Prevalence and antibiotic resistant *Escherichia coli* isolated from abattoir and aquaculture environment in Ebonyi State, South East Nigeria.

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Abstract

Background: The present study was carried out to evaluate the distribution and antibiotics profile of *Escherichia coli* from abattoir and aquaculture.

Methods: Abattoir and aquaculture effluents were randomly collected from various parts of Abakaliki in Ebonyi State. Bacterial detection was conducted using cultural and biochemical analysis. Susceptibility of the *E. coli* to antimicrobials was investigated using the Kirby−Bauer disk diffusion method.

Results: The microbial load from abattoirs ranges from $0.26\pm0.11x10^7$ to $4.08\pm0.11x10^7$ cfu/ml and aquacultures $0.40\pm0.04x10^7$ to $4.06\pm2.74x10^7$ cfu/ml differ significantly ($P<0.05$). Out of the total 44 *E. coli* isolates from abattoir, drainage shows the highest *E. coli* isolates (40.9 %) and waste water least (22.7 %), while of the 18 *E. coli* isolates from aquaculture, 55.6 % were from concrete pond, while 44.4 % were from earthen pond. The *E. coli* isolates showed reasonable susceptibility to cefeprime (62.5 %), followed by imipenem (50. 0 %). However, all the *E. coli* isolates were resistant to amoxicillin/clavulanic acid, cefixime, cefotaxime and tobramycin. *E. coli* MAR index range from 0.4 −0.9.

Conclusion: The high microbial load, antibiotic resistance and higher MAR index >2 is of public health concern and further demonstrates the need for adequate treatment and disposal of waste generated from abattoir and aquaculture.

Keywords: *E. coli,* abattoir, aquaculture, antibiotics

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Prévalence et escherichiacoli résistant aux antibiotiques isolés de l'abattoir et de l'environnement aquacole dans l'état d'Ebonyi , au sud-est du Nigéria

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Résumé

Contexte général de l'étude: La présente étude a été réalisée pour évaluer la distribution et le profil d'antibiotiques d'Escherichia *coli* provenant d'abattoirs et d'aquaculture.

Méthode de l'étude : Les effluents d'abattoir et d'aquaculture ont été collectés au hasard dans différentes parties d'Abakaliki dans l'état d'Ebonyi. La détection bactérienne a été effectuée à l'aide d'analyses culturales et biochimiques. La sensibilité d'*E. coli* aux antimicrobiens a été étudiée à l'aide de la méthode de diffusion sur disque de Kirby-Bauer.

Résultat de l'étude : La charge microbienne des abattoirs varie de $0,26\pm0,11\times10^{7}$ à $4,08\pm0,11\times10^{7}$ ufc /ml et les aquacultures de $0,40\pm0,04\times10^{7}$ à $4,06\pm2,74\times10^{7}$ ufc /ml diffèrent significativement ($P<0,05$). Sur le total de 44 isolats *d'E. coli provenant d'abattoir, le drainage montre les* isolats *d'E. coli* les plus élevés (40,9 %) et les eaux usées le moins (22,7 %), tandis que sur les 18 isolats *d'E. coli* provenant de l'aquaculture, 55,6 % provenaient d'étangs en béton, tandis que 44,4 % provenaient d'un étang en terre. Les isolats *d'E. coli* ont montré une sensibilité raisonnable au céféprime (62,5 %), suivi de l'imipénem (50,0 %). Cependant, tous les isolats *d'E. coli* étaient résistants à l'amoxicilline/acide clavulanique , au céfixime, au céfotaxime et à la tobramycine. L'indice *E. coli* MAR varie de 0,4 à 0,9.

Conclusion : La charge microbienne élevée, la résistance aux antibiotiques et l'indice MAR supérieur > 2 sont préoccupants pour la santé publique et démontrent en outre la nécessité d'un traitement et d'une élimination adéquats des déchets générés par les abattoirs et l'aquaculture.

Mots-clés : *E. coli, abattoir, aquaculture, antibiotiques*

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INTRODUCTION

Foodborne infections are widespread and growing public health problems in the world (1). Abattoir, otherwise known as slaughter house is a facility where animals are killed for consumption as food products (2). Like many other sewage types, effluents from the abattoir flow into water bodies such as ground water, streams, rivers, lakes and oceans thereby introducing enteric pathogens, excess nutrients and other contaminants into the water sources (3). Water pollution from abattoir effluents may cause substantial environmental and public health hazards especially in Nigeria, a country where abattoir effluents like most other wastewater are untreated before they are discharged (4, 5, 6, 7). The increasing demand for aquaculture products as a source of protein stimulates the propagation and expansion of aquaculture in many countries (8). Despite the high nutritional quality that links fish consumption to positive health effects in humans, the aquaculture system is tremendously vulnerable to pollution and run-offs from anthropogenic sources which contaminate fish products with microbiological hazards such as *E. coli* and *Salmonella* (9).

