Prevalence of Enterobacteria Species in Different Hospital Wards of a Tertiary Health Facility in Imo State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author ECI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors IEN and RNO managed the analyses of the study. Author IEN managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim: to assess the prevalence of Enterobacteria species in different hospital wards in a healthcare outfit in Imo State, Nigeria.
Study Design: The randomized complete block sampling design was adopted for the study.
Place and Duration of the Study: Sample: Imo State University Teaching Hospital, Federal Medical Centre, Owerri, Aboh-Mbaise General Hospital, and Imo State Specialist Hospital. Between May to October, 2021.
Methodology: Using Cochran formula, a total of 360 samples were collected for the study from bedpan, ward floor, bed cover, and staff gloves, at maternity, ICU, and surgical theatre wards. Sample were collected by swabbing the surfaces using sterile swab sticks soaked in saline water. Following standard microbiological procedures, which include bacteria culturing in MacConkey agar

to select for Enterobacteria, subculturing on salmonella-shigella agar and cetrimide agar to obtain pure cultures of specific bacteria species; the samples were subjected to morphological, and biochemical characterization. The prevalence was determined and compared for significance ($P = .05$).

Results: The investigation showed the presence of *Escherichia coli* (29%)*, Pseudomonas aeruginosa* (26.84%)*, Klebsiella pneumoniae* (25.11%)*,* and *Shigella* species (19.05%), in all the outfits investigated, with their percentage occurrences. The wards floor showed the highest number of Enterobacteria species (38.96%) while the staff gloves showed the least (9.96%). Based on the wards investigated, surgical theatre showed the highest prevalence rate (20.25±6.55) and ICU had the least prevalence rate (17.75±5.63). The prevalence pattern of Enterobacteria species were significantly ($P = .01$) dependent on the healthcare outfits. The prevalence pattern between the hospital wards was not significant ($P = .59$) and there was no significant interaction between the hospital wards and hospital equipment ($P = .84$).

Conclusion: Enterobacteria species was present in the healthcare outfits (bed pan, ward floor, bed cover and staff gloves). Its prevalence is dependent on these hospital outfits but not influenced by the wards or the interaction between wards and hospital outfits.

1. INTRODUCTION

The existence of hospital acquired infections dated as far back as before the inception of healthcare facilities [1]. Such infections are commonly associated with bacterial pathogens such as *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus* spp.*, Enterobacter* spp.*,* and *Acinetobacter* spp. [2]. Bacteria belonging to the Enterobacteriaceae family constitute a significant percentage of nosocomial pathogens [1]. Hospital acquired infection usually describes diseases acquired by patients placed under medical care; and it is identified from 48-72 hours following the patient's admission into the healthcare facility [3,1]. Hospital acquired infections include surgical wound infection, urinary tract infections (UTIs), blood stream infections (BSIs), pneumonia etc. [2]. Nosocomial or hospital acquired infection can result from either intrinsic factor (e.g., patient's age, underlying disease, injury, or lesion) or extrinsic factors (i.e., factors within the healthcare environment or staff) [1,4].

For organism to be associated with hospital acquired infections, it should exhibit these two features; it should be an established medically significant pathogen, and it should be capable of adapting to the severity of the hospital environment [2]. Enterobacteriaceae are Gram-negative, non-spore forming enteric bacilli. Members of this bacteria family are glucose fermenters, aerobes (occasionally facultative anaerobes), can reduce nitrates, and are oxidase negative [5]. They are usually motile, possessing petrichous flagella except for *Shigella* and *Klebsiella* species. Some of the members of Enterobacteria are established true pathogens (e.g., *Salmonella*, *Shigella*, *Yersinia* species and certain strain of *E. coli*), while most members are opportunistic or cause secondary infections of wounds/lesions, the respiratory and urinary tract, and the blood circulatory system [5]. As such, Enterobacteria species are frequently associated with nosocomial infections [6]. Members of Enterobacteriaceae family produces endotoxin, which is an important virulence factor with wide-range effects on the host. Their capsules are usually antiphagocytic, and they exhibit the ability to alternate either expressing or not expressing their capsule/flagella to avoid host immunity [6]. Enterobacteria are responsible for numerous diseases which are transmitted through unhealthy hygiene, healthcare personnel, and hospital equipment. Some of these diseases include urinary tract infection (mostly caused by *E. coli*), pneumonia (mostly caused by *K. pneumoniae*), abdominal sepsis (usually polymicrobial), meningitis, and endocarditis [6]. Enteric diseases are the predominant cause of illness in developing countries. Diarrhea and gastric infections are reported to be responsible for about 2.2 million global mortalities annually [7]. Pathogens associated with enteric disease pose an exceptional threat to children under the age of five, as it is linked to about 15 % of death of infants worldwide [7].

