International Journal of
Enteric
Pathogens

Int J Enteric Pathog. 2019 May;7(2):31-36

Original Article

http://enterpathog.abzums.ac.ir

doi 10.15171/ijep.2019.09

Occurrence and Antibiogram of Extended-Spectrum Cephalosporin- and Cephamycin-Resistant *Escherichia coli* in Asymptomatic University Students

Madubuike Umunna Anyanwu[1*], Chiamaka Felicitas Okpalanwa[2], Raymond Nduka Ugwuanyi[2]

[1]Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria
[2]Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria

***Corresponding Author:**
Madubuike Umunna Anyanwu, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria.
Tel: +234-8132662827;
Email: madubuike.anyanwu@unn.edu.ng

Published Online March 5, 2019

Keywords: *Escherichia coli*, Healthy university students, Cefoxitin resistance, Extended-spectrum β-lactam resistance, Extended-spectrum β-lactamase production

Abstract

Background: Apparently healthy individuals could serve as reservoirs and disseminators of extended-spectrum cephalosporin (ESC)- and cephamycin (cefoxitin, FOX)-resistant, and extended-spectrum β-lactamase-producing (ESBL-P) *Escherichia coli* which jeopardizes antibacterial therapy thereby posing a threat to the health of infected individuals/carriers.

Objectives: This study aimed to screen healthy asymptomatic students in the University of Nigeria, Nsukka (UNN) as potential reservoirs of ESC- and FOX-resistant and ESBL-P *E. coli* and to determine the antibacterial resistance profile.

Materials and Methods: Anal swabs were collected from 190 randomly selected healthy asymptomatic students of both genders in UNN between March and July 2018. ESC-resistant *E. coli* was isolated using MacConkey agar with 2 µg/mL ceftazidime. ESBL production was assessed by combination disc method while cephamycin resistance was determined using cefoxitin disc screening. Phenotypic resistance of the isolates was determined using disc diffusion method.

Results: Out of 190 samples, 20 (10.2%) demonstrated growth. Of these, 6 (30%) were FOX resistant (putative AmpC-producers) but none produced ESBL. The resistance of the isolates was 100% to ampicillin (AMP), 95% to ceftazidime (CAZ), tetracycline (TET) and sulfamethoxazole-trimethoprim (SXT), 30% to FOX and chloramphenicol (CHL), 85% to ciprofloxacin (CIP), enrofloxacin (ENR) and streptomycin (STR), and 65% to kanamycin (KAN). All the isolates were susceptible to meropenem (MEM). Among the 20 isolates, 1 (5%) was resistant to 2 classes of antibacterial agents while 19 (95%), including all the FOX-resistant strains, were resistant to ≥ 3 classes of antibacterial agents. The isolates exhibited 11 multiple antibacterial resistance patterns with AMP, CAZ, FOX, TET, CIP, ENR, STR, KAN, SXT being predominant.

Conclusion: Healthy asymptomatic students in UNN are potential reservoirs and disseminators of ESC- and cephamycin (FOX)-resistant *E. coli*.

Received January 8, 2019; **Revised** February 2, 2019; **Accepted** February 23, 2019

Background

Escherichia coli, a member of *Enterobacterales* is a natural inhabitant of the intestinal tract of humans and animals and thus found in various ecological niches such as soil, water, vegetables and so on.[1-3] *Escherichia coli* is a useful indicator organism for antimicrobial resistance surveillance (because it easily acquires and transfers resistance genes) and it is versatile, being facultatively pathogenic with the pathogenic variants associated with intestinal (enteritis) and extra-intestinal infections, including urinary tract infection, meningitis, peritonitis, and septicemia in humans.[1,2,4] Extended-spectrum cephalosporins (ESCs) which include the third (e.g., ceftazidime, cefpodoxime), fourth and fifth generation cephalosporins, and cephamycin (cefoxitin: FOX) are critically-important antibacterial agents of the highest priority in managing serious *E. coli* infections in humans and animals.[5-7] In *Enterobacterales*, extended-spectrum β-lactamases (ESBL) inhibited by clavulanic acid and Ampicillinase C (AmpC) which is not inhibited by clavulanic acid mediate resistance to ESCs, but cephamycin (FOX) resistance is conferred only by AmpC.[7] Inappropriate use of any ESC and/or non-ESCs β-lactams stimulates resistance against all generations of cephalosporins in *Enterobacterales*.[5,7] There is increased interest in ESC- and cephamycin-resistant *E. coli* strains because these organisms jeopardize ESC/cephamycin therapy, and exhibit resistance to all β-lactams (except carbapenems) and other classes of antibacterial agents including chloramphenicol (CHL), fluoroquinolones, trimethoprim and sulphonamide.[8-10] The World Health Organization (WHO) recently classified ESC-/cephamycin-resistant *Enterobacterales* as "critical priority 1 pathogens" that pose a threat to human and animal

