JOURNAL OF FOOD QUALITY & HAZARDS CONTROL
SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 8 (2021) 186-189

Acquired Antimicrobial Resistance Genes of *Escherichia coli* Obtained from Nigeria: *In silico* Genome Analysis

O. Nwaiwu [1*], H. Onyeaka [2]

1. School of Biosciences, University of Nottingham, Sutton Bonington Campus, LE12 5RD, United Kingdom
2. School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

HIGHLIGHTS

- A total of 107 antimicrobial resistance genes, which included genes that encode for 24 extended-spectrum beta-lactamases were detected.
- Twenty four strains harboured over 20 antimicrobial resistant genes.
- Acquisition of resistance genes in this set of *E. coli* genomes from Nigeria is intra-species.

Article type
Short communication

Keywords
Escherichia coli
beta-Lactamases
Drug Resistance; Microbial
Genome
Nigeria

Article history
Received: 9 Sep 2021
Revised: 3 Nov 2021
Accepted: 12 Nov 2021

ABSTRACT

Background: Antimicrobial resistance is a global problem with enormous public health and economic impact. This study was carried out to get an overview of acquired antimicrobial resistance gene sequences in the genomes of *E. coli* isolated from different food sources and the environment in Nigeria.

Methods: To determine the acquired antimicrobial-resistant genes prevalence, genome assemblies of 272 isolates were analyzed *In silico* with KmerResistance 2.2 software.

Results: A total of 107 antimicrobial resistance genes, which included genes that encode for 24 extended-spectrum beta-lactamases were detected. Potential multidrug resistance was found in 90% of the genomes analyzed. All strains analyzed contained at least one resistant gene sequence and had high similarity or homology (95% ID and above). Two strains harboured over 30 sequences of antimicrobial resistant genes, and in 24 strains over 20 genes were detected.

Conclusion: The resistant genes found in all the genomes analyzed were acquired intra-species and not inter-species. This provides an opportunity for further studies of the orthologous nature of the genes detected and the data obtained can help monitor the epidemiology of *E. coli* resistant genes in the food and environment.

Introduction

Escherichia coli, a member of the Enterobacteriaceae family, has earned a top spot in the microbe's world due to its notorious ability to cause infections in humans and animals and invade the efficacy of antibiotics (Poirel et al., 2018). It has attracted the attention of public health experts, stakeholders, and policymakers who are concerned about its impact on public health (Dadgostar, 2019; Hofer, 2019). Despite its pathogenic properties, *E. coli* also represents a significant part of the indigenous microbiota, and has been the primary microbe driving most research on biotechnological innovations (Yeung et al., 2019). Multidrug resistance is a big concern especially in the treatment of food-borne diseases of humans and animals (Palma et al., 2020). This issue is

* Corresponding author (O. Nwaiwu)
✉ E-mail: ogueri.nwaiwu@nottingham.ac.uk
ORCID ID: https://orcid.org/0000-0003-0794-0866

To cite: Nwaiwu O., Onyeaka H. (2021). Acquired antimicrobial resistance genes of *Escherichia coli* obtained from Nigeria: *in silico* genome analysis. *Journal of Food Quality and Hazards Control*. 8: 186-189.

DOI: 10.18502/jfqhc.8.4.8260 Journal website: http://jfqhc.ssu.ac.ir

compounded by their impressive ability to effortlessly act as a donor and as a recipient of resistance genes through accumulation, and then passing them on to other microbes, mostly through horizontal gene transfer (Partridge et al., 2018). Not surprisingly, in the last decades, a wide range of antimicrobial resistance genes have been identified in *E. coli* (Choi and Yoo, 2019).

This work focuses on the monitoring of *E. coli* in the environment, which is very important for food safety. Efficient surveillance will help ascertain the development of new strains or trends among different food sources and their environment. Thousands of genomes have been studied which enabled scientists and the public to gain more insights into different organisms and their antimicrobial resistance capabilities. However, most of the antimicrobial resistance genomic information available in the literature are from the developed world and there is now a need to report significant developments in less developed countries to help environmental monitoring. Hence, this study aimed at highlighting the antimicrobial resistance genes sequences present in the *E. coli* genomes obtained from Nigeria.

