

Isolation and Characterisation of Carbapenemase-Producing *Escherichia coli* Isolates from Fomites in Madonna University, Elele Campus, Rivers State, Nigeria

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Abstract

The rise of carbapenem-resistant bacteria exemplifies a significant worldwide health concern due to antibiotic resistance. This research examined the prevalence and antimicrobial resistance profiles of carbapenemase-producing *Escherichia coli* (CPE) obtained from fomites at Madonna University, Elele Campus, Rivers State, Nigeria. One hundred swab samples were obtained from commonly touched surfaces at five sites: hospital, school, church, boys' hostel, and girls' hostel. Samples were cultivated using conventional microbiological procedures, and antibiotic susceptibility was assessed utilizing the Kirby-Bauer disc diffusion method. The Modified Hodge Test was used to evaluate carbapenemase production. Of 100 samples, 8% produced *E. coli* isolates, with the hospital area exhibiting the greatest incidence at 12%. Antibiotic susceptibility tests demonstrated significant multidrug resistance (MDR), with all isolates displaying a multiple antibiotic resistance (MAR) index over 0.2. The highest MAR values (0.9–1.0) were recorded in isolates from the hospital vicinity. Resistance was particularly severe against beta-lactams (Ampiclox, Cefuroxime, and Cefotaxime), whereas Ofloxacin displayed the maximum effectiveness. Nonetheless, none of the isolates exhibited good results for carbapenemase synthesis. The results reinforce fomites' importance as potential MDR bacteria reservoirs and highlight the hospital area as a key contamination hotspot. The research underlines the critical need for increased hygiene standards, frequent environmental monitoring, and antibiotic stewardship to limit the emergence of resistant bacteria in academic contexts.

Keywords: Antibiotic resistance, Carbapenemase, Multidrug resistance, Fomites, Environmental contamination, Hygiene

Introduction

Antibiotic resistance, particularly the emergence of carbapenem resistance, poses a significant threat to global public health [1]. The increasing prevalence of carbapenemase-producing *Escherichia coli* (CPE) adds complexity to treating bacterial infections. In the specific context of academic institutions, the potential transmission and persistence of CPE on fomites represent an understudied area of concern. Antibiotic resistance in *Escherichia coli* has been on the rise globally, presenting a serious threat to the efficacy of antibiotic treatment regimens. The emergence of resistance to carbapenems, a class of beta-lactam antibiotics that are the most effective against both Gram-positive and Gram-negative bacteria and have a wide range of antibacterial activity, is especially concerning [2]. It is frequently regarded as a last resort for treating severe bacterial infections. For infection management and treatment measures to be successful, it is critical to understand the mechanisms and prevalence of carbapenem resistance in *E. coli*. For infections brought on by the most resistant bacteria, including *Enterobacteriaceae*, (*Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter spp.*, *Serratia spp.*, *Proteus spp.*), *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterococcus fecium*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Shigella spp.*, and *Mycobacterium tuberculosis*, carbapenem antibiotics are thought to be the first line of treatment [3].

Carbapenemase production in *E. coli* significantly limits treatment options, as these enzymes can hydrolyze carbapenem antibiotics [4]. This resistance mechanism is associated with increased mortality rates and poses challenges in both healthcare and community settings. Investigating the occurrence of CPE in non-clinical environments, such as academic institutions, is critical for a comprehensive understanding of the epidemiology of antibiotic-resistant strains. There is an urgent need for novel antibiotics. Nonetheless, this presents significant challenges. Pharmaceutical companies must allocate substantial financial resources and time towards scientific

inquiry, and despite these efforts, only one in five drugs that enter the preliminary human testing phase ultimately gain approval from the US Food and Drug Administration [5].

