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Prevalence of *Enterobacteriaceae* from Clinical Isolates in Federal Teaching Hospital Gombe, Nigeria

[1]Saleh, Z. M., [1]Umar, A. T.,[2]Baba, F. A., [1]Adamu, M. T.,[1]Yarma, A. A., [1]Isa, S., [3,4]Manga, M. M. and *[1]Garba, L.

[1]Department of Microbiology, Faculty of Science, Gombe State University, PMB 127, Tudun Wada Gombe, Gombe State, Nigeria

[2]Department of Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Gombe State University, PMB 127, Tudun Wada Gombe, Gombe State, Nigeria

[3]College of Medical Sciences, Gombe State University, PMB 127, Tudun Wada Gombe, Gombe State, Nigeria

[4]Department of Medical Microbiology and Immunology, Federal Teaching Hospital Gombe, P.O. Box 0037, Off Ashaka road, Gombe State, Nigeria

***Correspondence email:** lagarpak@gsu.edu.ng

Abstract

Enterobacteriaceae is a family of Gram-negative, oxidase-negative, and catalase-positive bacteria mostly found in Humans and animals' intestines. Some of these organisms are enteric opportunistic pathogens associated with urinary tract infections, respiratory tract infections, and wound infections, whereas others are regularly pathogenic for humans. This study aimed to determine the prevalence of Enterobacteriaceae in clinical samples from Federal Teaching Hospital Gombe between August 2022 and November 2022. A total of 420 non-duplicate isolates from various clinical samples were analyzed in the study. The isolates were identified based on cultural characteristics, Gram staining, and standard biochemical tests. Out of the 420 isolates identified, Escherichia coli was the most prevalent with 163(38.8%) isolates, followed by Klebsiella pneumoniae with 69(16.4%), Klebsiella oxytoca with 61(14.5%), Proteus spp. 28(6.7%), Citrobacter spp. 27(6.4%), Enterobacter spp. 22(5.2%), Serratia marcescens 13(3.1%), Providencia spp. 12(2.9%), Yersinia enterocolitica 11(2.6%), Morganella morganii 3(0.7%) and Salmonella spp. with 2(0.5%). Based on clinical specimens, urine had the highest percentage of isolates with 53.4%, followed by wound swab (19.1%), stool (10.1%), High vaginal swab (6.8%), Endocervical swab (3.6%), sputum (3.1%), blood (1.4%), Cerebrospinal fluid (1.0%) and semen (1.0%) and then pleural fluid (0.5%). In conclusion, Enterobacteriaceae clinical isolates were highly prevalent in Federal Teaching Hospital Gombe. Further research to assess the antimicrobial resistance profile of these clinical bacterial organisms in the study area is recommended for effective treatment options for bacterial infections.

Keywords: Pathogens, Enterobacteriaceae, prevalence, clinical samples, and identification.

INTRODUCTION

The family *Enterobacteriaceae* comprises many closely related bacteria that inhabit large intestines of man and animals, water, soil, and decaying materials. These bacteria are short Gram-negative rods, facultative anaerobes that grow on basic laboratory media like MacConkey agar and can be either motile or non-motile with peritrichous flagella. They ferment glucose to create either acid or acid and gas. They are catalase-positive and oxidase-negative. Moreover, they break down glucose and other carbohydrates under aerobic and anaerobic conditions (Bhatia and Ichhpujani, 2008).

The *Enterobacteriaceae* family includes many genera, such as Escherichia, Salmonella, Klebsiella, Enterobacter, Proteus, Shigella, and others (Carroll *et al.*, 2016). The number of genera and species is constantly increasing, with the greatest increase in percentages after 2005, when the number of genera increased from 40 to 68, and the number of species increased from 150 to 355 (Janda and Abbott, 2020). *Enterobacteriaceae* are medically important Gram-negative organisms that account for 80% of clinically relevant Gram-negative bacilli and 50% of septicaemia cases (Gillespie and Hawkey, 2006).

Enterobacteriaceae account for nearly one-third of all Intensive care unit (ICU) cases-acquired pneumonia, one-third of all ICU-acquired urinary tract infections, and 10 to 15% of ICU-acquired bloodstream infections.