health and against which new management strategies and researches documenting their occurrence in different reservoirs are urgently needed.[8]

The University of Nigeria, Nsukka (UNN) is a federal university located in Enugu State, Southeast Nigeria. The student population of UNN comprised individuals from various backgrounds and places within and outside the country. The use of antibacterial agents, including critically important ones (such as ESCs and FOX) is not controlled in Nigeria.[11,12] The third-generation cephalosporins (3GCs), particularly ceftazidime and cefpodoxime, are readily available over-the-counter and are often used as first-line therapy in the management of virtually all infections (diagnosed and undiagnosed) in humans and animals in Nigeria.[12,13] Therefore, recovered and asymptomatic healthy individuals may harbor ESC-/FOX-resistant and/or ESBL-P *E. coli*. These organisms harbored by these individuals could be discharged into the environment, thus serving as reservoirs and disseminators of genes encoding ESC/FOX resistance/ESBL production.[5,12] These genes present in the discharged *E. coli* could be acquired by horizontal transfer to other pathogenic human/animal bacteria, thereby complicating the infection and compromising antibacterial therapy.[12,14] Because of poor personal hygiene and environmental sanitation (defecation in open field/farmland and poor toilet facilities) in UNN students' hostels, these organisms could easily spread among the students (by direct contact with carriers and/or indirect contact with contaminated fomites and consumption/ingestion of contaminated food/drinking water) and the public thus posing a threat to the health of infected individuals.[5,14,15] Evidences support zoonotic transmission of ESC/FOX-resistant/ESBL-P *E. coli* from humans to animals and vice versa.[16]

In the available literature, there are numerous studies[17-19] on the occurrence and antibiogram of ESC-/FOX-resistant and ESBL-/AmpC-P *E. coli* in hospitalized (in- and out-) patients and the community, including Nigeria.[20-23] However, reports on the colonization of healthy asymptomatic individuals by these organisms are rather scanty.[17-19] Studies which assessed the occurrence of ESC-resistant/ESBL-/AmpC-P *E. coli* in asymptomatic healthy university students included that of Kader et al. in Saudi Arabia,[24] Chirindze et al in Mozambique,[25] Li et al in China[26] and Lonchel et al in Cameroun.[27] Recently, Chukwunwejim et al screened asymptomatic students in Nnamdi Azikiwe University (NAU) located in Anambra State, Southeast Nigeria and reported 7.3% ESBL-P *E. coli* occurrence.[13] The potential of healthy students in the University of Nigeria as reservoirs of ESC-/FOX-resistant and ESBL-P *E. coli* has remained uninvestigated.

Objective

Surveillance and determination of the antibiogram of ESC- and FOX-resistant *Enterobacterales* are crucial for controlling the spread of these organisms and for targeted empiric therapy in infected individuals. Therefore, this study was undertaken to screen asymptomatic healthy students in the University of Nigeria as potential reservoirs of ESC- and FOX-resistant and ESBL-P *E. coli*.

Materials and Methods

Study Site

This study was done in the UNN. UNN is a federal university located in Nsukka, Enugu State, Southeast Nigeria. It is geographically located at coordinates 6°51'24"N 7°23'45"E and has student population close to 40 000.

