https://dx.doi.org/10.4314/dujopas.v9i3b.12
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 9 No. 3b September 2023*

Colistin-Resistant *Escherichia coli* from Hospital and Community Nexus: Public and Environmental Health Concern

Abeni Beshiru[1*] and Kate Enoh Uwhuba[1]

[1]Department of Microbiology and Biotechnology,
College of Natural and Applied Sciences,
Western Delta University, Oghara,
Delta State Nigeria.

Email: bash_ab@rocketmail.com

Abstract
Antimicrobial resistance (AMR) is one of the greatest encounters for human health globally, which is associated with elevated morbidity and increased economic burdens. Colistin represents an important resort in treating severe infections caused by multidrug-resistant difficult-to-treat Gram-negative bacteria, including Escherichia coli. The study aimed to investigate the prevalence of colistin-resistant E. coli (CREC) in community and hospital sources and their associated risk factors. Eighty-five samples from hospital and community sources were screened using cultural and biochemical techniques and confirmed via Analytical Profile Index 20E. The antimicrobial susceptibility profile was determined using disc diffusion procedure and the phenotypic virulence factors were determined using standard culture protocols. The occurrence of CREC based on sample types includes cow carcass (26.7%), abattoir effluents (20%) and hospital effluents (8.6%). Pumpkin leaf, carrots and smoked fish were negative for CREC. The percentage distribution of CREC positive samples based on sample sources were slaughterhouses (77%) and hospital environment (33%) with open markets showing no case of CREC. Overall, CREC positive samples were 9/85 (10.6%). CREC demonstrated 100% ampicillin and trimethoprim/sulfamethoxazole resistance. The multiple antibiotics resistance profile of the Escherichia coli in this study ranged from 0.83 – 0.46. The phenotypic virulence distribution of CREC were lipase activity (33.3%), β-hemolytic activity (22.2%) and gelatinase activity (22.2%).The detection of CREC in both community and hospital environment allays a notable threat to human and animal health. Public enlightenment campaign and proper education against indiscriminate antibiotics usage as animal feed and growth promoters coupled with routine antibiotic resistance monitoring is crucial to curtail the dissemination of superbugs.

Keywords: Colistin, E. coli, Drug resistance, Virulence, Superbugs.

INTRODUCTION
Antimicrobial resistance (AMR) represents one of the utmost encounters for human health globally. AMR is an evolving health menace that linked with elevated morbidity and increased economic burdens (Phodha *et al*., 2019). Bacterial resistance especially the Gram-negative bacteria to multiple antibiotics have been associated to several infections worldwide and is predominantly significant in low and middle income countries where antibiotics misuse is high (Beshiru *et al*., 2017; Imanah *et al*., 2017; Igbinosa & Beshiru, 2019; Igbinosa *et al*., 2020a). Globally, the estimate of deaths linked to AMR stands at 700,000, and

*Author for Correspondence

projected to reach ten million by the year 2050 (O'Neill, 2014). However, the deaths attributable to AMR is projected to reach 4,150,000 by 2050 in Africa (O'Neill, 2014). Indiscriminate prescription and usage of antibiotics in veterinary and human medicine have been attributed to the spread of multidrug-resistant (MDR) bacteria as they demonstrate resistance to almost all commercially available antimicrobials (Igbinosa *et al*., 2022a; Beshiru & Igbinosa, 2023).

This rise in AMR translates to longer hospital stays, therapeutic complications, difficult-to-treat infections and amplified mortality in animals and human (Beshiru *at al*., 2016). The risk associated with AMR is more worrisome in nations with limited resources such as sub-Saharan Africa where common infections exist and last-resort antimicrobials are relatively unaffordable and scarce (Igbinosa *et al*., 2022b). In view of the enormous lack of novel antimicrobials active in the treatment of infections arising from MDR Gram-negative bacteria, there have been renewed interest in polymyxin antibiotics especially polymyxin E (colistin) whose usage were initially discontinued in human medicine in several countries (Karaiskos *et al*., 2019). Colistin acts by competitively disrupting the cell membrane integrity thereby resulting in leakage of important cellular components and ultimately bacterial cell death (Ma *et al*., 2022). The restriction of colistin a last-resort antibiotics in human medicine despite its effectiveness is due to patient safety concerns as it has been linked to nephrotoxicity and neurotoxicity potential (Falagas *et al*., 2010).

The therapeutic use of colistin regained global interest after the emergence of MDR *Enterobacteriaceae* (Igbinosa *et al*., 2020b; Ma *et al*., 2022). Colistin represent an important resort in treating severe, infections caused by MDR difficult-to-treat Gram-negative bacteria, including *Escherichia coli* (Igbinosa & Beshiru, 2018). Colistin was relatively reintroduced recently in human medicine within formation on colistin-resistant bacteria already on the rise (Falagas *et al*., 2010). The unfortunate increase of colistin resistance can be notably attributed to its misuse in veterinary medicine as therapeutics and growth promoters (El-Mokhtar *et al*., 2021). Several studies have implicated food animals and their products as a notable foundation of colistin resistance genes of zoonotic importance (Poirel *et al*., 2018). Resistance to colistin can be disseminated zoonotically or indirectly through the food platforms and subsequently cause difficult-to-treat diseases in human (Marshall & Levy, 2011).

