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Research Article

Phenotypic Detection of Metallo-β-Lactamase in Imipenem Resistant *E. Coli* Isolated from Drinking Water in Zaria, Nigeria

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ABSTRACT
Metallo-β-lactamase (MBL) producing bacteria pose a severe health risk globally. This study focused on phenotypic detection of MBL producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from drinking water from ABU Zaria, Kaduna State, Nigeria. The isolates (one hundred and eighty-four) were screened for carbapenem resistance. Of the isolates, 142 (77.2%) were *E. coli*, while 42 (22.8%) were *Klebsiella pneumoniae*. The imipenem resistant isolates (5) were all *E. coli* and were phenotypically screened for Metallo-β-lactamase production using the combination disc diffusion test (CDDT). Five (2.72%) *E. coli* isolates showed MBL production phenotypically while no MBL-production was detected in the *K. pneumoniae* isolates. The presence of MBL bacteria in drinking water highlights that water matrices are important routes of transmission of antibiotic-resistant bacteria.

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1.0 INTRODUCTION

Carbapenem resistance in *Enterobacteriaceae* has emerged as a threat of public health concern, since carbapenems were regarded as one of the few drugs of last resort for the treatment of infections caused by multidrug resistant pathogens (Ventola, 2015).

Carbapenem resistant *Enterobacteriaceae* (CRE) were assigned the highest threat level in 2013 and declared public health threat that requires urgent attention by the Centers for Disease Control and Prevention (CDC) (Fertas-Aissani *et al*. 2013). Invasive infections with MBL-producing isolates are also associated with a higher morbidity and mortality. (Walsh *et al*., 2005). The occurrence of an MBL-positive isolate in a hospital environment poses not only a therapeutic problem but is also a serious concern for infection control management (Lee *et al*. 2017). Detection usually involves preliminary screening for carbapenem resistance followed by phenotypic detection of MBL and confirmation by PCR detection accompanied by gene sequencing.

While the hospital environment has received serious attention as a major source of antibiotic-resistant pathogens, the spread of resistance genes thorough environmental compartments and as the site of antibiotic resistance evolution has only received attention recently (Quintela Baluja *et al*., 2015; Calfee, 2017). There is credible evidence that suggests raw water, treated water, effluents from hospitals, homes, industries as well as farm runoff contaminated with fecal material and chemicals are redistributors of drug-resistant pathogens to water bodies and other environmental compartments (Egervarn *et al*., 2017). Moreover, susceptible individuals may be exposed to these resistant bacteria through recreation in contaminated surface water, consuming contaminated drinking water and food (Andremont and Walsh, 2015).

Human activities around ABU, Zaria do not seem to abate but rather increase, the ABU Dam is under the threat of being gradually polluted. Given the critical role of water in sustaining life and the increasing threats posed by contamination, monitoring and evaluating the quality of drinking water sources is essential for safeguarding public health. Assessing the microbiological characteristics and determining the antimicrobial susceptibility pattern of water-borne isolates provide valuable insights into potential contamination sources and helps inform effective management strategies to ensure safe and sustainable water supply systems.

MATERIALS AND METHODS

Study Area

The Ahmadu Bello University Dam is the major source of water supply to the University community and other neighboring villages. Basically, the Dam was constructed to meet the water needs of ABU, Zaria community.

Sample Collection

A total of 312 water samples were collected using sterile screw-capped glass bottles (100 mL) (Haberecht *et al*., 2019) from Akenzua Hall, Booster Station, ICSA Ramat, Suleiman Hall, and Water Works in ABU Zaria over a period of nine months, January to September covering dry and rainy seasons respectively. Water samples were collected from the sampling points over the period. The cap of the sterile bottle was carefully removed and the water was fetched. (Cheesbrough 2006).

Isolation and Characterization of *E. coli* and *K. pneumoniae*

Water samples were inoculated onto the surface of freshly prepared MacConkey media. The spread plate method was used in inoculating dilutions on the surface of freshly prepared MacConkey agar. All inoculations were performed in duplicates and incubated at 37° C for 24 hours. Macroscopic examination for physical morphology was performed based on the size, color, texture, pigmentation, odor and consistency. Pink colonies (lactose fermenters) on MacConkey agar were subjected to the following biochemical tests; indole, methyl red, Voges Proskauer and citrate utilization tests. The isolates identified to be *E. coli* and *K. pneumoniae* were subjected to antibiotic susceptibility testing to determine MBL production.

Antibiotic susceptibility testing of *E. coli* and *K. pneumoniae*.

