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PREVALENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *KLEBSIELLA PNEUMONIAE* AND *PSEUDOMONAS AERUGINOSA* AMONG WOMEN WITH URINARY TRACT INFECTIONS ATTENDING ANTENATAL CARE IN KADUNA, NIGERIA

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ABSTRACT
This study determined the prevalence of Extended Spectrum B-lactamase (ESBL) and their resistance gene producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* among women with urinary tract infections attending antenatal care in Barau Dikko Teaching Hospital Kaduna, Nigeria. A total of 230 mid-stream urine samples were identified using cultural (MacConkey agar and Cetrimide agar respectively) and biochemical methods. Isolates were further screened for Extended Spectrum Beta-lactamase (ESBLs) production using double disc synergy test (DDST). Positive ESBLs isolates were further subjected to multiplex PCR for resistance gene determination. The overall UTIs prevalence was 22.17% (51/230) among which 14.78% (34/230) were *Klebsiella pneumonia* and 7.39% (17/230) were *Pseudomonas aeruginosa*. The overall prevalence of Extended spectrum β-lactamase was 0.87% (2/230) among which 11.76% (2/17) were *Klebsiella pneumoniae* and 14.29% (2/14) were *Pseudomonas aeruginosa*. Extended spectrum Beta-lactamase genes were detected only in *Klebsiella pneumonae* and not in *Pseudomonas aeruginosa*. The resistance genes identified in ESBLs producing *Klebsiella pneumonae* were bla_{SHV} and bla_{CTX-M}, both in equal proportion; bla_{TEM} was not detected. Antimicrobial susceptibility testing and ESBL production monitoring are therefore recommended in patients.

Keywords: Extended Spectrum Beta-lactamase, Prevalence, *Klebsiella pneumonae, Pseudomonas aeruginosa*.

INTRODUCTION
Extended Spectrum Beta Lactamases (ESBLs) is one of the groups whose appearance is due to consumption of third generation cephalosporins. Extended Spectrum Beta Lactamases has variants of primary enzymes namely; Temonera (TEM-1), TEM-2 and SulphurHydryl Variable (SHV-1). This variation is based on changes in one or more amino acids (Majeed and Aljanabi, 2019; Olowe *et al*., 2019).
In Nigeria, reports in literature have described the different epidemiological distributions of ESBLs producing organisms (Spadafino *et al*., 2014). One study reported prevalence of 44.6% in Enugu State (Olowe *et al*., 2012), another study reports a prevalence of 35% and an incidence of 9% in Nsukka (Afunwa *et al*., 2011). Some other studies have also reported a prevalence of 72.5% in Lagos state (Egbebi and Famurewa, 2011) and 15.4% in Kano State (Muhammad and Muhammad 2019).
Urinary tract infections (UTIs) are caused by the presence and growth of micro-organisms in the urinary tract and are the single common bacterial infection of mankind (Onwuezobe and Orok, 2015). In pregnancy, UTIs may involve the lower urinary tract or bladder (Almehdawi *et al*., 2017). The three clinical manifestations of UTIs in pregnancy are asymptomatic bacteriuria, acute cystitis and pyelonephritis (Oluwafemi *et al.,* 2018; Odoki *et al.,* 2019). Antibiotic resistance of urinary tract pathogens has been on the increase worldwide especially to commonly used antimicrobials (Aernan and Umeh, 2016). Resistance to extended spectrum beta-lactamse has been found among the strains of *Klebsiella pneumoniae* and *Escherichia coli* isolates that produce Beta-lactamases which are resistant to penicillins, cephalosporins and monobactams (azetronam) (Mohammad *et al.,* 2016). Beta-lactamases has been divided into four groups on the basis of substrate type and physical characteristics such as molecular weight and isoelectric point (Bush *et al*., 1995).
Bacteria belonging to the genus *Klebsiella* frequently cause human nosocomial infections in particular, the medically important species, *Klebsiella pneumoniae*, accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections (Almehdawi *et al*., 2017). The principal pathogenic reservoirs for transmission of *Klebsiella* sp are the gastrointestinal tract and the hands of hospital personnel. The genetic mutations that give rise to ESBLs broaden the parental resistance pattern to a phenotype that includes resistance to third generation cephalosporins (Akpan *et al*., 2019). *Klebsiella pneumoniae* is an important nosocomial pathogen that has the potential to cause severe morbidity and mortality.
Pseudomonas aeruginosa is an ubiquitous pathogen prevalent in hospital environments. It causes severe nosocomial infections, particularly among immunocompromised patients. People with respiratory, gastro-intestinal, urinary tract, and wound infections as well as burn victims, individuals with cancer, and patients hospitalized in intensive care units are affected by *P. aeruginosa* mostly due to nosocomial spread and cross contamination (Aernan and Umeh 2016). *Pseudomonas. aeruginosa* accounts for 10% of all hospital acquired infections, a site specific prevalence which may vary from one unit to another (Matar *et al*., 2005). *Pseudomonas aeruginosa* can develop resistance to antibiotics either through the acquisition of resistance genes on mobile genetic elements (i.e., plasmids) or through mutational processes that alter the expression and/or function of chromosomally encoded mechanisms (Davies and Davies, 2010). Both strategies for