Treatment options for these enteric organisms include beta-lactam antibiotics such as Penicillins, Cephalosporins, Carbapenems, and Monobactam, Aztreonam, in combination with beta-lactamase inhibitors, quinolones, Aminoglycosides, and Tigecycline (Thenmozhi *et al.*, 2014).

The extensive use of these beta-lactam antibiotics has caused the spread of resistant *Enterobacteriaceae*. The essential mechanism of resistance to beta-lactam antibiotics involves the production of beta-lactamases (especially ESßLs) that inactivate beta-lactam antibiotics by breaking down the amide bond of the antibiotic's beta-lactam ring, and this continues to be the leading cause of global beta-lactam antibiotics resistance among *Enterobacteriaceae* (Teklu *et al.*, 2019).

Antimicrobial resistance has been a serious clinical issue and a danger to the global healthcare system. The emergence of diverse resistance mechanisms, such as drug modification and their unavailability to microbial target sites, gives birth to microbial resistance (Ferri *et al.*, 2017; Garba *et al.*, 2021a). It is a concern that bacterial diseases are becoming more resistant to antimicrobial agents, which may be primarily due to an adaptable microbial genetic system under pressure from various control agents (Garba *et al.*, 2018). Antimicrobial resistance has been linked to the improper and excessive use of medicines in conjunction with the pharmaceutical firms' failure to create novel drugs, maybe due to legal and financial constraints. According to data from the Centers for Disease Control and Prevention (CDC), a number of bacteria pose a major risk to patients' safety from antibiotic resistance, as well as to the efficacy of treatments and the financial burden on government healthcare (Garba *et al.*, 2021b).

As reported by Morfin-otero *et al.* (2013), clinical isolates were essential components in the management and surveillance of infections in hospitals. Therefore, this study aimed to determine the prevalence of *Enterobacteriaceae* among clinical isolates obtained from the Federal Teaching Hospital, Gombe.

MATERIALS AND METHODS

Study location and sample collection

The study was conducted at the Department of Medical Microbiology/Immunology, Federal Teaching Hospital (FTH), Gombe. The Hospital was established in 1996 with a mandate to serve Gombe State as a referral tertiary healthcare facility. It has 500 beds capacity with about 90-95% occupancy rate.

Ethical clearance was obtained from the Health Research Committee of the Gombe State Ministry of Health (MOH/ADM/621/Vol.1/417). A total of 420 clinical isolates from various clinical samples, which include urine, wound swab, stool, blood, sputum, high vaginal swab, endocervical swab, semen, cerebrospinal fluid, and pleural fluid, were collected from the main Microbiology laboratory of the Federal Teaching Hospital and transported immediately to the research laboratory FTH, Gombe for analysis. Demographic information of the isolated sources was obtained from hospital records of individual patients.

Identification of bacterial isolates

The bacterial isolates were sub-cultured on MacConkey agar plates prepared according to the manufacturer's instruction and incubated at 35°C for 18 hrs. Suspected Enterobacteriaceae isolates were identified based on cultural characteristics, Gram staining, and biochemical tests according to Cheesbrough (2006), as described in the following subheadings:

Gram staining and microscopy

A smear of each colony of the isolates from an overnight culture was prepared by emulsifying the colony in a drop of sterile distilled water on a microscope slide, left to air-dry, and set over a Bunsen burner flame. After applying a crystal violet stain to the fixed smear for 60 seconds, it was quickly cleaned with clean water. After applying drops of Lugol's iodine and waiting for 60 seconds, clean water was used to cleanse the area once more. Acetone was used to quickly decolorize it, and clean water was then used to wash it. Subsequently, the smear was submerged in safranin for one minute and subsequently cleaned with fresh water. After wiping the slide's back with cotton wool, it was set in a draining rack to allow the smear to air-dry. Lastly, a microscopic examination was conducted using a light Microscope with the oil immersion objective (100x).