Sampling

This cross-sectional study was conducted between March and July 2018 after obtaining permission from the Research Committee of the Department of Pharmaceutical Microbiology and Biotechnology, UNN and the Medical Research Ethics Committee of the University of Nigeria. A total of 190 apparently healthy asymptomatic students in UNN were randomly selected. Students with diarrhea and/or any obvious symptoms of illness were excluded from the study. Each of the selected students who gave written free and informed consent in accordance with the declaration of Helsinki[28] was given a sterile swab stick and taught how to collect anal swab aseptically. They were asked to collect the samples in the morning and deliver to the researcher within 30 minutes to avoid deterioration. Each subject collected and submitted a non-duplicate anal swab; these swabs were transported on ice packs to the Microbiology Laboratory, Department of Pharmaceutical Microbiology and Biotechnology, UNN and processed on the day of collection.

Isolation and Identification of ESC-Resistant *Escherichia coli*

The anal swabs were cultured on MacConkey agar (MCA) with 2 µg/mL ceftazidime and incubated at 37°C for 24 hours in ambient air. One colony per sample with putative *E. coli* morphology (non-mucoid/slightly mucoid, medium-sized pinkish colonies) was purified by sub-culturing on plain MCA and incubated at 37°C for 24 hours. Pure cultures of the isolates were phenotypically characterized by subjecting them to various tests such as gram staining, citrate, indole and triple sugar iron agar tests and sub-culturing on eosin methylene blue agar followed by incubation at 37°C for 24 hours in ambient air.[29] The isolates with characteristic greenish metallic sheen appearance on eosin methylene blue agar (*E. coli*) were sub-cultured onto nutrient agar slants, incubated at 37°C for 24 hours and stored in refrigerator at 4°C as stock cultures until needed for further analysis.

Determination of Antibacterial Susceptibility of ESC-Resistant *Escherichia coli* Isolates From Students

Antibacterial resistance/susceptibility profiles of the

E. coli isolates were determined by the disc diffusion method on Mueller-Hinton agar (Oxoid, Basingstoke, UK) according to the guidelines of Clinical and Laboratory Standards Institute,[30] using 10 antibacterial agents (Oxoid, Basingstoke, United Kingdom) belonging to 6 classes: β-lactam/cephalosporin: ceftazidime (CTZ: 30 µg), ampicillin (AMP: 10 µg), and meropenem (MEM: 10 µg), tetracycline (TET: 30 µg), aminoglycosides: streptomycin (STR : 10 µg) and kanamycin (KAN: 30 µg), fluoroquinolones: enrofloxacin (ENR: 5 µg) and ciprofloxacin (CIP: 5 µg), phenicols: chloramphenicol (CHL; 30µg), and folate pathway antagonists: sulfamethoxazole/trimethoprim (SXT: 25 µg). *E. coli* ATCC (American Type Culture Collection) 25922 was used as the reference strain. The results of the antibacterial resistance/susceptibility testing were interpreted according to the CLSI guidelines for members of the family Enterobacteriaceae.[31] An isolate resistant to agents in ≥3 classes/categories was considered multidrug-resistant (MDR).[32]

Detection of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Strains

The isolates were screened for ESBL production using the combination disc method (cefpodoxime/clavulanic acid [10:1 µg] and cefpodoxime alone [10 µg]) on Mueller-Hinton agar following CLSI guidelines.[30,31] *E. coli* ATCC 25922 was used as the reference strain. An isolate that produced an inhibition zone with a diameter difference of 5 mm or more between the combination disc and cefpodoxime alone was considered as ESBL-producer.[30,31]

Assessment for Cephamycin Resistance

The isolates were evaluated for cephamycin resistance using FOX disc (30µg) screening on Mueller-Hinton agar following CLSI guidelines.[30] *E. coli* ATCC 25922 was used as the reference strain. An isolate that produced an inhibition zone with a diameter of less than or equal to 14 mm was considered FOX-resistant (putative AmpC-producer).[30,31]

Results

Out of 190 anal swabs cultured, 20 (10.5%) demonstrated growth of *E. coli*. None (0.0%) out of the 20 ESC-resistant *E. coli* isolates was ESBL-producer, but 6 (30%) exhibited FOX resistance hence putative AmpC-producers.

