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***ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF CITROBACTER AND ENTEROBACTER SPECIES FROM VAGINAL SWAB OF WOMEN ATTENDING ENUGU STATE UNIVERSITY TEACHING HOSPITAL, PARKLANE.***

**ABSTRACT**

This study aimed to determine the prevalence and antibiotic susceptibility patterns of Citrobacter and Enterobacter species isolated from vaginal swabs of women attending Enugu State University Teaching Hospital-ESUTH, Parklane. It employed a cross-sectional design involving mixed data which was carried out from 15th October -15th Nov 2024 in ESUTH. Ethical clearance and informed consent were obtained and a total of 70 samples were analyzed from women above 18 years using standard methods. Questionnaires were given to patients to fill out to obtain epidemiological data. Samples were then cultured on Eosin methylene blue (EMB) agar and susceptibility testing was done on nutrient agar using gram-negative antibiotic disks. The results of the study showed a prevalence of 21.4% for these opportunistic pathogens. Out of these, 15 participants (21.4%) tested positive for either Citrobacter (55%) or Enterobacter (45%). Seventy (70) samples were collected and analysed for this work. A majority of 25 participants (35.7%) of the women were of age between 26-30 years. 19 participants (27.1%) were between 21-25 years while 17.1% were between 31-35 years. The remaining 12 participants (17.1) % and 5 participants (7.2%) were between 15-20 and 36-40 years of age respectively. The study identified several risk factors contributing to vaginal infections, including hormonal changes, sexual activity, and antibiotic use, particularly among immunocompromised individuals. Antibiotic susceptibility testing demonstrated concerning resistance rates, with high levels of resistance observed against commonly prescribed antibiotics, such as ciprofloxacin (57%) and amoxicillin (60%). This is also similar to a study showing a 30-54% resistance of Citrobacter species, with fluoroquinolones having the least sensitivity. In conclusion, the findings indicate that Enterobacter and Citrobacter are prevalent diseases with growing multi-drug resistance trends. Recommendations include implementing educational programs, establishing antibiotic stewardship initiatives, and enhancing diagnostic techniques to improve clinical management of infections caused by Citrobacter and Enterobacter species. 9(12.9)

19(27.1)

25(35.7)

12(17.1)

5(7.2)

**INTRODUCTION**

**Background**

 Enterobacter and Citrobacter are gram-negative bacteria belonging to the family Enterobacteriaceae, which includes many common pathogens. These bacteria can cause a variety of infections in humans, including urinary tract infections, bloodstream infections, pneumonia, and wound infections [1]. Gram-negative enterobacteria (bacteria belonging to the family Enterobacteriaceae) such as Klebsiella, Enterobacter, Escherichia and Citrobacter are members of the normal intestinal microbiota of humans and animals, and species of these genera are usually harmless commensals [2]. They are also found in several other environments, such as wastewater, surface water, soil and foods, and they can play an important role in food spoilage [3]. Species of these genera, such as E. coli, K. pneumonia, E. cloacae and C. freundii are also among the most frequently occurring opportunistic pathogens in clinical infections.[3]

The vaginal microbiome is a complex ecosystem that plays a crucial role in maintaining reproductive health. Disruptions to this microbiome can lead to infections, including those caused by Citrobacter and Enterobacter species [4]. Understanding the antimicrobial susceptibility patterns of these bacteria is essential for the effective management of vaginal infections and the prevention of their spread [5]. Citrobacter and Enterobacter species can cause a variety of infections, including:

* Urinary tract infections (UTIs)
* Bacteremia
* Pneumonia
* Sepsis
* Meningitis

These infections can be particularly severe in immunocompromised individuals, such as those with HIV/AIDS, autoimmune disorders like systemic lupus erythematosus and cancer [6].