E. coli is reported as one amongst other bacterial species liable for the horizontal spread of antimicrobial resistance genes (Tang *et al*., 2021). Over the years, colistin acquired resistance has been frequent consequent of chromosomal mutation in regulatory pathways or via amplified capsular polysaccharide production (Olaitan *et al*., 2014). However, resistance to antibiotic agents is further complicated and spread faster via antimicrobial resistance gene encoded on transferable plasmids (Ma *et al*., 2022). Most antimicrobial resistant genes of *E. coli* are plasmid-mediated hence they have high potential of being transferred horizontally (Beshiru *et al*., 2023). *E. coli* can cause infections in animals from the environment or other infected populations (Poirel *et al*., 2018). The transferable plasmid encoding colistin resistance (mobilized colistin resistance-*mcr*) emerged in China in late 2015 (Poirel *et al*., 2018). This global emergence of CREC from different nexus has evoked public health attention. Hence, it is important to concurrently surveil and monitor the spread of CREC from diverse sources and environment. The study aimed to investigate the prevalence of CREC from community and hospital sources and their associated risk factors.

MATERIALS AND METHODS

Study Area

This study was conducted within Oghara town, Ethiope West Local Government Area, Delta State, Nigeria (Latitude: 5.63314, Longitude: 6.02819 5° 37′ 59″ North, 6° 1′ 41″ East). Oghara community is a home to various educational and health institutions which include Delta State Polytechnic in Otefe-Oghara, Western Delta University, Oghara, Nigeria Naval Logistic Headquarters, Oghara and Delta State University teaching hospital, Oghara.

Samples Collection

A total of 85 samples were collected and analyzed in this study. These include community samples (*n*= 25) gotten from open markets [pumpkin leaf - *Telfairia occidentalis* (*n*= 10), carrots - *Daucus carota* (*n*= 10), smoked fish (*n*= 5)] and slaughterhouses samples (*n*= 25) [cow carcass (*n*= 15), abattoir effluents (*n*= 10)]. Hospital-related samples were gotten from Delta State University teaching hospital, Oghara [Hospital effluents (*n*= 35)]. Sample collection did not require ethical approval as samples were not directly obtained from animals and humans. However, permission and informed consent were gotten from the abattoir workers and clinic institutions before sampling. The effluent specimens were collected in sterile sampling plastic containers at a depth approximately 500 mm and other samples were collected in sterile polythene bags. The samples were conveyed immediately on ice packs to the laboratory for analysis.

Isolation of Colistin-Resistant *Escherichia coli*

Twenty-five grams of pumpkin leaf, carrots and fish samples were weighed, severed into pieces and transferred to 225 mL sterile distilled water. The effluent samples were homogenized by shaking the sampling containers slightly before opening and 10 mL was transferred into 90 mL sterile distilled water. An aliquot of 1 mL from the stock samples was cultured in 10 mL Tryptone Soy Broth (TSB) (Merck, Darmstadt, Germany) at 37 ^{o}C for 24 hours. After incubation, 100 µL of each TSB culture was inoculated onto a Chromocult Coliform Agar (CCA) (Merck, Darmstadt, Germany) agar plate supplemented with 4 µg/mL colistin to screen for colistin resistance as recommended by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and adopted in previous studies (El-Mokhtar *et al*., 2021). Plates were incubated at 37 ^{o}C for 24 hours. Distinct blue or violet colonies were considered presumptive CREC isolates. The presumptive *Escherichia coli* isolates were purified on nutrient agar (Lab M, Lancashire UK) and stored on nutrient agar slants until ready for further use.

Biochemical Characterization and Identification of Colistin-Resistant *Escherichia coli*

Colonies were presumptively identified by colony pigmentation and Gram reactions using 3% potassium hydroxide (3% KOH) (Gram-negative) characteristics. Pure cultures were obtained by streaking a portion of an isolated colony on nutrient agar and incubated aerobically at 37 °C for 24 hours. The isolates were confirmed by oxidase test, catalase test, indole test, urease test, and hydrogen sulphide test. Isolates were further characterized biochemically using Analytical Profile Index (API) 20E (Biomérieux, France). The tests were performed according to the manufacturer's instruction for use. Data interpretation was performed using the Analytical profile index (API) database (V4.1) with the apiweb™ identification software.