Of the 20 ESC-resistant isolates, all (100%) were resistant to AMP, 6 (30%, 95% CI: 9.9-50.1) to CHL, 17 (85%, 95% CI: 69.4-100.0) to CIP, ENR and STR, 13 (65%, 95% CI: 44.1-85.9) to KAN while 19 (95%, 95% CI: 85.4-100.0), including the 6 FOX-resistant strains, were resistant to CAZ, TET and SXT (Table 1). All the isolates were susceptible to MEM. Among the 20 isolates, 1 (5%) was resistant to 2 classes of antibacterial agents whereas 19 (95%) were resistant to at least 4 classes of antibacterial agents (Table 2). The isolates exhibited 11 multiple antibacterial resistance patterns with AMP, CAZ, FOX, TET, CIP, ENR, STR, KAN, SXT being predominant (n = 5).

Discussion

The fact that 20 (10.5%) of the 190 non-duplicate anal swab samples cultured using MacConkey agar with 2µg/mL of CTZ (a 3GC) demonstrated growth, suggesting that a sizeable percentage of asymptomatic healthy students in UNN are colonized by ESC-resistant *E. coli*. Isolation of these ESC-resistant *E. coli* strains suggested that the isolates produced ESBL and/or AmpC.[7,10,12] It also suggested that either the students already harbored these organisms in their gut or they acquired them from their environment.[10] It equally suggested selection of CTZ probably due to the frequent use of 3GCs in the sampled individuals. This finding of ESC-resistant *E. coli* in healthy students calls for concern because these students are putative reservoirs and disseminators (by faecal shedding) of these organisms into the environment (students in UNN often defecate in open field/farms), thereby posing a threat to the health of individuals that get in direct and/or indirect (contaminated fomites such as books, bed and desk surfaces, etc) contact with them.[5,12] These are MDR organisms exhibiting cross-resistance to other classes of

Table 1. Antibacterial Susceptibility Profile of 20 Extended-spectrum Cephalosporin-resistant E. coli Isolates From Anal Swabs of Healthy University Students

Class of Antibacterial Agent	Antibacterial Agent (Concentration)	Number (Percent) of Isolates		
		Susceptible	Intermediately-Susceptible	Resistant, 95% CI
β-lactam	Ampicillin (10 µg)	0 (0.0)	0 (0.0)	20 (100)
	Ceftazidime (30 µg)	0 (0.0)	1 (5)	19 (95), 85.4-100.0
	Meropenem (10 µg)	20 (100)	0 (0)	0 (0.0), 0.0-0.0
	Cefoxitin (30 µg)	14 (70)	0 (0)	6 (30), 9.9-50.1
Tetracycline	Tetracycline (30 µg)	1 (5)	0 (0)	19 (95), 85.4-100.0
Fluoroquinolones	Ciprofloxacin (5 µg)	0 (0.0)	3 (15)	17 (85), 69.4-100.0
	Enrofloxacin (5 µg)	0 (0.0)	3 (15)	17 (85), 69.4-100.0
Aminoglycosides	Streptomycin (10 µg)	1 (5)	2 (10)	17 (85), 69.4-100.0
	Kanamycin (30 µg)	0 (0.0)	7 (35)	13 (65), 44.1-85.9
Phenicol	Chloramphenicol (30 µg)	14 (70)	0 (0)	6 (30), 9.9-50.1
Folate pathway antagonists	Sulphamethoxazole-trimethoprim (25 µg)	1 (5)	0 (0.0)	19 (95), 85.4-100.0

Table 2. Multiple Antibacterial Resistance Profile and Patterns of 20 Extended-spectrum Cephalosporin-resistant *E. coli* Isolates from Anal Swabs of Healthy University Students

No. of Antibacterial Class	Resistance pattern (Number of isolates)	Total number of Isolates (%)
2	AMP, CAZ, ENR (1)	1 (5)
4	AMP, CAZ, TET, STR, SXT (1)	19 (95)
5	AMP, CAZ, TET, STR, KAN, CHL, SXT (1)	
	AMP, CAZ, TET, CIP, ENR, STR, SXT (4)	
	AMP, CAZ, FOX, TET, CIP, ENR, STR, KAN, SXT (5)	
	AMP, CAZ, TET, CIP, ENR, STR, KAN, SXT (2)	
	AMP, CAZ, TET, CIP, ENR, KAN, SXT (1)	
	AMP, CAZ, FOX, TET, STR, KAN, CHL, SXT (1)	
	AMP, TET, ENR, STR, KAN, SXT (1)	
6	AMP, CAZ, TET, ENR, STR, KAN, CHL, SXT (2)	
	AMP, CAZ, TET, CIP, ENR, KAN, CHL, SXT (1)	