The antibiotic susceptibility pattern of the isolates was determined using the disc diffusion method. The antibiotics tested include meropenem (10 µg) and imipenem (10 µg) (CLSI, 2024). The standard inoculum was prepared by making a direct saline suspension of the colonies selected from a 24 hours culture. The suspension was compared to 0.5 McFarland standard. Sterile Mueller Hinton agar was prepared and seeded with 0.1 mL aliquot of the standardized inoculum using a sterile wire loop. The surface was allowed to dry by allowing it to stand for 5 minutes with the Petri dish lid in place. A sterile pair of forceps was used to place the antimicrobial discs on the surface of the medium and the plates were inverted and incubated aerobically at 37°C for 18 hours. After the incubation period, a ruler was used to measure the diameter of each zone of inhibition and it was recorded in millimeters (Chesbrough, 2006). All isolates that showed a zone of inhibition diameter of less than 19 mm were recorded as resistant while those with diameter above 23 mm were recorded as susceptible, for both imipenem and meropenem, respectively (CLSI, 2024).

Phenotypic confirmation of MBL-production by *E. coli*

All isolates that showed a zone of inhibition diameter of less than 19 mm were phenotypically screened for MBL-production using the Combination disk diffusion test (CDDT). The Combination Disc Diffusion Test (CDDT) for detection of Metallo-β-lactamases (MBL) is a phenotypic method used to identify bacteria that produce MBL enzymes. These enzymes hydrolyze carbapenem antibiotics such as Imipenem or Meropenem, leading to resistance. An imipenem disc soaked in 5µl of 0.5M EDTA and a 10µg imipenem disc without EDTA were placed on the Muller Hinton agar plates which was inoculated with the isolates. Plates were incubated for 24h at 37°C, an organism was considered MBL positive, if growth inhibition zone increase by 7mm or more in comparison with IMP disk alone (Shahcheraghi *et al*., 2010).

Data analysis

The data obtained from the seasonal distribution of the isolates and antibiotic susceptibility testing were subjected to statistical analysis using SPSS (Statistical Package for the Social Sciences). Inferential statistical technique Chi-square test was used compare seasonal distribution of the bacterial isolates, the association between organism and resistance, and the association between season and antibiotics. Significance level of $p < 0.05$ were considered significant.

RESULTS

Seasonal distribution of *E. coli* and *Klebsiella pneumoniae* isolated from the water samples is presented in Table 1. 70(44.9%) was recorded for *E. coli* in dry season while 72(46.1%) was recorded in rainy season. *K. pneumoniae* recorded 18(11.5%) and 24(15.4%) in dry and rainy season respectively. p-value, 0.76 represents association between season and distribution of isolates. The antibiotic susceptibility pattern of the isolates is presented in Table 2. *E coli* showed greater susceptibility to imipenem and meropenem (100%) in rainy season while resistance of 9.6% (5) was observed in dry season. *K. pneumoniae* showed susceptibility of 100% to imipenem in both seasons. The statistical analysis is presented in table 3**.** The combination disc diffusion test for MBL production by *E. coli* is as shown in Table 4. Out of the 184 isolates tested only 5(2.7%) were MBL producers. Phenotypic distribution of MBL based on sampling points is presented in figure 1. Suleiman Hall had 3 MBL producing *E. coli* while ICSA Ramat recorded 2. The remaining sampling points didn't record MBL producing isolates

Table 1: Seasonal distribution of *E. coli* and *K. pneumoniae* from water distribution channels

Isolate	Dry Season (n=156)	Rainy Season (n=156)	Total	p-value
Escherichia coli	70(44.9%)	72(46.1%)	142	
Klebsiella pneumoniae	18(11.5%)	24(15.4%)	42	
Total isolates	88	96	184	0.76

Table 2: Seasonal antibiotic susceptibility pattern of *E. coli* and *K. pneumoniae* isolated from water distribution channels

Organism	Antibiotic (ug)	Dry Season Susceptible n (%)	Dry Season Resistant n (%)	Rainy Season Susceptible n (%)	Rainy Season Resistant n (%)
Escherichia coli	Imipenem (10)	47(90.4)	5(9.6)	50(100)	0(0)
	Meropenem (10)	51(98)	1(2)	50(100)	0(0)
Klebsiella pneumoniae	Imipenem (10)	18(100)	0(0)	24(100)	0(0)
	Meropenem (10)	18(100)	0(0)	0(0)	24(100)

KEY: n; Total number of isolates tested

Table 3: Chi Square Analysis of the Association Between Organism type, Season and Antibiotic Resistance Among Isolates