According to World Health Organization (as cited by [8]), hospital acquired infection is a global

health challenge with huge economic consequence. About two million people suffers nosocomial infection annually [9]. Uneke and Ijeoma [10], estimated 25-40 % nosocomial prevalence in poor resource settings. This indicated that the prevalence of nosocomial infection is two to three folds higher in developing countries [11]. Nosocomial infection prevalence rate of 45.8 % have been recorded in Nigeria, with an incidence density of 26.7 infections per 1000 patients [12]. Azeez-Akande [12], also reported an infection rate of 21.1-35.6 % in patients admitted to the intensive care unit. Studies have indicated disparities in the distribution rate of nosocomial infection among hospital wards [13]. Ige et al. [13] reported over 48.3 % infection rate from surgical wards, 20.5 % from medical, 16.1 % from obstetrics and gynecology, and 15.1 % from pediatric ward. In the course of time, from 2005-2009, Ige et al. [13], recorded changes in the rates of infection within these wards, but surgical ward was consistently recorded as the ward with the highest nosocomial infection rate. Frequently nosocomial infections are due to extrinsic factors, especially from high-risk medical interventions, like the use of invasive devices (e.g., catheter) and surgical operations [1]. Nosocomial infections have caused increase in antimicrobial resistance, prolonged hospital stays, long term disability in patients, and increased mortality rate [14]. The issue of nosocomial infection has been aggravated by overcrowding in hospitals, inadequate staff, poor infection control policies, and lack of healthcare professionals [15]. Also, the duration of stay in the hospital adds considerably to the extent of the infection [5]. For proper prevention of nosocomial infections, it is necessary to identify the sources and causal organisms of the infection, in order to strategize and implement preventive practices. The prevalence pattern was investigated to ascertain if there is no significant difference in the number of Enterobacteria isolated from the various healthcare equipment and wards. Also, the non-interaction between the wards and equipment was tested at $P = .05$. This study is geared towards assessing the prevalence of Enterobacteria species in different hospital wards of a healthcare facility.

2. METHODOLOGY

2.1 Sampling Technique

The randomized complete block sampling design was adopted for the study. Using Cochran formula to determine the sample size when the population size is large but unknown. A total of 360 samples were collected for the study. The sources of variation in the study include the hospital wards and the hospital materials. The wards used in the study were maternity wards, surgical theatres, and intensive care units (ICU). The materials from which samples were collected include bed cover, bed pan, staff gloves, and ward floor.

2.2 Sample Collection

Swab samples were aseptically collected from various equipment in the wards and staff apparels. The items from which the samples were collected include floor, bed cover, bed pan, and hospital staff apparel (protective gown and hand gloves) within maternity ward, surgical theatres, and intensive care unit of each hospital. Three hundred and sixty (360) sterile swab tubes (appropriately labelled) were taken to the hospitals. Swabbing of the surface of each item was made using sterile swab soaked with saline water. Many portions as possible of each item were swabbed and more than one swab stick was used for each subject. The swabs were immediately conveyed to the laboratory [16].

2.3 Isolation of Enterobacteria Species

The swab sticks were used to inoculate sterile duplicates petri-dishes of MacConkey to select for Enterobacteria. Inoculated plates were then incubated in an incubator at 37 °C for 24 to 48 h. Plates were observed for growth and colonies recorded.

2.4 Phenotypic Characterization

2.4.1 Morphological characterization

The morphological identification of the bacterial culture was done following the methods of [17].

2.4.1.1 Gram staining

Thin smear of bacterial culture was made on clean glass slide, air dried and heat fixed. The smear was covered with crystal violet for 30 seconds. The slide was then washed with distilled water and the smear was covered with Gram iodine solution for 60 seconds, washed with distilled water and blot dried, air dried and observed under microscope. The shape and colour of the organisms were observed. Purple colour depicts Gram-positive bacteria while pink colour shows Gram-negative bacteria.