Materials and methods

To ascertain a wider perspective of antimicrobial resistant genes in *E. coli* strains in Nigeria, a search in the biosamples browser of the Genbank® database was performed. The terms "*Escherichia coli"* and "Nigeria" were used for the search. This yielded 446 documented genome assembly submissions. After examination, 272 strains, which had publicly available genome nucleotide sequences, were analyzed further. The genome assemblies were from strains, which included isolates from the endocervical, wound swabs, chicken, human stool, blood, cow milk, and water. To determine the prevalence of acquired antimicrobial-resistant genes, the genome assemblies were analyzed *in silico* with KmerResistance 2.2 software (Clausen et al., 2016, 2018), which can demonstrate high congruency between *in silico* and standard laboratory results. The host and gene databases selected were 'bacteria and resistance genes' while the identity threshold was the default setting of 70% with a depth correction of 10%. The sequences of antimicrobial resistant genes, which had similarities with reference strains were recorded. Homologous antimicrobial resistant gene sequences obtained after comparison with templates or reference genes were deposited in a public repository.

According to the developers (Clausen et al., 2016) of the software used, it was designed to avoid gaining multiple hits due to identical k-mers between genes in the database, in a way that each k-mer is first only assigned to the gene with the highest number of unique k-mer matches. Then after this, the k-mers mapping to the best hit are removed and the procedure is repeated with the remaining reads to obtain an estimate of both depth and coverage. The method is believed to be reliable and it has high concordance with traditional phenotypic susceptibility testing. To determine whether the strains analyzed are potentially multidrug resistant, classification was carried out based on previous work (Nwaiwu and Aduba, 2020) by checking how many antibiotic drug classes were present in each genome. This approach is in line with methods developed by others (Magiorakos et al., 2012).

Results and discussion

The output from the *in silico* analysis (Nwaiwu, 2021) carried out was used to identify reference genomes. The prevalence of the resistant genes is shown in Figure 1. Only genes that were found in 30 or more strains are displayed (Figure 1) and it was found that the gene *mdf*(A)- (Y08743) was present in all 272 strains analyzed. The gene was first characterized as a multidrug-resistant gene with an extraordinarily broad spectrum of drug resistance in *E. coli* (Edgar and Bibi, 1997). In addition to the common sulphonamide resistant genes *sul1* and *sul2* genes, *sul3* genes were present but was found in fewer strains. The predominant tetracycline gene *tet*(A)-(AJ517790) among other genes in that group was found in 171 strains whereas the gene *aph*(3")-Ib (AF321551), which was the most prevalent resistant gene to aminoglycosides was found in 168 strains (Figure 1).

Only five out of the 24 extended-spectrum beta-lactamases genes were detected in 30 or more strains and the predominant gene was $bla_{\text{TEM-1B}}$ (AY458016) which was found in 146 (53%) strains (Figure 1). This is average when compared to other reports of the prevalence of extended-spectrum beta-lactamases in *E. coli* and other Enterobacteriaceae in Nigeria, which is sometimes low or high (Jesumirhewe et al., 2020; Musa et al., 2020; Ojo et al., 2016; Tanko et al., 2020). The presence of $bla_{\text{CTX-M-15}}$ (AY044436) was found in less than half (103/272) of the total strains analyzed. Of note is that $bla_{\text{CTX-M-15}}$ was detected in isolates from both human and non-human derived strains e.g. water, chicken, human vaginal swab, urine, wound swab, blood, and cerebrospinal fluid. Other workers also found multidrug resistance in chickens and humans (Aworh et al., 2020). A consensus in the literature is that a predominant gene in one location may not necessarily be dominant in another.