developing drug resistance can severely limit the therapeutic options for treatment of serious infections (Ghaima *et al*., 2018; Rajivghandhi *et al.,* 2018; Behbani *et al*., 2019; Dabir *et al*., 2020).

MATERIALS AND METHODS

Sample site
The sample site of this study was Barau Dikko Teaching Hospital Kaduna, Kaduna State Nigeria, which lies between longitude 10° 31ˈ36.1ˈˈN and latitude 7°26ˈ31.5ˈˈ E (GPS). The samples were collected from Antennal Units in Barau Dikko Teaching Hospital Kaduna. It is a Hospital that received patients from and within the metropolis and across the state.

Sample size
The sample size of the study was determined using the Cochran's formula (Cochran 1977) to calculate a representative sample for proportion as follows:
$N=Z^2 P (1-P)/d^2$
Where, n_o is the sample size z is the selected critical value of desired confidence level, p is the estimated proportion of an attribute that is present in the population, q = 1 –p and d is the desired level of precision.
The sample size of these study was calculated using the prevalence rate of 15.4% in Kano state (Muhammad and Muhammad 2019).

N=Z2 P (1-P) /d2
N = 1.96²x 0.154 x (1-0.154) / (0.05) ²
N= 200
Therefore, 230 samples were convinient for this study.

Ethical Clearance
Ethical clearance was obtained from the Ethical Committee of Kaduna State Ministry of Health and Human Services Kaduna and Ethical Committee of Barau Dikko Teaching Hospital Kaduna.

Inclusion Criteria
Patients sent to the Microbiology laboratory with suspected cases of Urinary Tract Infection were included in the study.

Exclusion Criteria
Patients sent to the Microbiology laboratory with suspected cases other than Urinary Tract Infection were excluded in the study.

Consent form
Consent form was given to all patients that fulfill the inclusion criteria.

Administration of structured questionnaire
Structured questionnaire was administered to those patients that fulfil the consent form**.**

Sample Collection
A total of 230 urine samples were collected from antenatal patients of Barau Dikko Teaching Hospital, Kaduna. All the samples were collected and processed according to the standard laboratory procedure. The samples were inoculated in selective and differential media and incubated overnight at 37°C.

Cultural and Microscopic Characteristics
Isolates were identified by their morphological characteristics on MacConkey agar and Cetrimide agar. The appearance of Pink mucoid colonies and flat dry circle on MacConkey and yellow-green mucoid colonies after incubation at 37°C for 24h were processed for Gram staining. Colonies showing Gram negative short, plump, straight rods and smooth-mucoid respectively were characterized biochemically.

Gram Staining
Thin Smears were prepared from a 24h culture, air-dried and heat-fixed by passing the slide over a Bunsen flame for three quick successions. The smears were Gram stained and examined microscopically under oil immersion objective lens following the addition of oil immersion (Cowans and Still, 2004).

Biochemical test
For further reconfirmation, suspected colonies of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were subjected to a number of biochemical tests which includes; Indole test, Methyl Red - Voges-Proskauer test, citrate utilizations test, urease test, Catalase test, motility test, hydrogen Sulphide Production along with carbohydrate fermentation test (Glucose, Lactose, Sucrose, and Mannitol fermentation) (Cheesbrough, 2006).

Extended Spectrum Beta-lactamase Test
Double Disc Synergy test
The double disc synergy test (DDST) method described by CLSI (2000) was employed. Standardized inoculum of the test organisms was inoculated on Muller Hinton Agar (MHA) (BIOTECH, England) using sterile swab sticks. Amoxicillin/clavulanic acid disc (20µg + 10µg), was placed at the center of the inoculated MHA. Ceftazidime (30µg) and Cefotaxime (30µg) were placed 15mm center to center from the Amoxicillin/clavulanic acid discs. The plates were incubated at 37°C for 24h. After incubation, enhancement of zone of inhibition in either or both the Ceftazidime and Cefotaxime discs towards the Amoxicillin/clavulanic acid discs is indicative of ESBL production.
The enhancement is due to inhibition of ESBL by clavulanic acid and subsequent action of the extended spectrum cephalosporins.