2.4.1.2 Motility test

Clean cavity slide was taken and placed on a table with depletion upper side. A cover slip was taken, and wax was applied on its four corners. A loop-full of culture was transferred exactly at the center of the cover slip and the cavity slide was placed on the cover slip and pressed gently. The preparation was lifted gently so that the culture drop is suspended in the form of hanging drop. The edge was observed under a microscope.

2.4.2 Cultural characterization of isolates

Cetrimide and Salmonella-Shigella Agar were prepared in accordance with the manufacturer's instructions and incubated for 24 h, at 37 °C. [17].

2.4.3 Biochemical test for bacterial isolates

Pure cultures of the isolates were further subjected to Oxidase test, lactose fermentation test, and IMVIC test following the guideline of Chessbrough [18].

2.5 Data Analysis

Two-way ANOVA at P =.05 was used in comparing the data on the number of isolates across items and wards. Similarly, mean and standard error were determined using Eq.1, while percentage (%) prevalence was calculated as shown in Eq. 2 and 3.

$$mean = \frac{\sum fx}{\sum f}, \; Standard \; error = \frac{\sigma}{\sqrt{n}} \qquad (1)$$

Prevalence of Enterobacteria = Number of the isolates from an item / Total number of isolates ×100 (2)

Prevalence in wards = Number of isolates from each ward / Total number of isolates ×100 (3)

3. RESULTS AND DISCUSSION

3.1 Isolation of Enterobacteria Species from the Materials in Healthcare Centres

The Enterobacteria species were isolated from the study material using MacConkey agar, the result is represented in Fig. 1. It was observed that out of the 360 MacConkey agar plates inoculated, growth was observed in 231 (64.17 %) while the remaining 129 (35.83 %) plates showed no observable growth.

The number of Enterobacteria species isolated from these healthcare centres were further analysed in respect to the various wards. It was observed that sample materials from surgical theatre showed the highest number of Enterobacteria species ($\dot{x}$ = 20.25 ± 6.55). While samples from the ICU recorded the lowest number of Enterobacteria species ($\dot{x}$ = 17.75 ± 5.63). The number of Enterobacteria species observed in samples from the maternity wards ($\dot{x}$ = 19.75 ± 4.59) were higher than that observed from ICU samples ($\dot{x}$ = 17.75 ± 5.63) but below the observation made from samples collected from surgical theatre ($\dot{x}$ = 20.25 ± 6.55) (Fig. 2). This observation is in line with the reported higher prevalence (48.3 %) for surgical wards compared to the other wards in tertiary healthcare centres in southwestern Nigeria investigated by Ige et al. [13]. This report however contrasts the report by Azeez-Akande [12], which indicated higher numbers of isolates from ICU. Similarly, Tolera et al. [19] reported disparities in *Enterobacteriaceae* isolated from various wards; with higher number in Obstetrics/Gynaecology ward. The reason for such disparities as recorded in this research and by other studies has been attributed to poor hygienic condition of the hospital environment and inadequate waste disposal system in healthcare settings [14]. Furthermore, according to WHO [20], the financial status of the healthcare centres also plays a role in the prevalence rate of nosocomial infection.

Analysis of the number of Enterobacteria species isolation based on the healthcare equipment and surroundings (as represented in Fig. 3). showed that the floors of the wards recorded highest percentage of Enterobacteria species observed [Maternity ward floor 12.99 %, ICU floor 12.12 % and Surgical theatre ward floor 13.85 %]. This was followed by the samples from the wards' bed pan [Maternity (10.82 %), ICU (11.69 %), and surgical theatre (n = 13.42 %)]. Equal percentages of isolates were observed in samples collected from bed covers and gloves maternity wards (Maternity (5.19 % for bed cover and gloves), similarly ICU ward showed equal percentages of Enterobacteria species (3.46 % for bed cover and gloves). From the surgical wards' bed covers and gloves, the percentage of Enterobacteria species observed (4.76 %) were higher than those observed for gloves samples (3.08 %). This is summarized in Fig. 3. This research indicated that all the fomites sampled harboured the Enterobacteria, with higher prevalence reported from the ward floors and

bed pans than from the other items samples (Fig. 3). Olise and Simon-Oke [21] in their study on fomites as a possible vehicle of nosocomial infections affirmed that fomites serve as reservoir for pathogenic microorganisms, and therefore serve as possible vehicles for transmission of nosocomial infections within healthcare facilities. This report seems to be in agreement with the observations in this study. They however reported that doorknobs recorded the highest microbial contamination (22.76%); while light-

