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Antibiotic Resistance of the Pathogenic *E. coli* Isolates in the Food Samples Collected from the Government's Primary Schools, Kombo Central District, The Gambia

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Abstract

Antimicrobial-resistant infections cause the death of approximately 700,000 people annually around the world, and this rate is predicted to reach 10 million by 2050. The study aimed to determine the antibiotic resistance of the pathogenic E coli isolate in the food samples collected from the government's primary schools Kombo Central District. A cross-sectional study design was used, and 104 food samples were collected using the aseptic method. These food samples were analyzed in the laboratory using biochemical methods to assess the prevalence of pathogenic E. coli. Antibiotic resistance testing was performed on these isolated pathogenic *E. coli* using the Kirby-Bauer disk diffusion method. The E. coli isolates were 100 % resistant to Penicillin G, Cefotaxime, and Oxacillin and 80 % resistant to Amoxycillin/Clavulanic acid. Sulphamethoxazole/Trimethoprim was the most effective antibiotic, followed by Tetracycline. The prevalence of ESBL-Producing *E. coli* was 80% and all the tested isolates had multidrug-resistant abilities. According to the antimicrobial susceptibility pattern of the 10 pathogenic *E. coli* tested in this study, Tetracycline and Sulphamethoxazole/Trimethoprim should thus be considered encouraging antimicrobial agents in treated *E. coli* infections in the Gambia.

Keywords: Antibiotic-resistant; Pathogenic *E. coli*; Food samples; Government Primary school; The Gambia

1. Introduction

Antibiotics are among the most commonly prescribed drugs used in both human medicine and in farm animals, resulting in the selection of multiple drug-resistant bacteria (10,18;). Infections with resistant bacteria are difficult to treat, causing severe illness and requiring costly and sometimes toxic alternatives, such as antibiotics as a last resort. Drugs of last resort, such as vancomycin against gram-positive bacteria and colistin against gram-negative bacteria, have been the most reliable therapeutic agents against multidrug-resistant bacteria (20). However, bacterial strains resistant to these antibiotics have been isolated worldwide (20). This resistance can result from a chromosomal gene mutation but comes mainly from horizontal transfer from external gene sources (18). The development of novel antibiotics is not likely to solve the problem and it is probably only a matter of time before they will also be ineffective. Since bacteria will inevitably find ways of resisting conventional antibiotics, alternative approaches are urgent (18). Antimicrobial resistance in *E. coli* is consistently highest for antimicrobial agents that have been in use for the longest time in human and veterinary medicine, such as ampicillin. However, in the past two decades, increases have been observed in the emergence and spread of multidrug-resistant bacteria, including strains resistant to newer antibiotics such as fluoroquinolones and extended-spectrum cephalosporins (19)

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Antimicrobial-resistant infections cause the death of approximately 700,000 people annually around the world, and this rate is predicted to reach 10 million by 2050. There has been a noticeable increase in antimicrobial resistance in gram-negative bacteria such as *E. coli* over the years (17). According to (15), worldwide, several antimicrobial-resistant food-borne pathogens have emerged recently in the food-production chain, which can transmit to humans and cause infections. *E. coli* resistance to commonly utilized antimicrobials is one of them and is creating trouble for the healthcare system worldwide. *E. coli* is considered a useful indicator of antimicrobial resistance for a wide range of bacteria due to its medical importance and its presence in a wide range of hosts (1,11).

A study by (8) shows that *E. coli* was resistant to the following antibiotics, ceftriaxone (79%) and penicillin (79%), followed by nalidixic acid (67%), sulfamethoxazole/trimethoprim (64%), ampicillin (44%), and oxytetracycline (31%) and 3% of the isolates showed resistance to azithromycin resistance. The result of the study (7) using 6 different antibiotics to test for antibiotic resistance of *E. coli* isolates from raw beef shows a rising resistance by the *E. coli* isolates to conventionally utilized first-line antibiotics. The resistance ranged from 60% in tetracycline, 80% in erythromycin, and 85% in amoxicillin. The high rates of antibiotic resistance of *E. coli* are a public health concern as organisms with resistant plasmids and genes can pass on the resistance to previously susceptible organisms, thus leading to a clone of multi-drug resistant superbugs which are difficult to control.

