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Prevalence of *Klebsiella pneumoniae* and *Acinetobacter baumannii* in Urine Samples of Pregnant Women in South-East, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Pregnancy induces so many changes in the woman's body that likely predisposes them to *Klebsiella pneumoniae* and *Acinetobacter baumannii* which causes Urinary Tract Infections (UTIs).
Aims: To determine the prevalence of *Klebsiella pneumoniae* and *Acinetobacter baumannii* in urine samples of pregnant women attending ante-natal clinics at three health institutions within the study area.
Study Design: This study was a descriptive cross-sectional study.
Place and Duration of Study: Department of Microbiology Federal University Teaching Hospital, Owerri, Teaching Hospital Orlu and General Hospital Okigwe, Imo State between October 2021 and February 2022.

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Methodology: Urine samples were collected aseptically from 408 pregnant women between 18-45 years and analyzed by culturing onto the surface of freshly prepared MacConkey and Chromogenic agar plates for the isolation of *Klebsiella pneumoniae* and *Acinetobacter baumannii* respectively and confirmed by appropriate biochemical tests.
Results: From the results, there was a prevalence rate of 145/408 (35.54%) for both isolates with *Klebsiella pneumoniae* being more dominant with a prevalence rate of 96/408 (23.53%) as against *Acinetobacter baumannii* that was 49/408 (12.01%). The highest prevalence of the isolates was seen in the Owerri zone with a prevalence rate of 37 (9.07%) for *Klebsiella pneumoniae* and 23 (5.64%) for *Acinetobacter baumannii*, respectively. The highest age-specific prevalence of the isolates within the three zones was observed in the 24–29-year-olds with a prevalence rate of 42 (10.29%) in the Owerri zone. In contrast, the prevalence of both organisms was higher in pregnant women in their third trimester of pregnancy with a prevalence rate of 72 (17.65%) and in multigravida women with a prevalence rate of 217 (53.2%).
Conclusion: The results from this study showed that these isolates are becoming more predominant in causing both symptomatic and asymptomatic urinary tract infections in pregnant women, hence regular check-ups and proper treatments of urinary tract infections in pregnant women should be encouraged.

Keywords: Prevalence; urinary tract infection; Klebsiella pneumoniae; Acinetobacter baumannii; Urine; pregnant women; Owerri.

1. INTRODUCTION

Urinary Tract Infections (UTIs) account for a significant percentage of community and healthcare-acquired infections affecting 2–10% of pregnant women in both underdeveloped, developed and developing countries, and they may be symptomatic or asymptomatic [1]. UTI can be described based on the part of the tract affected; for the upper tract it is pyelonephritis, while for the lower tract it is cystitis or urethritis [2]. Untreated or undertreated bacteriuria in pregnancy, regardless of symptoms, increases the risk of negative outcomes, including increased maternal and newborn morbidity and death [3]. As a result, screening and treating pregnant women for bacteriuria has become a standard aspect of prenatal treatment.

Urinary tract infection occurs in about 8% of all pregnant women and peaks in about 90% during the period of about 24 weeks (about 5 and a half months) of gestation [4]. About 70% of pregnant women have urethral dilatation which remains until delivery in addition to having glucosuria which enhances bacterial growth in urine [5]. Symptoms associated with urinary tract infection include pain or burning (discomfort) sensation when urinating, cramps or pain in the lower abdomen, the need to urinate more often than usual, turbid and foul-smelling urine, pressure or tenderness in the area of the bladder and when bacteria spread to the kidneys, there can be back pain, chills, fever, nausea and vomiting [1].

Globally, the prevalence of UTIs in pregnant women is between 13% - 33% with symptomatic bacteriuria occurring in 1% - 18% [6], while asymptomatic cases are observed in 2%-45% in different populations [7] among females. The rate of prevalence has varied including epidemiological and cross-sectional research in developing and under-developed nations. Epidemiological surveillance of outpatient urine culture offers relevant insights into the changing prevalence and even antimicrobial drugs that are susceptible to specific uropathogens [6], particularly in patients presenting nonspecific symptoms. The most significant organisms causing UTI in pregnant women include *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacteriaceae* (ESKAPE) [8]. Because of their distinct resistance properties, *Acinetobacter baumannii* and *Klebsiella pneumoniae* are becoming more difficult to combat. They are non-lactose-fermenting, catalase-positive, non-motile, non-fastidious, oxidase-negative, and aerobic gram-negative coccobacilli are all members of this genus. Clinically, *A. baumannii* and *K. pneumoniae* are important because they cause UTIs and nosocomial infections and are inherently resistant to a broad range of antimicrobials, with a considerable risk of acquiring resistance. They are found in 42% of healthcare personnel and patients' hands, according to studies [9]. Their capacity to tolerate desiccation allows them to persist for months in

inert things, facilitating their spread in hospitals and environments.