Antibiotic	X^2	P-value	Association
Imipenem[a]	2.13	0.144	Not significant
Meropenem[a]	65.40	0.001	Highly Significant
Imipenem[b]	3.55	0.059	Not significant
Meropenem[b]	21.99	0.000003	Highly Significant

KEY: superscript a = organism versus resistance, Superscript b = season versus antibiotics

Table 4: Combination disc diffusion test for MBL production by *E. coli* isolated from water samples isolated in Zaria, Nigeria

	Zone of Inhibition (mm)	
Isolate Lab Code	IMP	IMP + EDTA
E. coli SH3	0	7
E. coli IM9	0	8
E. coli SM7	0	8
E. coli IM5	0	10
E. coli SH4	0	8

KEY: IMP = Imipenem, EDTA = Ethylenediaminetetraacetic Acid

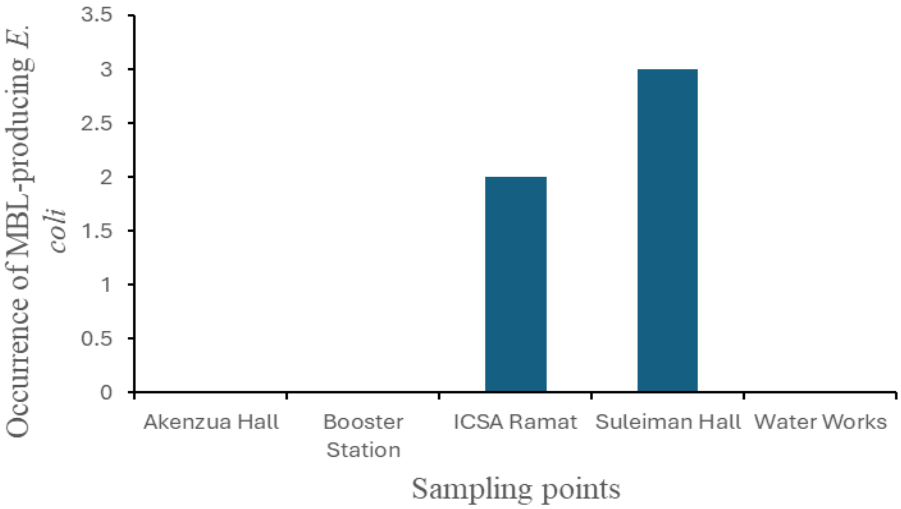

Figure 1: Phenotypic distribution of MBL based on sampling points

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DISCUSSION

Escherichia coli and *Klebsiella* spp. are coliforms and their presence in water is an indication of faecal contamination. In this study, *E. coli* was more frequently isolated than *Klebsiella pneumoniae* which is the case in most studies. In this study, 70(44.9%) was recorded for *E. coli* in dry season while 72(46.1%) was recorded in rainy season. *K. pneumoniae* recorded 18(11.5%) and 24(15.4%) in dry and rainy season respectively. These bacteria usually get to the water treatment plant most likely as a result of run-off from domestic dwellings, from human and/or animal faeces, or it could also be from farmlands from animal droppings used as manure that gets to the Kubanni river which is the ABU Dam primary source of water. Microbial contamination may also originate from distribution pipes after treatment. The seasonal differences observed in the distribution of isolates was not significant statistically. The greater susceptibility of 50(100%) observed against imipenem and meropenem in rainy season against *E coli* in this study strongly agrees with the findings of Beshiru *et al*. (2024) which reported 100% susceptibility of ESBL *E. coli* from surface water sources in Edo State to imipenem and meropenem. The works of Zhaojun *et al*. (2017), Chavda *et al.* (2016) and Jude *et al*. (2023) strongly argue in favor of this current study that carbapenems such as imipenem and meropenem are regarded as some of the few drugs of last resort for the treatment of infections caused by multidrug resistant pathogens. An important observation in this study is that *K. pneumoniae* was more resistant to meropenem than *E. coli.* The higher resistance observed in *K. pneumoniae* compared to *E. coli* may be attributed to its greater ability to acquire and express carbapenems' genes which hydrolyze third carbapenems, enhanced biofilm-forming ability, and the protective role of its polysaccharide capsule. These factors, combined with its higher adaptability to environmental stress and potential exposure to anthropogenic contamination sources, may account for its increased resistance profile in the studied water samples. Findings revealed by Pitout *et al*. (2008) and Nordmann *et al*. (2011) strongly support this observation. A highly significant association between organism type and meropenem resistance was observed, with higher resistance observed in *K. pneumoniae.* It was also observed in this current study that resistance to meropenem was higher in rainy season than dry season. This could be attributed to mobilization of antibiotic residues from