According to the study by(2) on the Prevalence of antibiotic-resistant e coli in drinking water sources in Hangzhou city, a total of 200 *E. coli* isolates from drinking water sources were examined. Among them, 99 isolates (49.50%) were resistant to at least one of the 18 antibiotics tested, the most frequently resisted antibiotic was tetracycline (42.00%), followed by ampicillin (29.00%), piperacillin (27.00%), trimethoprim/sulfamethoxazole, (25.50%), and chloramphenicol (19.00%). Isolates were more susceptible to aztreonam (94.00%), amoxicillin/clavulanate (96.00%), ceftazidime (98.00%), and amikacin (99.00%). No isolates were resistant to imipenem or meropenem. Additionally, 48 isolates (24.00%) exhibited multiple resistance, and six isolates were found to be resistant to all classes of antibiotics. Notably, 75.51% of multiple-resistant *E. coli* (37/49) in their study were resistant to tetracycline and β-lactams concurrently.

2. Methods

2.1. Study Design

A cross-sectional mixed-method study design was conducted in the government's primary schools in the Kombo Central District. The study was done from November 2022 to September 2023.

2.2. Study Setting

The study was conducted in nineteen government primary schools in the Kombo Central District. Kombo Central is a district in the West Coast Region Two in The Gambia. It is located at latitude 13° 15 '00.0" N (13.2500000°) and longitude 16° 40' 00.0" W (-16.6666700°) with a total population of 142,831 (5). Kombo Central has a total of 25 primary schools, among which 19 were government schools, 5 private, and 1 grant-aided school. The district was divided into three clusters namely Brikama, Jamisa, and Bakary Sambouya. Each cluster was headed by a cluster monitor who was responsible for helping the schools achieve the best possible teaching and learning and the highest possible standards for pupils. Each of the schools was headed by a headmaster/mistress.

2.3. Data Collection Methods

The food samples collection form was adopted from (Gilbert et al., 2000), and it was used during sample collection and laboratory analysis. Information such as the sample number, the sample name, the place where the samples were collected, the date, the time it was collected, and the time it arrived in the laboratory was recorded on it. Each Sample had its form. The food samples were put into sterile containers, the containers were labeled with identifier codes kept in a cool box containing ice packs, and transported within 1-3 hours to the University of the Gambia Biology Laboratory for analysis. A food sample collection form was filled out for each sample.

2.4. Reliability and Validity of Instruments

Training on collection was done with data collectors, which was coordinated by my supervisor and the lecturer who did the laboratory analysis and 10 food samples were also collected and analysed at the laboratory. The aseptic technique was used throughout all sampling, handling, and analysis procedures by using sterile materials and flaming. Samples were kept in a cool box containing ice packs and transported to the University of the Gambia biology laboratory within one to three hours. Microbial analysis was conducted within one to three hours after the sample collection to avoid

unpredictable changes. The microbial analysis involved negative controls for the reliability of the results. Culture Media was prepared aseptically based on the manufacturer's instructions.

2.5. Antibiotic resistance analysis of the *E. coli*

The susceptibility of the *E. coli* isolates to 6 commonly utilized antibiotics such as Amoxycillin/Clavulanic acid (2:1) (AMC30), Penicillin G (P10), Cefotaxime (CTX30), Sulphamethoxazole/Trimethoprim (SXT25), Tetracycline (TE30) and Oxacillin (OX1) was done to determine antibiotic resistance of the in isolated *E coli*. The antibiotics were from OXOID LTD, UK. Analysis was done using the disk diffusion method on Müller Hinton agar as described in the approved standard, eleventh edition of Clinical and Laboratory Standards Institute (CLSI) for antimicrobial disk susceptibility tests for bacteria and yeasts, testing volume 32 (3). This test was carried out on the *E. coli* isolate that manifested high pathogenicity.

2.6. Data Management

The food samples were collected in a sterile container, labeled, and kept in a cool box containing ice packs. When the samples arrived at the laboratory, they were prepared and cultured, and a warning label was pasted on the incubator and cool box so that no one could open it without permission.

2.7. Data Analysis

The laboratory analysis was done as described in 3.8.1-3.8.5. The results from the food samples analysis were used to determine the prevalence of *E coli*. The prevalence of pathogenic *E coli* and antibiotic-resistant E. *coli* was also determined. The data was then exported to Statistical Package for the Social Sciences (SPSS) software version 20.0 for analysis. Descriptive statistics, such as frequency and percentage, were computed, and the data were presented in tables

2.8. Ethical Procedures

Approval was obtained from the University of the Gambia Department of Public and Environmental Health Research Committee. Before the data collection, permission was obtained from the regional education director of West Coast Region Two to conduct the research in Kombo Central District primary schools. The director was verbally informed and issued a supportive letter obtained from the Department of Public and Environmental Health research committee. The director then gave the researcher a permission letter (19 copies), which the researcher took to the headmaster/mistress of each school within the study area before the date of data collection to solicit support and cooperation. Before the commencement of the study, schools were visited to gather information on the number of food handlers in each school, which was obtained from the food handlers' register book of each school. Also, the respondents were informed both verbally (for those who cannot read) and through the participant's information sheet (for those who can read) about the objective of the study as well as the confidentiality of their information and their right to participate or not to participate. The food vendors were informed that a sample of their food would be collected for analysis before the commencement of the study.