In many hospitals, infections of the respiratory system, urinary tract, bloodstream (septic), post-surgical (wound) infections, and pneumonia are among the most often reported diseases caused by these bacteria [10]. Although the prevalence of these organisms has been detected in several Nigerian hospitals, there are no mechanisms in place to ameliorate the issue, such as surveillance studies that give trustworthy data in the state. As a result, investigations to determine the prevalence of these organisms in Imo state are required to close the knowledge gap and provide more foundation for empirical treatment. This research will look at the distribution of *Klebsiella pneumoniae* and *Acinetobacter baumannii*, which was found in urine samples of pregnant women in the state.

2. METHODOLOGY

2.1 Study Area

The research was conducted in Imo state Nigeria. The state is divided into three zones, namely; Owerri, Orlu and Okigwe. Hospitals and health centers in these three zones were used as sample collection centers and analyzed at the federal university teaching hospital Owerri, Imo state.

The inclusion criteria included pregnant women within the study area who gave informed consent to participate in the study. Pregnant women without informed consent and who had taken antibiotics within the last 2 weeks of sample collection were excluded.

2.2 Sample Collection

Four hundred and eight (408) mid-stream urine samples of healthy pregnant women between the ages of 18-45 years in Imo state were collected in a sterile, dry, leak-proof container and labelled properly. Using the sampling formula from Charan & Biswas [11]. The samples were divided into the three geopolitical zones comprising Okigwe (87), Orlu (121) and Owerri (200). With the aid of a questionnaire, demographic parameters including age, gestational age of pregnancy, and questions related to clinical features of *K. pneumoniae, Acinetobacter* and urinary tract infection were issued to them. The collected urine samples were placed in a cold box and transported to the microbiology laboratory of Federal University Teaching Hospital Owerri for analysis.

2.3 Isolation of Organisms

The Chromagar^{tm} (Paris, France) and MacConkey Agar (Titan Biotech Ltd, India) were prepared according to the manufacturer's instruction and were used for primary isolation of *Acinetobacter baumannii* and *Klebsiella pneumoniae* respectively by streaking the urine sample unto the surface of the prepared media [12]. Colonies from culture plates of both organisms were subcultured onto nutrient agar plates, pure cultures were made with repeated streaking and incubated at 37°c for 24 hours.

2.4 Identification of Isolates

Identification and confirmation of the organisms from the culture plates of the pure colonies was done by morphological, cultural characteristics and biochemical tests adopted from Cheesbrough, (2006) and the result was recorded accordingly. *Klebsiella pneumoniae* appeared as large, mucoid pink colonies without retaining the primary stain and was confirmed to be non-motile, citrate positive, catalase positive and oxidase negative.

Acinetobacter baumannii appeared as round, smooth pink colonies on the chromogenic agar without also retaining the primary stain and was also confirmed to be catalase positive, non-motile, citrate positive and urease negative.

2.5 Data Analysis and Interpretation

Statistical analysis was done using SPSS, chi-square for categorical data and ANOVA for variables that were more than two.

3. RESULTS AND DISCUSSION

3.1 Total Distribution of the Isolates within the Study Area

Out of the four hundred eight (408) urine samples of the pregnant women examined, hundred and forty-five (145) representing 35.57% of the population was infected with either *A. baumannii* or *K. pneumoniae* with a mixed infection of about 1.97%. While two hundred and sixty-three (263) representing 64.43% of the population were uninfected.

The overall pattern of distribution of *A. baumannii* and *K. pneumoniae* examined within the study

area is shown in Table 1 below. Both organisms were found in all the zones sampled with an overall prevalence of 35.57%. Of the number examined in each zone, the Owerri zone recorded the highest occurrence of 14.71%, followed by the Orlu zone; 11.03% while the lowest percentage occurrence was observed in the Okigwe zone with 9.80%. The total distribution of *K. pneumoniae* and *A. baumannii* showed that urinary infection was not dependent on demography with p=0.28. Statistical analysis revealed that there is no correlation between mixed infection and against zones of infection with P=.05.