Antimicrobial Susceptibility Testing

The confirmed CREC isolates were subjected to antimicrobial susceptibility testing to determine the AMR patterns using the Kirby-Bauer disc diffusion method. Suspension containing approximately 10^5 colony forming units (CFU)/mL was prepared accordingly and 50 µL of the suspension were deposited and aseptically spread on Mueller-Hinton agar (MHA) plates (Lab M, Lancashire, United Kingdom). The antibiotics disc tested include ceftazidime (30 µg), aztreonam (30 µg), ampicillin (10 µg), amoxicillin-clavulanate (20/10 µg), meropenem (10 µg), gentamicin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), nitrofurantoin (300 µg) and trimethoprim/sulfamethoxazole (1.25/23.75 µg) (Oxoid, Basingstoke, Hampshire, United Kingdom). Plates were incubated for 24 hours at 37 °C. Diameter of zones of inhibition were measured and interpreted according to Clinical Laboratory Standards Institute (CLSI) (2020).

Multiple Antibiotic Resistance Index

Multiple antibiotic resistance index (MARI) of CREC was elucidated as follows: $\text{MAR index} = \frac{a}{b}$ where "a" represents total antibacterial agents that an isolate is resistant and "b" is represents, antibacterial agents that an isolate was exposed (Igbinosa *et al*., 2023a). Isolates with MAR index ≥0.2 was considered to be of high risk. Isolates resistant to ≥3 antibiotic class were considered multidrug-resistant (Igbinosa *et al*., 2023b).

Determination of Phenotypic Virulence

Colistin-resistant *E. coli* isolates were cultivated on tryptone soy agar (TSA) (Merck, Darmstadt, Germany). The recovered colonies on TSA were suspended in 3.0 mL of TSB (Merck, Darmstadt, Germany). The turbidity of colonies suspension was adjusted to 0.5 McFarland standards approximately 10^8 cells/mL. Lipase activity was determined by inoculating 5.0 mL of the sample suspension on TSA and incubated for 48 h at 37 ^{o}C. Positive lipase activity was indicated by a clear halo surrounding the growth of lipase producing bacterium. Gelatinase activity was determined by inoculating 5.0 mL sample of the suspension on gelatin medium and incubated for 48 h at 37 ^{o}C. Zones of clearance in the media indicated the proliferation of gelatin-liquefying microorganisms. Haemolytic activity was determined by inoculating 5.0 mL sample suspension on sheep blood agar plate and incubated for 48 h at 37 ^{o}C. Haemolytic activity was spotted by clear colourless zones surrounding the colonies which indicate lyses of the red blood cells. Phenotypic virulence was determined using the protocol adapted from previous studies of Beshiru *et al*. (2018); Beshiru *et al*. (2022).

Data Analysis

All data were analyzed using Microsoft Excel 2013 and SPSS 21.0. The prevalence and occurrence of the bacteria were expressed in percentage (%).

RESULTS AND DISCUSSION

The Occurrence and Distribution of Colistin-Resistant *E. coli*

Suspected isolates that were Gram negative, catalase positive, indole positive, oxidase negative, urease negative and hydrogen sulphide negative were confirmed as *E coli* using API-20E. The occurrence of CREC based on API-20E identification is shown in Table 1. The occurrence of CREC based on sample types were cow carcass (26.7%), abattoir effluents (20%) and hospital effluents (8.6%). Pumpkin leaf, carrots, and smoked fish had no CREC. The highest prevalence occurred in cow carcass samples while there was no occurrence

observed in smoked fish and carrots samples. The percentage distribution of CREC positive samples based on sample types is shown in Figure 1. The distribution observed were cow carcass (44.3%), abattoir effluents (22.7%) and hospital effluents (33%).

Table 1: Occurrence of colistin-resistant *E. coli* based on sample types

Samples	Samples assessed	Colistin-resistant *E. coli* positive samples
Pumpkin leaf	10	0
Carrots	10	0
Smoked fish	5	0
Cow carcass	15	4
Abattoir effluents	10	2
Hospital effluents	35	3
Total	85	9 (10.6%)

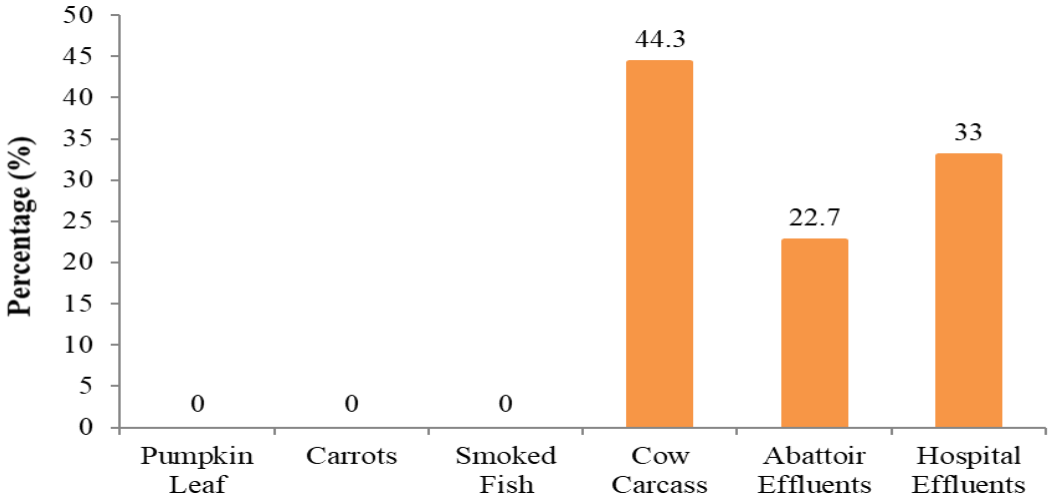

Figure 1: Percentage distribution of colistin-resistant *E. coli* positive samples based on sample types.

