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Original Research Article

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

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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 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 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.

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

13 1. INTRODUCTION

14
15 Urinary Tract Infection (UTI), which is caused by the presence and growth of microorganisms in the
16 urinary tract, is perhaps the single most common bacterial infection of mankind. Numerous reports have
17 suggested that UTI can occur in both male and female patients of any age with bacterial counts as low as
18 cfu/ml of urine [1].

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

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

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

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

60 2. MATERIAL AND METHODS

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62 Following reception of ethical approval from the Medical Advisory Committee of Ahmadu Bello University
63 Health Service, Zaria, a total of 50 mid-stream urine samples were collected from consented pregnant

64 women (previously confirmed to be pregnant by human chorionic gonadotrophin immunochromatographic
65 test) attending antenatal ward of Ahmadu Bello University Health Centre, Zaria in screw-capped universal
66 bottles and transported to laboratory for analysis.

67 The portion of the urine samples were dispensed into sterile test-tubes and centrifuged at the speed of
68 1500rpm for 15 minutes. The supernatant was discarded and the sediments were inoculated on the
69 surface of prepared MacConkey agar and incubated at 37°C for 24 hours [9]. Following the incubation,
70 *Klebsiella* species were isolated based on their macroscopic cultural characteristics (Plate 1). The
71 suspected isolates were subjected to Gram's staining to determine the Gram potential and morphology of
72 the isolated organisms. *Klebsiella* species were preliminarily identified based on their microscopic
73 features as Gram negative bacilli (rod shaped), non-sporulating, and non-motile bacteria [10].
74

75 2.1 Biochemical Characterization of the isolated *Klebsiella* species

76 The following biochemical tests were employed in order to characterize the isolates up to their species
77 level by comparing their reactions with that of the known taxa as documented in Bergey's manual of
78 determinative keys in Bacteriology: Catalase, Methyl Red, Voges Proskauer, Indole, Citrate Utilization,
79 motility, Oxidase, Urease, Nitrate Reduction, Esculin Hydrolysis and Triple Sugar Iron tests, as well as
80 Lactose, Maltose, Sucrose and Glucose fermentations [10, 11].
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82 2.2 Antimicrobial Sensitivity Testing (Kirby-Bauer Disc Diffusion Method)

83 Kirby-Bauer disc diffusion method for antimicrobial sensitivity testing was employed. Muller Hinton agar
84 was freshly prepared according to the manufacturer's specifications and dispensed in sterile petri-dishes
85 and allowed to solidify. A 0.5 McFarland standard of the test organism suspension was aseptically
86 inoculated onto the surface of Muller Hinton agar using sterile forceps, and was allowed to diffuse for
87 5minutes. The commonly used antibiotics discs were mounted on the surface of the inoculated plates with
88 the aid of a sterile forceps and incubated at 37°C for 24 hours. Antibiotics used include ampicillin (10µg),
89 erythromycin (15µg), ciprofloxacin (5µg), doxycycline (30µg) and gentamicin (10µg). Following the
90 incubation, zones of growth inhibition were measured to the nearest millimeter (mm) and recorded as
91 described by CLSI [12] and adopted by Umar *et al.* [13].
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93 3. RESULTS AND DISCUSSION

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95 A total of 20 *Klebsiella* species and others were isolated from the total of 50 samples collected and
96 characterized biochemically from pregnant women attending selected medical center in Northern Nigeria,
97 20 isolates were microscopically and biochemically identified as *Klebsiella* species (Table 1).

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

101 Table 3 and Figure 1 show the highest susceptibility of *Klebsiella* species with ciprofloxacin (100%) and
102 gentamicin (100%) whereas the least susceptibility occurred with ampicillin (0%). The antibiotics that were
103 found to be more effective were ciprofloxacin and gentamicin.
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105 **Table 1: Prevalence of the *Klebsiella* species from urinary tract of pregnant women attending**
106 **selected medical centre in Northern Nigeria**

Organism Isolated	Frequency of Isolates	Prevalence (%)
<i>Klebsiella species</i>	5	10
Other Isolates	23	46

No Growth	22	44
TOTAL	50	

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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.