3.2 Age-related Prevalence of the Isolates within the Study Area

The distribution of the organisms with respect to the age of the women examined is shown in Table 2. Results showed that pregnant women between the 24-29 years age bracket had the highest prevalence of the organisms with a prevalence rate of 10.29% while pregnant women between 42 years and above had the least prevalence of 1.72%. From the result below, it was further observed that the prevalence rate of *A. baumannii* was higher in pregnant women between the ages of 36-41 in the three zones with a rate of 3.19% while *K. pneumoniae* occurred more in pregnant women between the 24-29 age brackets amongst the three zones with a rate of 7.35%. Further analysis is in Table 2. Revealed that *K. pneumoniae* occurred more between the 24-29 age brackets in the three zones with similar prevalence rates while *A. baumannii* occurred more between the 36-35 age brackets of Owerri zone with a prevalence rate of 34.78% and Orlu zone recorded zero prevalence of *A. baumannii* between the 18-23 age brackets. From the results, it can be deduced that infection and mixed infection were not dependent on both age and zones studied, thus the relationship between the prevalence as against age and zones showed no correlation with a p-value of 0.30.

3.3 Prevalence of the Isolates with Relation to Age of Pregnancy and Number of Births

From Table 4 below, the prevalence of both organisms was highest in pregnant women in their third trimester of pregnancy with a rate of 17.65%, followed by those within their second trimester and first trimester with a rate of 13.48% and 4.41% respectively. The prevalence of *K. pneumoniae* was higher within the third trimester of pregnancy (12.75%) while the prevalence of *A. baumannii* was higher (5.64%) within the second trimester of pregnancy. Mixed infection occurred at a rate of 1.22% within the second trimester. This shows that infection was not dependent on the trimester of pregnancy with a P value of 0.10.

Based on the information provided in Table 4, the prevalence of both organisms was higher in multigravida women with a rate of 14.46% for *K. pneumoniae* and 5.64 % for *A. baumannii*.

Prevalence and mixed occurrence in the overall infection show that infection was not dependent on the gravidity of pregnancy with a P value of 0.12. Relationship against gravidity in pregnancy showed no correlation between them with a P value of 0.16.

4. DISCUSSION

The criteria for prevalence selection were determined after the isolation and identification of the organisms in the urine samples of pregnant women within the three zones. Pregnant women are at a higher risk of having UTIs due to the physiologic changes in pregnancy. However, in the majority of these women, the infections are asymptomatic. The results from this study revealed that *A. baumannii* and *K. pneumoniae* are endemic in the three zones of Imo state with an overall prevalence of 35.57% (145/408) of the population studied, with *K. pneumoniae* possessing an infection rate of 23.53% and *A. baumannii* possessing an infection rate of 12.01% and a mixed infection of about 1.97%. The level of infection however varied with some zones having higher levels of infection than others. This difference in the prevalence rate may be influenced by peculiar ecological characteristics, mode of living and the degree of exposure [13]. This trend of results may not be unconnected to the fact that most of the local governments in these zones studied have rural villages and community arrangements. A close look at the prevalence of these organisms in the three zones of Imo state studied shows that Owerri has the highest prevalence of 14.71% out of the 408 sampled; this might be due to the fact that Owerri has the highest number of population sampled due to development, better opportunities and migration of most pregnant women from the rural settings to urban settings for proper ante-natal and counselling, but the high prevalence level was not significant ($p<0.05$). This prevalence no matter how low

Table 1. Total distribution (%) of infections among zones studied

Zones	No. of samples N (%)	No of persons Infected N (%)	No Infected with *Klebsiella pneumonia* N (%)	No Infected with *Acinetobacter baumannii* N (%)	No of Mixed infection *Kp/Ab* N (%)
Okigwe	87 (21.3)	40 (9.80)	26 (6.37)	14 (3.43)	3 (0.74)
Orlu	121 (29.7)	45 (11.03)	33 (8.09)	12 (2.94)	1 (0.25)
Owerri	200 (49.0)	60 (14.71)	37 (9.07)	23 (5.64)	4 (0.98)
Total	**408 (100)**	**145 (35.54)**	**96 (23.53)**	**49 (12.01)**	**8 (1.97)**

** Kp/Ab = Klebsiella pneumoniae and Acinetobacter baumannii*

Table 2. Prevalence (%) of the isolates in relation to age of the pregnant women studied

AGE (YEARS)	No. of Samples N (%)	No of persons Infected N (%)	No Infected with *Klebsiella pneumonia* N (%)	No Infected with *Acinetobacter baumannii* N (%)	No of Mixed infection *Kp/Ab* N (%)
18-23	63 (15.4)	27 (6.62)	18 (4.41)	9 (2.21)	3 (0.74)
24-29	117 (28.7)	42 (10.29)	30 (7.35)	12 (2.94)	2 (0.49)
30-35	128 (31.4)	34 (8.33)	22 (5.39)	12 (2.94)	2 (0.49)
36-41	74 (18.1)	35 (8.58)	22 (5.39)	13 (3.19)	1 (0.25)
42 and above	26 (6.4)	7 (1.72)	40 (0.98)	3 (0.74)	0
Total	408 (100)	145 (35.54)	96 (23.52)	49 (12.01)	8 (1.97)