Over the years, there has been substantial increase in the spread of MDR Gram-negative bacteria and resulting upsurge of colistin usage as last resort drugs. Previous studies have adopted similar protocol to ours by supplementing culture media with 4 µg/mL colistin concentrations in screening CREC (El-Mokhtar *et al*., 2021). Similarly, other authors have agreed that the colistin resistance gene is mainly detected in *Enterobacteriaceae* and has a moderate level of resistance with minimum inhibitory concentration values varying from 4 to 16 mg/L (Skov *et al*., 2016). The prevalence (10.6%) CREC in this study is higher in comparison to the prevalence (0.09%) of CREC strains detected in Tunisia (Mezghani-Maalej *et al*., 2012) and the prevalence (8.3%) of CREC previously reported in Nigeria (Olowe *et al*., 2018). However, higher prevalence of CREC (26.5%, 14.0% and 0.9%) has been detected in pigs, chickens, and cattle respectively (Zhuge *et al*., 2019). This high prevalence agrees with this study as most of the resistant isolates observed were from environments related to farm animals.

The distribution of CREC based on sample sources is shown in Table 2 with slaughterhouses (24%) and hospital environment (8.6%) having the bulk of distributions. The highest occurrence was observed in slaughterhouses (24%) while no occurrence was observed at open market. The percentage distribution of CREC positive samples based on sample sources is shown in Figure 2. The distribution for open market recorded 0%,

slaughterhouses (77%) and hospital environment (33%). Overall, the total CREC positive samples was (10.6%).

Table 2: Occurrence of colistin-resistant *E. coli* based on sample sources

Samples	Samples assessed	Colistin-resistant *E. coli* positive samples
Open markets	25	0
Slaughterhouses	25	6
Hospital environment	35	3
Total	85	9 (10.6%)

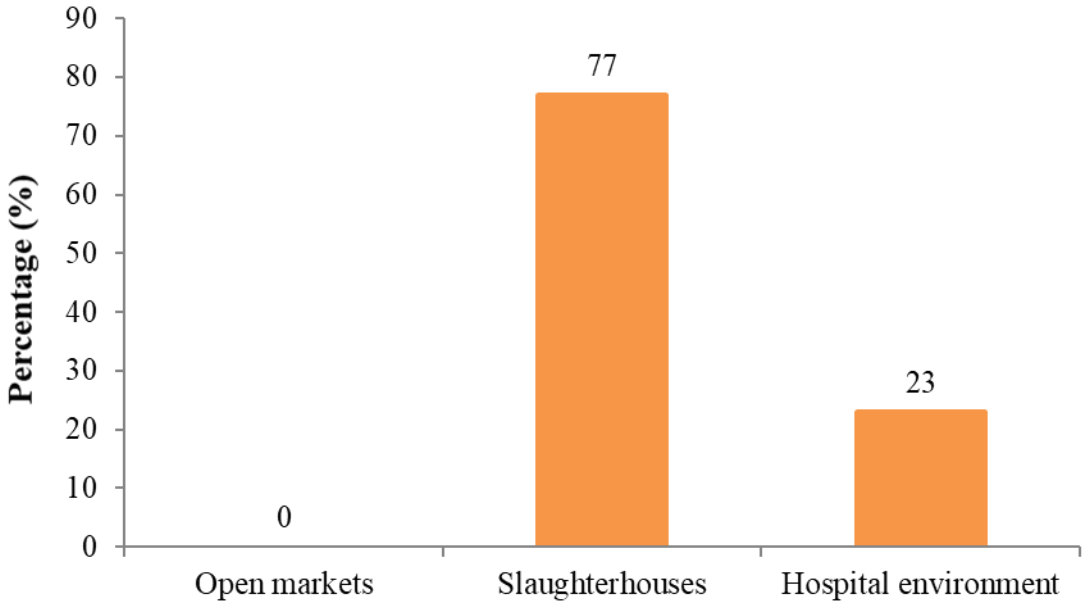

Figure 2: Percentage distribution of colistin-resistant *E. coli* positive samples based on sample sources