Escherichia coli is among the most challenging *Enterobacteriaceae* group of bacterial meat contaminant worldwide (1). *E. coli* is a common inhabitant of human and animal intestinal tract. It is a Gram-negative facultative aerobic organism and the most common in the family Enterobacteriaceae (5). *E. coli* is the most common pathogen leading to uncomplicated cystitis, and also results in other extraintestinal illnesses, including pneumonia, bacteremia, and abdominal infections such as spontaneous bacterial peritonitis (9). The detection of pathogenic organisms as well as the incidence of proteolytic and lipolytic bacteria in a water body is suggestive of impending health hazards and public health concern. Micronutrients in abattoir wastewater sustain the prevalence of pathogenic and entropic organisms that constitute biohazards in water bodies. Antibiotic resistance is a pandemic that requires global health solutions (11). The emergence of antimicrobial resistance among bacterial pathogens demands a local understanding of the epidemiological situation. This information is needed both for clinical treatment decision-making purposes as well as for the revision of current care guidelines (12). Wastewaters are considered hotspots for antibiotic resistant bacteria and horizontal gene transfer among related and unrelated bacterial species (13). Abattoir effluents are potential carriers of resistant pathogenic bacteria and could be contributing to the global spread of these strains in the environments (14). As a consequence of the use of antibiotics in aquaculture, antibiotic resistance is induced in the surrounding bacteria in the column water, sediment, and fish-associated bacterial strains (15). Antibiotics are widely used in intensive fish farming, which in turn increases the emergence of antimicrobial-resistant bacteria in the aquatic environment (8). Pathogenic *E. coli* has become alarming and livestock acquired infection is now one of the leading cause infections globally. Antimicrobial resistance aggravates the already difficult treatment of bacterial infections. Due to many factors influencing antibiotic resistance, the correct choice of antimicrobial management remains debatable. Hence, this has necessitated the research in the distribution and antibiotics susceptibility profile of pathogenic *E. coli* from abattoir and aquaculture effluents in Abakaliki, Ebonyi State.

MATERIALS AND METHODS

Approval and consent for the study were obtained from the owners of the various abattoir and aquaculture farms. The study was conducted from March 2020 to June 2020 at the Microbiology Laboratory of Ebonyi State University Abakaliki, Nigeria.

Sample Collection and Procession

Fifty (50) samples were collected from abattoir waste-water and aquaculture environment within Abakaliki Metropolis, Ebonyi State. The waste water from the abattoir were obtained from drainage, butcher table and wash water. All samples were collected aseptically using universal sterile container. Containers were filled leaving a top space of about 2.5cm. Samples were processed and incubated within 5 hours of sampling. Samples were transported in isothermal boxes with ice to the laboratory of Applied Microbiology Department, Ebonyi State University for *Escherichia coli* analyses.

Isolation, Enumeration, and Identification of *Escherichia coli*

The isolation, identification and enumeration of *E. coli* were carried out using standard microbiological/biochemical methods (16, 17).

Antibiotics Sensitivity Testing

Susceptibility patterns of the isolated *E.*

coli were tested against a wide range of antibiotics namely; Imipenem (10 µg), cefoxitin (30 µg), cefotaxime (30 µg), cefeprime (30 µg), meropenem (10 µg), tobramycin (10 µg) ceftazidime (30 µg) and amoxicillin clavulanic acid (30 µg) on Muller Hinton Agar (Oxoid, UK) using Kirby and Bauer disc diffusion methods of determining susceptibility (16). All the antibiotics disk were procured from Oxoid limited (Oxoid, UK).

Multiple Antibiotic Resistance (MAR) Index

MAR index was determined by following the procedure described by Ayandele ***et al.*** (18).

MAR Index for an isolate =

$$= \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics against which isolate was tested}}$$

Statistical Analysis

The percentage frequency of occurrence of the *E.coli* isolated from abattoir and aquaculture environment was calculated using Frequency (%) = $^{n}/_{N} \times {}^{100}/_{1}$

Where *n* = Number of occurrence of bacteria species, *N* = Total number of bacteria isolated.

Experimental data was presented as mean±standard deviation, while one way ANOVA procedure was used to analyze statistical difference in the data generated.

RESULTS

The result from abattoir shows that samples from location designated AB_3 had significantly ($P<0.05$) high microbial load ($4.08\pm0.11\times10^7$ cfu/ml), followed by AB_2 from waste water ($3.54\pm0.65\times10^7$ cfu/ml), while samples from AB_2 waste water recorded the lowest microbial load ($0.26\pm0.11\times10^7$ cfu/ml)(Table 1). No significant variation ($p>0.05$) was observed with microbial load from sample location designated AB_2 from butchers table, waste water and drainage (Table 1). No significance difference ($p>0.05$) was observed with microbial load of waste water and drainage from sample AB_5 and AB_6, microbial load of waste water and butcher table from sample AB_6, microbial load of drainage and butcher table from sample AB_1 and AB_6 (Table 1).

