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Isolation and Molecular Detection of Bacteria from the Urinary Tract of Pregnant Women Attending Health Centres in Imo State, Nigeria Using Polymerase Chain Reaction (PCR)

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Abstract

Urinary tract infection is one of the most prevalent bacterial infections in pregnant women and can be a serious problem for mother and foetus. Therefore, early and accurate diagnosis of the causative bacterial infections is important for optimal management. The purpose of this study was to isolate and identify bacterial pathogens from the urinary tract of pregnant women attending health clinics in Imo State, Nigeria, using traditional microbiological methods and polymerase chain reaction (PCR)-based detection. A cross-sectional study design was employed and midstream urine samples were obtained from pregnant women attending prenatal clinics in selected health centres in Imo State. Samples were cultivated on appropriate bacteriological media for isolation and initial identification. Confirmed isolates were subjected to DNA extraction and PCR amplification with species-specific primers for the bacterial pathogens. The most frequently isolated species were *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. PCR detection was more sensitive and more specific than the standard culture techniques. The study emphasises the utility of PCR based detection for rapid and accurate identification of urinary tract infections in pregnant women. Identification of uropathogens early will improve treatment outcomes and prevent complications of UTIs in pregnancy.

Keywords: Urinary Tract Infection; Pregnancy; Molecular Identification; Uropathogens; PCR; Nigeria

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Introduction

Urinary tract infections (UTIs) are one of the most prevalent bacterial diseases among pregnant women globally. Physiological and structural changes of pregnancy expose women to colonisation and infection of the urinary system by bacteria. Hormonal alterations, ureteral dilatation and urine stasis contribute to enhanced bacterial proliferation inside the urinary system [1].

UTIs in pregnancy can occur as silent bacteriuria, cystitis or pyelonephritis. Untreated infections can lead to major consequences including early delivery, low birth weight, maternal sepsis, and higher perinatal mortality. Thus, early detection and accurate identification of the pathogenic bacteria are important components of prenatal healthcare [2].

Urine culture and other basic microbiological techniques continue to be the mainstay of UTI diagnosis. However, these methods can be time-consuming and sometimes fail to identify fastidious or slow growing organisms. Molecular diagnostic techniques, such as polymerase chain reaction (PCR), have developed as potent instruments for rapid and sensitive identification of bacterial infections. PCR enables the amplification of certain DNA sequences and hence the identification of a microbe even in very little amount [3].

UTI in pregnancy is connected with many kinds of bacteria. *Escherichia coli* is the most frequent pathogen responsible for the majority of community acquired UTIs. Other prominent uropathogens include *Klebsiella species*, *Proteus species*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus species* [4].

Urinary tract infections (UTIs) remain a major public health problem in Nigeria, particularly in pregnant women who frequent prenatal clinics. Though these infections are common, molecular-based diagnostic investigations are still restricted in many parts of the world. Imo State is located in the south-eastern part of Nigeria and has many health centres where antenatal care services are rendered. However, few studies have used molecular techniques such as PCR for the detection of uropathogenic bacteria among pregnant women [5].

Thus, the study aimed at isolating bacterial pathogens from the urinary tract of pregnant women attending health centres in Imo state, Nigeria and detection of these organisms using polymerase chain reaction (PCR) based molecular techniques.

Materials and Methods

Study Area

The study was conducted in Imo State, Nigeria. Imo State comprises several Local Government Areas with a network of primary healthcare centres and secondary healthcare facilities that provide antenatal services to pregnant women. These facilities serve as major access points for maternal healthcare delivery and formed the basis for participant recruitment in this study.

Study Design

This study was a **cross-sectional, laboratory-based study** designed to determine the bacterial isolates associated with urinary tract infections (UTIs) among pregnant women attending antenatal clinics in selected health facilities in Imo State.

Study Population

The study population consisted of pregnant women attending antenatal clinics in selected healthcare facilities across Imo State, Nigeria.

Sample Size Determination

A total of **80 (n = 80)** pregnant women were recruited for this study based on eligibility criteria and willingness to participate.

Inclusion Criteria

Pregnant women attending antenatal clinics who provided informed consent to participate in the study were included.

Exclusion Criteria

Pregnant women who were currently on antibiotic therapy at the time of sample collection or those who declined participation were excluded from the study.