A shift in CTX-M enzymes spread and increasing occurrence of the emerging $bla_{\text{CTX-M-27}}$ (Castanheira et al., 2021) was suggested after it was found in two strains from chicken by others (Ayeni et al., 2020). This data

[DOI: 10.18502/jfqhc.8.4.8260]

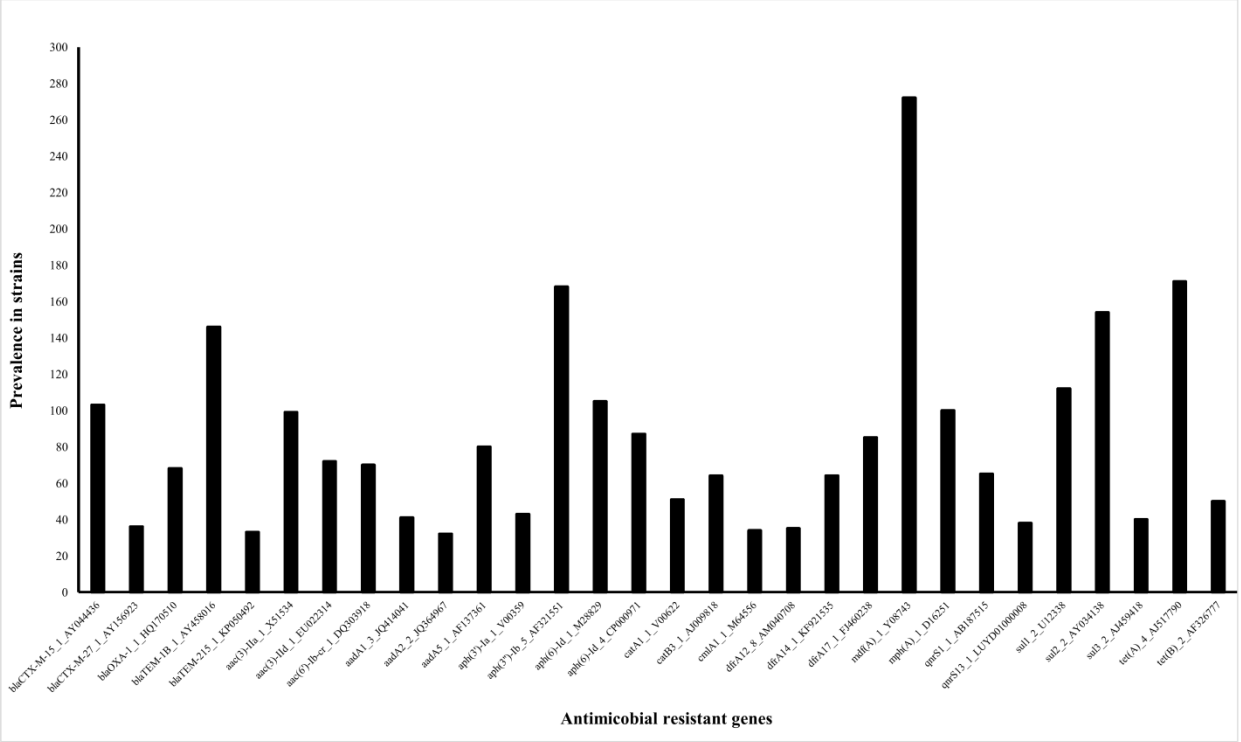

Figure 1: Prevalence of acquired antimicrobial resistance genes in genomes of *Escherichia coli* obtained from Nigerian strains. Genes shown occurred in 30 isolates or more.

shows that the gene sequence is also present in strains obtained from a wound (strain NHA52; AAXCOM000000000), and endocervical (NHA016; AAXCQR000000000) swabs. Other CTX-M gene family found included $bla_{\text{CTX-M-14, 17, 24, 27, 55, 65, 216}}$. The template sequence detected varied among strains analyzed but only strains of *E. coli* was detected for all samples. The isolate 14EC001 was the most referenced strain predicted in 35 isolates followed by strain Ecol_AZ147 (CP018995.1) in 25 genomes. Other reference genomes predicted for 10 or more isolates include *E. coli* strains WAT (CP012380.1), WCHEC4533 (CP028589.1), and ATCC 8739. The strain NHA 065 (AAXCOV000000000) had the reference genome from an *E. coli* strain (CP031653.1) isolated in Liverpool, United Kingdom, which suggests strain mobility. When the genomes were checked for possible multidrug resistance, it was found that up to 244 of out of the 272 (89.7%) assemblies studied harboured up to three sequences of different antimicrobial resistant drug classes which is a very big concern. These characteristics may have contributed immensely to the decreased number of effective treatment regimens and increased the risk of poor clinical outcomes (Hinić et al., 2015).