### 1.1.1 Vaginal Infections

Vaginal infections are common among women of all ages. They can be caused by a variety of pathogens, including bacteria, fungi, and viruses [7]. The most common bacterial causes of vaginal infections include:

* Gardnerella vaginalis
* Escherichia coli
* Streptococcus agalactiae
* Staphylococcus aureus

Citrobacter and Enterobacter species are less common causes of vaginal infections but are increasingly being recognized as significant pathogens in this setting [8]. This is greatly associated with microbial dysbiosis of the intestinal, vaginal and even seminal ecosystem which is greatly linked to infertility and requires in-depth studies to provide more evidence [9].

Infections caused by Enterobacteriaceae, particularly Citrobacter and Enterobacter species, have emerged as significant health concerns in clinical settings [10]. These bacteria are known to inhabit various environments, including the human gastrointestinal tract, and can lead to opportunistic infections, particularly in immunocompromised individuals [11]. The vaginal microbiome plays a crucial role in women's reproductive health, and dysbiosis can facilitate the growth of pathogenic microorganisms, increasing the risk of infections [12].

In Nigeria, there is a growing prevalence of bacterial infections among women, yet limited data exists on the specific contribution of Citrobacter and Enterobacter species in this context [13]. Understanding the isolation and sensitivity patterns of these organisms from vaginal swabs can provide valuable insights into their role in gynaecological infections and inform appropriate therapeutic strategies [14]. This study aims to investigate the prevalence of Citrobacter and Enterobacter species in vaginal swabs collected from women attending Enugu State University Teaching Hospital, Parklane, and to assess their antibiotic sensitivity profiles, ultimately contributing to improved clinical management and patient outcomes.

## 1.4 Significance of the Study

The significance of this study lies in its potential contributions to both clinical practice and public health:

* **Understanding Pathogen Dynamics**: By investigating the prevalence and antibiotic resistance patterns of Citrobacter and Enterobacter species, this study will enhance the understanding of these opportunistic pathogens in the context of vaginal infections. This is crucial as these bacteria are increasingly recognized as significant pathogens in clinical settings [8,10].
* **Improving Clinical Management**: The findings from this study will provide valuable insights into the effective management of vaginal infections caused by these bacteria. Understanding their susceptibility profiles will guide clinicians in choosing appropriate empirical treatment options, particularly in immunocompromised populations at increased risk for such infections [6].
* **Public Health Implications**: The study's outcomes may inform public health strategies aimed at controlling the spread of antibiotic-resistant infections. Given the global concern regarding antimicrobial resistance [15], understanding local resistance patterns is vital for developing targeted interventions [16].
* **Contribution to Local Data**: This research will fill a significant gap in the existing literature regarding the prevalence of Citrobacter and Enterobacter species in Nigeria. Limited data currently exists on their specific contribution to vaginal infections in this context [17], and this study aims to address that gap.
* **Foundation for Future Research**: The findings of this study may serve as a foundation for further research on microbial dysbiosis and its implications for reproductive health, especially regarding the interplay between different bacterial species in the vaginal microbiome [9].

By addressing these aspects, the study aims to contribute to a better understanding of the role of Citrobacter and Enterobacter species in vaginal infections and their implications for women's health in Nigeria.

**1.5 Aim**:

To Isolate Citrobacter and Enterobacter species from high vaginal swabs of women attending Enugu State University Teaching Hospital, Parklane.

**1.6 Specific Objectives:**

· **To determine the prevalence of Citrobacter and Enterobacter species among the study population.**

· **To establish the antibiotic susceptibility patterns of isolated Citrobacter and Enterobacter species.**

· **To determine the correlation between the presence of these bacteria and any associated clinical symptoms.**

· **To determine the correlation between the presence of these bacteria and some epidemiological patterns.**

**MATERIALS AND METHOD**

**Study Design**

This study adopted a prospective cross-sectional study conducted among women attending the outpatient clinic as well as those admitted into the female medical ward (in-patients) of ESUT Parklane, Enugu, Nigeria. It employed the mixed method design using quantitative and qualitative data to achieve its aim and objectives.