Biochemical tests

Motility, Indole and Urease tests

In test tubes, motility indole and urease (MIU) agar slant was made. The test organism was introduced into the medium using a sterile straight wire loop, and the mixture was then incubated for 24 hours at 35℃. Following the incubation period, 0.5 ml of Kovac's reagent was added to the test tube and gently shaken. The medium was then checked for turbidity and colour change. Within ten minutes, a crimson ring began to form at the interface between the reagent and the inoculated media (Cheesbrough, 2006).

Citrate test

Test tubes were used to prepare Simmon's citrate agar. The test organism was streaked down the slope and stabbed into the medium's butt using a sterile straight wire loop. The media was then incubated for 24 hours at 35℃ (Cheesbrough, 2006).

Kligler iron agar test

Kligler Iron Agar was prepared in test tubes and inoculated with the test organism using a wire loop by streaking the slant and stabbing the butt of the medium. The tubes were incubated at 37°C for 24 hours and observed (Cheesbrough, 2006).

RESULTS

Table 1 shows the occurrence of isolates in relation to age group and gender of patients whose samples were used. Out of the 420 isolates collected, a higher occurrence of the isolates was obtained from female samples, with 213 (59.20%), compared to those collected from male samples with 147 (40.80%) isolates. In terms of age group, the age range of 31-40 years had a higher percentage of isolates corresponding to 19.0%, followed by 21-30 years (16.3%), and then 41-50 years (14.9%).

Table 1: Percentage Occurrence of Isolates Based on Patients Age and Gender

Variables	Occurrence	Percentage (%)
Age Group		
0-10	32	10.8
11-20	29	9.8
21-30	48	16.3
31-40	56	19.0
41-50	44	14.9
51-60	21	7.1
61-70	38	12.9
>70	27	9.2
Gender		
Male	147	40.8
Female	213	59.2

Table 2 shows fifteen (15) members of *Enterobacteriaceae* identified based on Gram staining and biochemical tests. The identified bacteria include *Escherichia coli*, *Klebsiella* spp. *Citrobacter* spp. *Enterobacter* spp. *Proteus* spp. *Shigella* spp. *Salmonella* spp. *Providencia* spp. *Yersinia enterocolitica*, *Morganella morganii* and *Serratia marcescens*.

Table 2: Gram Reactions and Biochemical Characteristics of the *Enterobacteriaceae*

GR	LF	M	C	I	U	KIA				Bacteria identified
						Slope	Butt	Gas	H_2S	
-	+	+	-	+	-	Y	Y	+	-	*Escherichia coli*
-	+	-	+	-	+	Y	Y	+	-	*Klebsiella pneamoniae*
-	+	-	+	+	+	Y	Y	+	-	*Klebsiella oxytoca*
-	-	-	+	+	+	Y	R	+	+	*Proteus vulgarus*
-	-	+	+	-	+	Y	R	+	+	*Proteus mirabilis*
-	+	+	+	-	-	Y	Y	+	-	*Enterobacter* spp.
-	+	+	+	-	-	Y	Y	+	+	*Citrobacter freundii*
-	+	+	+	+	-	Y	Y	+	-	*Citrobacter koseri*
-	+	+	+	-	+	Y	R	+	-	*Serratia marcescens*
-	-	+	-	+	+	Y	R	-	-	*Yersinia enterocolitica*
-	-	-	-	+	-	Y	R	-	-	*Shigella* spp.
-	-	+	-	-	-	Y	R	-	+	*Salmonella typhi*
-	-	+	-	-	-	Y	R	+	-	*Salmonella paratyphi*
-	-	+	+	+	-	Y	R	-	-	*Providencia* spp.
-	-	+	-	+	+	R	Y	-	-	*Morganella morganii*

Key: GR = Gram reaction, LF=Lactose fermentation, M= Motility, C= Citrate, I=Indole, U=Urease, KIA= Kligler Iron Agar, + =Positive, - =Negative, Y=Yellow (acid reaction), R=Red (alkaline reaction).

The Prevalence of *Enterobacteriaceae* is presented in Table 3. Out of 420 isolates, *E. coli* was the most predominant, with 38.8%, followed by *K. pneumoniae* with 16.4%, and then *K. oxytoca* with 14.5%. However, *M. morganii* and *Salmonella* spp. were the least prevalent organisms, with 0.7% and 0.5%, respectively.