Fomites refer to inanimate objects or surfaces that may contain or transmit faecal pathogens [6]. Inanimate objects, often referred to as fomites, are recognized for their role in the transmission of human pathogens. This can occur through direct contact, where surfaces come into contact with the mouth, or indirectly, through contaminated fingers leading to oral transmission [7]. Common fomites found indoors include countertops, handrails, doorknobs, light switches, mobile phones, and more. Fomites present a significant issue in healthcare settings, including hospitals, doctor's offices, and care homes. The presence of these microorganisms suggests a bio-transfer potential, indicating they could be transferred to other substrates where growth might occur, such as on food, inanimate objects, or the human body [8]. A study revealed that picornaviruses were detected on 20 percent of toys in paediatric office waiting rooms that were tested, indicating a viral risk associated with fomites in these high-traffic environments that accommodate numerous individuals daily [9]. Additionally, rotavirus was found to be present on almost half of the sampled surfaces in a pediatric unit, with fomites harboring high viral loads being thermometers and play mats, both of which come into direct contact with large numbers of children [10].

However, the study focuses on fomites within Madonna University, Elele campus, Nigeria as a potential reservoir for carbapenemase-producing *Escherichia coli* (CPE), addressing a research gap in understanding the environmental dynamics of carbapenem-resistant strains.

Materials and Methods

Study area

The research was conducted out at Madonna University, Elele, Rivers state, Nigeria from September 2023 to October 2023. The geographical coordinates for Elele are approximately 5.1018° N latitude and 6.8190° E longitude. The Elele campus is equipped with a range of facilities to support both academic and extracurricular activities with the presence of state-of-the-art classrooms and lecture halls, libraries, On-campus accommodations, sporting facilities, teaching hospital, churches all designed to facilitate effective learning and moral upbringing. Madonna University, across all its campuses, has a student enrollment ranging between 10,000 to 14,999, classifying it as a large-sized institution. Swab samples were taken from five designated facilities.

Sample gathering and processing

This study used an experimental research approach. The study was conducted in five locations (school area, hospital area, church area, boys hostel, and girls hostel) within Madonna University. These locations included common contact points such as tables, chairs, shelves, floors, windows, door handles, drawer handles, computer keyboards, socket switches, air conditioners/fans, and hospital beds. A deliberate sampling strategy was used. A total of 100 sterile swab stick samples were utilized to swab five different Madonna University sites. Following proper labelling with reference numbers, they were sent to the pharmaceutical microbiology laboratory. The samples were cultured for 18 to 24 hours after being inoculated in a nutrient broth medium.

Isolation and isolate identification

The Eosin Methylene Blue agar (TM MEDIA, Titan biotech LTD, Rajasthan, India) was inoculated with the turbidity-marked growth from the nutrient broth and incubated for 24 hours at 35°C. The *E. coli* isolate is identified by the appearance of a green metallic sheen colony on the agar plate. The bacterial isolates were submitted to normal microbiological techniques, such as morphological features of the colonies, to distinguish between Gram-negative and positive bacteria. To further identify and characterize the isolates, biochemical analyses were performed. Gram staining (negative), the Indole test (positive), the Catalase test (positive), and the Oxidase test (negative) were among the biochemical tests that were performed. Bergey's Determinative Bacteriology Manual (1994) was used in order to compare the isolates' morphological and biochemical traits [11].

Antibiotic sensitivity testing (AST)

Kirby-Bauer's disc diffusion technique was used to determine the isolates' antibiotic sensitivity, and the results were interpreted in accordance with Clinical Laboratory Standard [12]. The inoculum's broth culture was standardized after 18 to 24 hours (modified to 0.5 McFarland Standard 108 cfu/ml). Using a sterile swab stick, the produced standardized inoculum was seeded onto Mueller-Hinton susceptibility agar plates and let dry for five to ten minutes. A total of twelve (12) antibiotics, including Ampiclox (10 µg), Cefuroxime (30 µg), Ceftriaxone Sulbactam (45 µg), Nalidixic acid (30 µg), Cefexime (5 µg), Ofloxacin (5 µg), Imipenem/Cilastatin (10/10 µg), Cefotaxime (25 µg), Nitrofurantoin (300 µg), Levofloxacin 5 µg, Gentamycin (10 µg), and Amoxicillin Clavulanate (30 µg) were used in the Gram-negative antibiotics susceptibility disc (Celtec, Diagnostic, Belgium Inc.) [13].