AMP: Ampicillin, CAZ: Ceftazidime, TET: Tetracycline, STR: Streptomycin, KAN: Kanamycin, CHL: Chloramphenicol, ENR: Enrofloxacin, CIP: Ciprofloxacin, FOX: Cefoxitin, SXT: Sulfamethoxazole-trimethoprim.

antibacterial agents thus could compromise empirical therapy in infected individuals/carriers.[7,12,14]

The 10.5% ESC-resistant *E. coli* occurrence in this study is higher than 9% ESC-resistant *E. coli* occurrence in 150 stool samples of healthy students in a Cameroonian University.[27] However, it is lower than 12.7% ESC-resistant *E. coli* occurrence in stool specimens of 275 healthy students in a Mozambican University.[25] Differences in the result of the studies could be related to variation in the type of sample collected, concentration and type of ESC used for primary isolation, degree of contamination of students' food, drinking water and/or environment, level of hygiene practiced by the students, and usage of ESCs in the study areas. In the current experiment, 2 µg/mL CTZ in MCA was used for the primary isolation whereas MCA with 1.5 µg/mL cefotaxime and 1 µg/mL ceftriaxone was used for primary isolation in the studies conducted in Cameroon[27] and Mozambique,[26] respectively. Possible sources of these ESC-resistant *E. coli* in the sampled students were nosocomial following the previous visitation to hospitals, contact with contaminated fomites in the school environs or elsewhere, contact with carriers/infected individuals (humans and/or animals), and/or consumption/drinking of contaminated food/water.[10,17] However, the use of non-ESC β-lactam antibiotics has also been reported to stimulate ESC resistance in *Enterobacterales*.[7,17]

In this study, 0.0% occurrence of ESBL production was observed suggesting that none of the ESC-resistant *E. coli* isolates was an ESBL-producer. This finding may not be surprising because of the health status (asymptomatic and healthy) of the sampled students. However, since the medical history of the sampled students was not assessed, it would be difficult to attribute the observed zero ESBL-P *E. coli* occurrence to non-use of antibiotics in the last 6 months or visitation to hospital in the past 3 months prior to sampling. These factors have been reported as risk factors for colonization of healthy individuals with ESBL-P enterobacteria.[25] Nonetheless, the absence of ESBL production in this study may suggest that these students have not acquired ESBL-P strain of *E. coli*. It also suggested that these students might not have been treated with ESC/CTZ, thus the isolates exerted no preference to CTZ. Therefore, finding the CTZ resistance without ESBL production in this study suggested selection of oxyimino-cephalosporin (3GC) by other mechanisms of β-lactam resistance such as production of other β-lactamases, change in cell wall permeability, expression of active efflux pumps, gene mutations and/or alteration of penicillin binding protein receptors.[2,7,33] However, production of AmpC could be the mechanism by which the 6 FOX-resistant isolates in this study preferred to CTZ since resistance to ESCs is mediated majorly by ESBL and/or AmpC which confers FOX resistance.[7,10,11,25] The finding of 30% FOX-resistant (putative AmpC-producers) *E. coli* in this study also calls for concern because AmpC-P enterobacteria are MDR as also observed in the current experiment. Chirindze et al.[25] also used FOX disc screening and observed 46% FOX-resistance among 35 non-duplicated ESC-resistant/ESBL-P *E. coli* isolates from healthy students in a university in Mozambique.

