry samples (droppings=10.4%, cloacal swab=33.3%) when compared with isolates from cow (dungs=5.26%, rectal swab=16.6%). With no trace of carbapenem usage in Agriculture in Abraka or elsewhere in Nigeria, means that the observed resistance of the *E. coli* to carbapenem through production of carbapenemase and MBL, is not due to use of carbapenem usage in animal husbandry in the region. Molecular testing for the presence of resistance genes was not carried out for the isolates, and was therefore a limitation of the present study.

This observation has great public health implication, since human and animals from some of the sampling area, especially in rural areas use the same source of water for drinking, washing, cooking and irrigation of crops. The fresh cattle faeces which contain MDR can contaminate water, fruits, and food and may become a source of environmental ESBL and carbapenemase-producing *E. coli* in the community. Isolates harbouring ESBL, carbapenemase, and other related genes can transfer the gene by the lateral and horizontal mechanism. This study can also provide a clue as to how patients with no previous treatment with carbapenem or travel to endemic area harbour isolates of *Klebsiella pneumoniae*

and *E. coli* in some hospitals in another state in Nigeria as earlier reported.

CONCLUSION

The study showed that there were high sales of unregulated antibiotics and high prevalence of resistance of strains of *Escherichia coli* isolated from fresh and dry faecal samples of cattle and poultry in selected farms in Abraka, Delta state of Nigeria. The isolates produced ESBL, carbapenemase, and metallo beta-lactamase and were highly resistant to commonly used beta-lactam and non beta-lactam antibiotics used in the area. Poultry droppings harbour resistant *E. coli* more than cow dungs. The high level of free cattle range, lack of effective disposal of poultry wastes, indiscriminate use of antibiotics in rearing of both animals, poor personal hygiene of food handlers and lack of functional infection control in the area pose a high danger of transmission of these multi-drug resistant bacteria to human living in the surroundings and beyond.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENTS

We acknowledged the supports of Departments of Microbiology of Delta State University Abraka and Bayero University Kano, for providing logistics and control strain of *E. coli* (ATCC 25922) used for the study.

REFERENCES

- Adesokan, H. K., Akanbi, I. O., Akanbi, I. M., & Obaweda, R. A. (2015). Pattern of antimicrobial usage in livestock animals in south-western Nigeria: The need for alternative plans. *The Onderstepoort Journal of Veterinary Research*, 82(1), 816. doi:10.4102/ojvr.v82i1.816
- Aibinu, I. E., Ohaegbulam, V. C., Adenipekun, E. A., Ogunsola, F. T., Odugbemi, T. O., & Mee, B. J. (2003). Extended-spectrum β -lactamase enzymes in clinical isolates of *Enterobacter* species from Lagos, Nigeria. *Journal of Clinical Microbiology*, 41(5), 2197-2200. doi:org/10.4314/njhbs.v2i2.11484
- Birgy, A., Bidet, P., Genel, N., Doit, C., Decré, D., Arlet, G., & Bingen, E. (2012). Phenotypic screening of carbapenemases and associated β -lactamases in carbapenem-resistant Enterobacteriaceae. *Journal of Clinical Microbiology*, 50(4), 1295–1302. doi: 10.1128/JCM.06131-11
- Braykov, N. P., Eisenberg, J. N. S., Grossman, M., Zhang, L., Vasco, K., Cevallos, W., ... Levy, K. (2016). Antibiotic Resistance in animal and environmental samples associated with small-scale poultry farming in Northwestern Ecuador. *mSphere*, 1(1), e00021-15. doi: 10.1128/mSphere.00021-15
- Clinical and Laboratory Standards Institute. (2011). *Performance standards for antimicrobial susceptibility testing (M 100-S21)*. Wayne, USA: CLSI.
- Clinical and Laboratory Standards Institute. (2016). *Antimicrobial susceptibility testing guideline*. Wayne, USA: CLSI.
- Costa, D., Vinué, L., Poeta, P., Coelho, A. C., Matos, M., Sáenz, Y., ... Torres, C. (2009). Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates in faecal samples of broilers. *Veterinary Microbiology*, 138(3-4), 339–44. doi: 10.1016/j.vetmic.2009.03.029
- Diallo, A. A., Brugère, H., Kérourédan, M., Dupouy, V., Toutain, P.-L., Bousquet-Mélou, A., ... Bibbal, D. (2013). Persistence and prevalence of pathogenic and extended-spectrum beta-lactamase-producing *Escherichia coli* in municipal wastewater treatment plant receiving slaughterhouse wastewater. *Water Research*, 47(13), 4719–29. doi: 10.1016/j.watres.2013.04.047
- Dierikx, C., van Essen-Zandbergen, A., Veldman, K., Smith, H., & Mevius, D. (2010). Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. *Veterinary Microbiology*, 145(3-4), 273–8. doi: 10.1016/j.vetmic.2010.03.019
- Egea, P., López-Cerero, L., Torres, E., Gómez-Sánchez, M. D. C., Serrano, L., Navarro Sánchez-Ortiz, M. D., ... Pascual, A. (2012). Increased raw poultry meat colonization by extended spectrum beta-lactamase-producing *Escherichia coli* in the south of Spain. *International Journal of Food Microbiology*, 159(2), 69–73. doi: 10.1016/j.ijfoodmicro.2012.08.002
- Horton, R. A., Randall, L. P., Snary, E. L., Cockrem, H., Lotz, S., Wearing, H., ... Coldham, N. G. (2011). Fecal carriage and shedding density of CTX-M extended-spectrum β -lactamase-producing *Escherichia coli* in cattle, chickens, and pigs: Implications for environmental contamination and food production. *Applied and Environmental Microbiology*, 77(11), 3715–9. doi: 10.1128/AEM.02831-10
- Iroha, I., Adikwu, M., Esimone, C., Aibinu, I., & Amadi, E. (2009). Extended spectrum beta lactamase (ESBL) in *E. coli* isolated from a tertiary hospital in Enugu State, Nigeria. *Pakistan Journal of Medical Sciences*, 25(2), 279-282.
- Kim, H. K., Park, J. S., Sung, H., & Kim, M. N. (2015). Further modification of the modified hodge test