Our findings agree with comparable studies, which reported a higher prevalence of CREC in livestock's (0.02% to 20.6%) as compared to the 1.3% to 19% in retail meat and 0.08% to 2% in hospital settings (Hasman *et al*., 2015; Kluytmans *et al*., 2016). Higher CREC in our study could be probably due to the continuous and increasing exposure of animals to colistin as feed additives (particularly bacteria colonized in the gastrointestinal tract of animals), growth promoter and therapeutic purposes. These bacteria could contaminate animal products and meat during slaughterhouse processing and spread to humans via food chain or to workers through direct animal contact. In further agreement with this study, previous study has reported a noteworthy upsurge in the prevalence of colistin resistance over a period of a 3 years surveillance (El-Mokhtar *et al*., 2021). The differences observed among the studies can be attributed to differences in study location, geographical conditions, and climate. Additionally, the relatively high prevalence of colistin resistance in livestock and retail meat could be ascribed to the influence of *mcr-1* mediated resistance making food animals a notable reservoir for transmission to humans (Skov & Monnet, 2016) and possible transmission within the food chain. This poses significant health threat to humans that largely consume the products, animal and workers. This makes routine colistin resistance surveillance crucial in both community and hospital environment.

Antimicrobial Resistance Profile of Colistin-Resistant *E. coli* Isolates

The AMR profile of CREC is shown in Table 3. The observed resistance profile was ceftazidime (88.9%), aztreonam (66.7%), ampicillin (100%), amoxicillin-clavulanate (66.7%), meropenem (22.2%), gentamicin, tetracycline (77.8%), chloramphenicol (55.6%), nitrofurantoin (66.7%) and trimethoprim/sulfamethoxazole (77.8%). The highest resistance was demonstrated towards ampicillin with a resistance rate of 100%. No resistance was observed towards ciprofloxacin and gentamicin.

Table 3: Antimicrobial resistance profile of colistin-resistant *E. coli*

Antimicrobial class	Antibiotics	Resistance profile of CREC(*n*=9)		
		Sensitive (%)	Intermediate (%)	Resistance (%)
Cephalosporins	CAZ	1 (11.1)	0 (0)	8 (88.9)
Monobactams	AZT	3 (33.3)	0 (0)	6 (66.7)
Penicillins	AMP	0 (0)	1 (11.1)	8 (88.9)
B-lactam combination agents	AMC	1 (11.1)	2 (22.2)	6 (66.7)
Carbapenems	MEM	6 (66.7)	1 (11.1)	2 (22.2)
Aminoglycosides	GEN	8 (88.9)	1 (11.1)	0 (0)
Tetracyclines	TET	2 (22.2)	3 (33.3)	4 (44.4)
Fluoroquinolones	CIP	8 (0)	1 (11.1)	0 (0)
Phenicols	CHL	4 (44.4)	1 (11.1)	4 (44.4)
Nitofurans	NIT	3 (33.3)	0 (0)	6 (66.7)
Folate pathway inhibitors	SXT	0 (0)	2 (22.2)	7 (77.8)

Key: CAZ: ceftazidime (30 µg), AZT: aztreonam (30 µg), AMP: ampicillin (10 µg), AMC: amoxicillin-clavulanate (20/10 µg), MEM: meropenem (10 µg), GEN: gentamicin (10 µg), TET: tetracycline (30 µg), CIP: ciprofloxacin (5 µg), CHL: chloramphenicol (30 µg), NIT: nitrofurantoin (300 µg) and SXT: trimethoprim/sulfamethoxazole (1.25/23.75 µg).

Substantial proportion of the isolates were highly resistant to commercially available antibiotics with100% resistance demonstrated towards ampicillin comparable to previous study (Ma *et al*., 2022). These authors reported higher resistance profile of 90.9% towards amoxicillin/clavulanate and ceftazidime while lower resistance towards meropenem (33.3%) and gentamicin (11.1%) was equally reported. However, in this study all the isolates were susceptible to ciprofloxacin. In agreement with our study, high resistance rates of CREC isolates to antibiotics commonly used in veterinary therapy including ampicillin, tetracycline and chloramphenicol have been reported (Zhang *et al*., 2021). The high resistance rates correlate with other reports on isolates in Nigeria (Shettima *et al*., 2020).

Table 4: Multiple antimicrobial resistance index of colistin-resistant *E. coli*

Isolate code	Number of antibiotics	Resistance phenotype	MAR Index
AES1	9	CAZ/AZT/AMP/AMC/MEM/TET/CHL/NIT/SXT	0.82
HES4	8	CAZ/AZT/AMP/AMC/TET/CHL/NIT/SXT	0.73
HES30	8	CAZ/AZT/AMP/MEM/TET/CHL/NIT/SXT	0.73
CCS6	7	CAZ/AZT/AMP/AMC/TET/NIT/SXT	0.64
CCS11	7	CAZ/AMP/AMC/TET/CHL/NIT/SXT	0.64
HES15	7	CAZ/AMP/MEM/TET/CHL/NIT/SXT	0.64
CCS4	5	CAZ/AZT/AMP/AMC/SXT	0.46
AES8	5	AZT/AMP/AMC/TET/SXT	0.46
CCS10	5	CAZ/AMP/AMC/MEM/SXT	0.46

Key: CAZ: ceftazidime (30 µg), AZT: aztreonam (30 µg), AMP: ampicillin (10 µg), AMC: amoxicillin-clavulanate (20/10 µg), MEM: meropenem (10 µg), TET: tetracycline (30 µg), CHL: chloramphenicol (30 µg), NIT: nitrofurantoin (300 µg) and SXT: trimethoprim/sulfamethoxazole (1.25/23.75 µg).

