***Original Research Article***

# Antibiotic Susceptibility Profile of Klebsiella Species Isolated from Pregnant Women with Dysuria Attending Selected Medical Centre in Northern Nigeria


## ABSTRACT

Urinary Tract Infection (UTI) is caused by the presence and growth of microorganisms in the urinary tract. Klebsiella infection is perhaps one of the common bacterial infections of mankind capable of causing urinary tract infections.

**Aims:** Antibiotic susceptibility profile of Klebsiella species isolated from pregnant women with dysuria attending selected Medical Centre in Northern Nigeria was carried out

**Place and Duration of Study:** The study was conducted at the Microbiology unit, Nigerian Institute of Leather and Science Technology, Zaria, and Ahmadu Bello University Health Service, Kaduna, Nigeria, between July 2010 and February 2011.

**Methodology:** Fifty samples of mid-stream urine were collected from consented pregnant women complaining of painful urination, attending selected medical centre in Northern Nigeria and biochemically characterized using standard microbiological methods. The isolates were subjected to antimicrobial sensitivity test using Kirby-Bauer disc diffusion technique.

**Results:** The prevalence of Klebsiella species was found to be 5 (10%). The klebsiellae species identified biochemically includes *Klebsiella oxytoca* and *K. pneumoniae*. The highest antimicrobial susceptibility in Klebsiella species was recorded in ciprofloxacin (100%) and gentamicin (100%). Resistance was recorded in ampicillin (0%). The antibiotics that were found to be more effective such as ciprofloxacin and gentamicin are regarded to be the first line drugs of choice for the treatment of urinary tract infections caused by Klebsiella species. Thus, continuous monitoring of antibacterial susceptibility before antibiotic prescription is important in order to monitor any emergence of resistance to the commonly active antimicrobials.

**Conclusion:** *Klebsiella oxytoca* and *K. pneumoniae* were isolated amongst the screened pregnant women with overall prevalence of 10%. From the result obtained, ciprofloxacin and gentamicin were found to be the first line drugs of choice for the treatment of urinary tract infections due to klebsiellae species isolated, and ampicillin recorded less activity.



*Keywords: Antibacterial, Biochemical characterizations, Klebsiella, Urinary Tract Infection*

## 1. INTRODUCTION

Urinary Tract Infection (UTI), which is caused by the presence and growth of microorganisms in the urinary tract, is perhaps the single most common bacterial infection of mankind. Numerous reports have suggested that UTI can occur in both male and female patients of any age with bacterial counts as low as cfu/ml of urine [1].

Klebsiella is one of the common bacterial pathogens capable of causing urinary tract infections. The common pathogenic species of the genus *Klebsiella includes: Klebsiella pneumoniae, Klebsiella ozanae, Klebsiella oxytoca, Klebsiella rhinoscleromatis, Klebsiella terrigina, Klebsiella planticola* and *Klebsiella granulomatis* [2].

In female human subjects, urinary tract has an important association with the reproduction organ because of its proximity. In the non-pregnant state, the uterus lies just behind and partly over the bladder while in the pregnant state, the enlarging uterus affects all the tissues of the urinary tract at various times. This is why Urinary Tract Infection (UTI) is common in females. The highest incidence of urinary tract infection (UTI) occurs in the child-bearing age and this has a direct relationship to sexual activity and aging [3]. UTI may be asymptomatic in many cases, while it may be accompanied by dysuria (painful urination), cystitis (inflammation of urinary bladder) and pyelonephritis in other patients. The urethras and bladders normally prevent urine from basking up towards the kidneys and the flow of urine in the bladder helps wash bacteria out of the body, in men, the prostate gland produces secretions that slow bacterial growth. In both sexes, immune defenses also prevent infections. Despite these safeguards, and mechanisms, infection still occur [4]. Bacterial infections of the urinary tract are commonly seen in out-patients, hospitalized patients and apparently healthy populations [5]. A common source of infection in the catheters and tubes, placed in the bladder. A person, who cannot void, is unconscious or critically ill, often need a catheter that stays in place for a long time. Some people, especially the elderly or those with nervous system disorders who lose bladder control, may need a catheter for life. Bacteria on the catheter can infect the bladder, so hospital staff takes special care to keep the catheter sterile and remove it as soon as possible [4]. In infants and children, the clinical presentation of UTI is often with non-specific clinical signs such as fever; irritability and vomiting that are also commonly seen in many acute self-limiting childhood viral illness. UTI may be the signal of serious underlying congenital anomaly such as obstruction that, if no revealed will lead not only to more serious illness but also to renal damage. The infection may also be associated with progressive loss of kidney function either in association with renal dysphasia or with recurrent episodes of acute pyelonephritis [6].