 **Study Area**

The study area is at Enugu State Teaching Hospital Parklane in Enugu, Nigeria's capital. Enugu is a well-known city in southeastern Nigeria, particularly as an educational and medical hub. The hospital is a large healthcare centre that serves a diverse population from both urban and rural locations.

**Study Population**

This study's population covered all female patients at Enugu State University Teaching Hospital, Parklane, with a focus on adult women above the age of 18 who present with vaginal symptoms. This includes both inpatients and outpatients seeking medical care at the hospital. The study focused on this age range because adult women are more vulnerable to and have a higher risk factor for vaginal and urinary tract disorders caused by Enterobacter and Citrobacter species.

 **Sample Size**

Determining an appropriate sample size is crucial for ensuring the validity and reliability of research findings. In this study, we utilized the Cochrane sample size formula to estimate the required sample size for assessing the isolation and sensitivity of Citrobacter and Enterobacter species from vaginal swabs of women attending Enugu State University of Technology, Teaching Hospital, Parklane.

Using the Cochrane Sample Size Formula

**n=√(N×Z² ×p×q) ÷ (E² × (N-1)+ Z²×p×q)**

where N= estimated population size of ESUT =2000

Z= Confidence Interval= 1.96 or 95%

p = estimated proportion of the population = 10% or 0.1

q = estimated proportion of population not being studied= 90% or 0.9

E= margin of error = 0.056

**SS = 70 participants**

Based on these parameters, the calculated sample size was determined to be 70 participants, which will provide a robust basis for analyzing the prevalence and antibiotic sensitivity patterns of the targeted bacterial species in the study population.

 **Inclusion and Exclusion Criteria**

***Inclusion criteria:***

* Women above the age of eighteen present with vaginal symptoms in Enugu State University Teaching Hospital, Parklane, both inpatient and outpatient cases.
* Women who gave their informed consent for their sample to be collected

***Exclusion criteria:***

* Women under the age of eighteen.
* Women not experiencing any vaginal symptoms, menstruating women and pregnant women.
* Men and children

**Materials**

· Sterile vaginal swabs

· Transport medium (e.g., Stuart's transport medium)

· Eosin methylene blue (EMB) media

 Nutrient agar plates

· Biochemical test reagents (indole, methyl red, hydrogen peroxide, urease)

· Antibiotic disks (appropriate for Gram-negative bacteria)

· Incubator

· Bunsen burner or alcohol lamp

· Inoculation loop

· Sterile Petri dishes

· Gram stain reagents (crystal violet, neutral red, lugol’s iodine)

· Microscope, slides and cover slips.

**Data Collection**

The data collection procedure for the study consisted of multiple stages. This procedure assured complete and reliable data collection by utilising questionnaires for demographic variables, laboratory testing, and data collectors.

***Questionnaires***

Questionnaires were used to collect demographics, medical history, and clinical presentations for Enterobacter and Citrobacter infections. The essential sections include demographic information on the women and their families including their age, gender, place of residence, and signs and symptoms which they experience.

***Sample Collection***

Prior to the collection of samples, informed consents were carefully obtained from the parents or guardians to ensure they understood the study's goal, procedures, potential dangers, and benefits. A trained laboratory scientist also aided in the collection of the sample using a sterile swab stick and properly labelled and transported to the laboratory for analysis.

***Culture and Isolation***

1. The collected swab was inoculated onto EMB agar plates by streaking in different quadrants.
2. The plates were then incubated aerobically at 37°C for 24-48 hours.
3. Characteristics and colony morphology of Enterobacter and Citrobacter species (black colonies on EMB agar plates) were observed and reported.
4. Suspicious colonies were then selected for further purification by sub-culturing on nutrient agar plates [18].