Molecular Detection of Resistance Genes

Genomic DNA extraction
An overnight suspension of the bacterial isolates was prepared in a sterile 2 mLependorf tube containing 1.8 ml of brain heart infusion broth. The tubes were centrifuged at 14000 rpm for 1 minute. The supernatant was discarded and the cells were resuspended in 300uL of nuclease free water. DNA was extracted from the suspension using Qiagen DNA extraction kit following the manufacturer's instructions

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Primers

Table 1: Primer sets for amplification of extended spectrum beta-lactamase genes (ESBL)

Gene	Primer sequence (5' 3')	Amplicons size (bp)	References
BlaTEM	F-GTATCCGCTCATGAGACAATAACCCTG	918	Alkhudhairy and Alshammari, 2020
	R-CCAATGCTTAATCAG TGAGGCACC		
BlaSHV	F-CGCCTGTGTATTATCTCCCTGTTAGCC	842	Alkhudhairy and Alshammari, 2020
	R-TTGCCAGTGCTCGAT CAGCG		
BlaCTX-M	F-CGCTTTGCGATGTGCAG	550	Alkhudhairy and Alshammari, 2020
	R-ACCGCGATATCGTTG GT		

Key:
TEM: Temonera, SHV: Sulphur Hydryl Variable, CTX-M: Cefotaxime

DNA Amplification
PCR assays was performed in a volume of 20 microliter containing 2 microliter of DNA (template) extracted from serum, 16 microliter of water, 1 microliter of each forward primer (SHV, TEM and CTX-M) and 1 microliter of each (SHV, TEM and CTX-M) reverse primer were all added to the premix. Each run contained a positive control, as well as a no-template (water) negative control. Amplification of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was performed by multiplex PCR, and 2 microliters of the first-round PCR product was used as the template for the second-step PCR. PCR conditions of Extended Spectrum Beta-lactamase gene was as follows: Pre- Denaturation: 5min at 94°C, Denaturation: 30sec at 94°C, Annealing: 30sec at 52°C, Extension: 1min at 72°C 35 cycles, Final extension: 5min at 72°C as carried out in the work of (Olowe *et al*., 2012).

Agarose Gel Electrophoresis
An aliquot 1.5g of powder gel was transferred into 100mL of buffer (Tris acetate EDTA). Solution was heated in a microwave until agarose gel was completely dissolved and was allowed to cool for 15-30min at room temperature. Gel casting tray was prepared by sealing ends of gel chamber with tape. Appropriate number of combs was placed in gel tray to create wells. 5ul of ethidium bromide was added to cooled gel and poured into gel tray and was allowed to solidify. Combs were removed and placed in electrophoresis chamber and covered with buffer (Tris acetate EDTA) as carried out in the work of (Olowe *et al*., 2012). DNA bands were visualized using UV light box.

RESULTS

Table 1: Colonial and Biochemical Characterization of Bacterial Isolates from Urine Samples of Antenatal Patients Attending Barau Dikko Teaching Hospital Kaduna

Colonial characteristics	Gram reaction	Biochemical characteristics								Sugar fermentation				Probable Bacteria
		In	MR	VP	MT	Ur	Ca	H_2S	Ci	Glu	Lac	Suc	Mn	
Pink mucoid colonies on Maconkey agar	-ve	-	-	+	-	+	-	-	+	+	+	+	+	*Klebsiella pneumoniae*
Yellow-green on Cetrimide agar	-ve	-	-	-	-	+	-	+	-	-	-	+		*Pseudomonas aeruginosa*

KEY: In=Indole, MR=Methyl-red, VP=Voges-proskauer, MT=Motility, Ur=Urease, Ca=Catalase, H_2S=Hydrogen-sulphide, Ci=Citrate, Glu=Glucose, Lac=Lactose, Such=Sucrose, Mn=Mannitol, -ve=Negative, +ve=Positive

Table 2 shows the occurrence of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (14.78% and 7.39%), from ESBLs profile (11.76% and 14.29%), multidrug resistance (50% and 82%) among pregnant women with UTI in Kaduna

Table 2: Occurrence of Drug resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and their ESBLs Profile in Urine of Pregnant Women Attending Antenatals

Bacteria	Sample size (n)	Number isolates Positive (%)	Resistant	ESBLs (%)
Klebsiella pneumonia		34 (14.78)	17(50)	2(11.76)
Pseudomonas aeruginosa		17 (7.39)	14(82)	2(14.29)
Total	230	51 (22.17)		0.87

KEY:
ESBL=Extended spectrum beta-lactamase

Table 3 shows a 0.8% prevalence of both *Klebsiella pneumoniae* and Pseudomonas *aeruginosa* with ESBLs production capacity.