Conclusion

Overall, the study outlines acquired genes in *E. coli* from different sources and provides an opportunity for further comparisons with strains implicated in food-borne outbreaks. The prevalence of antimicrobial resistant genes is a food safety concern and information highlighted in this work may help local scientists and the government to initiate a more robust surveillance program. The resistant genes found in all the genomes analyzed were acquired intra-species and not inter-species and this provides a starting point for further studies of the orthologous nature of the genes detected. Epidemiologists and health officers in charge of disease control can use the information generated to develop a preventive approach by monitoring emerging antimicrobial-resistant patterns to drive better use of antibiotics of animal food production and human treatment in hospitals. The finding that *E. coli* strains from cow milk harboured fewer antimicrobial resistant genes than other sources, provides useful information that indicates post-process contamination and exposure of food products to the environment, which is common in Nigeria may aid resistance genes acquisition. The data can contribute to an initial holistic

[DOI: 10.18502/jfqhc.8.4.8260]

epidemiological analysis to track emerging events of antimicrobial resistant genes, and trends in Nigeria where antibiotics abuse is a huge concern.

Author contributions

O.N. designed the study and carried out the work; O.N. and H.O. analyzed the data and wrote the manuscript. Both authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships, which have or could be perceived to have influenced the work reported in this article.

Acknowledgements

Authors acknowledge the Center for Genomic Epidemiology, Denmark for use of k-mer software. This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Aworh M.K., Kwaga J., Okolocha E., Harden L., Hull D., Hendriksen R.S., Thakur S. (2020). Extended-spectrum ß-lactamase-producing *Escherichia coli* among humans, chickens and poultry environments in Abuja, Nigeria. *One Health Outlook*. 2: 8. [DOI: 10.1186/s42522-020-00014-7]

Ayeni F.A., Falgenhauer J., Schmiedel J., Schwengers O., Chakraborty T., Falgenhauer L. (2020). Detection of *bla*$_{CTX-M-27}$-encoding *Escherichia coli* ST206 in Nigerian poultry stocks. *Journal of Antimicrobial Chemotherapy*. 75: 3070-3072. [DOI: 10.1093/jac/dkaa293]

Castanheira M., Simner P.J., Bradford P.A. (2021). Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. *JAC-Antimicrobial Resistance*. 3: dlab092. [DOI: 10.1093/jacamr/dlab092]

Choi J.-K., Yoo J.-H. (2019). Increasing antimicrobial resistance of *Escherichia coli* makes antimicrobial stewardship more important. *Journal of Korean Medical Science*. 34: e236. [DOI: 10.3346/jkms.2019.34.e236]

Clausen P.T.L.C., Aarestrup F.M., Lund O. (2018). Rapid and precise alignment of raw reads against redundant databases with KMA. *BMC Bioinformatics*. 19: 307. [DOI: 10.1186/s12859-018-2336-6]

Clausen P.T.L.C., Zankari E., Aarestrup F.M., Lund O. (2016). Benchmarking of methods for identification of antimicrobial resistance genes in bacterial whole genome data. *Journal of Antimicrobial Chemotherapy*. 71: 2484-2488. [DOI: 10.1093/jac/dkw184]

Dadgostar P. (2019). Antimicrobial resistance: implications and costs. *Infection and Drug Resistance*. 12: 3903–3910. [DOI: 10.2147/IDR.S234610]

Edgar R., Bibi E. (1997). MdfA, an *Escherichia coli* multidrug resistance protein with an extraordinarily broad spectrum of drug recognition. *Journal of Bacteriology*. 179: 2274–2280. [DOI: 10.1128/jb.179.7.2274-2280.1997]