plugins (4.88%) and pillowcase (4.88%) recorded the least, in contrast to our report. Similarly, Bereket et al. [1] in their study, reported extrinsic factors (such as staff hygiene and hospital practices) are high risk factors of hospital acquired infection. Furthermore, according to Farahani et al. [22], medical equipment used in hospitals plays an essential role in the transmission of infectious agents to patients and the occurrence of hospital-acquired infections.
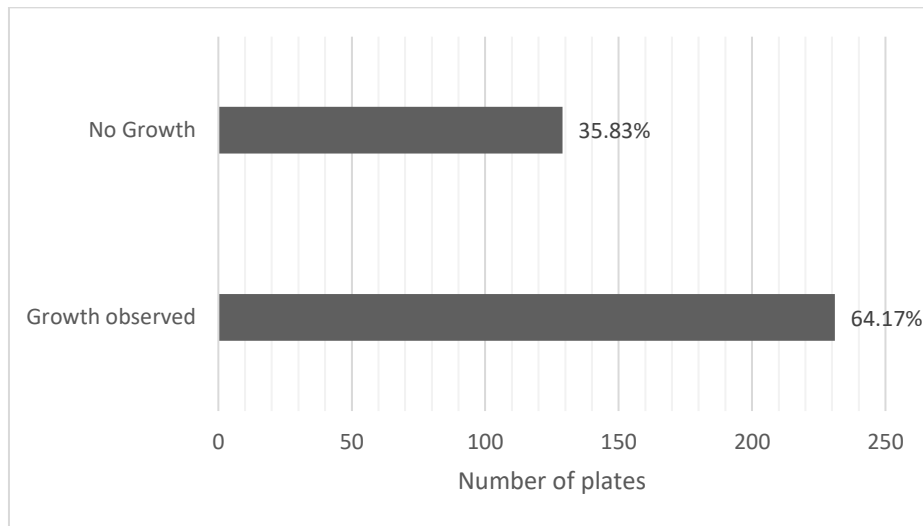

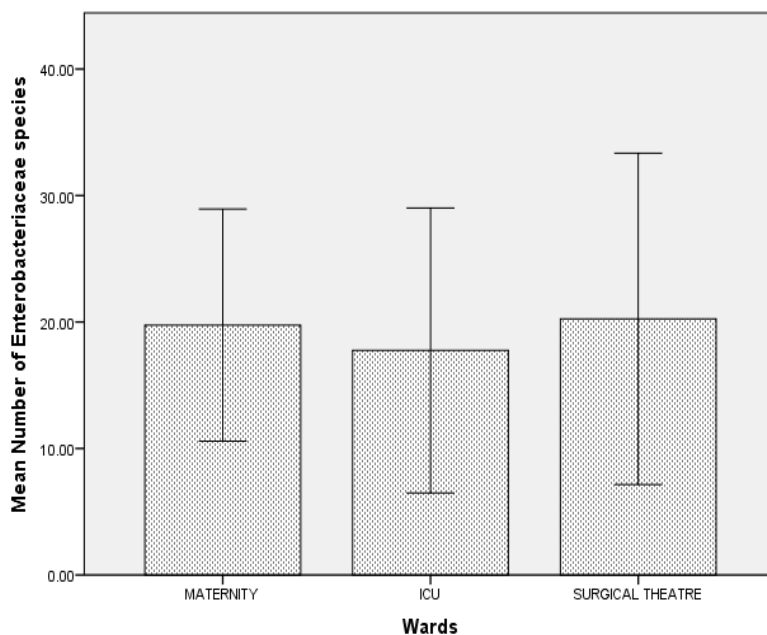**Fig. 1. The percentage of enterobacteria isolated from hospital samples cultured on macconkey agar**