Urinary Tract Infections caused by Klebsiella is one of the most devastating and fulminating disease that usually causes high rate of maternal mortality and morbidity in pregnant women, whom immunity is low, especially in rural areas of developing countries like Nigeria. Urinary Tract Infections (UTI) are among the most common infections afflicting man. Urinary Tract Infections (UTIs) in young and adult women are usually uncomplicated, but are often recurrent and cause considerable morbidity. Urinary tract infection in pregnant women, elderly patients and catheterized patients warrant special attention because of their association with increased morbidity and possibly with increased mortality [7]. The antibiotic resistant strains of Klebsiella produce extended spectrum ß-lactamase (ESBL) enzymes that destroy penicillin or cephalosporin classes of drugs, thereby conferring resistance to other commonly used antibiotic drug classes as well. These common bacteria, when they produce these enzymes are much harder to kill with antibiotics. The antibiotic resistance problem is likely to become widespread, as such it often become difficult to select an appropriate antibiotic therapy for urinary tract infection [8].

The aim of this study is to isolate, biochemically characterize and to determine the antibiotic susceptibility profile of Klebsiella species isolated from pregnant women attending selected medical center in Northern Nigeria.

## 2. MATERIAL AND METHODS

Following reception of ethical approval from the Medical Advisory Committee of Ahmadu Bello University Health Service, Zaria, a total of 50 mid-stream urine samples were collected from consented pregnant women suspected with various symptoms of urinary tract infections (previously confirmed to be pregnant by human chorionic gonadotrophin immunochromatographic test) attending antenatal ward of Ahmadu Bello University Health Centre, Zaria in screw-capped universal bottles and transported to laboratory for analysis. Only consented and volunteered pregnant women of various age groups suspected with symptoms of urinary tract infections such as cystitis or dysuria were enrolled in the study. Written consent forms were signed by the patients (Appendix 1). Non pregnant women, asymptomatic pregnant women and those symptomatic but not volunteered to participate in the study were excluded.

The portion of the urine samples were dispensed into sterile test-tubes and centrifuged at the speed of 1500rpm for 15 minutes. The supernatant was discarded and the sediments were inoculated on the surface of prepared Cysteine Lactose Electrolyte Deficient (CLED) agar, then the suspected colonies of Klebsiella were sub-cultured on MacConkey agar and incubated at 37°C for 24 hours [9]. Following the incubation, Klebsiella species were isolated based on their macroscopic cultural characteristics (Plate 1). The suspected isolates were subjected to Gram's staining to determine the Gram potential and morphology of the isolated organisms. Klebsiella species were preliminarily identified based on their microscopic features as Gram negative bacilli (rod shaped), non-sporulating, and non-motile bacteria [10].

### 2.1 Biochemical Characterization of the isolated Klebsiella species

The following biochemical tests were employed in order to characterize the isolates up to their species level by comparing their reactions with that of the known taxa as documented in Bergey's manual of determinative keys in Bacteriology: Catalase, Methyl Red, Voges Proskauer, Indole, Citrate Utilization, motility, Oxidase, Urease, Nitrate Reduction, Esculin Hydrolysis and Triple Sugar Iron tests, as well as Lactose, Maltose, Sucrose and Glucose fermentations [10, 11].