Key: Kp/Ab = Klebsiella pneumoniae and Acinetobacter baumannii*

Table 3. Prevalence (%) of the isolates with respect to age of pregnant within the three zones studied

Zones **Age (Years)**	**No. of samples N (%)**	**No of persons infected N (%)**	**No Infected with *Klebsiella pneumonia* N (%)**	**No Infected with *Acinetobacter baumannii* N (%)**	**No of Mixed infection *Kp/Ab* N (%)**
Okigwe	N= 87	N=40	N=26	N=14	N=3
18-23	18 (20.7)	12 (30.0)	7 (26.92)	5 (35.71)	1 (50.0)
24-29	22 (25.3)	14 (35.0)	10 (38.46)	4 (28.57)	1 (50.0)
30-35	21 (24.1)	6 (15.0)	5 (19.23)	1 (7.14)	0
36-41	17 (19.5)	6 (15.0)	3 (11.54)	3 (21.42)	1 (50.0)
42 and above	9 (2.2)	2 (5.0)	1 (3.85)	1 (7.14)	0
Orlu	N=121	N=45	N=33	N=12	N=1
18-23	17 (14.0)	4 (8.8)	4 (12.12)	0	0
24-29	28 (23.1)	13 (28.9)	10 (30.30)	3 (25.0)	0
30-35	40 (33.1)	12 (26.7)	9 (27.27)	3 (25.0)	1
36-41	25 (20.7)	14 (31.1)	9 (27.27)	5 (41.67)	0
42 and above	11 (9.1)	2 (4.4)	1 (3.03)	1 (8.33)	0
Owerri	N=200	N=60	N=37	N=23	N=5
18-23	28 (14.0)	11 (18.3)	7 (18.91)	4 (17.39)	2 (40.0)
24-29	67 (33.5)	15 (25.0)	10 (27.02)	5 (21.74)	2 (40.0)
30-35	67 (33.5)	16 (26.7)	8 (21.62)	8 (34.78)	1 (20.0)
36-41	32 (16.0)	15 (25.0)	10 (27.02)	5 (21.74)	0
42 and above	6 (2.0)	3 (5.00)	2 (5.41)	1 (4.35)	0
Total	408 (100)	145 (35.54)	96 (23.52)	49 (12.01)	8 (1.97)

Key Kp/Ab = Klebsiella pneumoniae and Acinetobacter baumannii*

Table 4. Occurrence (%) of the isolates with respect to gestational age of pregnancy

Trimester (gestational age)	No. of samples N (%)	No of persons infected N (%)	No Infected with *Klebsiella pneumonia* N (%)	No Infected with *Acinetobacter baumannii* N (%)	No of Mixed infection *Kp/Ab* N (%)
1st Trimester (0-13 weeks)	68 (16.7)	18 (4.41)	12 (2.94)	6 (1.47)	1 (0.24)
2nd Trimester (14-27 weeks)	151 (37.0)	55 (13.48)	32 (7.84)	23 (5.64)	5 (1.22)
3rd Trimester (28-40 weeks)	189 (46.3)	72 (17.65)	52 (12.75)	20 (4.90)	2 (0.49)
Total	408 (100)	145 (35.54)	96 (23.52)	49 (12.01)	8 (1.97)

Key: Kp/Ab = Klebsiella pneumoniae and Acinetobacter baumannii

Table 5. Prevalence (%) of the isolates with respect to gravidity and parity

Parity	No. of samples N (%)	No of persons infected N (%)	No Infected with *Klebsiella pneumonia* N (%)	No Infected with *Acinetobacter baumannii* N (%)	No of Mixed infection *Kp/Ab* N (%)
Primagravida	118 (28.9)	39 (9.56)	22 (5.39)	17 (4.17)	1 (0.25)
Multigravida	217 (53.2)	82 (20.09)	59 (14.46)	23 (5.64)	6 (1.47)
Grand-multi Gravid	73 (17.9)	24 (5.88)	15 (3.67)	9 (2.21)	1 (0.25)
Total	408 (100)	145(35.54)	96 (23.52)	49 (12.01)	8 (1.97)

Key Kp/Ab = Klebsiella pneumoniae and Acinetobacter baumannii*

could be attributed to factors such as the nature of pregnancy, trimester and period of pregnancy, lack of sensitization and awareness, age, ignorance, poor living conditions, inadequate sanitation, level of contact with the organism, inappropriate medication, and the indiscriminate use of antibiotics.