Fig. 2. Bar chart showing the number of enterobacteria species isolated from various wards in the healthcare facility
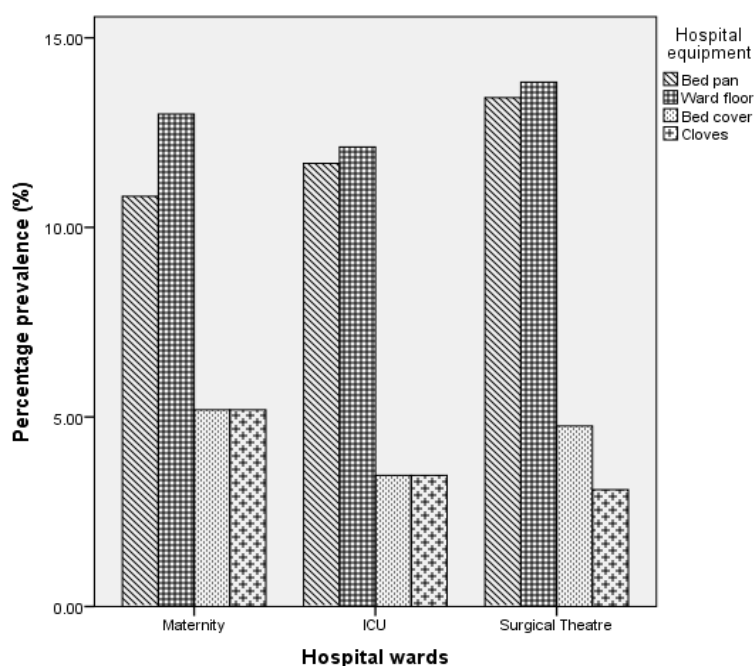*Mean ± S.E.M = Mean values ± Standard error of means of four experiments*

Fig. 3. Bar chart showing the number of enterobacteria isolated from the various equipment in each ward

3.2 Identification and Characterization of Enterobacteria Isolates using Microscopic, Cultural, and Biochemical Assay

The result for the microscopic and cultural assay are represented in Table 1. It was observed that the isolates when viewed under a microscope were pink to reddish rod-shaped organisms (Appendix Fig. 1a). Isolates growth on MacConkey agar produced brownish colonies for *Pseudomonas aeruginosa*; pink colonies was observed for *Escherichia coli* while *Klebsiella pneumoniae* produced pinkish-red colonies; and transparent colonies were observed for *Shigella spp.* (Appendix Fig. 1b). Isolates on Salmonella-Shigella agar showed inhibited growth for *P. aeruginosa*; reduced pinkish colonies for *E. coli*; inhibited growth for *K. pneumoniae*, and colourless colonies for *Shigella spp*. Table 1. Culturing of isolates on Cetrimide Agar showed inhibited growth for *E. coli*, *K. pneumoniae* and *Shigella spp. P. aeruginosa* showed growth which gave a green, fluorescent radiation when viewed under UV rays.

The results from the biochemical assay showed that *P. aeruginosa* tested positive for oxidase test and lactose fermentation test, but negative for indole, methyl red and VP test. *E. coli* tested positive for indole, methyl red and lactose fermenting test; but a negative result was observed for oxidase and VP test. *K. pneumoniae* tested positive for only VP and lactose fermenting test; a negative result was observed for the other tests (oxidase, methyl red, and indole test). For *Shigella spp.* a positive result was observed only for indole test, all other tests yielded negative results (Table 1).

Ssekitoleko et al. [18] reported similar pathogens in their study on the role of medical equipment in the spread of nosocomial infections. Similar bacterial pathogens were also reported to cause multi-antibiotic resistant nosocomial infections by Baka et al. [2]. According to Bereket et al. [1], bacteria belonging to the *Enterobacteriaceae* family constitute a significant percentage of nosocomial pathogens. The study also noted that pathogens responsible for nosocomial infections are mostly medically established Gram-negative bacteria; of which the most frequently reported are *P. aeruginosa*, *E. coli*, and *Enterococcus.* Similarly, in line with the finding this study, Ige et al. [13], reported that majority of the isolates (78.3 %) collected from tertiary hospitals in Ibadan were Gram-negative bacteria; of which most were Enterobacteria species (*Klebsiella spp.*, *E. coli*, *P. aeruginosa*, *Pseudomonas* sp, and *Proteus* spp.).