The multiple antibiotics resistance (MAR) profile of CREC is shown in Table 4. It was observed that 1/9 (11.1%) was resistant to nine antibiotics, 2/9 (22.2%) was resistant to eight antibiotics, 3/9 (33.3%) was resistant to seven antibiotics, and 3/9 (33.3%) was resistant to five antibiotics. The MAR profile of the CREC in this study ranged from 0.83 – 0.46. All the isolates demonstrated resistance to at least five antibiotics and demonstrated an MAR index ≥ 0.46.

Mishra *et al*. (2013) reported that MAR index ≥0.2 designates high-risk contamination sources while MAR index ≥0.4 is allied with fecal contamination source. Furthermore, MAR index >0.2 portrays isolate from high-risk sources of contamination with antibiotic misuse. Therefore, high MAR index in this study necessitates adequate surveillance and measures to curb the spread of AMR.

Distribution of Phenotypic Virulence Factors

The phenotypic virulence distribution of CREC was shown in Figure 3. In total the virulence factors observed were lipase activity 3/9 (33.3%), β-hemolytic activity 2/9 (22.2%) and gelatinase activity 2/9 (22.2%).

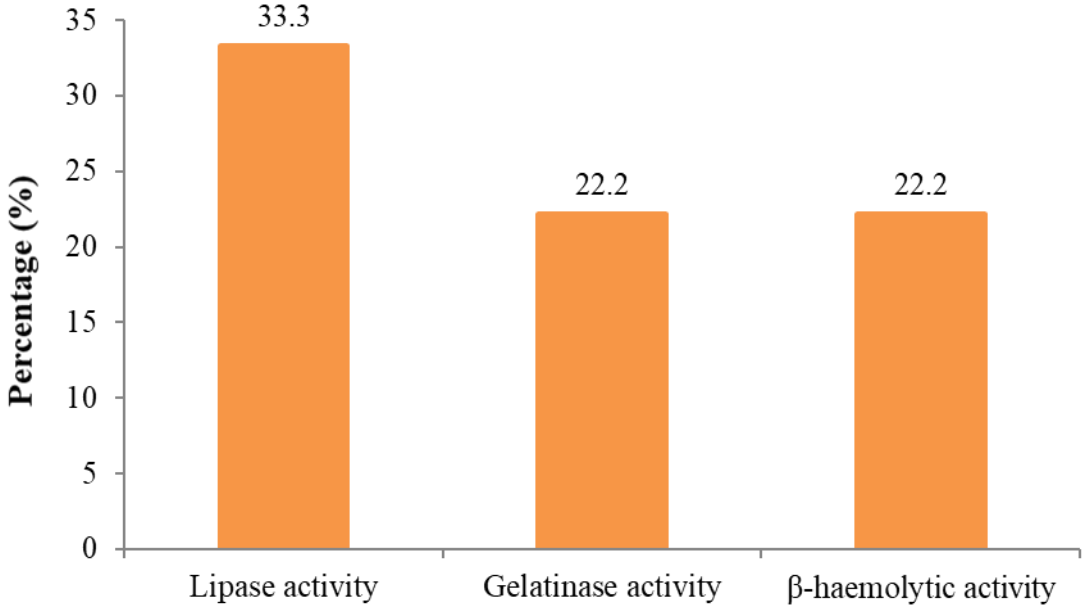

Figure 3: Distribution of colistin-resistant *E. coli* phenotypic virulence factors.

Lower rate of hemolysin production has been reported in previous studies (Mittal *et al*., 2014). Similarly, El-Mosallamy *et al*. (2015) have reported lower gelatinase activity. Hemolysin is a crucial virulence factor, which targets multiple pathways of the host and significantly influences infection (Beshiru & Igbinosa, 2018). Gelatinases are critical virulence factor of *E. coli* liable for numerous disease pathogenicity. Therefore, the linkage of isolates in this study to virulence factors further aggravates public and environmental health concern other than solely the spread of AMR.

CONCLUSION

The detection of CREC in both community and hospital environment allays a notable threat to public and environmental health. Therefore, routine AMR monitoring and surveillance towards critical antibiotics such as colistin classes of clinically relevant antimicrobials should

be implemented. These findings also highlight on the necessity for continuous studies to ascertain the factors that promote colistin resistance and the requisite to reinforce regulations specific to antimicrobial usage among farm personnel to reduce colistin resistance in the community. Public enlightenment campaign and proper education against indiscriminate antibiotics usage as animal feed and growth promoters is crucial to curtail the spread of AMR.