The absence of ESBL production among 20 non-repetitive ESC-R *E. coli* isolates (from 20 students) in this study contrasted the reports of Lonchel et al and Chirindze et al stating that 6 and 12.7% non-duplicate faecal ESC-resistant *E. coli* isolates from 150 and 275 healthy students in a university in Cameroon[27] and Mozambique,[25] respectively, were ESBL-producers. It also contrasted the results of other studies in which the carriage rate of faecal ESBL-P *E. coli* was 13.1% among 426 students in Saudi Arabia[24] and the occurrence of faecal ESBL-P Enterobacteriaceae (*Klebsiella pneumoniae* and *E. coli*) was 11.3% among 53 students in China,[34] respectively. However, the result (0% occurrence of faecal ESBL-P *E. coli*) of this study is similar to 0.0% faecal ESBL-P *E. coli* occurrence in anal swabs of 1998 healthy persons in Indonesia[35] and 517 asymptomatic young adults (army

recruits) in France.[36] Elsewhere, the occurrence of faecal ESBL-P *E. coli* was reported to be 0.1-69.3% among healthy individuals.[17-19] It is worth to note that Chukwunwejim et al used double disc synergy test method and detected 20 (7.3%) ESBL-P *E. coli* strains among 63 *E. coli* isolates from stool specimens of 273 asymptomatic students in NAU located in Anambra State, southeastern Nigeria.[13] The variation in the results of the studies may reflect the differences in the number of samples analyzed and usage of ESCs and other antibacterial agents in the various study areas. In this experiment, combination disc method employing cefpodoxime and cefpodoxime-clavulanic acid combination was used. This method is recommended for phenotypic detection of ESBL production though it is not as sensitive as the molecular method.[12,31] However, increasing the sample size might have increased the likelihood of detecting ESBL-P strains among ESC-R *E. coli* isolates from students in UNN. However, the absence of ESBL-P *E. coli* in asymptomatic students in UNN should be maintained by instituting antibacterial stewardship programs. This is because if ESBL-P *E. coli* strains eventually emerge in healthy students, it will spread fast in human and animal population in the school and the country thereby posing a serious threat to public health.

Almost all the isolates (95%) in this study were MDR because of the fact that they were ESC-/FOX-resistant strains.[7,11,12,14,17] This portends danger in future antibacterial therapy in the sampled individuals because of limited options for therapy.[5,7,11,14] Interestingly, none of the MDR isolates in this study exhibited resistance to MEM (a carbapenem) which is a last-resort drug for managing infections associated with MDR *Enterobacterales*.[7,11,12] This finding suggested that MEM may not have been abused in human clinical practice in Nigeria or that the sampled students have not been treated previously with a carbapenem.

Conclusion

This study has shown that ESC- and FOX-resistant (putative AmpC-P) *E. coli* are harbored by a sizeable percentage (10.5%) of asymptomatic healthy students in UNN although colonization of healthy students in UNN by ESBL-P *E. coli* strains seems to be uncommon. UNN students colonized by ESC-/FOX-resistant *E. coli* strains are potential disseminators of these organisms into the environment. This portends serious danger to public health because these are MDR organisms capable of jeopardizing antimicrobial therapy in infected individuals. The spread of ESC-/cephamycin-resistant *E. coli* strains could have a huge impact on the ecology and epidemiology of antibacterial resistance in Nigeria. Therefore, attention should be paid to the use of ESCs and FOX in Nigeria. However, further studies to determine the genes encoding β-lactamases and AmpC in the isolates are recommended.

Authors' Contributions

This paper was extracted from the Bachelor of Pharmacy project of RNU. MUA conceptualized and designed the study, CFO and MUA supervised the study; MUA, CFO and RNU participated in sample collection and processing. RNU and MUA collated and analyzed the data. MUA wrote and edited the manuscript. All authors read and approved the final manuscript..

Ethical Approval

Permission to conduct this study was obtained from the Research Committee of the Pharmaceutics Department, UNN and the Medical Research Ethics Committee of the University of Nigeria.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Financial Support

None.

Acknowledgments

This paper was adopted from Raymond Ugwuanyi's B. Pharm. project. The authors would like to express their gratitude to the Department of Pharmaceutics, UNN, for providing the equipment used in this study.