Table 1. Microscopic, cultural, and biochemical assay of Enterobacteria isolates

| Isolate ID | Morphology | | Gram Stain | Growth on MacConkey | Growth Salmonella-Shigella agar | Growth on Cetrimide agar | Biochemical test | | | | | Probable organism |
	Shape	Motility					Oxidase	Indole	Methyl red	VP	Lactose	
MW1	Rod	+	Negative	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
MW2	Rod	-	Negative	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
MW3	Rod	-	Negative	Red colony	Inhibited growth	Inhibited growth	-	-	-	+	+	*K. pneumoniae*
MW4	Rod	-	Negative	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
MW5	Rod	+	Negative	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
MW6	Rod	-	Negative	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
MW7	Rod	+	Negative	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
MW8	Rod	+	Negative	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
IC1	Rod	+	Negative	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
IC2	Rod	+	Negative	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
IC3	Rod	+	Negative	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
IC4	Rod	-	Negative	Red colony	Inhibited growth	Inhibited growth	-	-	-	+	+	*K. pneumoniae*
IC5	Rod	+	Negative	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
IC6	Rod	-	Negative	Red colony	Inhibited growth	Inhibited growth	-	-	-	+	+	*K. pneumoniae*
IC7	Rod	+	Negative	Brown	Inhibited	Green	+	-	-	-	+	*P.*

Isolate ID	Morphology		Gram Stain	Growth on MacConkey	Growth Salmonella-Shigella agar	Growth on Cetrimide agar	Biochemical test					Probable organism
	Shape	Motility					Oxidase	Indole	Methyl red	VP	Lactose	
				colony	growth	fluorescent under UV						*aeruginosa*
IC8	Rod	-	Negative	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
ST1	Rod	-	Negative	Red colony	Inhibited growth	Inhibited growth	-	-	-	+	+	*K. pneumoniae*
ST2	Rod	-	Negative	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
ST3	Rod	+	Negative	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
ST4	Rod	+	Negative	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
ST5	Rod	+	Negative	Pink colony	Reduced pink colony	Inhibited growth	-	+	+	-	+	*E. coli*
ST6	Rod	+	Negative	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*
ST7	Rod	-	Negative	Transparent	Brown colony	Inhibited growth	-	+	-	-	-	*Shigella spp.*
ST8	Rod	+	Negative	Brown colony	Inhibited growth	Green fluorescent under UV	+	-	-	-	+	*P. aeruginosa*

+ = Positive result; - = Negative result

The percentages of the Enterobacteria species isolated from the samples was represented in Fig. 4. It was observed that the species *E. coli* showed the highest percentage (29.00 %) of growth on nutrient media after inoculation. *P. aeruginosa* showed the second highest growth percentage (26.84 %), *K. pneumoniae* showed 25.11 % growth while *Shigella spp.* showed the lowest percentage growth (19.05 %). Disparities have been reported to exist in percentage of the different isolates observed in different hospital, as well as in different wards with a health facility by previous authors. This assertion was also observed in this study. For instance, in this present study, *E. coli* showed the highest percentage occurrence (29.00 %), followed by *P. aeruginosa* (26.84 %), while *Shigella* species had the least (19.05 %). In their study on bacterial nosocomial infections and antimicrobial susceptibility pattern, Tolera et al. [19] reported *Staphylococcus aureus* as the most common isolate (18.5%), followed by *Escherichia coli* (16.7%). Similarly, Pirozu et al. [23] reported *Staphylococcus epidermidis* (43.33%) and *Escherichia coli* (16.66%), as the most isolated bacteria. These observed disparities could be attributed to variations in the sampled items/specimens by the various authors.