Table 3: Percentage of ESBL Genes among Isolated Bacteria from Urine Samples of Antenatal Patients Attending Barau Dikko Teaching Hospital Kaduna

Bacteria	Number tested (%) (n=230)	TEM (%)	SHV (%)	CTX-M (%)
Klebsiella pneumonia	2(0.87)	0(0)	2(100)	2(100)
Pseudomonas aeruginosa	2(0.87)	0(0)	0(0)	0(0)
Total	4	0(0)	2(100)	2(100)

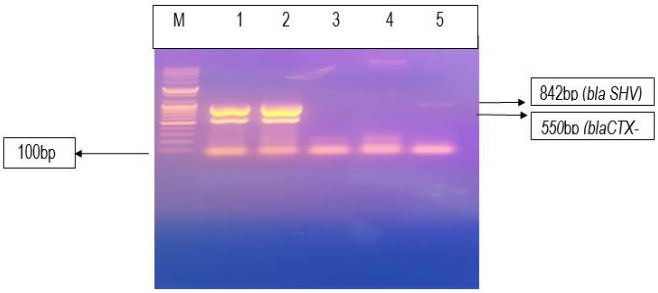

Plate 1: Agarose gel electrophoresis of *bla* CTX-M, *bla* SHV and *bla* TEM (multiplex PCR). M (BIONEER DNA Marker, 100bp), 1-2 (Positive sample wells, 3-4(Negative sample wells), 5(Negative control)

DISCUSSION

Urinary tract infections (UTIs), which occur as a result of colonization of urine and the invasion of any structure of the urinary tract by microorganisms such as bacteria, viruses, yeasts and protozoa, have been recognized as a common infection of human (Chijioke *et al*., 2016; Almehdawi *et al*., 2017). Among individuals presenting clinical symptoms of UTIs, females have been reported to show increased susceptibility, especially during pregnancy with significant maternal and fetal risks (Almehdawi *et al*., 2017; Vaishali and Aarti, 2017). The results of the present study indicated an overall UTIs prevalence of 22.17% among pregnant women attending antenatal care facility in Barau Dikko Teaching Hospital Kaduna. Different authors have reported widely varying bateriuria among pregnant women. Muhammad and Muhammad (2019) reported an incidence rate of 15.8% UTIs among pregnant women in Kano state, Northern Nigeria. In a 2016 study conducted among pregnant women in Owerri, South-eastern Nigeria, a 25% bacteriuria was recorded by Onuorah *et al*. (2016). According to a study conducted by Oladipo (2013), a UTIs prevalence of 56.7% among pregnant women in South-western Nigeria was recorded. In another study, 61.5% prevalence of UTIs among pregnant women in Aba, South-eastern Nigeria was recorded by Ezeigbo *et al*. (2016). Immunological, physiological, anatomical and hormonal factors have been identified as responsible for incidences of urinary tract infections among pregnant women (Samuel *et al*., 2016). Almehdawi *et al*. (2017) indicated that shortened urethra, absence of prostatic secretion, contamination of urinary tract with fecal flora increase the risk of UTIs among women, conditions which are further exacerbated in pregnancy due to concomitant hemodilution, urinary stasis, decreased urine concentration and glycosuria, all of which favour bacterial colonization and growth within the urinary tracts. The weakened immune mechanisms associated with pregnancy also make pregnant women more susceptible to infection with bacterial pathogens (Almukhtar, 2018). Urinary tract and virginal trauma during sexual intercourse have also been put forward as probable causes of increased UTIs among women (Tamalli *et al*., 2013). The seeming disparity in prevalence of UTIs recorded in this study and those of Oladipo (2013) and Ezeigbo *et al*. (2016) which recorded higher prevalence may be due to the number of bacterial pathogens identified and the culture media used. In this study, only two organisms were of experimental interest, namely *Klebsiella* sp and *Pseudomonas aeruginosa* while in the studies by the aforementioned authors a wider range of bacterial pathogens were isolated and identified. Furthermore, the need to isolate only two bacterial pathogens, *Klebsiella* sp and *Pseudomonas aeruginosa*, necessitated the use of selective media (MacConkey agar and Cetrimide agar) in this study, which only allowed for the growth of specific organisms. In studies carried out by other authors, general purpose media were utilized in addition to selective media for the isolation of a wider panel of bacterial pathogen. Furthermore, increased incidences of UTIs have been associated with humid climate, poor hygienic and environmental conditions, and non-availability of portable water supply (Ezeigbo *et al*., 2016).