Table 3: Percentage Occurrence of *Enterobacteriaceae*

Bacterial Isolates	Occurrence	Percentage (%)
E. coli	163	38.8
K. pneumoniae	69	16.4
K. oxytoca	61	14.5
Proteus spp.	28	6.7
Salmonella spp.	2	0.5
Enterobacter spp.	22	5.2
Shigella spp.	9	2.1
S. marcescens	13	3.1
Providencia spp.	12	2.9
Citrobacter spp.	27	6.4
Y. enterocolitica	11	2.6
M. morganii	3	0.7
Total	**420**	**100.0**

Table 4 shows the distribution of *Enterobacteriaceae* amongst various clinical samples. It was observed that urine samples had the highest percentage of identified *Enterobacteriaceae* with the corresponding value of 53.4%. This was followed by wound swabs with 19.1%, stool with 10.1%, and then high vaginal swabs with 6.8%. However, cerebrospinal fluid, semen, and pleural fluid samples had the lowest percentage of occurrence, with 1.0%, 1.0% and 0.5%, respectively.

Table 4: Percentage Occurrence of *Enterobacteriaceae* based on Clinical Specimen

	Occurrence per Clinical specimen										
Bacterial isolates	**U**	**St**	**WS**	**HVS**	**B**	**CSF**	**ECS**	**Sp**	**Sm**	**PF**	**Total (%)**
E. coli	94	19	23	12	3	0	8	1	1	0	161 (38.9)
K. pneumoniae	26	1	17	6	1	3	1	8	3	1	67 (16.2)
K. oxytoca	37	2	12	3	1	0	3	3	0	0	61 (14.7)
Proteus spp.	11	2	10	4	0	0	1	0	0	0	28 (6.8)
Citrobacter spp.	14	5	6	2	0	0	0	0	0	0	27 (6.5)
Enterobacter spp.	14	1	3	1	1	0	0	1	0	0	21 (5.1)
S. marcescens	9	2	1	0	0	0	1	0	0	0	13 (3.1)
Providencia spp.	5	4	3	0	0	0	0	0	0	0	12 (2.9)
Y. enterocolitica	7	1	1	0	0	1	0	0	0	1	11 (2.7)
Shigella spp.	4	2	2	0	0	0	1	0	0	0	9 (2.2)
M. morganii	0	3	0	0	0	0	0	0	0	0	3 (0.7)
Salmonella spp.	0	0	1	0	0	0	0	0	0	0	1 (0.2)
Total (%)	**221 (53.4)**	**42 (10.1)**	**79 (19.1)**	**28 (6.8)**	**6 (1.4)**	**4 (1.0)**	**15 (3.6)**	**13 (3.1)**	**4 (1.0)**	**2 (0.5)**	**414 (100.0)**

Key: U=urine, St=stool, WS=wound swab, HVS=high vaginal swab, B=blood, CSF=cerebrospinal fluid, ECS=endocervical swab, Sp=sputum, Sm=semen, and PF=pleural fluid

DISCUSSION

This study had shown a high occurrence of *Enterobacteriaceae* in females. Previous reports have indicated a high occurrence of these organisms among female patients compared to male subjects (Prasad *et al.*, 2016; Eltai *et al.*, 2018; Mohamed *et al.*, 2020), which can be attributed to the fact that females visit healthcare facilities more often than males (CDC, 2006).

The 420 isolates of *Enterobacteriaceae* were identified as *E. coli*, *K. pneumoniae*, *K. oxytoca*, *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp., *Shigella* spp., *Serratia marcescens*, *Yersinia enterocolitica*, *Morganella morganii* and *Providencia* spp. This finding corroborates well with that of Yusha'u *et al.* (2010), who reported the same organisms except *Providencia* spp.