3. Results

Table 1 shows biochemical detection of *E. coli* and analysis of pathogenic and biofilm-producing *E. coli* from different food samples. Of the 34 isolated *E. coli*, 25 (73.5 %) were found to be pathogenic and biofilm-producing, while 9 (26.5 %) were non-pathogenic and non-biofilm-producing *E. coli*. Out of the 25 pathogenic *E. coli* isolates, 8 (32.0% %) were found to be highly pathogenic/strong biofilm producers, 11 (44%) were intermediate pathogenic/biofilm producers and 6 (24%) were low/weak pathogenic/biofilm producers (Table 3) (Plate 5). Percentage-wise, "gorong" soup has the highest prevalence (75.0%) of pathogenic *E. coli,* followed by steamed fish sauce (44.4%), Cake (33.3%), and Puff-puff 33%. Fish balls and "Acheke" were not contaminated with pathogenic *E. coli*. However, all three 3 *E. coli* isolates from Ice were highly pathogenic.

Table 2 shows the result of the antibiotic resistance test. High antibiotic resistance was observed among the 10 tested pathogenic/biofilm-producing *E. coli* (Plate 1). All the tested *E. coli* isolates show 100 % resistance toward 3 of the antibiotics (Penicillin G (P10), Cefotaxime (CTX30) and Oxacillin (OX1). While 8 of the pathogenic/biofilm-producing *E. coli* were resistant to Amoxicillin/Clavulanic acid (2:1) (AMC30). The most effective antibiotic is Sulphamethoxazole/Trimethoprim (SXT25) with 80% sensitivity, followed by Tetracycline (TE30) with 70% sensitivity. The most antibiotic-resistant *E. coli* was from sample 90 ("Ebbe" sample type), which was resistant to all 6 tested antibiotics, as shown in plate 1D. Five different resistotypes of *E. coli* were observed in this study (Table 3). All the 10 tested pathogenic *E. coli* exhibited multidrug-resistant (MDR) patterns. MDR *E. coli* were resistant to as few as 3 and as many as 5 antimicrobial classes.

Table 1 Biochemical detection of *E. coli* and analysis of pathogenic and biofilm-producing *E. coli*

Sample Type	***E. coli* positive n (%)**	**Pathogenic/biofilm producing *E. coli***			**Non-pathogenic/ biofilm producing**
		High	**Moderate**	**Low**	
Bean sauce (n=19)	6 (31.6)	0 (0.0)	2 (10.5)	0 (0.0)	4 (21.1)
Steam fish sauce (n=18)	9 (50.0)	1 (5.6)	3 (16.7)	4 (22.2)	1 (5.6)
Gorong soup (n=4)	3 (75.0)	1 (25.0)	2 (50.0)	0 (0.0)	0 (0.0)
Fish ball (n=7)	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	2 (28.6)
Ebbe (n=13)	4 (30.8)	1 (7.7)	1 (7.7)	1 (7.7)	1 (7.7)
Puff-puff (n=12)	4 (33.3)	1 (8.3)	2 (16.7)	1 (8.3)	0 (0.0)
Fish pie (n=6)	1 (16.7)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
Cakes (n=3)	1 (33.3)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
Acheke (n=3)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)
Local beverage (ice) (n=19)	3 (15.8)	3 (15.8)	0 (0.0)	0 (0.0)	0 (0.0)
Total	34 (32.7)	8 (7.7)	11 (10.6)	6 (5.8)	9 (8.7)