The relative prevalence of *Klebsiella* sp and *Pseudomonas aeruginosa* in the urine samples of the pregnant women is given in Table 4.2. *Klebsiella* sp and *Pseudomonas aeruginosa* have been variously identified in urine cultures of patients presenting with clinical symptoms of urinary tract infections (Oluwafemi *et al*., 2018; Odoki *et al*., 2019). In the present study, a prevalence of 14.78% infection with *K. pneumoniae* was recorded among urine samples collected from the pregnant women. This closely agrees with the findings of Oladipo (2013), Tamalli *et al*. (2013), Chijioke *et al*. (2016), Almehdawi *et al*. (2017), and Umar *et al*. (2018) who reported prevalence of 16.8%, 13.3%, 17.2%, 17.3%, and 10%, respectively, for *K. pneumoniae* in urine cultures of pregnant women.

In a study by Mohammad and Mohammed (2019), *Pseudomonas aeruginosa* accounted for 14.8% of identified bacterial pathogen in the urine cultures of pregnant women in Kano, Northern Nigeria. Prevalence of 13.0% *P. aeruginosa* in urine samples obtained from

pregnant women attending antenatal clinics in Aba, South-eastern Nigeria as recorded by Ezeigbo *et al*., (2016). According to Oladipo (2013), a prevalence of 2.6% was recorded in urine samples collected from pregnant women in Ile-Ife, Southwestern Nigeria. In another study, Farouk *et al*. (2019) reported 15.8% prevalence for *P. aeruginosa* among pregnant women in Kano, Northern Nigeria. The results from the present study showed that the prevalence of *Pseudomonas aeruginosa* among the pregnant women was 7.39%, a value marginally lower than most of the aforementioned authors. The differences in prevalence of both *Klebsiella* sp and *Pseudomonas aeruginosa* recorded in this and other studies may be due to differences factors including but not limited to socio-economic status, environmental condition, social habit, personal hygiene and educational level of the women (Almukhtar, 2018; Farouk *et al*., 2019; Gebremariam *et al*., 2019).

Urinary tract infections predispose both pregnant women and neonates to adverse outcomes. The expectant mother, with UTI, has increased risks of pyelonephritis, premature labour, preterm rupture of the membranes, pre-eclampsia and anaemia of pregnancy. Neonates may develop fatal sepsis, experience intrauterine growth retardation and low birth weight (Ballen *et al*., 2014; Valentina and Srirangaraj, 2016).
The results of this study showed the overall prevalence of ESBL producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was 11.76% and 14.29% respectively (Table 4.3). Akpan *et al*. (2019) reported a 19.2% and 9.6% prevalence, respectively, for ESBL producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolates in urine samples obtained from pregnant women attending antenatal care in a primary health care center in South-south Nigeria. A 2015 study by Anuradha recorded a 17.02% and 0.0%, respectively, ESBL producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in urine samples. According to Kasango *et al*. (2018), 24% prevalence for ESBL producing *Klebsiella pneumoniae* was recorded in Uganda, 15.3% of ESBL producers among isolates of *Klebsiella pneumoniae* was obtained from urine samples of pregnant women in Indonesia by Thapa *et al*. (2015). Chander and Shrestha (2013) also recorded 16.55% prevalence of ESBL producing *Klebsiella pneumoniae* in urine samples in Nepal. Majeed and Aljanabi showed that ESBL producing *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were identified at prevalence of 22.2 and 37.1%, respectively in a 2019 study conducted in Iraq.