References

1. Vila J, Saez-Lopez E, Johnson JR, et al. Escherichia coli: an old friend with new tidings. FEMS Microbiol Rev. 2016;40(4):437-463. doi:10.1093/femsre/fuw005
2. Poirel L, Madec JY, Lupo A, et al. Antimicrobial resistance in *Escherichia coli*. Microbiol Spectr. 2018;6(4). doi:10.1128/microbiolspec.ARBA-0026-2017
3. Adeolu M, Alnajar S, Naushad S, R SG. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. Int J Syst Evol Microbiol. 2016;66(12):5575-5599. doi:10.1099/ijsem.0.001485
4. Tadesse DA, Zhao S, Tong E, et al. Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. Emerg Infect Dis. 2012;18(5):741-749. doi:10.3201/eid1805.111153
5. Abraham S, Jordan D, Wong HS, et al. First detection of extended-spectrum cephalosporin- and fluoroquinolone-resistant *Escherichia coli* in Australian food-producing animals. J Glob Antimicrob Resist. 2015;3(4):273-277. doi:10.1016/j.jgar.2015.08.002
6. World Health Organization (WHO). Critically important antimicrobials for human medicine. 5th ed. Geneva: WHO; 2017.
7. van Duijkeren E, Schink AK, Roberts MC, Wang Y, Schwarz S. Mechanisms of bacterial resistance to antimicrobial agents. Microbiol Spectr. 2018;6(1). doi:10.1128/microbiolspec.ARBA-0019-2017
8. World Health Organization (WHO). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. WHO; 2017. http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25FebET_NM_WHO.pdf.
9. Logan LK, Braykov NP, Weinstein RA, Laxminarayan R. Extended-Spectrum beta-Lactamase-Producing and Third-Generation Cephalosporin-Resistant Enterobacteriaceae in Children: Trends in the United States, 1999-2011. J Pediatric