Hinić V., Ziegler J., Straub C., Goldenberger D., Frei R. (2015). Extended-spectrum β-lactamase (ESBL) detection directly from urine samples with the rapid isothermal amplification-based eazyplex® SuperBug CRE assay: proof of concept. *Journal of Microbiological Methods*. 119: 203-205. [DOI: 10.1016/j.mimet.2015.10.015]

Hofer U. (2019). The cost of antimicrobial resistance. *Nature Reviews Microbiology*. 17: 3-3. [DOI: 10.1038/s41579-018-0125-x]

Jesumirhewe C., Springer B., Allerberger F., Ruppitsch W. (2020). Whole genome sequencing of extended spectrum β-lactamase genes in *Enterobacteriaceae* isolates from Nigeria. *PLoS One*. 15: e0231146 . [DOI: 10.1371/journal.pone.0231146]

Magiorakos A.-P., Srinivasan A., Carey R.B., Carmeli Y., Falagas M.E., Giske C.G., Harbarth S., Hindler J.F., Kahlmeter G., Olsson-Liljequist B., Paterson D.L., Rice L.B., et al. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*. 18: 268-281. [DOI: 10.1111/j.1469-0691.2011.03570.x]

Musa B.M., Imam H., Lendel A., Abdulkadir I., Gumi H.S., Aliyu M.H., Habib A.G. (2020). The burden of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in Nigeria: a systematic review and meta-analysis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 114: 241-248. [DOI: 10.1093/trstmh/trz125]

Nwaiwu, O. (2021). Output from KmerResistance data base 2.2 of acquired antimicrobial resistance genes from Nigerian *E. coli* genomes", Mendeley Data, V1. [DOI: 10.17632/jkjcmtzvps.1]

Nwaiwu O., Aduba C.C. (2020). An *in silico* analysis of acquired antimicrobial resistance genes in *Aeromonas* plasmids. *AIMS Microbiology*. 6:75-91. [DOI: 10.3934/microbiol.2020005]

Ojo O.E., Schwarz S., Michael G.B. (2016). Detection and characterization of extended-spectrum β-lactamase-producing *Escherichia coli* from chicken production chains in Nigeria. *Veterinary Microbiology*. 194: 62–68. [DOI: 10.1016/j.vetmic.2016.04.022]

Palma E., Tilocca B., Roncada P. (2020). Antimicrobial resistance in veterinary medicine: an overview. *International Journal of Molecular Sciences*. 21: 1914. [DOI: 10.3390/ijms21061914]

Partridge S.R., Kwong S.M., Firth N., Jensen S.O. (2018). Mobile genetic elements associated with antimicrobial resistance. *Clinical Microbiology Reviews*. 31: e00088-17. [DOI: 10.1128/CMR.00088-17]

Poirel L., Madec J.-Y., Lupo A., Schink A.-K., Kieffer N., Nordmann P., Schwarz S. (2018). Antimicrobial resistance in *Escherichia coli*. *Microbiology Spectrum*. 6. [DOI: 10.1128/microbiolspec.ARBA-0026-2017]

Tanko N., Bolaji R.O., Olayinka A.T., Olayinka B.O. (2020). A systematic review on the prevalence of extended-spectrum beta lactamase-producing Gram-negative bacteria in Nigeria. *Journal of Global Antimicrobial Resistance*. 22: 488–496. [DOI: 10.1016/j.jgar.2020.04.010]

Yeung A.W.K., Tzvetkov N.T., Gupta V.K., Gupta S.C., Orive G., Bonn G.K., Fiebich B., Bishayee A., Efferth T., Xiao J., Silva A.S., Russo G.L., et al. (2019). Current research in biotechnology: exploring the biotech forefront. *Current Research in Biotechnology*. 1: 34-40. [DOI: 10.1016/j.crbiot.2019.08.003]

[DOI: 10.18502/jfqhc.8.4.8260]