Table 3 gives the prevalence of ESBL genes, *TEM, SHV,* and *CTX-M*, in isolates of the uropathogens. In *Klebsiella pneumoniae*, two of the assayed genes were detected (*SHV* and *CTX-M*) while none was detected in *Pseudomonas aeruginosa*. The result further showed that the *SHV* and *CTX-M* genes were found to be concomitant in *Klebsiella pneumoniae*. The presence of the β-Lactamase genes, *SHV* and *CTX-M*, in *Klebsiella pneumoniae* agrees with the findings of Olowe *et al*., (2012) and Majeed and Aljanabi (2019). In isolates of *Pseudomonas aeruginosa*, none of the three β-Lactamase genes were detected (Table 4.6). This observation closely agrees with those of Lee *et al*. (2005) who reported that *Pseudomonas aeruginosa* did not harbor some of ESBL genes including *TEM, SHV,* and *CTX-M*. However, the present result on *Pseudomonas aeruginosa* is at variance with those of several authors who reported the presence of these genes in clinical isolates of *Pseudomonas aeruginosa* (Ghaima*et al*., 2018; Rajivgandhi*et al*., 2018; Behbahani *et al*., 2019; Dabir *et al*., 2020). These authors identified *TEM, SHV,* and *CTX-M* genes in clinical isolates *Pseudomonas aeruginosa*. This may be attributable to the fact that β-Lactamase activity is also mediated by genes other than the three that were assayed for in this study. Zafer *et al*. (2014) identified five genes that encode for ESBL producing *P. aeruginosa* resistance among cancer patients in Egypt, namely bla-$_{VIM-2}$, bla-$_{OXA-10}^{-}$, bla-$_{VEB-1}$, bla-$_{NDM}^{-}$, bla-$_{IMP-1}^{-}$ like genes. Jiang *et al*. (2006) identified *bla$_{OXA-10}$* and *bla$_{VEB-1}$* genes in clinical isolates of *Pseudomonas aeruginosa*, while *PSE-1*, *OXA-10*, *OXA-1*, *OXA-30*, *OXA-2*, and *OXA-17* genes were identified by Lee *et al*. (2005) in isolates of *Pseudomonas aeruginosa*.

Conclusion

Klebsiella pneumonae and *Pseudomonas aeruginosa* are pathogens which colonize the urinary tracts and/or urine of pregnant women attending Barau Dikko Teaching Hospital, Kaduna with prevalence of 14.78 and 7.39 %, respectively
The prevalence of Extended Spectrum Beta-lactamase was 11.76% for *Klebsiella pneumoniae* and 14.29% for *Pseudomonas aeruginosa*.

Extended spectrum β-lactamase genes were detected only in *Klebsiella pneumonae* and not in *Pseudomonas aeruginosa*.
The resistance genes identified in ESBL producing *Klebsiella pneumonae* were bla$_{SHV}$ and bla$_{CTX-M}$, both in equal proportion; bla$_{TEM}$ was not detected.

Recommendations

i. Necessary health care should be provided to very vulnerable subset of pregnant women who are at increased risk of infection with urinary tract infection. This will ensure better maternal and neonatal outcomes during pregnancy and after child delivery.
ii. More studies should be undertaken to establish national baseline data on prevalence of ESBL producing uropathogens as well as the distributions and trends of the genes associated with this form of antimicrobial resistance. This will assist in monitoring the emergence of these genes and their spread within other microbial species.

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REFERENCES

Aernan, P.T and Umeh, E.U. (2016): Molecular Characterization of Extended-Spectrum Beta Lactamase (ESBLs) genes in *Pseudomonas aeruginosa* from Pregnant Women Attending a Tertiary Health Care Centre in Makurdi, Central Nigeria. *International Journal of Scientific and Research Publications*, 6**(10)**: 19-24

Afunwa, R. A., Odimegwu, D. C., Iroha, R. I. and Esimone, C. O. (2011). Antimicrobial Resistance Status and Prevalence Rate of Extended Spectrum Beta-Lactamase (ESBL) Producers Isolated from a Mixed Human Population. Basic Medical Science. 11(**2**): 91- 96.

Akpan, I., Atting, I., Umoh, G and Bassey, E. (2019). Prevalence of Common Diseases in a Perturbed Wetland Community of

Okorombokho in the Niger-Delta Region, *Nigerian.Journal of Quality in Health Care & Economics,* 2(**2**): 000117.

Al-Khudhairy, M.K and Al-Shammari, M. MM. (2020). Prevalence of metallo-β-lactamase-producing Pseudomonas aeruginosa isolated from diabetic foot infections in Iraq. New Microbes and new Infections. 35

Almehdawi, K. A., Ali, R.H and Ismail, F.F. (2017). Bacteriuria in Pregnant and Non-Pregnant Women in Benghazi A comparative Study. *Journal of Pharmacy and Biological Sciences,* 12: 133-137.