Result from aquaculture pond revealed a significant ($p<0.05$) difference in their microbial burden showing that PW_2 earthen pond had significantly ($P<0.05$) high microbial load ($4.06\pm2.74\times10^7$ cfu/ml), followed by PW_1 earthen pond ($2.56\pm0.23\ \times10^7$ cfu/ml), while samples from PW_4 concrete ponds recorded the lowest microbial load ($0.40\pm0.04\times10^7$ cfu/ml) as shown in Table 2. No significant variation ($p>0.05$) was observed with microbial load from PW_6 concrete and earthen pond (Table 2).

The result of the prevalence of E. coli showed that AB_3 (33.3 %) had the highest frequency of *E. coli* isolates from drainage, followed by AB_4 (22.2 %), AB_2 (16.2 %), while AB_1 (5.6 %) shows the least prevalence (Figure 1). AB_5 (30.0 %) showed the highest frequency of *E. coli* isolates from waste water, followed by AB_1 (20.0 %), AB_2 (20.0 %), while AB_3, AB_4 and AB_6 (10.0 %) shows the least percentage prevalence (Figure 1). There was also highest frequency of *E. coli* isolates from butcher table from AB_3 (37.5 %), followed by AB_4 (25.0 %), while AB_2 and AB_5 (6.3 %) had the least prevalence (figure 1).

Also, PW_3 (30.0 %) showed the highest *E. coli* from concrete pond, followed by PW_2 (20.0 %), while others (PW_1, PW_4, PW_5 and PW_6) recorded the least with 10.0 % prevalence respectively (figure 2). PW_1 and PW_3 (25.0 %) had the highest *E. coli* from earthen pond, while PW_2, PW_4, PW_5 and PW_6 recorded the least with 12.5 % prevalence each (figure 2).

The result of the antibiotics susceptibility of *E. coli* to the respective antibiotics revealed that the *E. coli* isolates showed highest resistance to amoxicillin/clavulanic acid , cefixime ,cefotaxime and tobramycin at 100 %, , while imipenem recorded the least at 25.0%. Highest susceptibility was observed with cefeprime (62.5 %), followed by imipenem (50. 0 %) (Table 3).

The result of the multiple antibiotics resistance index of the isolates ranges from 0.4 to 0.9 with the mean value of 0.6 (Table 4). The isolate from AB_1 drainage recorded the highest MARI of 0.9 while all the wastewater samples recorded MARI of 0.7 except the one collected from AB_2 whose MAR index was 0.5. The result of the multiple antibiotics resistance index of the *E. coli* isolates from aquaculture environment ranges from 0.5 to 0.7 (Table 4).

DISCUSSION AND CONCLUSION

The findings of the study demonstrated that Escherichia coli existed in high densities in samples in the study area with waste water from AB_3 designated sampling showing the highest microbial load $4.08\pm0.11\times10^7$ cfu/ml, while samples from AB_2 waste water recording the least microbial load $0.26\pm0.11\times10^7$ cfu/ml

(Table1). High bacterial load obtained is due to poor techniques of meat handling and non-hygienic practices used by the butchers. The variations of bacterial load observed in different sample locations were attributed to the hygiene standard in the processing and handling of meat. The result of the present study is in conformity with the findings of Joseph *et al* (19), Atlabachew et al (20) , Gufe *et al*. (14), Egwu *et al*. (21) , Ogunlade *et al*. (22) and Olawale *et al*. (23), who contended that the presence of bacteria in high densities obviously constitute a serious public health hazard as the presence of these microorganisms is associated with water borne diseases. The result from aquaculture samples shows that the sample point designated as PW_2 earthen pond shows the highest microbial load ($4.06\pm2.74x10^7$ cfu/ml) and differs significantly at ($P<0.05$), it was immediately followed by PW_1 earthen pond (2.56 ± 0.23 x10^7 cfu/ml), while samples from PW_4 concrete ponds recorded the lowest microbial load ($0.40\pm0.04x10^7$ cfu/ml) (Table 2). Higher microbial load obtained in earthen pond might be attributed to the use of animal manure in the fertilization of the pond. Pond water is known as an ideal culture medium for the proliferation of bacterial pathogens causing bacterial infection in fish and an important cause of food poisoning (24). High microbial load as obtained from our study corroborates work as reported by other authors (25, 26, 27).