### 2.2 Antimicrobial Sensitivity Testing (Kirby-Bauer Disc Diffusion Method)

Kirby-Bauer disc diffusion method for antimicrobial sensitivity testing was employed. Muller Hinton agar was freshly prepared according to the manufacturer's specifications and dispensed in sterile petri-dishes and allowed to solidify. A 0.5 McFarland standard of the test organism suspension was aseptically inoculated onto the surface of Muller Hinton agar using sterile swabs. The commonly used antibiotic discs were mounted on the surface of the inoculated plates with the aid of sterile forceps, and were allowed to diffuse for 5minutes prior to incubation. The plates were incubated at 37°C for 24 hours. Antibiotics used include ampicillin (10µg), erythromycin (15µg), ciprofloxacin (5µg), doxycycline (30µg) and gentamicin (10µg). Following the incubation, zones of growth inhibition were measured to the nearest millimeter (mm) and recorded as described by CLSI [12] and adopted by Umar *et al*. [13].

## 3. RESULTS AND DISCUSSION

A total of 5 Klebsiella species and others were isolated from the total of 50 samples collected and characterized biochemically from pregnant women attending selected medical center in Northern Nigeria, 5 isolates were microscopically and biochemically identified as Klebsiella species (Table 1).

Table 2 shows the biochemical characterizations of the bacterial isolates obtained from pregnant women attending selected medical center in Northern Nigeria. The klebsiellae isolated were *Klebsiella oxytoca* and *K. pneumoniae*.

Table 3 and Figure 1 show the highest susceptibility of Klebsiella species with ciprofloxacin (100%) and gentamicin (100%) whereas the least susceptibility occurred with ampicillin (0%). The antibiotics that were found to be more effective were ciprofloxacin and gentamicin.

**Table 1: Prevalence of the Klebsiella species from urinary tract of pregnant women attending selected medical centre in Northern Nigeria**

| Organism Isolated | Frequency of Isolates | Prevalence (%) |
|---|---|---|
| *Klebsiella species* | 5 | 10 |
| Other Isolates | 23 | 46 |
| No Growth | 22 | 44 |
| TOTAL | 50 | |

Urinary tract infections due to Klebsiella species recorded an overall prevalence of 10% (Table 1). This is relatively low prevalence compared to the studies of Oko *et al*. [14] and Aiyegoro [15] who reported a prevalence of 45.8% bacteriuria in pregnant women. *Klebsiella oxytoca* and *Klebsiella pneumoniae* were the organisms isolated from pregnant women attending Ahmadu Bello University Health Service. Even the prevalence recorded in this study may be due to the immune status of the pregnant women and some antenatal procedures that pregnant women often exposed to, such as frequent collection of high vaginal swabs and endocervical swabs by sharing unsterilized speculum during sample collection. This agrees with the findings of Martin *et al*. [16], Kevic [17] and Högenauer *et al*. [18] who reported *Klebsiella oxytoca* and *K. pneumoniae* as common bacterial species capable of causing nosocomial infections in immunocompromised individuals, especially during first trimester of pregnancy. This work suggests that Klebsiella species is one of the aetiologic agents of urinary tract infection among the set of people screened during the time of this study.

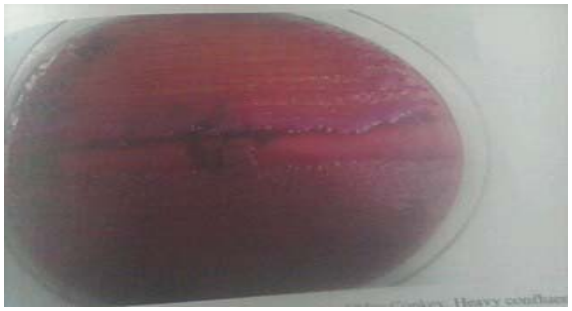

**Plate 1: Preliminary macroscopic morphology of Klebsiella species on McConkey agar**

**Table 2: Biochemical characterizations of the bacterial isolates obtained from pregnant women attending selected medical centre in Northern Nigeria**

| Microscopy | CAT | MR | VP | IND | CIT | MT | OXI | URE | NIT | ESC | TSI | Sugar Fermentation | | | | INFERENCE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | LAC | MAL | SUC | GLU | |
| Gram-negative non sporing rods | + | − | + | − | + | − | − | + | + | + | A/A +Gas | + | + | + | + | *Klebsiella pneumoniae* |
| Gram-negative non sporing rods | + | − | + | + | + | − | − | + | + | + | A/A +Gas | + | + | + | + | *Klebsiella oxytoca* |
| Gram-negative rods | − | + | − | + | − | + | − | + | − | − | K/A+$H_2S$ | − | − | + | + | Non-klebsiellae isolates |