Screening for carbapenemase production

The Modified Hodge test was used to check for carbapenemase production in isolates that had imipenem-resistance or intermediate zones, or 16 mm. Five millilitres of broth were used to create a 0.5 McFarland dilution of the indicator strain, *Escherichia coli* susceptible isolate. A Mueller Hinton agar plate was streaked with a 1:10 dilution in the form of a lawn. In the middle of the test area was a disc containing 10 µg of meropenem. From the disk's edge to the plate's edge, the test organism was streaked in a straight line. For 16–24 hours, the plate was incubated at 35°C at room temperature. The outcomes were noted [14].

Analysis of data

The Student's t-test was used to assess the statistical differences in the isolates' incidence in the various sites. Statistical significance was defined as a two-tailed P value of less than 0.05. With Graph Pad Prism 5, all statistical analyses were carried out.

Results

Data presented in Table 1 displays the comprehensive count of samples gathered from various fomites including table surfaces, chair surfaces, slabs, rails, door knobs, and toilet seat surfaces. These samples were collected from five distinct locations within Madonna University, Elele Campus.

Table 1: Collection of Sample

Sample Location	No. of Collected Samples
Hospital Area	25
Church Area	10
School Area	15
Boys' Hostel	30
Girls' Hostel	20
Total	100

The data presented in Table 2 shows the Prevalence of *E. coli* isolates from five different locations in Madonna University, Elele campus. The percentage prevalence results of *E. coli* isolates within different locations in the university environment illustrated that the highest number of *E. coli* isolates found to be at the hospital area with 12% prevalence. The statistical test carried out using the student “t” test method showed no significant difference between the number of *E. coli* and non-*E. coli* isolates in the location with P value = 0.05

Table 2: Prevalence Of *E. coli* Isolates

S/N	Location	No of <i>E. coli</i> Isolates (% prevalence)	No. of Non <i>E. coli</i> Isolates (% prevalence)
1.	Girls hostel	1(5%)	19(95%)
2.	Boys hostel	2(6.67%)	28(93.33%)
3.	Hospital Area	3(12%)	22(88%)
4.	Church area	1(10%)	9(90%)
5.	School area	1(6.67%)	14(93.33%)
6.	Total prevalence	8(8%)	92 (92%)

$$\% \text{ Prevalence} = \frac{\text{No of Escherichia coli isolates}}{\text{Total No of Isolates}} \times 100$$

The data presented in Table 3 showcases the antibiotic resistance profile of various isolates against different antibiotics. The result shows different activities of the antibiotics towards the isolates. The isolates were resistant, susceptible, and intermediate in their response to the actions of the antibiotic. The total number of antibiotics used was 12 in a single multiple antibiotic disc.

Table 3: Antimicrobial Susceptibility Pattern

Antibiotics	No of Susceptible isolates	No of Intermediate isolates	No of Resistant isolates
Ampiclox ACX 10µg	0	0	8
Cefuroxime CXM30µg	0	0	8
Ceftriaxone Sulbactam CRO 45µg	0	0	8
Nalidixic acid NA 30µg	0	0	8
Cefexime ZEM 5µg	0	0	8
Ofloxacin OFX 5µg	7	0	1
Imipenem/Cilastatin IMP 10/10µg	1	0	7
Cefotaxime CTX 25µg	0	0	8
Nitrofurantoin NF 300µg	0	0	8
Levofloxacin LBC 5µg	3	1	4
Gentamycin GN 10µg	0	0	8
Amoxicillin Clavulanate AUG 30µg	0	0	8