- for detecting metallo- β -lactamase-producing carbapenem-resistant Enterobacteriaceae. *Annals of Laboratory Medicine*, 35(3), 298–305. doi: 10.1016/j.ijantimicag.2015.09.010
- Korzeniewska, E., Korzeniewska, A., & Harnisz, M. (2013). Antibiotic resistant *Escherichia coli* in hospital and municipal sewage and their emission to the environment. *Ecotoxicology and Environmental Safety*, 91, 96–102. doi: 10.1016/j.ecoenv.2013.01.014
- Motayo, B., & Akinduti, P. (2014). Antibigram and plasmid profiling of carbapenemase and extended spectrum Beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* in Abeokuta, South western, Nigeria. *African Health Sciences*, 13(4), 1091–1097. doi:10.4314/ahs.v13i4.33
- Ojo, O. E., Fabusoro, E., Majasan, A. A., & Dipeolu, M. A. (2016). Antimicrobials in animal production: Usage and practices among livestock farmers in Oyo and Kaduna States of Nigeria. *Tropical Animal Health and Production*, 48(1), 189–97. doi: 10.1007/s11250-015-0939-8
- Olowe, O. A., Grobbel, M., Büchter, B., Lübke-Becker, A., Fruth, A., & Wieler, L. H. (2010). Detection of blaCTX-M-15 extended-spectrum β -lactamase genes in *Escherichia coli* from hospital patients in Nigeria. *International Journal of Antimicrobial Agents*, 35(2), 206–207. doi:10.1016/j.ijantimicag.2009.10.004
- Sayah, R. S., Kaneene, J. B., Johnson, Y., & Miller, R. (2005). Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. *Applied and Environmental Microbiology*, 71(3), 1394–1404. doi: 10.1016/j.bcab.2012.02.001
- Smith, J. L., Drum, D. J. V., Dai, Y., Kim, J. M., Sanchez, S., Maurer, J. J., ... & Lee, M. D. (2007). Impact of antimicrobial usage on antimicrobial resistance in commensal *Escherichia coli* strains colonizing broiler chickens. *Applied and Environmental Microbiology*, 73(5), 1404–1414. doi: 10.1128/science.1190719
- Yusuf, I., & Adam, R. U. (2014). Adopting Chennai declaration strategies in the prevention and control of the spread of multidrug-resistant hospital-acquired bacterial infections in Nigeria: A call to action. *Journal of Global Antimicrobial Resistance*, 3(2), 203–204. doi: 10.1016/j.jgar.2014.03.003
- Yusuf, I., Arzai, A., & Haruna, M. (2014). Detection of multi drug resistant bacteria in major hospitals in Kano, North-West, Nigeria. *Brazilian Journal of Microbiology*, 45(3), 791–798. doi: 10.1590/S1517-83822014000300005
- Yusuf, I., Arzai, A., Yushau, M., & Garba, L. (2015). Comparing Ebola virus disease and antimicrobial resistance outbreaks in Nigeria - A cross sectional survey of awareness level of health care workers and members of community. *Antimicrobial Resistance and Infection Control*, 4(Suppl 1), P1. doi: 10.1186/2047-2994-4-S1-P1