*CAT= catalase; MR= methyl red; VP= Voges Proskauer; IND= indole; CIT= citrate; MT= motility; OXI= oxidase; URE= urease; NIT= nitrate reductase; ESC= esculin hydrolysis; TSI= triple sugar iron; Lac= lactose; MAL= maltose; SUC= sucrose; GLU= glucose; A= alkaline; K= acid; $H_2S$= hydrogen sulfide; + = positive;* − = negative

The preliminary microscopy, colonial morphology, coupled with biochemical characterizations (Table 2) were used for the isolation of the bacterial isolates up to their species level using Bergey's Manual for Bacteriology as guideline as described by Buchanan and Gibbons [11]. Gram negative non-motile rod shaped bacteria that showed positive reaction to catalase, Voges Proskauer, urease, citrate, nitrate reductase and esculin, which produce alkaline on the butt and slants of triple sugar iron medium, capable of fermenting lactose, maltose, sucrose and glucose were identified as *Klebsiella pneumoniae*. Similar bacterial isolates that are indole positive in addition to the features of *K. pnuemoniae* were identified as *Klebsiella oxytoca.* All other isolates that did not conform to the aforementioned biochemical characteristics were identified as non klebsiellae isolates.

**Table 3: Antibiotic susceptibility profile of *Klebsiella species* isolated from pregnant women attending selected medical centre in Northern Nigeria (n=5).**

| Antimicrobials | Disc potency (µg) | Susceptibility (%) | Resistance (%) |
|---|---|---|---|
| Ampicillin | 10 | 0 (0) | 5 (100) |
| Ciprofloxacin | 5 | 5 (100) | 0 (0) |
| Doxycycline | 30 | 3 (60) | 2 (40) |
| Erythromycin | 15 | 1 (20) | 4 (80) |
| Gentamicin | 10 | 5 (100) | 0 (0) |

The antimicrobial susceptibility of the antibiotics used showed relatively higher level of susceptibility of *Klebsiella oxytoca* and *K. pneumoniae* to ciprofloxacin (100%) and gentamicin (100%) respectively (Table 3 and Figure 1). This conformed to the work of Nwanze [19] and Awoniyi [20], who reported that klebsiella isolates showed relatively higher level of susceptibility to ciprofloxacin and gentamicin. Antibiotic resistance observed in this study is a sufficient cause for serious concern, especially with resistance to ampicillin (0%). The resistance may be due to lateral acquisition of resistance plasmid. However, Martin *et al*. [16] reported that *Klebsiella oxytoca* is capable of acquiring antibiotic resistance, and the isolates have been shown to produce extended-spectrum beta-lactamases and carbapenemases that deactivate penicillin derivatives.