ACKNOWLEDGEMENT
We express gratitude to the Departments of Microbiology and Biotechnology, Western Delta University for their support and encouragement during the period of investigation.

CONFLICT OF INTEREST
The authors declare no competing interest.

REFERENCES
Beshiru, A. and Igbinosa, E.O. (2018). Characterization of extracellular virulence properties and biofilm-formation capacity of *Vibrio* species recovered from ready-to-eat (RTE) shrimps. *Microbial Pathogenesis*, **119**: 93-102.

Beshiru, A. and Igbinosa, E.O. (2023). Surveillance of *Vibrio parahaemolyticus* pathogens recovered from ready-to-eat foods. *Scientific Reports,* **13**(1): 4186.

Beshiru, A., Igbinosa, I.H. and Igbinosa, E.O. (2016). An investigation on antibiogram characteristics of *Escherichia coli* isolated from piggery farms in Benin City, Nigeria. *Annals of Science and Technology*, **1**(1): 8-12.

Beshiru, A., Igbinosa, I.H. and Igbinosa, E.O. (2018). Biofilm formation and potential virulence factors of *Salmonella* strains isolated from ready-to-eat shrimps. *PLoS ONE,* **13**(9): e0204345.

Beshiru, A., Igbinosa, I.H., Omeje, F.I., Ogofure, A.G., Eyong, M.M. *et al*. (2017). Multi-antibiotic resistant and putative virulence gene signatures in *Enterococcus* species isolated from pig farms environment. *Microbial Pathogenesis,* **104**: 90-96.

Beshiru, A., Okoh, A.I. and Igbinosa, E.O. (2022). Processed ready-to-eat (RTE) foods sold in Yenagoa Nigeria were colonized by diarrheagenic *Escherichia coli* which constitute a probable hazard to human health. *Plos ONE,* **17**(4): e0266059.

Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing (CLSI)*. 30th edn.Clinical and Laboratory Standards Institute. 2020. CLSI supplement M100.

El-Mokhtar, M.A., Daef, E., Mohamed-Hussein, A.A.R., Hashem, M.K. and Hassan, H.M. (2021). Emergence of nosocomial pneumonia caused by colistin-resistant *Escherichia coli* in patients admitted to chest intensive care unit. *Antibiotics,* **10**: 226.

El-Mosallamy, W.A., Desouky, S.M., El-Azum, A.A.A. and El Hamid, H.S.A. (2015). Detection of some virulence factors and pyelonephritis–associated pilus (pap) encoding operon gene in uropathogenic *Escherichia coli*. *Egyptian Journal of Medical Microbiology,* **24**(3): 37–43.

Falagas, M.E., Rafailidis, P.I. and Matthaiou, D.K. (2010). Resistance to polymyxins: mechanisms, frequency and treatment options. *Drug Resistance Updates,* **13**: 132-138.

Hasman, H., Hammerum, A., Hansen, F., Hendriksen, R., Olesen, B. *et al*. (2015). Detection of mcr-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveillance,* **20**(49): 30085.

Igbinosa, E.O., Beshiru, A. and Odjadjare, E.E.O. (2020a). Diversity, antimicrobial characterization and biofilm formation of *Enterococci* isolated from aquaculture and slaughterhouse sources in Benin City, Nigeria. *Ife Journal of Science,* **22**(3): 51-63.

Igbinosa, E.O., Beshiru, A., Igbinosa, I.H. and Okoh, A.I. (2022b). Antimicrobial resistance and genetic characterisation of *Salmonella enterica* from retail poultry meats in Benin City, Nigeria. *LWT Food Science and Technology,* **169**: 114049.

Igbinosa, E.O., Beshiru, A., Igbinosa, I.H., Cho, G.S. and Franz, C.M.A.P. (2023a). Multidrug-resistant extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* from farm produce and agricultural environments in Edo State, Nigeria. *Plos ONE,* **18**(3): e0282835.

Igbinosa, E.O., Beshiru, A., Igbinosa, I.H., Ogofure, A.G., Ekundayo, T.C. *et al*. (2023b). Prevalence, multiple antibiotic resistance and virulence profile of methicillin-resistant *Staphylococcus aureus* (MRSA) in retail poultry meat from Edo, Nigeria. *Frontiers in Cellular and Infection Microbiology,* **13**: 183.

Igbinosa, E.O., Okeigbemen, R., Beshiru, A. and Akinnibosun, O. (2022a). Detection and characterization of antibiotic resistance of Gram negative bacteria from hospital and non-hospital environments in Benin City Nigeria. *University of Lagos Journal of Basic Medical Sciences,* **9**: (1-2)

Igbinosa, E.O. and Beshiru, A. (2019). Characterization of antibiotic resistance and species diversity of staphylococci isolated from apparently healthy farm animals. *African Journal of Clinical and Experimental Microbiology,* **20**(4): 289-298.