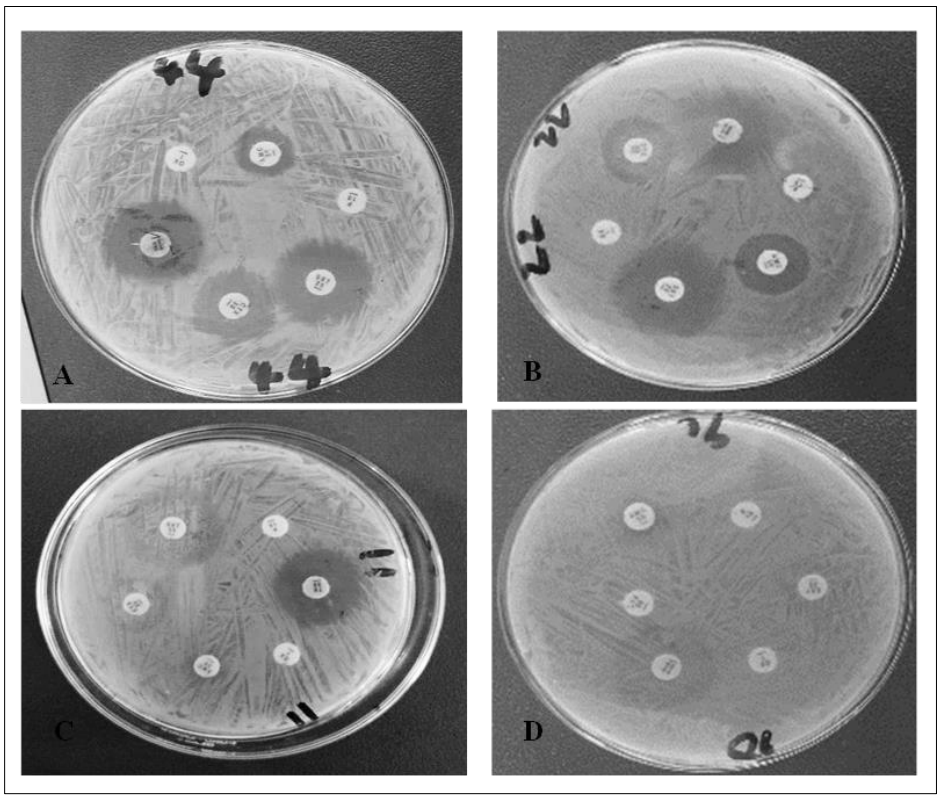

Plate 1 Isolated Pathogenic E. coli showing multiple antimicrobial resistance

- Antimicrobial resistance pattern of *E. coli* isolate 44 from "ice" sample type.
- Antimicrobial resistance pattern of *E. coli* isolate 22 from fish pie sample type.
- Antimicrobial resistance pattern of *E. coli* isolate 11 from "Gorong soup" sample type.
- Antimicrobial resistance pattern of *E. coli* isolate 90 from "Ebbe" sample type, showing complete resistance to all the tested antibiotics

Table 2 Antibiotic resistance pattern of the 10 most pathogenic *E. coli* isolates

Antimicrobial agent	Antimicrobial class	Disk potency (µg or unit)	Zone diameter interpretation (mm)			Resistant pattern of E. coli isolates n (%)		
			Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
Amoxycillin/Clavulanic acid (2:1) (AMC30)	β-lactams	30	≤ 13	14-17	≥18	8 (80.0)	2 (20.0)	0 (0.0)
Penicillin G (P10)*	Penicillins	10	≤ 19	20-27	≥28	10 (100.0)	0 (0.0)	0 (0.0)
Cefotaxime (CTX30)	Cephalosporin	30	≤ 22	23-25	≥26	10 (100.0)	0 (0.0)	0 (0.0)
Sulphamethoxazole/Trimethoprim (SXT25)	Folate pathway inhibitors	25	≤ 10	11-15	≥16	1 (10.0)	1 (10.0)	8 (80.0)
Tetracycline (TE30)	Tetracyclines	30	≤ 11	12-14	≥15	2 (20.0)	1 (10.0)	7 (70.0)
Oxacillin (OX1)*	β-lactams	1	≤ 10	11-12	≥13	10 (100.0)	0 (0.0)	0 (0.0)

Table 3 Resistotypes of E. coli isolates and multidrug resistance patterns

Number	Resistotypes/antimicrobial resistance pattern	Number of isolates
3	CTX, P, OX	1
4	CTX, P, OX, AMC	6
4	CTX, P, OX, TE	1
5	CTX, P, OX, AMC, TE	1
6	CTX, P, OX, AMC, TE, SXT,	1