Untreated abattoir effluent constitutes potential reservoir for transmission of pathogenic strains of multiple antibiotic-resistant bacteria by pollution of surface and ground water sources (28). Antibiotics are widely used in intensive fish farming, which in turn increases the emergence of antimicrobial-resistant (AMR) bacteria in the aquatic environment (8). Antimicrobial resistance threatens infectious disease management outcomes, especially in developing countries (29). *E. coli* isolates shows highest resistances to amoxicillin/clavulanic acid, cefixime, cefotaxime and tobramycin at 100 %, followed by meropenem and ceftazidime (Table 3). High resistance of antibiotics of human value as obtained in our study was previously reported by other authors both in Nigeria and elsewhere (Datok *et al*. (30) and Wu *et al*. 31). However, contrary report have been obtained from several authors (Mapanguy *et al*. 32, Aabed *et al*. 33, Montagnani *et al*. 34 and Isac *et al* 35)

Multiple antibiotic resistance index (MARI) is an effective, valid, and cost-effective method that is used in source tracking of antibiotic resistant organisms (36). MARI is an important analysis to check antibiotic resistance and health risk factors (37). Organisms which have MAR indices of greater than $\geq$0.2 confirm the presence of multidrug-resistant genes originating from the environment where there is an abuse of these drugs and also that the plasmids contain one or more resistance genes, each encoding a single antibiotic resistance phenotype (38. 39). From the abattoir and aquaculture samples analyzed, *E. coli* MAR indices were greater than 0.2. It was an evidence that all the *E. coli* isolates originated from potentially dangerous sources where antibiotics are frequently used and possibly was introduced through fecal contamination. The result of the study agrees with work of Adinortey *et al*. (40); Afunwa *et al*., (37) and Kusunur *et al*. (41). The result from the study authenticated that there are high level of exposure to antibacterial agents in cat fish reared/sold within Abakaliki metropolis. The study implicated not just the presence highly pathogenic *E. coli* but also highly resistance pathogens in abattoir and aquaculture wastes. Hence wastewater from abattoir and aquaculture should be treated before discharge into soil and water bodies. Steps must be taken now in other to avoid a health crisis in the anticipated future.

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REFERENCES

1. Tadese, N.D., Gebremedhi, E.Z., Moges, F., Borana, B.M., Marami, L.M., Sarba, E.J., Abebe, H., Kelbesa, K.A., Atalel, D. and Tessema, B. Occurrence and antibiogram of *Escherichia coli* O157 : H7 in raw beef and hygienic practices in abattoir and retailer shops in Ambo town, Ethiopia. *Veterinary Medicine International*. 2021; 1: 1-12.
2. Dike-Ndudim, J.N., Ugwuegbu, R.N., Amah, H.C., Ndubueze, C.W. and Anikwo, E.S. Identity and antibiogram of bacterial isolates from Owerri Modern Abattoir, Imo State, Nigeria *Journal of Advances in Microbiology.* 2021; *21(10): 43-54.*
3. Nafarnda, W. D., Ajayi, I. E., Shawulu, J. C., Kawe, M. S., Omeiza, G. K., Sani, N. A., Tenuche, O.Z., Dantong, D. D. & Tags, S. Z.

Bacteriological Quality of Abattoir Effluents Discharged into Water Bodies in Abuja, Nigeria. ISRN Veterinary Science, 2012; 15.