Infect Dis Soc. 2014;3(4):320-328. doi:10.1093/jpids/piu010
10. Apostolakos I, Franz E, van Hoek A, et al. Occurrence and molecular characteristics of ESBL/AmpC-producing *Escherichia coli* in faecal samples from horses in an equine clinic. J Antimicrob Chemother. 2017;72(7):1915-1921. doi:10.1093/jac/dkx072
11. Alonso CA, Zarazaga M, Ben Sallem R, Jouini A, Ben Slama K, Torres C. Antibiotic resistance in *Escherichia coli* in husbandry animals: the African perspective. Lett Appl Microbiol. 2017;64(5):318-334. doi:10.1111/lam.12724
12. Anyanwu MU, Ugwu IC, Onah CU. Occurrence and antibiogram of generic extended-spectrum cephalosporin-resistant and extended-spectrum beta-lactamase-producing enterobacteria in horses. Mac Vet Rev. 2018;41(2):123-132. doi:10.2478/macvetrev-2018-0015
13. Chukwunwejim C, Eze PM, Ujam NT, et al. Incidence of community acquired ESBL-producing bacteria among asymptomatic University students in Anambra State, Nigeria. Eur J Biol Res. 2018;8(3):138-147. doi:10.5281/zenodo.1314719
14. Rawat D, Nair D. Extended-spectrum beta-lactamases in Gram Negative Bacteria. J Glob Infect Dis. 2010;2(3):263-274. doi:10.4103/0974-777x.68531
15. Bengtsson-Palme J, Kristiansson E, Larsson DGJ. Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiol Rev. 2018;42(1). doi:10.1093/femsre/fux053
16. Valentin L, Sharp H, Hille K, et al. Subgrouping of ESBL-producing *Escherichia coli* from animal and human sources: an approach to quantify the distribution of ESBL types between different reservoirs. Int J Med Microbiol. 2014;304(7):805-816. doi:10.1016/j.ijmm.2014.07.015
17. Woerther PL, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum beta-lactamases in the community: toward the globalization of CTX-M. Clin Microbiol Rev. 2013;26(4):744-758. doi:10.1128/cmr.00023-13
18. Storberg V. ESBL-producing Enterobacteriaceae in Africa - a non-systematic literature review of research published 2008-2012. Infect Ecol Epidemiol. 2014;4. doi:10.3402/iee.v4.20342
19. Kawamura K, Nagano N, Suzuki M, Wachino J, Kimura K, Arakawa Y. ESBL-producing *Escherichia coli* and its rapid rise among healthy people. Food Safety. 2017;5(4):122-150. doi:10.14252/foodsafetyfscj.2017011
20. Ogbolu DO, Alli OA, Olanipekun LB, Ojo OI, Makinde OO. Faecal carriage of extended-spectrum beta-lactamase (ESBL)-producing commensal *Klebsiella pneumoniae* and *Escherichia coli* from hospital out-patients in Southern Nigeria. Int J Med Medical Sci 2013;5(3):97-105.
21. Iroha IR, Okoye E, Osigwe CA, Moses IB, Ejikeugwu CP, Nwakaeze AE. Isolation, phenotypic characterization and prevalence of ESBL-producing *Escherichia coli* and *Klebsiella* species from orthopedic wounds in National Orthopedic Hospital Enugu (NOHE), South East Nigeria. J Pharma Care Health Sys. 2017;4(4):1-5. doi:10.4172/2376-0419.1000184
22. Iroha IR, Adikwu MU, Amadi ES, Aibinu I, Esimone CO. Characterisation of extended spectrum beta-lactamase pproducing *Escherichia coli* from secondary and tertiary hospitals in South Eastern Nigeria. Res J Microbiol. 2008;3(7):514-519. doi:10.3923/jm.2008.514.519
23. Inwezerua C, Mendonca N, Calhau V, Domingues S, Adeleke OE, Da Silva GJ. Occurrence of extended-spectrum beta-lactamases in human and bovine isolates of *Escherichia coli* from Oyo state, Nigeria. J Infect Dev Ctries. 2014;8(6):774-779. doi:10.3855/jidc.3430
24. Kader AA, Kumar A, Kamath KA. Fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in patients and asymptomatic healthy individuals. Infect Control Hosp Epidemiol. 2007;28(9):1114-1116. doi:10.1086/519865
25. Chirindze LM, Zimba TF, Sekyere JO, et al. Faecal colonization of *E. coli* and *Klebsiella* spp. producing extended-spectrum beta-lactamases and plasmid-mediated AmpC in Mozambican university students. BMC Infect Dis. 2018;18(1):244. doi:10.1186/s12879-018-3154-1
26. Li B, Zhong Y, Fu XC, et al. Duration of stool colonization in healthy medical students with extended-spectrum-beta-lactamase-producing *Escherichia coli*. Antimicrob Agents Chemother. 2012;56(8):4558-4559. doi:10.1128/aac.00171-12
27. Lonchel CM, Meex C, Gangoue-Pieboji J, et al. Proportion of extended-spectrum ss-lactamase-producing Enterobacteriaceae in community setting in Ngaoundere, Cameroon. BMC Infect Dis. 2012;12:53. doi:10.1186/1471-2334-12-53
28. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053
29. Cheesebrough M. District laboratory practice in tropical countries Part 2. Cambridge: Cambridge University Press; 2000:63-70.
30. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically (CLSI Standard M07). 11th ed. Wayne, PA: CLSI; 2018.
31. Clinical and Laboratory Standards Institute (CLSI). Performance standard for antimicrobial susceptibility testing (CLSI Standard M100). 28th ed. Wayne, PA: CLSI; 2018.
32. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-281. doi:10.1111/j.1469-0691.2011.03570.x
33. Livermore DM, Brown DF. Detection of beta-lactamase-mediated resistance. J Antimicrob Chemother. 2001;48 Suppl 1:59-64.
34. Ho PL, Wong RC, Chow KH, Yip K, Wong SS, Que TL. CTX-M type beta-lactamases among fecal *Escherichia coli* and Klebsiella pneumoniae isolates in non-hospitalized children and adults. J Microbiol Immunol Infect. 2008;41(5):428-432.
35. Severin JA, Lestari ES, Kloezen W, et al. Faecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae among humans in Java, Indonesia, in 2001-2002. Trop Med Int Health. 2012;17(4):455-461. doi:10.1111/j.1365-3156.2011.02949.x
36. Janvier F, Merens A, Delaune D, Soler C, Cavallo JD. [Fecal carriage of third-generation cephalosporins-resistant Enterobacteriaceae in asymptomatic young adults: evolution between 1999 and 2009]. Pathol Biol (Paris). 2011;59(2):97-101. doi:10.1016/j.patbio.2010.07.012