***Identification***

1. Gram staining was performed on the purified cultures to confirm Gram-negative bacilli morphology.
2. Biochemical tests were conducted (urease, and coagulase to determine pathogenicity) to differentiate Enterobacter and Citrobacter species.
3. The outcomes were then referred to standard biochemical identification charts for confirmation.

***Antibiotic Susceptibility Testing***

1. A standardized inoculum of the isolated bacteria was prepared.
2. They were then inoculated onto nutrient agar plates
3. Antibiotic disks were inoculated into the plates according to the Kirby-Bauer method.
4. The plates were incubated at 37°C for 18-24 hours.
5. Results were measured by the zones of inhibition around the antibiotic disks and interpreted according to CLSI guidelines. [19]

### 3.7.3 Laboratory Precautions

* Maintaining of aseptic techniques throughout the procedure to prevent contamination.
* The use of appropriate personal protective equipment (PPE).
* Proper disposal of contaminated materials.

**3.8 Data Recording and Analysis**

**3.8.1 Documentation:**

All results from culture and antimicrobial susceptibility tests were meticulously recorded in laboratory logs.

### 3.8.2 Data Analysis

Data were analyzed using SPSS version 20 software. Descriptive statistics were used to describe the socio-demographic characteristics of the study participants. The prevalence of Citrobacter and Enterobacter species was determined. Antibiotic susceptibility patterns were analyzed and presented as percentages.

**3.9 Ethical Considerations**

 Prior to the start of the study, informed consent were obtained from the women, which included a detailed explanation of the study's objective, procedures, potential dangers, and benefits. Furthermore, the Enugu State University Teaching Hospital's ethical review board will review and approve the study protocol to ensure that it meets ethical standards. The confidentiality of the participants were ensured by employing unique IDs for samples and data rather than personal information.

 **RESULTS**

**4.1 Isolation and Sensitivity of Citrobacter and Enterobacter Species from Vaginal Swab of Women Coming to ESUT, Parklane.**

Seventy (70) samples were collected and analysed for this work. Majority (35.7%) of the women were of age between 26-30 years. 27.1% were between 21-25 years while 17.1% were between 31-35 years. The remaining 12.9% and 7.2% were between 15-20 and 36-40 years of age respectively.

51.4% of the women were married while the remaining 48.6% were still single. 91.4% of the women had a university degree; thus they are educated. 92.9% had only one sexual partner while the rest had more than one. 48.6% of the women had history of sexually transmitted infections.

|  |  |
| --- | --- |
| Demographics |  |
| **Age**15-2021-2526-3031-3536-40**Marital status**Single Married Divorced **Educational level**FSLCO’levelB.Sc/B.A**Employment status**UnemployedSelf employedEmployed **Number of sexual partners**12**History of sexual transmitted infections**YesNo  | 9(12.9)19(27.1)25(35.7)12(17.1)5(7.2)34(48.6)36(51.4)006(8.6)64(91.4)26(37.1)19(27.1)25(35.7)65(92.9)5(7.1)34(48.6)36(51.4) |

81.4% of the women had never heard of Citrobacter and Enterobacter bacteria before in their life time. All the study participants noted that they don’t know these bacteria’s can cause illness nor are they aware of the risk factors of these bacteria’s. half of the study population had been diagnosed of vaginal infection before.

|  |  |
| --- | --- |
| Knowledge and awareness |  |
| **Have you heard of Citrobacter and Enterobacter bacteria**Yes No **Do you know what these bacteria can cause disease?**Yes No **Are you aware of the risk factors for infections caused by these bacteria?**Yes No **Have you ever been diagnosed with a vaginal infection?**Yes No | 13(18.6)57(81.4)0(0)70(100)0(0)70(100)50(50)50(50) |