Igbinosa, I.H. and Beshiru, A. (2018). Detection and antibiotic-resistance of *Salmonella* species and *Escherichia coli* from selected captive animals in Ogba Zoological Garden. *Nigerian Journal of Pure and Applied Sciences,* **31**(1): 3171-3079.

Igbinosa, I.H., Beshiru, A., Egharevba, N.E. and Igbinosa, E.O. (2020b). Distribution of Enterobacteria in ready-to-eat food in cafeterias and retail food outlets in Benin City: Public health implications. *Journal of Community Medicine and Primary Health Care,* **32**(2): 80-94.

Imanah, E.O., Beshiru, A. and Igbinosa, E.O. (2017). Antibiogram profile of *Pseudomonas aeruginosa* isolated from some selected hospital environmental drains. *Asian Pacific Journal of Tropical Disease,* **7**(10): 604-609.

Karaiskos, I., Lagou, S., Pontikis, K., Rapti, V. and Poulakou, G. (2019). The "old" and the "new" antibiotics for MDR Gram-negative pathogens: for whom, when, and how. *Frontiers in Public Health,* **7**: 151.

Kluytmans, M.F., Huizinga, P., Bonten, M.J., Bos, M., De Bruyne, K. *et al*. (2016). Presence of *mcr*-1-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009. *Eurosurveillance,* **21**(9): 12-18.

Ma, J., Tang, B., Lin, J., Ed-Dra, A., Lin, H. *et al*. (2022). Genome assessment of carbapenem and colistin-resistant *Escherichia coli* from patients in a Sentinel Hospital in China. *Cells,* **11**: 3480.

Marshall, B.M. and Levy, S.B. (2011). Food animals and antimicrobials: impacts on human health. *Clinical Microbiology Reviews,* **24**: 718.

Mezghani-Maalej, S., Rekik-Meziou, M., Mahjoubi, F. and Hammami, A. (2012). Epidemiological study of Enterobacteriaceae resistance to colistin in Sfax (Tunisia). *Medicine Malpractice and Infection,* **42**: 256–263.

Mishra, M., Patel, A.K. and Behera, N. (2013). Prevalence of multidrug resistant *E. coli* in the river Mahanadi of Sambalpur. *Current Research in Microbiology and Biotechnology,* **1**(5): 239-244.

Mittal, S., Sharma, M. and Chaudhary, U. (2014). Study of virulence factors of uropathogenic *Escherichia coli* and its antibiotic susceptibility pattern. *Indian Journal of Pathology and Microbiology,* **57**(1): 61–64.

O'Neill J. (2014). *Review on antimicrobial resistance*. Antimicrobial resistance: tackling a crisis for the health and wealth of nations, UK.

Olaitan, A.O., Morand, S. and Rolain, J.M. (2014). Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Frontiers in Microbiology,* **5**: 643.

Olowe, O. A., Olowe, R. A., Oluremi, A. S. and Adefioye, O. J. (2018). A novel report of colistin-resistant *Escherichia coli* carrying *mcr*-1 gene from animal and human fecal samples in Nigeria. *Pan African Journal of Life Sciences,* **1**: 7-10.

Phodha, T., Riewpaiboon, A., Malathum, K. and Coyte, P.C. (2019). Annual relative increased in inpatient mortality from antimicrobial resistant nosocomial infections in Thailand. *Epidemiology and Infection,* **147**: e133.

Poirel, L., Madec, J.Y., Lupo, A., Schink, A.K., Kieffer, N. *et al*. (2018). Antimicrobial resistance in *Escherichia coli*. *Microbiology Spectrum,* **6**(4): ARBA-0026-2017.

Shettima, S. A., Tickler, I. A., Dela Cruz, C. M. and Tenover, F. C. (2020). Characterisation of carbapenem-resistant Gram-negative organisms from clinical specimens in Yola, Nigeria. *Journal of Global Antimicrobial Resistance,* **21**: 42–45.

Skov, R.L. and Monnet, D.L. (2016). Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. *Eurosurveillances,* **21**(9): 30155.

Tang, B., Ma, Y., He, X., Zhou, Q., Chang, J. *et al*. (2021). Similar antimicrobial resistance of *Escherichia coli* strains isolated from retail chickens and poultry farms. *Foodborne Pathogens and Disease,* **18**: 489-496.

Zhang, S., Chen, S., Abbas, M., Wang, M., Jia, R. *et al*. (2021). High incidence of multi-drug resistance and heterogeneity of mobile genetic elements in *Escherichia coli* isolates from diseased ducks in Sichuan province of China. *Ecotoxicology and Environmental Safety,* **222**: 112475.

Zhuge, X., Ji, Y., Tang, F., Sun, Y., Jiang, M. *et al*. (2019). Population structure and antimicrobial resistance traits of avian-origin *mcr*-1-positive *Escherichia coli* in Eastern China, 2015 to 2017. *Transboundary and Emerging Diseases,* **66**: 1920–1929.