4. Adegoke, A.A., Madu, C.E.,Aiyegoro, O.A., Stenström, T.A. and Okoh, A.I. Antibiogram and beta-lactamase genes among cefotaxime resistant *E. coli* from wastewater treatment plant. *Antimicrobial Resistance and Infection Control*. 2020; 11(2): 1-12.
5. Adebami, G.E., Fasiku, S.A., Solomon, O.D. and Babalola, B.A. Physicochemical and microbial evaluations of different fish ponds' wastewaters and the antibiotics profiles of isolated bacteria. *Ethiopian Journal of Environmental Studies and Management.* 2021; 13(4): 509 – 521.
6. Adesina, A.O., Ogunyebi, A.L., Fingesi, T.S. and Oludoye, O.O. Assessment of Kara abattoir effluent on the water quality of Ogun River, Nigeria. *Journal of Applied Science and Environmental Management.* 2018; 22 (9): 1465 –1470.
7. Elemile, O.O., Raphael, D.O., Omole, D.O., Oloruntoba, E.O., Ajayi, E.O. and Ohwavborua, N.A. Assessment of the impact of abattoir effluent on the quality of groundwater in a residential area of Omu-Aran, Nigeria. *Environmental Sciences Europe.* 2019; 31(16): 1-10.
8. Dewi, R.R., Hassan, L., Daud, H.M., Matori, M.F., Nordin, F., Ahmad, N.I. and Zakaria, Z. Prevalence and antimicrobial resistance of *Escherichia coli*, *Salmonella* and Vibrio derived from farm-raised red hybrid tilapia (*Oreochromis* spp.) and Asian Sea Bass (Lates calcarifer, Bloch 1970) on the West Coast of Peninsular Malaysia. *Antibiotics.* 2022;11(136): 1-21.
9. Cabello, F.C. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology*. . 2006; 8: 1137–1144.
10. Tainter, C.R. and Mueller, M. *Escherichia coli*. StatPearls Publication, San Diego, USA. P. 1. 2021
11. Hernando-Amado, S., Coque, T.M., Baquero, F. and Martínez, J.L. Antibiotic resistance: Moving from individual health norms to social norms in one health and global health. *Frontiers in Microbiology*. 2020; 11(1914): 1-20.
12. Haindongo, E.H., Funtua, B., Singu, B., Hedimbi, M., Kalemeera, F., Hamman, J., Vainio, O., Hakanen, A.J. and Vuopio, J. Antimicrobial resistance among bacteria isolated from urinary tract infections in females in Namibia, 2016–2017. *Antimicrobial Resistance and Infection Control*. 2022; 11(33): 1-8.
13. Edward, K.C., Ibekwe, V.I., Amadi E.S. and Umeh, S.I. Prevalence and antibiotic susceptibility pattern of *Escherichia coli* isolated from abattoir wastewaters in Abia State, Nigeria. *International Research Journal of Public and Environmental Health*. 2020; 7 (5): 140-148.
14. Gufe, C., Ndlovu, M.N., Sibanda, Z., Makuvara, Z. and Marumure, J. Prevalence and antimicrobial profile of potentially pathogenic bacteria isolated from abattoir effluents in Bulawayo, Zimbabwe. *Scientific African*. 2021; 14: 1-10.
15. Pepi, M. and Focardi, S. Antibiotic-resistant bacteria in aquaculture and climate change: A challenge for health in the Mediterranean area. *International Journal of Environmental Research and Public Health.* 2021; 18 (57230): 1-31.
16. Cheesbrough, M. District laboratory practice in tropical countries, Part 2. Cambridge University Press, Cambridge, UK. 2006; 23-78, 137-159.
17. Abrahim, A., Sergelidis, D., Kirkoudis, I., Anagnostou, V., Kaitsa-Tsiopoulou, E., Kazila, P. and Papa, A. Isolation and antimicrobial resistance of *Staphylococcus* spp. in freshwater fish and Greek market places. *Journal of Aquatic Food Product Technology*. 2010; 19: 93–102.
18. Ayandele, A.A., Oladipo, E.K., Oyebisi, O. and Kaka, M.O. Prevalence of multi-antibiotic resistant *Escherichia coli* and *Klebsiella* species obtained from a tertiary medical institution in Oyo State, Nigeria. *Qatar Medical Journal* 2020; 9: 1-6.
19. Joseph, M.O., Ibrahim, B., Zaky, S.K., S. Abdulkadir and Auta, I.K. Bacterial assessment of effluents from selected abattoirs into adjoining water bodies in Kaduna Metropolis. *Science World Journal.* 2021;*16(1): 1-6.*
20. Atlabachew, T. and Mamo, J. Microbiological quality of meat and swabs from contact surface in butcher shops in Debre Berhan, Ethiopia. 2021; 1-11.
21. Egwu, O.C., Wahab, O.O., Dickson, M.A., Ucheoma, E.U. , Osuji C., Akudo, O.C., Amanabo, M. and Samuel, U.C. Ecological risk assessment of toxic elements, microbial loads, parasites and antinutritional factors in *Telfairia occidentalis* grown on sewage contaminated soil. *Acta Ecologica Sinica*. 2021; 1: 1-10.
22. Ogunlade, M.T., Babaniyi, B., Afolabi, J.F. and Babaniyi, G.G. Physicochemical, heavy metals and microbiological assessment of wastewater in selected abattoirs in Ekiti State, Nigeria. *Journal of Environmental Treatment Techniques*. 2021; *9*(4): 788-795.
23. Olawale, S.I., Busayo, O.M., Olatunji, O.I., Mariam, M. and Olayinka, O.S. Plasmid profiles and antibiotic susceptibility patterns of bacteria isolated from abattoirs wastewater within Ilorin, Kwara, Nigeria. *Iranian Journal of Microbiology.* 2020; *12(6)*: *547-555.*
24. Eze, V.C. and Ogbaran, I.O. Microbiological and physicochemical characteristic of fish pond water in Ughelli, Delta State Nigeria.

International Journal of Current Research. 2010; 8: 082‒087.