|  |  |
| --- | --- |
| **RISK FACTORS** |  |
| **How often do you use antibiotics?**Regularly SeldomOnly under prescription Never **Do you have any underlying health conditions (e.g., diabetes, HIV/AIDS)?**Yes No **Do you use hormonal contraceptives (e.g., birth control pills, IUD)?**Yes No **Do you have a history of vaginal douching?**Yes No **Do you have a history of multiple sexual partners?**Yes No **SYMPTOMS** **Vaginal discharge**Yes No Not sure **Vaginal itching or burning**Yes No**Pain during sex**Yes No **Lower abdominal pain**Yes No Not sure **Urinary urgency or frequency**Yes No Not sure **Fever**Yes No **HEALTHCARE SEEKING BEHAVIOUR** **If you experienced symptoms, when did you seek medical attention?**Immediately When it worsened**What treatment did you receive?**Antibiotic No treatment **Were you tested for Citrobacter or Enterobacter?**Yes No Not sure | 2(2.9)39(55.7)28(40.0)1(1.4)0(0)70(100)51(72.9)19(27.1)43(61.4)27(38.6)48(68.6)22(31.4)24(34.3)27(38.6)19(27.1)43(61.4)27(38.6)26(37.1)44(62.9)21(30.0)32(45.7)17(24.3)19(27.1)13(18.6)38(54.3)31(44.3)39(55.7)58(82.9)12(17.1)70(100)0(0)17(24.3)14(20.0)39(55.7) |

**Wet prep**

Key\_

A=1-5WBCs

B=6-10WBCs

C=>10WBCs

The result of the wet prep shows that greater percentage of the wet prep only had + yeast cells (65%) and 1-5 WBCs.

**Prevalence**

Out of the 70 samples that were analysed, 15 tested positive for either Citrobacter (55%) or Enterobacter (45%). The rest of the samples were negative.

**Table 3:** **Antibiotic Susceptibility Pattern by Percentage**

The following drugs showed greater percentage of resistance; Ciprofloxacin (57%), meropenem (55%), pefloxacin (68%), ceftriaxone (55%) and amoxicillin (60%).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|   |   C | SN | NF | CT | %CIP | OF | MP | PF | GN | AX |
| **Resistance**  | 35 | 49 | 45 |  55 | 57 | 47 | 55 | 68 | 49 | 60 |
| **Sensitive** | 65 | 51 | 55 | 45 | 43 | 53 | 45 | 32 | 51 | 40 |

Key:

|  |
| --- |
| SN= Streptomycin |
| GN= Gentamicin |
| CIP= Ciprofloxacin |
| C= Chloramphenicol |
| OF= Ofloxacin |
| MP= Meropenem |
| PF= Pefloxacin |
| CT= Ceftriaxone |
| AX= AmoxicillinNF= Nitrofurantoin |

**Discussion and Conclusion**

This study aimed to investigate the prevalence and antibiotic susceptibility patterns of Citrobacter and Enterobacter species isolated from vaginal swabs of women attending Enugu State University Teaching Hospital, Parklane. The findings revealed significant insights into the role of these opportunistic pathogens in vaginal infections, highlighting the public health implications associated with their increasing prevalence and antibiotic resistance.

Out of the 70 samples analyzed, 15 (21.4%) tested positive for either Citrobacter (55%) or Enterobacter (45%). This prevalence underscores the importance of recognizing these bacteria as potential pathogens in the context of vaginal infections, particularly given that they are often overlooked compared to more common pathogens. The demographic data indicated that the majority of participants were educated women, yet a concerning 81.4% had never heard of Citrobacter and Enterobacter, suggesting a gap in awareness regarding these opportunistic pathogens and their potential health impacts.

The findings from this study highlight the significant presence of Citrobacter and Enterobacter species in vaginal swabs collected from women attending Enugu State University Teaching Hospital, Parklane. With a prevalence of 21.4%, these opportunistic pathogens are increasingly recognized as contributors to vaginal infections, even though they are often overshadowed by more common bacterial pathogens. The demographic data indicated that a majority of participants were educated women, yet a notable 81.4% had never heard of these bacteria, indicating a critical gap in awareness about their potential health impacts.