Almukhtar, S.H. (2018). Urinary tract infection Among Women Aged (18-40) years old in kirkuk city, Iraq. Department of Clinical Nursing Sciences, College of Nursing, University of Mosul, Iraq. 12: 248-254.

Anuradha, R.K., Sathyavathi, R.B., Muneeswara, T., Hemalatha, R., Sudhakar, G. P., Geetha, P., Kodanda, K and Reddy, (2015). Effect of social and environmental determinants on overweight and obesity prevalence among adolescent school children. *Indian Journal of Endocrinology and Metabolism*, 19: 283-287.

Ballen, V., Saez, E., Benmessaoud, R., Houssain, T, Alami, H., Barkat, A., Kabiri, M., Moraleda, C., Bezad, R., Vila, J., Bosch, J., Bassat, Q and Soto, S.M., (2014). First report of a *Klebsiella pneumoniae* ST466 strain causing neonatal sepsis harbouring the blaCTX-M-15 gene in Rabat, Morocco. *FEMS Microbiology Letters*, 362**(1)**: 1-4

Behbahani, M.R., Keshavarzi, A., Pirbonyeh, N., Javanmardi, F., Khoob, F. and Emami A. (2019). Plasmid-related β-Lactamase Genes in *Pseudomonas aeruginosa* Isolates: A Molecular Study in Burn Patients. *Journal of Medical Microbiology*; 68**(12)**: 1740-1746.

Bush, K., Jacoby, G.A. and Medeiros A.A. (1995). A functional classification scheme for B-lactamases and it's correlation with molecular structure. *Antimicrobial agents and chemotherapy,* 39:1211-1233.

Chander, A and Shrestha, C. D. (2013). Prevalence of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* urinary isolates in a tertiary care hospital in Kathmandu, Nepal. 25**(6)**:487

Cheesebrough, M. (2006). *District Laboratory Practical in Tropical Countries.* Part two, 2 edition Cambridge University press.

Chijioke, A. N., Vivian, N. N and Christian, U. O. (2016). Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Various Clinical Specimens in South East Nigeria. Department of Microbiology, Imo State University 0f Owerri, Nigeria. 3**(2)**: 00054

Cowans, S.T., Steel, K.J. (2004). Mnual for the Identification of MedicaL Bacvterias (3rd edition). Cambridge University Press. London.

Egbebi, A. O. and Famurewa, O. (2011). Prevalence of Extended Spectrum Beta- Lactamase (ESBL) production among Klebsiella Isolated in Some Parts of South West Nigeria. *Journal of Micrbiology Biotechnology and Research,*16(**2**):64-68.

Ezeigbo, O.R., Nnadozie, R.I.A., Asuoha-Chuks, N., Ojiako, V.U., Awurum, I. N and Ugochukwu, M. G. (2016). Incidence of Urinary Tract Infection (UTI) Among Pregnant Women Attending Antenatal Clinics at Some Selected Hospitals in Aba, Southeastern Nigeria. *International Current of Microbiology and Applied Sciences,* 5**(1):**193-199.

Dabir, S., Mohammadi, J., Alizadeh, A., Karimi, F., Nori, K and Mahmoudi, S. (2020). Investigation of the Prevalence of *CTX-M-1* Beta-Lactamase gene in Pseudomonas aeruginosa Strains Isolated from Urinary Tract Infections in Zanjan Hospitals, Iran. *South Asian Research Journal of Biology and Applied Biosciences*, 2**(2)**: 20-24

Davies, J and Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Review*, 74: 417–33.

Farouk, S.N., Ibrahim, U.I., Abdullahi, Y., Bello, A.J and Muhammad A. (2019). Prevalence of *Klebsiella pneumoniae* Isolated from Pregnant Women with Urinary Tracts Infections. *Archives of Urology*, 2**(1)**: 32-38.

Gebremariam, G., Legese, H., Woldu, Y., Araya, T., Hagos, K and Wasihun, A.G. (2019). Bacteriological profile, risk factors and antimicrobial susceptibility patterns of symptomatic urinary tract infection among students of Mekelle University, Northern Ethiopia. *BMC Infectious Diseases*, 19: 950.

Ghaima, K.K., Abdulhassan, A.A and Mahdi, Z.H. (2018). Molecular Study of Extended Spectrum Beta-Lactamase (ESBL) Genes in *Pseudomonas aeruginosa* Isolated from Burns. *Biochemical Cell Archive*, 18**(1)**: 721-727

Jiang, X., Zhang, Z., Li M., Zhou, D., Ruan, F. and Lu Y. (2006). Detection of Extended-Spectrum β-Lactamases in Clinical Isolates of *Pseudomonas aeruginosa*. *Antimocrobial Agents and Chemotherapy*, 2990-2995.