25. Njoku, O.E., Agwa, O.K. and Ibiene, A.A. An investigation of the microbiological and physicochemical profile of some fish pond water within the Niger Delta region of Nigeria. *African Journal of Food Science.* 2015; 9(3): 155‒162.
26. Osuntokun, O.T., Muniru , A.A. and Morenike , K.T. Bacteriological assessment of African catfish (*Clarias gariepinus*) isolated from earthen and concrete fish Pond. *Asian Journal of Biochemistry, Genetics and Molecular Biology*. 2021: 6(1): 17‒34
27. Abdulkadir A., Abubakar, M.I. and Abdulkadir O.J. Investigative study on the bacteriological, physical and chemical profiles of aquaculture waters: insights into health hazards for fish and human. 2021
28. Akpan, S.N., Odeniyi, O.A., Adebowale, O.O., Alarape, S.A. and Adeyemo, O.K. Antibiotic resistance profile of Gram‒negative bacteria isolated from Lafenwa abattoir effluent and its receiving water (Ogun River) in Abeokuta, Ogun state, Nigeria. *Onderstepoort Journal of Veterinary Research.* 2020; 87(1): a1854.
29. Bekoe, S.O., Hane‒Weijman, S., Trads, S., Orman, E., Opintan, J., , M., Frimodt‒Møller, N. and Styrishave, B. Reservoir of antibiotic residues and resistant coagulase negative *Staphylococci* in a healthy population in the Greater Accra Region, Ghana. *Antibiotics*. 2022; *11(*1): 119.
30. Datok, D.W., Ishaleku, D., Tsaku, P.A., Agya, E.O. and Adoga, M.P. Multidrug resistance to commonly prescribed antibiotics in *Escherichia Coli* isolated from barbecued beef (Suya) sold in a Nigerian City. *Pan African Medical Journal*. 2021; 39(50): 1‒8.
31. Wu, D., Ding, Y., Yao, K., Gao, W. and Wang, Y. Antimicrobial resistance analysis of clinical Escherichia coli isolates in neonatal ward. *Frontiers in Pediatrics. . 2021; 9: 1-7.*
32. Mapanguy, C.C.M., Adedojaa, A., Keckaa, L.G.V., Vouvounguia, J.C., Nguimbib, E., Velavana, T.P. and Ntoumi, F. High prevalence of antibiotic‒resistant *Escherichia coli in* Congolese students. *International Journal of Infectious Diseases*. 2021;103: 119–123.
33. Aabed, K., Moubayed, N. and Alzahrani, S. Antimicrobial resistance patterns among different *Escherichia coli* isolates in the Kingdom of Saudi Arabia. *Saudi Journal of Biological Sciences*. 2021; 28(7): 3776‒3782.
34. Montagnani, C., Tersigni, C., D'Arienzo, S., Miftode, A., Venturini, A., Bortone, B., Bianchi, L., Chiappini, E., Forni, S., Gemmi, F. and Galli, L. *Infection and Drug Resistance*. 2021;14: 2341–2348.
35. Isac, R., Basaca, D.G., Olariu, I.C., Stroescu, R.F., Ardelean, A.M., Steflea, R.M., Gafencu, M., Chirita‒Emandi, A., Bagiu, I.C., Horhat, F.G., Vulcanescu, D., Ionescu, D. and Doros, G. Antibiotic resistance patterns of uropathogens causing urinary tract infections in children with congenital anomalies of kidney and urinary tract. *Children*. 2021; 8(585): 1‒11.
36. Sandhu, R., Dahiya, S. Sayal, P. Evaluation of multiple antibiotic resistance (MAR) index and Doxycline susceptibility of *Acinetobacter* species among inpatients. *Indian Journal of Microbiology Research*. 2016; 3(3): 299–304.
37. Afunwa, R.A., , J., Afunwa, E.C., Udeh, A.S., Oli, A.N. and Unachukwu, M. Multiple antibiotic resistant index of gram‒negative bacteria from bird droppings in two commercial poultries in Enugu, Nigeria. *Open Journal of Medical Microbiology.* 2020*;* 10 (4): 171‒181.
38. Riaz, S., Faisal, M. and Hasnain, S. Antibiotic susceptibility pattern and multiple antibiotic resistance (MAR) calculation of extended spectrum β-lactamase (ESBL) Producing *Escherichia coli* and *Klebsiella* Species in Pakistan. *African Journal of Biotechnology*. 2011; 10: 6325‒6331.
39. Ejiofor, S.O., Edeh, A.D., Ezeudu, C.E., Gugu, T.H. and Oli, A.N. Multi‒drug resistant acute otitis media amongst children attending out‒patient clinic in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, South‒East Nigeria. *Advances in Microbiology*. 2016; 6: 495‒501.
40. Adinortey, C.A., Aheto, D.W., Boateng, A.A. and Agbeko, R. Multiple antibiotic resistance‒coliform bacteria in some selected fish farms of the central region of Ghana. *Scientifica*. 2020;1: 1‒11.
41. Kusunur, A.B., Kuraganti, G.K., Mogilipuri, S.S., Vaiyapuri, M., Narayanan, S.V. and Badireddy, M.R. Multidrug resistance of *Escherichia coli* in fish supply chain: A preliminary investigation. *Journal of Food Safety*. 2022; 1:1.