Amongst the *Entrobacteriaceae* identified, *E. coli* was the most predominant, with 38.8%, followed by *K. pneumoniae,* with 16.4%. This is comparable to a study conducted by Hamid *et al.* (2019) in Sudan, where the predominant organisms were *E. coli* (43.3%) and *K. pneumoniae* (22.1%). However, in another study conducted in Osogbo, Nigeria, by Tolulope *et al.* (2020), *E. coli* (23.4%) and *Citrobacter* spp. (22.6%) were found to be the most predominant. The differences observed could be a result of environmental factors and changes in the time frame during which the study was conducted.

The high percentage of these organisms in the urine samples is justified by the fact that urine is the most dominant sample frequently sent for laboratory diagnosis. Tula and Iyaho (2020), who conducted their study in Adamawa, Nigeria, have also reported urine and Wound swabs having the largest number of *Enterobacteriaceae* isolates.

Nosocomial infections have been documented in about 1.40 million people worldwide, or roughly 9% of all patients (Tarigan *et al.*, 2023). As per the WHO research, nosocomial infections are a problem in fifty-five (55) hospitals, which accounts for 8.70% of hospitals across Europe, the Middle East, the Pacific, and Southeast Asia (Hapsari *et al.*, 2018). Endogenous factors, such as the normal flora of patients, or exogenous factors, such as contaminated materials or devices within the hospital, could be the cause of nosocomial infections, which remain a major problem and one of the leading causes of morbidity and mortality in medical facilities (Gill *et al.*, 2019). According to published research, the family *Enterobacteriaceae* members such as *Escherichia coli, Klebsiella pneumoniae*, *Enterobacter* spp., and other bacteria like *A. baumannii* and *Pseudomonas aeruginosa,* are the most frequently reported nosocomial pathogens (Vaiyapuri *et al.*, 2019; Michael *et al.*, 2023a; Michael *et al.*, 2023b).

In addition to Gram-negative bacteria, certain Gram-positive bacteria have also been connected to a number of nosocomial infections. For example, *Staphylococcus aureus* was found to be highly prevalent among Gram-negative nosocomial bacteria, as reported by Yang *et al.* (2023). Approximately 60% of nosocomial infections are caused by aerobic Gram-negative bacteria, whereas only 30% of nosocomial infections are associated with Gram-positive bacteria (Saba and Balwan, 2023). Moreover, fungi and viruses account for 7% of nosocomial illnesses (Saba and Balwan, 2023). Additionally, a study by Nimer (2022) discovered that nosocomial samples contained both Gram-positive and Gram-negative bacteria, with respective prevalence rates of 43% and 57%.

Gram-negative bacterial pathogens pose a significant threat because they are increasingly developing resistance to the majority of available antibiotics as well as several other multiple drugs. These bacteria can transfer genetic elements that enable other bacteria to develop drug resistance, and they have the innate ability to find new methods to be resistant (Archibald & Jarvis, 2011). Antimicrobial resistance poses a significant clinical risk to the world's healthcare system. Microbial resistance arises as a result of the development of many resistance mechanisms, including drug alteration and their inaccessibility to microbial target sites (Ferri *et al.*, 2017; Garba *et al.*, 2021a). The fact that bacterial illnesses are growing more resistant to antimicrobial treatments is worrying. This could be mainly because different control agents are putting strain on an adaptable microbial genetic system (Garba *et al.*, 2018).

The inappropriate and excessive use of therapeutic agents in conjunction with pharmaceutical industries' seeming lack of innovation in medication research due to regulatory obstacles and financial concerns has been linked to antimicrobial resistance. The effectiveness of treatments, the financial burden on government healthcare systems, and patient safety from antibiotic resistance are all seriously threatened by some bacteria, according to statistics from the Centers for Disease Control and Prevention (CDC) (Garba *et al.*, 2021b).

CONCLUSION

This study identified some members of *Enterobacteriaceae* from different clinical specimens in Federal Teaching Hospital Gombe. The study highlighted the high prevalence of *E. coli* and *Klebsiella* spp. among *Entrobacteriaceae*, which suggests the potential spread of hospital-acquired infections due to these bacteria.

Recommendation

Further research to assess the antimicrobial resistance profile of these clinical bacterial organisms in the study area is recommended for effective treatment options for bacterial infections.

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