Ethical Approval

Ethical approval for this study was obtained from the Imo State University Teaching Hospital Research Ethics Committee in Imo State, Nigeria. Informed consent was obtained from all participants before sample collection. Participation was entirely voluntary, and confidentiality of participants' information was strictly maintained throughout the study. All procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki for biomedical research involving human subjects.

Sample Collection

Clean-catch midstream urine samples were collected aseptically from consenting participants into sterile, wide-mouthed universal containers. Participants were adequately instructed on proper perineal hygiene and urine collection techniques to minimize contamination.

All samples were immediately transported to the Microbiology Laboratory of the Department of Medical Laboratory Science under appropriate conditions for analysis.

Bacterial Isolation and Culture

Urine samples were cultured using the standard calibrated loop technique on the following media: Cysteine Lactose Electrolyte Deficient (CLED) agar, MacConkey agar and Blood agar

The inoculated plates were incubated aerobically at **37°C for 24 hours**.

Significant bacteriuria was defined as a colony count of **$\geq 10^5$ colony-forming units (CFU)/mL**, in accordance with standard microbiological guidelines.

Identification of Bacterial Isolates

Bacterial isolates were identified using standard microbiological techniques, which included: Observation of colony morphology, Gram staining reaction and Biochemical tests. The biochemical test included are:

- Catalase test
- Coagulase test
- Indole test
- Citrate utilization test
- Urease test
- Triple Sugar Iron (TSI) test

These methods were used for the identification of bacterial isolates to species level.

DNA Extraction

Genomic DNA was extracted from confirmed bacterial isolates using a standard bacterial DNA extraction protocol. The extracted DNA served as the template for polymerase chain reaction (PCR) amplification.

Polymerase Chain Reaction (PCR) Detection

PCR amplification was performed using species-specific primers targeting conserved bacterial genes.

PCR Reaction Mixture

The PCR reaction mixture contained:

- DNA template
- Forward primer
- Reverse primer
- DNA polymerase enzyme
- Deoxynucleotide triphosphates (dNTPs)
- PCR buffer

Thermal Cycling Conditions

The PCR cycling conditions included:

- Initial denaturation at **94°C**
- Denaturation at **94°C**
- Annealing at primer-specific temperatures
- Extension at **72°C**
- Final extension step as required

Agarose Gel Electrophoresis

PCR products were analyzed using agarose gel electrophoresis. The amplified DNA fragments were visualized under ultraviolet (UV) illumination and compared with a molecular weight marker to confirm the presence of target bacterial DNA.

Data Analysis

Data obtained were analyzed using descriptive statistics and presented in tables as frequencies and percentages. Results were expressed in simple proportions for bacterial distribution and detection rates.

Results

Prevalence of Bacterial Isolates in Urine Samples of Pregnant Women.

A total of 80 urine samples (N = 80) collected from pregnant women were analyzed using culture and PCR methods. The results of bacterial isolation are presented in Table 1.

The results show that 70 (87.5%) of the urine samples were culture-positive, indicating a high prevalence of bacteriuria among the pregnant women studied. *Escherichia coli* was the most frequently isolated organism, accounting for 34 (42.5%) of all isolates, making it the predominant uropathogen. Other organisms identified included *Klebsiella pneumoniae* (17.5%), *Staphylococcus aureus* (10.0%), *Proteus mirabilis* (10.0%), and *Pseudomonas aeruginosa* (7.5%).

Comparison of Culture and PCR Detection Methods

The diagnostic performance of culture and PCR methods is presented in Table 2.

PCR detected bacterial pathogens in 78 (97.5%) of samples, while culture detected 70 (87.5%), indicating that PCR had a higher detection rate. This suggests that PCR is more sensitive than culture methods, particularly in identifying infections that may be missed due to low bacterial load or fastidious organisms.

Table 1: Distribution of bacterial isolates among pregnant women (N = 80).

Organism Isolated	Frequency (n)	Percentage (%)
Escherichia coli	34	42.5%
Klebsiella pneumoniae	14	17.5%
Proteus mirabilis	8	10.0%
Pseudomonas aeruginosa	6	7.5%
Staphylococcus aureus	8	10.0%
Total Positive Isolates	70	87.5%
No Growth (Negative Samples)	10	12.5%
Total Samples	80	100%

Table 2: Comparison of culture and PCR detection methods (N = 80).