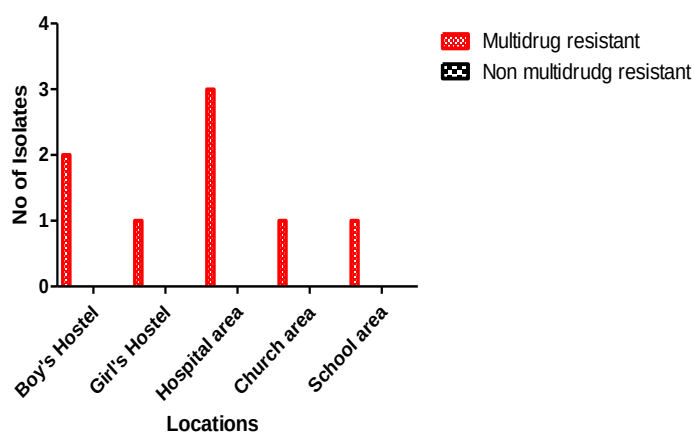
The result presented in Table 4 shows the resistant pattern of all isolates with their percentage. From all indications, HA137, HA139, CA185, and SA96 had the highest MAR index of 0.9. BH189 and BH190 had the lowest with an index of 0.7. All the isolates were multidrug resistant judging by their MAR Index which is greater than 0.2.

Table 4: Resistant pattern and MAR index of *E. coli*

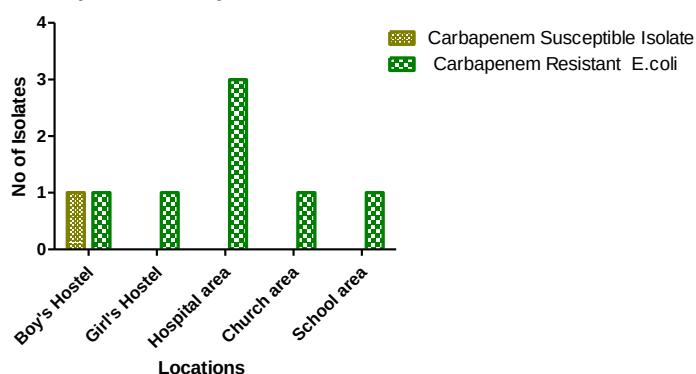
S/N	Isolate Identity	No. of Antibiotics used	No. of Resistant Antibiotic	MAR Index
1	HA136	12	10	0.8
2	HA137	12	11	0.9
3	HA139	12	11	0.9
4	GH180	12	12	1.0
5	CA185	12	11	0.9
6	BH189	12	9	0.7
7	BH190	12	9	0.7
8	SA96	12	11	0.9

MAR index = (No. of resistant antibiotic/Total No. of Antibiotic used); MAR index > 0.2 is MDR

The established standard MAR index designates a MAR index ≥ 0.2 as indicative of multi-drug resistance (MDR). All the isolates surpassed this threshold, placing them within the MDR category as can be seen in Figure 1. Specifically, isolates from the hospital area showcase the highest number of multidrug-resistant strains amongst the sources examined whereas isolates from the girls' hostel, boys' hostel, and church area exhibited the lowest number of multidrug-resistant isolates.

Multidrug Resistant Patten of the Isolates**Figure 1: Multidrug-resistant pattern of the isolates**

The graph in Figure 2 illustrates a higher count of isolates resistant to carbapenem compared to those susceptible. From the results presented from all locations, there are a higher number of carbapenem-resistant isolates than the susceptible isolates.

Carbapenem activity on the Isolates**Figure 2: Carbapenem activity on the isolates**

The data presented in Table 5 illustrates that none of the *E. coli* isolates was found to produce carbapenemase enzyme.