Table 1 : **Microbial load (x10^7 cfu/ml) from abattoir samples**

Sample code	Waste Water	Drainage	Butcher Table
AB_1	0.76 ± 0.02^a	1.86 ± 0.28^b	1.29 ± 0.01^b
AB_2	3.54 ± 0.65^a	1.31 ± 0.27^b	0.26 ± 0.11^c
AB_3	4.08 ± 0.11^a	1.60 ± 0.66^b	3.18 ± 1.44^c
AB_4	0.40 ± 0.33^a	2.20 ± 0.28^b	1.04 ± 0.05^c
AB_5	1.19 ± 0.65^a	1.32 ± 0.04^a	2.22 ± 0.85^b
AB_6	1.19 ± 0.37^a	1.50 ± 0.98^a	1.92 ± 0.33^a

Key: AB= Abattoir, Values were mean ± standard deviation (SD),
Values with no common superscripts within the same row are significantly different at $p < 0.05$,
Values with same superscripts within same row are not significantly different ($P > 0.05$).

Table 2: **Microbial load (x10^7cfu/ml) from aquaculture environment**

Sample Code	Concrete Pond	Earthen Pond
PW_1	0.66 ± 0.19^a	2.56 ± 0.23^b
PW_2	1.02 ± 0.02^a	4.06 ± 2.74^b
PW_3	1.50 ± 0.25^a	0.70 ± 0.14^b
PW_4	0.40 ± 0.04^a	1.50 ± 0.09^b
PW_5	1.92 ± 0.03^a	2.22 ± 0.88^b
PW_6	1.86 ± 0.03^a	1.29 ± 0.01^a

Key: PW= Pond water, Values were mean ± standard deviation (SD),
Values with no common superscripts within the same row are significantly different at $p < 0.05$,
Values with same superscripts within same row are not significantly different ($P > 0.05$).

Table 3: Antibiotics sensitivity pattern of the *E. coli* isolates

Antibiotics	Resistance (%)	Intermediate (%)	Susceptibility (%)
IPM	2(25.0)	2(25.0)	4(50.0)
AMC	8(100)	0(0)	0(0)
CXM	8(100)	0(0)	0(0)
CTX	8(100)	0(0)	0(0)
TOB	8(100)	0(0)	0(0)
FEP	3(37.5)	0(0)	5(62.5)
MEM	6(75.0)	0(0)	2(25.0)
CAZ	6(75.0)	0(0)	2(25.0)

Key: IPM = Imipenem, CTX= Cefotaxime, CXM= Cefixime, FEP = Cefeprime,
MEM = Meropenem, TOB = Tobramycin, CAZ = Ceftazidime,
AMC= Amoxicillin/clavulanic acid.

Table 4: Multiple Antibiotics Resistance Index (MARI) of the *E. coli* Isolates

Sample	Sample Code	MARI	ANTIBIOTICS
Drainage	AB_1	0.9	IPM, AMC, CTX, TOB, FEP, MEM, CAZ
	AB_2	0.6	AMC, CTX, TOB, MEM, CAZ
	AB_3	0.4	AMC, CTX, TOB
	AB_4	0.6	AMC, CTX, TOB, MEM, CAZ
	AB_5	0.5	AMC, CTX, TOB, MEM
	AB_6	0.7	AMC, CTX, TOB, FEP, MEM, CAZ
Waste Water	AB_1	0.7	AMC, CTX, TOB, FEP, MEM, CAZ
	AB_2	0.5	AMC, CTX, TOB, CAZ
	AB_3	0.7	AMC, CTX, TOB, FEP, MEM, CAZ
	AB_4	0.7	AMC, CTX, TOB, FEP, MEM, CAZ
	AB_5	0.7	AMC, CTX, TOB, FEP, MEM, CAZ
	AB_6	0.7	AMC, CTX, TOB, FEP, MEM, CAZ
Butchers Table	AB_1	0.5	AMC, CTX, TOB, CAZ
	AB_2	0.5	AMC, CTX, TOB, MEM
	AB_3	0.6	AMC, CTX, TOB, MEM, CAZ
	AB_4	0.6	AMC, CTX, TOB, MEM, CAZ
	AB_5	0.6	AMC, CTX, TOB, MEM, CAZ
	AB_6	0.6	AMC, CTX, TOB, MEM, CAZ
Concrete pond	PW_1	0.5	AMC, CTX, TOB, MEM
	PW_2	0.6	AMC, CTX, TOB, MEM, CAZ
	PW_3	0.6	AMC, CTX, TOB, MEM, CAZ
	PW_4	0.6	AMC, CTX, TOB, FEP, CAZ
	PW_5	0.7	AMC CTX, TOB, FEP, MEM, CAZ
	PW_6	0.7	AMC, CTX, TOB, FEP, MEM, CAZ
Earthen pond	PW_1	0.5	AMC, CTX, TOB, CAZ
	PW_2	0.6	AMC, CTX, TOB, MEM, CAZ
	PW_3	0.5	AMC, CTX, TOB, CAZ
	PW_4	0.6	AMC, CTX, TOB, FEP, CAZ
	PW_5	0.6	AMC, CTX, TOB, MEM, CAZ
	PW_6	0.7	AMC, CTX, TOB, FEP, MEM, CAZ
	Mean	0.6	