Detection Method	Frequency (n)	Percentage (%)
Culture Positive	70	87.5%
PCR Positive	78	97.5%
False Negatives (Culture missed cases)	8	10.0%
Total Samples	80	100%

Distribution Pattern of Bacterial Isolates

The distribution shows a predominance of Gram-negative bacteria compared to Gram-positive bacteria.

- Gram-negative bacteria (*E. coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa*) accounted for 62 (77.5%) of isolates.
- Gram-positive bacteria (*Staphylococcus aureus*) accounted for 8 (10.0%) of isolates.

Discussion

The study was carried out to determine the prevalence and bacteriological profile of urinary tract infections (UTI) in pregnant women using conventional culture and PCR based detection methods. Results show a significant burden of bacteriuria, a clear prevalence of Gram-negative uropathogens and a better diagnostic performance of PCR compared to culture methods [6].

The overall prevalence of bacteriuria was 87.5% (70/80) which is much higher than the global estimates of asymptomatic bacteriuria in pregnancy that are usually quoted at 2-10% in affluent settings. This variation may be a reflection of the differential in antenatal screening coverage, hygienic standards and healthcare access in low resource regions. UTIs remain a prevalent obstetric complication in African settings because of late booking into prenatal care and absence of routine screening programmes and similar high incidence have been documented in these settings [7]. Urinary stasis and ureteral dilatation, physiological changes during pregnancy, further predispose women to ascending infections.

The bacteriological profile showed that *Escherichia coli* was the most common organism isolated (42.5%), followed by *Klebsiella pneumoniae* (17.5%), *Staphylococcus aureus* (10.0%), *Proteus mirabilis* (10.0%) and *Pseudomonas aeruginosa* (7.5%). *E. coli's* dominance is consistent with its global emergence as the dominant uropathogen, aided by virulence elements such as fimbrial adhesins that mediate uroepithelial colonisation. This is consistent with earlier studies that have attributed 40–70% of UTI in pregnancy to *E. coli* [8].

Enterobacteriaceae are involved in community and healthcare-associated urinary infections, as suggested by the presence of other Gram-negative organisms such *Klebsiella pneumoniae* and *Proteus mirabilis*. Isolated *Staphylococcus aureus* may be contamination or genuine infection while the presence of *Pseudomonas aeruginosa* signals complex or healthcare-associated UTI. These findings highlight the microbiological variety of UTIs in pregnancy and the need for ongoing surveillance of uropathogens [9].

PCR performed better than culture in terms of diagnostic performance with a higher detection rate (97.5% versus 87.5%), showing its improved sensitivity. This is in line with recent research showing that molecular diagnostics are more efficient in detecting low bacterial loads and fastidious species that may be missed by conventional culture techniques [10]. However, while PCR is quite sensitive, it does not yield any information on antimicrobial susceptibility, which is still necessary to guide therapy in the face of developing antimicrobial resistance.

The study also showed the dominance of gram-negative bacteria

(77.5%) over gram-positive organisms (10.0%) in line with the global epidemiological patterns of UTIs. Enterobacteriaceae are the most common because of their gastrointestinal origin and their capacity to climb the urinary tract. This pattern has substantial clinical implications especially with respect to the rising antibiotic resistance among Gram-negative uropathogens, which has been extensively reported in recent surveillance studies [11].

The significant prevalence of bacteriuria found in this study has clinical implications for routine antenatal screening for UTIs to prevent problems such as pyelonephritis, premature delivery and low birth weight. Early diagnosis and adequate treatment are important to prevent maternal and foetal morbidity. The study results further suggest that the addition of molecular diagnostic approaches to routine culture techniques may be beneficial for improving detection accuracy, especially in high-risk groups [12].

Limitations of this study include the single-centre design and small sample size, which may restrict generalisability. Furthermore, antibiotic susceptibility testing was not conducted, which limited interpretation of resistance patterns. Future studies that sample from many centres and profile resistance would better inform empirical treatment guidelines [13].

Conclusion

This study revealed a significant prevalence of urinary tract infections among pregnant women with *Escherichia coli* being the major pathogen. PCR has a higher diagnostic sensitivity than culture techniques. These findings emphasise the necessity of integrated diagnostic strategies and systematic prenatal screening to improve maternal and foetal outcomes.

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