Table 5: Carbapenemase-producing isolates

Location (Isolate ID)	<i>E. coli</i> isolates	Carbapenemase-producing
Boys Hostel (189 & 190)	2	0
Girls Hostel (180)	1	0
Hospital Area (136,137,139)	3	0
Church Area (185)	1	0
School Area (96)	1	0

Discussion

E. coli's patterns of antibiotic resistance continue to be a major global public health concern, resulting in severe health issues such as extended hospital stays and treatment failure [15, 16]. The comprehensive sampling from various fomites across five distinct locations within Madonna University provides a solid basis for understanding environmental contamination. The highest number of samples (30) was collected from the Boys' Hostel, likely due to the dense population and frequent contact with fomites. Conversely, the Church Area had the lowest number of samples (10), reflecting its limited exposure to human activities compared to other locations. This distribution suggests that areas with high human interaction, like hostels and hospitals, are critical zones for microbial surveillance, particularly for pathogens such as *E. coli*.

This result is consistent with other studies reporting a higher prevalence of *E. coli* in hospitals [17, 18]. The presence of faecal coliforms, including *E. coli*, in this analysis, indicates the presence of faecal pollutants on the doorknobs, according to a prior study by Navab-Daneshmand et al. [19]. This might be because most people take cleanliness for granted and do not understand that hand washing is a fundamental method of preventing the spread of infectious agents, which leads them to contaminate their hands with faecal and urinal material after using the lavatory. This result is consistent with the findings of Ikede *et al.* [20], who identified *E. coli* isolates from hospital door knobs and handles.

This work is in line with the work of Agu et al., [21] who isolated *E. coli* from fomites in hospitals in Ado-Ekiti, Ekiti State, Nigeria. The most prevalent bacteria in HAIs, according to another research conducted in Africa are *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella*, and *Staphylococcus aureus* [22]. These three microbes have a high level of antibiotic resistance in addition to being simpler to move than others. However, compared to the others, they are more resilient to sterilization and disinfection techniques. Certain traits have caused certain microbes to be more prevalent than others [23].

The prevalence data indicates that the hospital area had the highest percentage of *E. coli* isolates (12%). This aligns with the environment's role as a healthcare setting, where fomites are exposed to patients carrying pathogenic microbes. The statistical analysis using the student "t" test showed no significant difference between the number of *E. coli* and non-*E. coli* isolates ($P = 0.05$). This suggests that while *E. coli* contamination is notable, other non-*E. coli* microorganisms are also prevalent. Interestingly, the Girls' Hostel and Boys' Hostel exhibited relatively low prevalence (1% and 2%, respectively), possibly reflecting better hygiene practices or lower direct contamination sources compared to the Hospital Area. The highest prevalence of *E. coli* and multidrug resistance highlights the hospital area as a critical hotspot for contamination and potential pathogen transmission. Lower prevalence and resistance rates in the hostels and church areas suggest comparatively better hygiene or less exposure to contaminated fomites. Moderate prevalence in school areas suggests an environment that balances high foot traffic with controlled hygiene practices.

E. coli isolated from various fomites at different locations in the University, showed differences in antibiotic sensitivity patterns. The results demonstrate significant resistance among isolates, with a multidrug-resistant (MDR) phenotype evident in all isolates based on their MAR (Multiple Antibiotic Resistance) Index. The resistance observed includes: Ampiclox (ACX), Cefuroxime (CXM), Cefotaxime (CTX), and Amoxicillin/Clavulanate (AUG) which showed consistent resistance across isolates, reflecting the inefficacy of these antibiotics in combating *E. coli*. This work is in line with the work of Rakhalaru et al [24] who isolated *E. coli* isolates resistant to β -lactam antibiotics from fomites in rural residents in South Africa. Levofloxacin (LBC) showed intermediate activity, indicating potential but limited effectiveness. Ofloxacin (OFX) exhibited the highest susceptibility among the antibiotics tested, suggesting its potential as a treatment option. This observation collaborates with that of (Naqid *et al.*, [25] who reported that *E. coli* is more sensitive to fluoroquinolones. This result is in agreement with a study conducted at a tertiary care hospital in Gujarat, India by Goswami *et al.*, [26] who found that *Escherichia coli* isolates were more sensitive to fluoroquinolones (levofloxacin and ciprofloxacin) more than the other antibiotics tested. These trends support earlier findings that significant proportions of isolates of Gram-negative bacteria in clinical and environmental environments are resistant to several antibiotics [27–29]. This suggests that illnesses brought on by these bacteria cannot be treated with these medications. According to reports, enormous amounts of antibiotics are used annually in clinical and environmental settings, which, among other reasons, significantly contributes to the rising incidence of bacteria resistant to antibiotics [30].