The study indicated that the prevalence of antibiotic resistant Enterobacteria species is significantly different across the various hospital outfits they were isolated from (P = .01). The difference in their prevalence rate based on the wards the species were isolated from did not vary significantly (P = .59). The interaction between hospital wards and hospital equipment did not significantly (P = .84) influences the prevalence rate of antibiotics resistant Enterobacteria species isolated (Table 2).
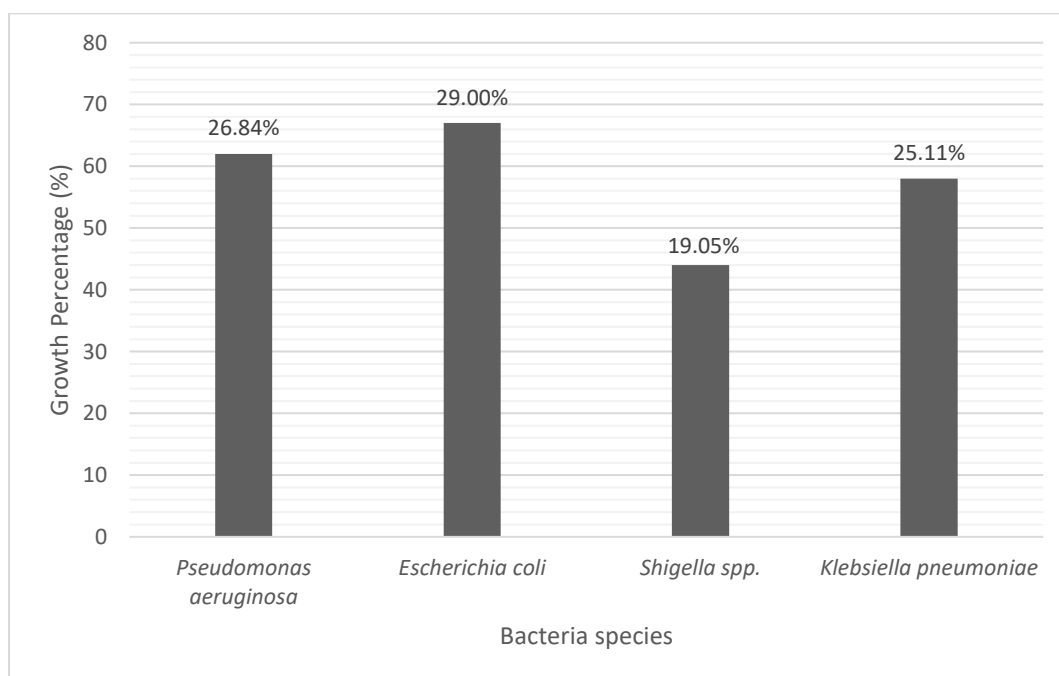

Fig. 4. Growth percentages of the various enterobacteria identified from the study

Table 2. ANOVA: Two-factor with replication

Source of Variation	SS	df	MS	F	P-value	F crit
Equipment	278.2292	3	92.74306	28.11579	1.52E-04	2.866266
Wards	3.5	2	1.75	0.530526	0.592824	3.259446
Interaction	8.833333	6	1.472222	0.446316	0.842712	2.363751
Within	118.75	36	3.298611			
Total	409.3125	47				

4. CONCLUSION

There is presence of Enterobacteria in hospital equipment (bed pan, bed cover, staff gloves) and environment (ward floors) from maternity ward, ICU, and surgical theatre. The species present were *P. aeruginosa, E. coli*, *K. pneumoniae*, and *Shigella spp.* The prevalence rate of these isolates was significantly different across the hospital equipment; but did not vary significantly across the various hospital wards. The interaction between hospital wards and hospital equipment did not significantly influences the prevalence rate of Enterobacteria isolated. The human resource for health should be educated on infection control, transmission-based precaution (transmission mechanisms of nosocomial pathogens), and proper hygiene in healthcare settings.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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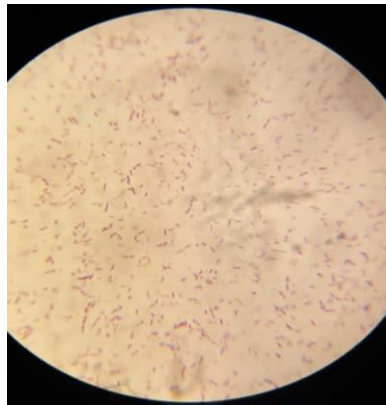Available:https://doi.org/10.5812/gct.93160

APPENDIX

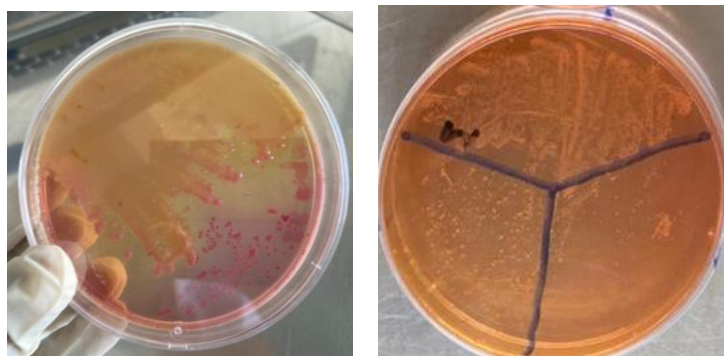**Fig. 1a. Microscopic imagine of isolate after gram staining**

Fig. 1b. Isolates on MacConkey agar showing reddish-pink, brownish and colourless colonies

Fig. 2. Biochemical assay: isolates reaction to indole production test