The low prevalence of 12.01% of *A. baumannii* infection observed in this study is almost in tandem with the 14.0% reported by Nwadike et al. [14] among pregnant women in a university college hospital Ibadan, 10.0% recorded by Bashir, et al. [15] in a study conducted in three different hospitals in Kano state, Nigeria alongside 16.0% recorded by Musyoki, et al. [16] in Kenyatta Kenya. The result was in contrast with a higher prevalence of 30.7% recorded by Iregbu et al. [17] in Lagos Nigeria, and at total variance with the 6.1% prevalence rate of *A. baumannii* recorded by Obadare et al. [18] at Ile-Ife, Nigeria, 6.0% reported by Egwu, et al. [19] in Abakaliki, Nigeria, and 8.5% by Odewale, et al. [20].

The infection rate of 23.53% *Klebsiella pneumoniae* observed in this study was in conformity with that of Nwachukwu et al [21] and Egwu et al, [22] who observed the prevalence of *Klebsiella pneumoniae* at 24.0% as one of the organisms causing urinary tract infections in pregnant women in Onitsha and Abakaliki respectively, Moftian et al (32) who reported 23.54% rate in Iran, and Suraiya, et al. [23] who observed a prevalence rate of 23.73% in a tertiary hospital in Bangladesh, but was slightly in contrast with the 19.1% recorded by Damilola, et al. [24] amongst 407 samples in Ondo south West Nigeria. Olamijulo et al. [10] reported a prevalence of 14.6% in a study carried out on 556 pregnant women in Lagos University Teaching Hospital, Nigeria while Afunwa, et al. [25] recorded a 20.0% prevalence of *K. pneumoniae* in a study conducted on pregnant women attending the ante-natal clinic in Nike district of Enugu. However, the result is also at variance with Umeaku, et al. [26] who recorded a low prevalence of 6% amongst 50 samples in Owerri, Imo state. This finding however confirmed the involvement of both pathogens in causing UTIs in pregnant women.

The distribution of the isolates in relation to maternal age shows a higher frequency of occurrence between the age range of 24–29 at 10.29% and the least occurrence in the age range of 42 and above at 1.72%. This is similar to the findings from Anozie, et al. [27] and Okonko et al. [28] in South-Western Nigeria, whose findings showed the highest prevalence rate of 58.6% and 17.9% respectively amongst pregnant women between the ages of 25-30 years but in contrast with the research published by Akobi, et al. [29] and Akerele, et al. [30] who showed a high prevalence of *Klebsiella pneumoniae* in pregnant women between 30-35 years. The reason for the prevalence in this age group could be because many women in this age group are likely to be multiparous, ignorant, engaged in regular sexual activity and could be using one form of contraceptive or another.

The distribution of the isolates with respect to gestational age was found to be highest in the third trimester. This agrees with the findings of Okonko, et al. [28] and Afunwa, et al. [25]. It however differs from the findings of Amala and Nwokah [31] who reported a higher prevalence of *K. pneumoniae* and *A. baumannii* in the first and second trimesters. These differences may be because of fluctuations in urinary progesterone and oestrogen in the different trimesters of pregnancy [31]. The increased prevalence in the third trimester could be due to the increased size of the uterus, urethral dilation, increased bladder volume and urethral tone, which may lead to stasis and encourage bacterial growth in the urine [4].

The distribution of isolates with gravidity in this study shows a higher prevalence among the multigravida participants compared to primigravida and grand multigravida. This finding agrees with a study conducted by Akpan, et al. [32] but contrasted with the study showed by Nwachukwu, et al. [21] who recorded an increased prevalence of *K. pneumoniae* and *Acinetobacter spp* in the grand-multi gravida and primigravida, respectively. This is due to the subsiding of pelvic organs associated with high parity leading to the widening of the urethral orifice and predisposing the woman to micro-organisms which may progress to symptomatic urinary tract infection. The second highest group in this study was the primigravida group, this may be induced by malaria in pregnancy, due to impaired immunity during the first pregnancy making the primigravida prone to infection [33,34].

5. CONCLUSION

The findings of this study recorded a moderate prevalence of bacteriuria in the study area

among the participants which calls for concern due to the possible effect of bacteriuria on the fetus. Also having most of this prevalence being asymptomatic, there is a need to educate pregnant women on personal and environmental hygiene and the need for regular medical check-ups, laboratory screening and treatment with the right antibiotics when infections are identified to prevent birth complications.

CONSENT

As per international standards and university standards, patient(s) written consent was collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standards written ethical approval was collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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