The MAR index ranged from 0.7 to 1.0, far exceeding the threshold of 0.2 for multidrug resistance. Isolates HA137, HA139, CA185, and SA96 had the highest MAR index (0.9), highlighting their ability to resist nearly all tested antibiotics. Results show that all isolates were multi-drug resistant as they had a MAR index > 0.2 . A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used [31]. The highest MAR index of 1.0 was obtained from GH180, followed by a MAR index of 0.9 which was obtained from 4 different locations i.e. (HA136, HA139, CA185, and SA96). The lowest MAR index value of 0.7 was obtained from the locations; BH189, and BH190. The result aligns with previous reports whose study revealed the presence of multidrug resistance in *E. coli* with MAR index > 0.2 , indicating their source to be from high-risk sources, being previously exposed to antibiotics [32–33]. For the study carried out, the prevalence of multidrug resistance is illustrated in Table 4 where eight *E. coli* isolates were investigated and results revealed that 100% of the isolates were MDR. From all indications, HA137, HA139, CA185, and SA96

had the highest MAR index of 0.9. BH189 and BH190 had the lowest with an index of 0.7. Multiple antibiotic resistance index reveals the spread of bacterial resistance in a given population [33,34]. MARI value greater than or equal to 0.2 indicates the high-risk source of contamination where antibiotics are often used. Similarly, several studies have reported the occurrence of MDR *E. coli* on inanimate hospital surfaces, Ibrahim *et al.* [35] and Shams *et al.* [36] reported the prevalence of 92.2% and 83% MDR *E. coli* respectively. This work is in line with the report of Wuna *et al.* [37] who also isolates MDR *E. coli* from medical equipment and surfaces from selected hospitals in Minna, Nigeria with a prevalence of 86.4%. This work is in line with the work of Rakhalaru *et al.* [24] who isolated MDR *E. coli* isolates from fomites in rural residents in South Africa and that of Bassey *et al.* [38] from different fomites in Cross River University of Technology Medical Centre Calabar, Nigeria.

Carbapenem resistance was notably high, as illustrated in Figure 2. Despite this, none of the *E. coli* isolates tested positive for carbapenemase production. This suggests that resistance mechanisms may involve efflux pumps, porin modifications, or other non-enzymatic factors rather than carbapenemase enzymes [39]. The absence of carbapenemase-producing isolates, especially in high-risk areas like the Hospital Area, is a positive finding. However, the presence of carbapenem-resistant isolates remains concerning, indicating a potential risk of therapeutic failure with carbapenem-based treatments.

Conclusions

This study highlights the presence of multidrug-resistant *Escherichia coli* on fomites within Madonna University, Elele Campus, with the highest prevalence observed in the hospital area. Despite significant antibiotic resistance among isolates, none were found to produce carbapenemase. The findings emphasize the potential role of fomites in the transmission of antibiotic-resistant bacteria, underscoring the need for stringent hygiene practices and routine microbial surveillance. Continuous monitoring and antimicrobial stewardship programs are essential to mitigate the spread of resistant strains and safeguard public health.

Ethical approval

Not applicable.

Conflict of interest

No conflict of interest was recorded.

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Contribution of authors

OOJ: Conceptualization, Supervision, editing; JMG: Conceptualization, editing; CEC: Supervision; VEO: Writing and Editing; VIE: Writing and Editing; CNO: Writing and Editing; OAN: Writing and Editing.

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