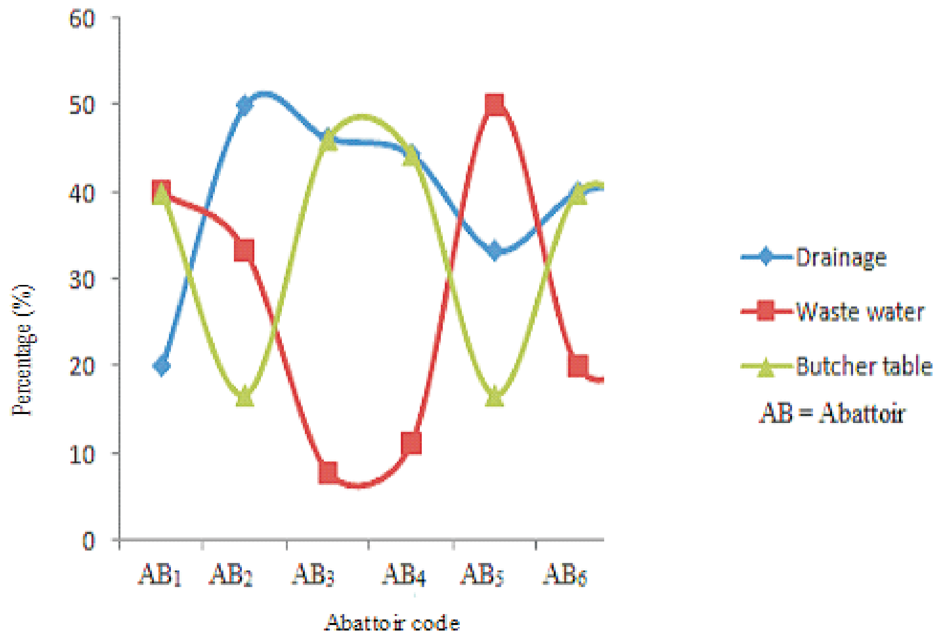

Figure 1: Prevalence of *E. coli* across the different abattoir sample sources

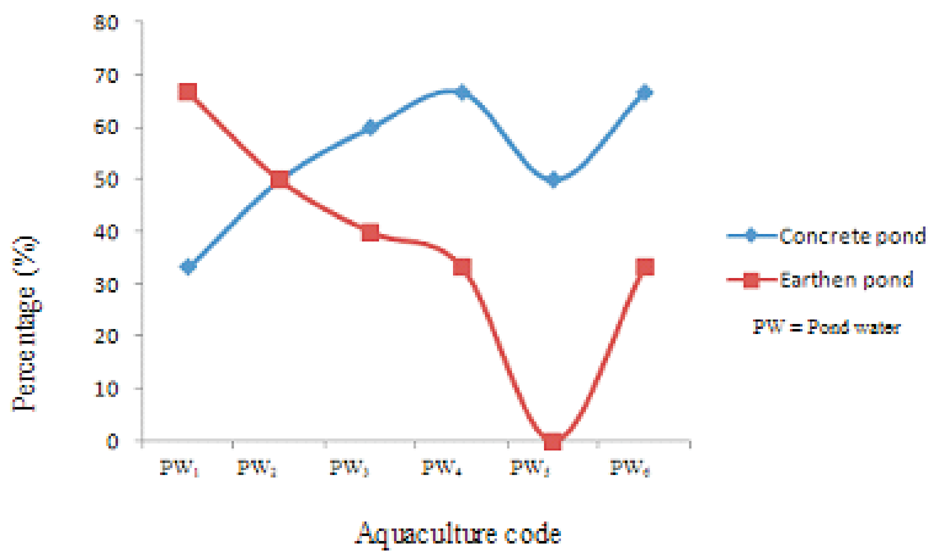

Figure 2: Prevalence of *E. coli* across the different aquaculture sample sources