pharmaceutical waste, hospital effluents and agricultural runoff (livestock antibiotics) by rainfall to water systems, increased environmental contamination from surface runoff, sewage overflow, and wastewater discharge. Statistically, there was significant association between season and meropenem resistance. Works of Berendonk *et al*. (2015) and Karkman *et al*. (2018) on environmental AMR studies are in line with the findings of this study. MBLs are a group of β-lactamase enzymes which need one or two zinc in their active site to cleave the amide bond of the β-lactam ring to inactive β-lactam antibiotics (Bebrone *et al*. 2013). MBL-producing *E. coli* exhibited a prevalence of 2.7%, detected in 5 out of 184 isolates while MBL-production was not detected in *K. pneumoniae*. The detection of MBL-producing *E. coli* in ICSA Ramat and Suleiman Hall but not *Klebsiella* may be attributed to the higher prevalence of *E. coli* in fecally contaminated water, differences in ecological survival, and variability in the distribution of carbapenemase genes among Enterobacteriaceae. Additionally, the relatively lower abundance of *Klebsiella* isolates in the samples may have reduced the likelihood of detecting MBL producers. The prevalence of 2.7% of MBL-producing *E. coli* slightly differs from the work of Agbo *et al*. (2015) that reported 2.6% MBL-producing *E. coli* from household water sources in Enugu, Nigeria. This finding raised a serious concern that water environment can act as reservoir for MBL producing Enterobacteriaceae. The detection of MBL-producing isolates in drinking water may be attributed to contamination from wastewater sources, horizontal gene transfer among environmental bacteria, selective pressure from antibiotic residues and heavy metals, as well as survival of resistant bacteria within biofilms in water distribution systems. In this current study MBL *E. coli* was isolated from 2 sampling points while not detected in 3 sampling points, this may reflect spatial variation within the water distribution system. Factors such as biofilm formation within pipelines, differences in residual disinfectant levels, localized contamination, and variations in microbial load may contribute to the uneven distribution of resistant bacteria across the sampling points.

CONCLUSION

The phenotypic detection of Metallo-β-lactamase in *E. coli* (5 of 184 isolates; 2.7%) isolated from water samples shows the role played by environmental samples in the horizontal spread of antibiotic resistance genes. The overall implication of these resistant bacteria in tap water is that the MBL bacteria can spread among residents of Ahmadu Bello University, Zaria who use the water for drinking and other domestic purposes. Therefore, there is need for routine monitoring of water quality and the need for improvement in the treatment process in ABU water treatment plant.

Conflict of interests

No potential conflict of interest was reported by the author(s).

REFERENCES

Agbo, E. C., & Eze, E. A. (2015). Phenotypic detection of metallo-beta-lactamase production in imipenem-resistant isolates of *Escherichia coli* and *Klebsiella* species isolated from hospital and environmental sources in Nsukka, Enugu State, Nigeria. *Journal of Biology and Nature, 2*(4), 135–141.

Andremont, A., & Walsh, T. R. (2015). The role of sanitation in the development and spread of antimicrobial resistance. *AMR Control*, 68–73.

Bebrone, C., Bogaerts, P., Delbrück, H., Bennink, S., Kupper, M. B., & De Castro, R. (2013). GES-18, a new carbapenem-hydrolyzing GES-type beta-lactamase from *Pseudomonas aeruginosa* that contains Ile80 and Ser170 residues. *Antimicrobial Agents and Chemotherapy, 57*(1), 396–401. https://doi.org/10.1128/AAC.01712-12

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M. N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., & Martinez, J. L. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology, 13*(5), 310–317. https://doi.org/10.1038/nrmicro3439

Beshiru, A., Isokpehi, N. A., Igbinosa, I. H., Akinnibosun, O., Ogofure, A. G., &

Igbinosa, E. O. (2024). Extended-spectrum beta-lactamase (ESBL)- and non-ESBL producing *Escherichia coli* surveillance in surface water sources in Edo State, Nigeria: A public health concern. *Scientific Reports, 14*, 21658. https://doi.org/10.1038/s41598-024-21658

Calfee, D. P. (2017). Trends in community versus healthcare-acquired methicillin-resistant *Staphylococcus aureus* infections. *Current Infectious Disease Reports, 19*(12), 48.