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

127 **Table 2: Biochemical characterizations of the bacterial isolates obtained from pregnant women attending selected medical centre in**
 128 **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	+	+	+	+	<i>Klebsiella pneumoniae</i>
Gram-negative non sporing rods	+	-	+	+	+	-	-	+	+	+	A/A +Gas	+	+	+	+	<i>Klebsiella oxytoca</i>
Gram-negative rods	-	+	-	+	-	+	-	+	-	-	K/A+H ₂ S	-	-	+	+	Non-klebsiellae isolates

129 *CAT= catalase; MR= methyl red; VP= Voges Proskauer; IND= indole; CIT= citrate; MT= motility; OXI= oxidase; URE= urease; NIT= nitrate*
 130 *reductase; ESC= esculin hydrolysis; TSI= triple sugar iron; Lac= lactose; MAL= maltose; SUC= sucrose; GLU= glucose; A= alkaline; K= acid;*
 131 *H₂S= hydrogen sulfide; + = positive; - = negative*

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

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 142 **Table 3: Antibiotic susceptibility profile of *Klebsiella* species isolated from pregnant women**
 143 **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)

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 146 The antimicrobial susceptibility of the antibiotics used showed relatively higher level of susceptibility of
 147 *Klebsiella oxytoca* and *K. pneumoniae* to ciprofloxacin (100%) and gentamicin (100%) respectively (Table
 148 3 and Figure 1). This conformed to the work of Nwanze [19] and Awoniyi [20], who reported that klebsiella
 149 isolates showed relatively higher level of susceptibility to ciprofloxacin and gentamicin. Antibiotic
 150 resistance observed in this study is a sufficient cause for serious concern, especially with resistance to
 151 ampicillin (0%). The resistance may be due to lateral acquisition of resistance plasmid. However, Martin
 152 *et al.* [16] reported that *Klebsiella oxytoca* is capable of acquiring antibiotic resistance, and the isolates
 153 have been shown to produce extended-spectrum beta-lactamases and carbapenemases that deactivate
 154 penicillin derivatives.

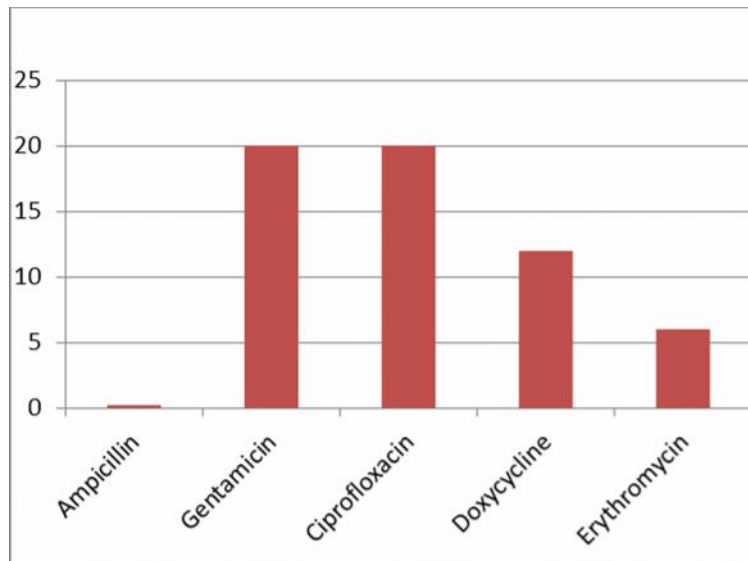


Fig.1: Antibiotic susceptibility of *Klebsiella* species

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158 The high resistance showed by some antibiotics could be due to earlier exposure of the isolates to the
 159 drug which may have enhanced resistant development [20]. It could also be due to practices of self-
 160 medication and indiscriminate use of this antibiotics and the acquiring of plasmid encoded resistant genes
 161 [21]. The findings have no doubt highlighted the need for constant monitoring of susceptibility of specific
 162 pathogens in different populations of commonly used antimicrobial susceptibilities and to assist clinician
 163 in the rational choice of antibiotics therapy to prevent misused or over use of the antibiotics.

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166 **4. CONCLUSION**

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168 *Klebsiella oxytoca* and *K. pneumoniae* were isolated amongst the pregnant women attending Ahmadu
 169 Bello University Health Service Clinic with total prevalence of 10%. From the result obtained, ciprofloxacin
 170 and gentamicin can serve as drugs of choice for the management of UTI caused by the *Klebsiella* species
 171 but ciprofloxacin was found to be generally more effective and ampicillin recorded less activity. *Klebsiella*
 172 *oxytoca* and *K. pneumoniae* were isolated amongst the pregnant women attending Ahmadu Bello
 173 University Health Service Clinic with total prevalence of 10%. From the result obtained, ciprofloxacin and
 174 gentamicin can serve as drugs of choice for the management of UTI caused by the *Klebsiella* species but
 175 ciprofloxacin was found to be generally more effective and ampicillin recorded less activity.

176 Constant and frequent review of antibiotic susceptibility of *Klebsiella* should be carried out to check for
 177 possible emergence of resistance by the organism. There is need for continuous monitoring of bacterial
 178 antibiotic susceptibility before antibiotic prescription in order to ensure adequate treatment of urinary tract
 179 infection (UTI) caused by the bacteria, because blind treatment is not economically wise.

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181 **CONSENT**

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

184

185 **ETHICAL APPROVAL**

186

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

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