4. Discussion

The indiscriminate use of antimicrobials in human medicine, veterinary, and agricultural purposes has led to antimicrobial resistance becoming a worldwide concern (9). In this study, an antibiotic resistance test was done for 10 of the most pathogenic *E. coli* isolates based on Congo red analysis results. All the isolates (100%) were resistant to Penicillin G, Cefotaxime, and Oxacillin. While 80 % were resistant to Amoxycillin/Clavulanic acid (2:1) antibiotics. Tan et al. (16) have recorded a similar level of resistance to Penicillin (85.7 %). A similar result (92.7% resistance) for Cefotaxime was recorded by Sarba et al. (13). However, the results are in contradict what Dubravka et al (4) reported, where they observed 100 % susceptibility to Cefotaxime and Amoxicillin-clavulanic acid. Considering that 8 of the *E. coli* isolates were resistant to the β-lactamase class of antibiotics, it means that all 8 *E. coli* isolates were Extended-Spectrum beta-lactamase (ESBL) producing *E. coli*. This high prevalence of *ESBL*-producing *E. coli* points to the abuse of the β-lactamase class of antibiotics in the Gambia. According to Wu et al. (21), multidrug-resistant (MDR) *E. coli* are *E. coli* isolates that are resistant to at least three distinct antibiotic families. Going by this definition, the detection of MDR *E. coli* was 100 % in this current study. Five classes (resisotype) of MDR *E. coli* were detected in this study. Dubravka et al. (4) also recorded 4 classes of MDR *E. coli* in their study. Multidrug-resistant nature of the *E. coli* isolates in this study points to their disease-causing ability. According to Wu et al. (21), multidrug-resistant bacterial infections are associated with high mortality and serious public health problems.

The most effective antibiotic in the study is Sulphamethoxazole/Trimethoprim, showing a susceptibility prevalence of 80 %. This result is in line with the report by Dubravka et al (4), who recorded 84 % susceptibility for Sulphamethoxazole/Trimethoprim. The study is also comparable to what Sarba et al. (13) reported on Sulphamethoxazole/Trimethoprim (100 %) susceptibility. The result is also close to the results of Shecho et al. (14), where they observed 92.3% susceptibility to Sulphamethoxazole/Trimethoprim. Sulphamethoxazole/Trimethoprim is a non-β-lactam antimicrobial agent and a folate pathway inhibitor class of antibiotic.

This study detected low, but some resistance toward Tetracycline (20 %). The results for Tetracycline resistance in this study are in line with what Dubravka et al (4) reported (16 % resistance for tetracycline). Sarba et al. (13) reported a resistance level of 46.3 % for Tetracycline, which is higher than the prevalence recorded in this study. Similarly, a 45% resistance prevalence of Tetracycline was reported by Ružauskas et al. (12). All these results support the idea that reducing the prescription/abuse of antibiotics is vital in controlling resistant *E. coli*, including extended-spectrum β-lactamase producers (21).

5. Conclusion

Most of the *E. coli* strains in this study were resistant to beta-lactam antibiotics and, therefore, extended-spectrum beta-lactamase (ESBL) producing *E. coli*. Tetracycline and Sulphamethoxazole/Trimethoprim were the most effective antibiotics in the study and both are non-beta lactamase agents. According to the antimicrobial susceptibility pattern of the 10 pathogenic *E. coli* tested in this study, Tetracycline and Sulphamethoxazole/Trimethoprim should thus be considered encouraging antimicrobial agents in treated *E. coli* infections in the Gambia.

Compliance with ethical standards

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Author contributions

Fatoumata Njim, Evelyn A. Uyamadu, and Abdoulie Nanko designed the study. Fatoumata Njim was involved in the study design and data collection. Lamin B.S. Dibba performed Laboratory analysis. Lamin B.S Dibba interpreted the result. Fatoumata Njim wrote the main body of the manuscript. Evelyn A. Uyamadu and Abdoulie Nanko edited and revised the manuscript. All authors approved the final version of the manuscript

Data availability

The datasets generated and/or analysed during the current study are not publicly available due to privacy and ethical concerns, neither the data nor the source of the data can be made available, but are available from the corresponding author on reasonable request.

Disclosure of conflict of interest

The authors declare no competing interests.

Statement of ethical approval

Approval was obtained from the University of the Gambia Department of Public and Environmental Health Research Committee. Before the data collection, permission was obtained from the Regional Education Director of West Coast Region Two to conduct the research in Kombo Central District primary schools

Statement of informed consent

Respondents were informed through both verbal (for those who cannot read) and the participant's information sheet (for those who can read) about the objective of the study as well as the confidentiality of their information and their right to participate or not to participate.

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