Chavda, K. D., Chen, L., Fouts, D. E., Sutton, G., Brinkac, L., & Jenkins, S. G. (2016). Comprehensive genome analysis of carbapenemase-producing *Enterobacter* spp.: new insights into phylogeny, population structure and resistance mechanisms. *mBio, 7*(6), e02093-16.

Cheesbrough M (2000) *District Laboratory Practice in Tropical Countries* Low Price Edition part 2. Cambridge University Press, England pp. 101-103

Cheesbrough M. (2006). District laboratory practice in tropical countries. Second Edition, Part 2. Cambridge University Press, Edinburgh building, Cambridge. Published in the United States of America by Cambridge University Press, New York; pp. 138–141

Egervärn, M., Englund, S., Ljunge, M., Wiberg, C., Finn, M., & Lindblad, M. (2017). Unexpected occurrence of transferable extended-spectrum cephalosporinase-producing *Escherichia coli* in Swedish surface waters used for drinking water supply. *Science of the Total Environment, 587–588*, 466–472.

Fertas-Aissani, M. E., Messai, Y., Alouache, S., & Bakour, R. (2013). Virulence profile and antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from different clinical specimens. *Pathologie Biologie, 61*(5), 209–216.

Haberecht, H.B., Nealon, N.J., Gilliland, J.R., Holder, A.V., Runyan, C., Oppel, R.C., Ibrahim, H.M., Mueller, L., Schrupp, F., Vilchez, S., Antony, L., Scaria, J. and Ryan, E.P. (2019). Antimicrobial-resistant *Escherichia coli* from environmental waters in Northern Colorado. *Journal of Environmental Public Health*.

Jude, Y. N., Sarah, A. J., Yusuf, A. A., Veronica, D., Zakari, H., Caroline, C. M., Amayi, D., Ujah, O., Gloria, A. G., Nanya, A. C., Joseph, A. A., & Samirah, A. D. (2023). Molecular detection of carbapenem-resistant *Klebsiella pneumoniae* isolated from clinical specimens in Jos, Nigeria. *Microbes and Infectious Diseases, 4*(2), 555–562.

Karkman, A., Do, T. T., Walsh, F., & Virta, M. P. J. (2018). Antibiotic-resistance genes in waste water. *Trends in Microbiology, 26*(3), 220–228. https://doi.org/10.1016/j.tim.2017.09.005

Lee, C. R., Lee, J. H., Park, K. S., Kim, Y. B., Jeong, B. C., & Lee, S. H. (2017). Antimicrobial resistance of hypervirulent *Klebsiella pneumoniae*: Epidemiology, Hypervirulence-Associated Determinants and Resistance Mechanisms. *Frontiers in Cellular and Infectious Microbiology*, 7, 483.

Nordmann, P., Naas, T., & Poirel, L. (2011). Global spread of carbapenemase-producing Enterobacteriaceae. *Emerging Infectious Diseases, 17*(10), 1791–1798. https://doi.org/10.3201/eid1710.110655

Pitout, J. D. D., & Laupland, K. B. (2008). Extended-spectrum beta-lactamase-producing Enterobacteriaceae: An emerging public health concern. *The Lancet Infectious Diseases, 8*(3), 159–166. https://doi.org/10.1016/S1473-3099(08)70041-0

Quintela-Baluja, M., Chan, C. W., Alnakip, M. E., Abouelnaga, M., & Graham, D. W. (2015). Sanitation, water quality and antibiotic resistance dissemination. In A. Méndez-Vilas (Ed.), *The battle against microbial pathogens: Basic science, technological advances and educational programs* (pp. 965–975). Formatex Research Center

Shahcheraghi, F., Nikbin, V.S., and Feizabadi, M. (2010). Identification and genetic characterization of metallo-beta-lactamase producing strains

of *Pseudomonas aeruginosa* in Tehran, Iran. *New Microbiologica*. 33:243–248

Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. *P & T, 40*(4), 277–283.

Walsh, T.R., Toleman, M.A., Poirel, L. and Nordmann, P. (2005). Metallo-ß-lactamases: the quiet before the storm? *Clinical Microbiology Reviews*. 18:306–25.

Zhaojun, C., Daojun, Y., Songzhe, H., Hui, Y., Lei, Z., Yanping, W., Wenhui, Z., Liping, S., & Shuchang, C. (2017). Prevalence of antibiotic-resistant *Escherichia coli* in drinking water sources in Hangzhou City. *Frontiers in Microbiology, 8*, 1133. https://doi.org/10.3389/fmicb.2017.01133