The study identified various risk factors associated with vaginal infections, including hormonal changes, sexual activity, and antibiotic usage. These factors are consistent with existing literature that emphasizes the complexity of the vaginal microbiome and its susceptibility to disruptions [20]. The association of infections with immunocompromised states, including those with HIV/AIDS, underscores the need for targeted interventions in vulnerable populations.

Antibiotic susceptibility testing revealed alarming resistance patterns. Notably, a high percentage of resistance was observed against commonly prescribed antibiotics, including ciprofloxacin (57%), meropenem (55%), and amoxicillin (60%). These findings are consistent with global trends of increasing antimicrobial resistance among Enterobacteriaceae, posing challenges for effective treatment options [21,22]. The emergence of multidrug-resistant strains necessitates urgent attention and action from healthcare providers and policymakers. The study by [23], showed that Aminoglycosides had a better spectrum of antimicrobial sensitivity (70.2%) with 85.2% isolates sensitive to amikacin. Fluoroquinolones had poor activity against Citrobacter (46% sensitive). Ureidopenicillins had poor efficacy (15.4%). This showed a marked similarity with this current study indicating a significant multi-drug resistance of these opportunistic microorganisms.

Similarly, the link between antimicrobial resistance and virulence factors is critical, as these traits often co-occur, complicating treatment strategies (24). The presence of virulence factors in Citrobacter and Enterobacter species, coupled with their resistance profiles, indicates a need for ongoing surveillance and research into the molecular mechanisms underlying these characteristics. Understanding these dynamics will be vital for developing effective therapeutic strategies and infection control measures.

The study's findings also highlight the need for improved diagnostic techniques and awareness programs regarding the risks associated with Citrobacter and Enterobacter species [25]. Enhanced education among healthcare professionals and the general public can lead to better prevention strategies and management of infections [20].

The interplay between virulence factors and antimicrobial resistance is crucial, as these traits often co-occur, complicating treatment strategies [26]. Understanding the molecular mechanisms underlying these characteristics will be vital for developing effective therapeutic strategies and infection control measures.

##  Conclusion

In conclusion, this study underscores the importance of recognizing Citrobacter and Enterobacter species as significant pathogens in vaginal infections. The prevalence of these bacteria, coupled with alarming antibiotic resistance patterns, highlights an urgent need for enhanced awareness, education, and research. Despite the participants’ high educational levels, the lack of knowledge regarding these pathogens indicates a critical gap that must be addressed to improve health outcomes.

## Recommendations

Based on the findings, the following recommendations are proposed:

* **Increase Awareness and Education**: Implement educational programs targeting both healthcare professionals and the general public to raise awareness about Citrobacter and Enterobacter species. This should include information on their role in vaginal infections and the importance of seeking medical attention for symptoms.
* **Antibiotic Stewardship**: Establish antibiotic stewardship initiatives within healthcare facilities to promote the rational use of antibiotics. This can help mitigate the development of antibiotic resistance and improve treatment outcomes.
* **Regular Surveillance**: Conduct ongoing surveillance of antimicrobial resistance patterns among Citrobacter and Enterobacter species in clinical settings. This data will inform empirical treatment choices and public health policies.
* **Improve Diagnostic Techniques**: Invest in advanced diagnostic methods to enhance the identification of these species in clinical specimens, facilitating timely treatment and reducing complications.
* **Quality Control in Laboratories**: Ensure strict adherence to quality control procedures in laboratories for antimicrobial susceptibility testing to guarantee accurate results.
* **Promote Research**: Encourage further research into the molecular mechanisms of virulence and resistance in Citrobacter and Enterobacter species to inform novel therapeutic strategies.
* **Public Health Campaigns**: Launch campaigns focusing on hygiene, safe sexual practices, and prudent antibiotic use to reduce the incidence of infections.
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