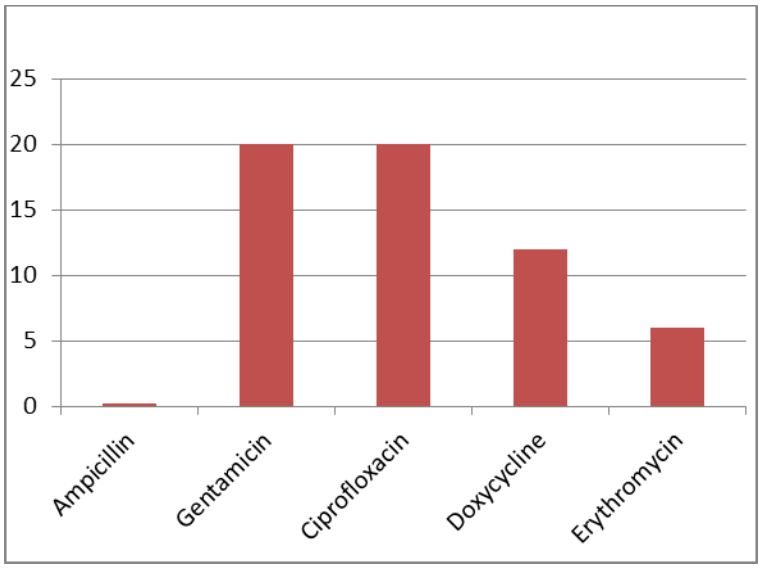


**Fig.1: Antibiotic susceptibility of *Klebsiella species***

The high resistance showed by some antibiotics could be due to earlier exposure of the isolates to the drug which may have enhanced resistant development [20]. It could also be due to practices of self-medication and indiscriminate use of this antibiotics and the acquiring of plasmid encoded resistant genes [21]. The findings have no doubt highlighted the need for constant monitoring of susceptibility of specific pathogens in different populations of commonly used antimicrobial susceptibilities and to assist clinician in the rational choice of antibiotics therapy to prevent misused or over use of the antibiotics.

## 4. CONCLUSION

*Klebsiella oxytoca* and *K. pneumoniae* were isolated amongst the pregnant women attending Ahmadu Bello University Health Service Clinic with total prevalence of 10%. From the result obtained, ciprofloxacin and gentamicin can serve as drugs of choice for the management of UTI caused by the Klebsiella species but ciprofloxacin was found to be generally more effective and ampicillin recorded less activity. *Klebsiella oxytoca* and *K. pneumoniae* were isolated amongst the pregnant women attending Ahmadu Bello University Health Service Clinic with total prevalence of 10%. From the result obtained, ciprofloxacin and gentamicin can serve as drugs of choice for the management of UTI caused by the Klebsiella species but ciprofloxacin was found to be generally more effective and ampicillin recorded less activity.
Constant and frequent review of antibiotic susceptibility of Klebsiella should be carried out to check for possible emergence of resistance by the organism. There is need for continuous monitoring of bacterial antibiotic susceptibility before antibiotic prescription in order to ensure adequate treatment of urinary tract infection (UTI) caused by the bacteria, because blind treatment is not economically wise.

## CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report.

## ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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## APPENDIX


**NIGERIAN INSTITUTE OF LEATHER AND SCIENCE TECHNOLOGY (NILEST)**

Formerly, Federal College of Chemical and Leather Technology (CHELTECH)

(FEDERAL MINISTRY OF SCIENCE AND TECHNOLOGY)

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*Our Ref:* NILEST/PER.532/VOL.I

*Your Ref:*

*Date:* 19th May, 2016

### PATIENTS' CONSENT FORM

**PATIENT/PATIENT'S PARENT OR GUARDIAN**

I have been asked to give consent for myself/daughter/family member (strike out what is not applicable) to participate in this research study which will involve collection of urine sample in the completion of the research study. I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily for myself/my child/my family member (strike out what is not applicable) to participate as a participant in this study. The results obtained thereby may be used in any way to improve the understanding and management of the urinary tract infections among pregnant women in our hospitals and community in general. Participation is strictly voluntary and refusal to participate in the research study will not in any way affect my right and benefit in this clinic/hospital.

Parent' s age_____ Sex_____

Signature of Patient/Patient' s guardian/Parent (if the patient is under 18 years) _____

Date _____

**STATEMENT BY THE WITNESS**

I have witnessed the accurate reading of the consent form to the families of the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely and individual data will be treated with strict confidentiality.

Name of witness_____ AND Thumb print of participant

Signature of witness _____

Date _____

**STATEMENT BY THE RESEARCHER/PERSON TAKING CONSENT**

I have accurately read out the information sheet to the patient or families of the potential participant, and to the best of my ability made sure that the person(s) understands that the following will be done:

1. Samples of urine will be collected using sterile container.
2. The samples will be analyzed in the laboratory to isolate *Klebsiella species*, and to determine the drugs for the treament.
3. The findings may be documented for public enlightenment and medical intervention by the concerned authorities.

My names are Mustapha Umar (NILEST/PER/532), Division of Microbiology, Department Science Laboratory Technology, Zaria, Kaduna state, Nigeria. I am currently carrying out a research titled Antibiotic Susceptibility Profile of Klebsiella Species Isolated from Pregnant Women with Dysuria Attending Selected Medical Centre in Northern Nigeria. I confirm that the patient/parent/patient' s guardian was given an opportunity to ask questions about the study, and all the questions asked by him/her have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher/person taking the consent UMAR, M.

**Appendix 1: Patients' informed consent form**