Kasango, S.D., Lutoti, S., Wewedru, I., Aboce, E and Calmax, D.A. (2018). Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum Beta Lactamase Producers in Gram-negative Urine Isolates at MBN Clinical Laboratories, Kampala Uganda.

Lee, S., Park, Y.L., Kim, M., Lee, H.K., Han, K., Kang, C.S. and Kang M.W. (2005). Prevalence of Ambler class A and B beta-lactamases among clinical isolates of *Pseudomonas aeruginosa* in Korea. *The Journal of Antimicrobial Chemotheraphy*; 56**(1)**:122-127

Majeed, H.T and Aljanabi, A.A.J. (2019). Antibiotic Susceptibility Patterns and Prevalence of Some Extended Spectrum BetaLactamases Genes in Gram-Negative Bacteria Isolated from Patients Infected with Urinary Tract Infections in Al-Najaf City, Iraq. *Avicenna Journal of Medical Biotechnology*, 11**(2)**: 192-201

Matar, G.M., Chaar, M.H., Araj, G.F., Srour, Z., Jamaleddine, G. and Hadi, U. (2005). Detection of a highly prevalent and potentially virulent strain of *Pseudomonas aeruginosa* from nosocomial infections in a medical center. BMC Microbiology. **5**(29):1-7.

Mohammad, L., Abolfazl, G and Mohammad S.D. (2016). Prevalence of Extended Spectrum Beta-Lactamase Producing *Klebsiella pneumoniae* isolates in nosocomial and community-Acquired Urinary Tract infections. Cellular and molecular research center, Shahrekord University of Medical Sciences, Shehrekord, IR Iran. 9**(3).**

Muhammad, A and Muhammad, S. A. (2019). Prevalence of Urinary Tract Infection among Pregnant Women in Kano, Northern Nigeria. Archives of Reproductive Medicine and Sexual Health. 2:23-29.

Odoki, M., Aliero, A.A., Tibyangye, J., Maniga, J.N., Wampande, E., Kato, C.D., Agwu, E and Bazira J. (2019): Prevalence of Bacterial Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, *Uganda International Journal of Microbiology,* 8.

Oladipo, A. O. (2013). Prevalence of urinary tract infections (UTIs) among pregnant women attending antenatal clinic at Ile–Ife, South Western Nigeria. *Journal of Microbial & Biochemical Technology,* 5:4

Olowe, O.A., Oladipo, G.O., Makanjuola, O.A and Olaitan, J.O. (2012). Prevalence of Extended Spectrum Beta-lactamases (ESBLS) Carrying Genes in *Klebsiella sp* from Clinical Samples at Ile-Ife, South Western Nigeria. *International Journal of Pharmaceutical, Medical and Biological Sciences*, 1**(2)**:131-138

Oluwafemi, TT, Akinbodewa, AA, Ogunleye, A and Adejumo, OA. (2018). Urinary tract infections and antibiotic sensitivity pattern of uropathogens in a tertiary hospital in South West, Nigeria. *Sahel Medical Journal*, 21:18-22.

Onuorah, S., Ogbonna, V and Obika, I. (2016). Prevalence of Asymptomatic Bacteriuria among the Pregnant Women Receiving Antenatal Care at Federal Medical Centre Owerri, Nigeria. *Journal of Clinical Medicine*, 4**(1)**: 1 - 5.

Onwwuezobe, A.I and Orok, F.E. (2015). Extended Spectrum Beta-Lactamase Producing Uropathogenes in Asymptomatic pregnant women attending Antenatal care in Urban Community secondary Health facility. *African Journal of clinical and Experimental Microbiology*, 16**(1)**:49-53.

Rajivgandhia, G., Muthuchamy, M., Govindan, R., Muthu, P and Natesan, M. (2018). Detection of ESBL genes from ciprofloxacin resistant Gram-negative bacteria isolated from urinary tract infections (UTIs). 2:5-13.

Samuel, O., Victoria, O and Ifeanyi, O. (2016). Prevalence of asymptomatic bacteriuria among the pregnant women receiving antenatal care at Federal Medical Centre Owerri, Nigeria. *Universal Journal of Clinical Medicine*, 4:1-5.

Spadafino, J.T., Cohen, B., Liu, J and Larson, E. (2014). "Temporal trends and risk factors for extended-spectrum beta-lactamase producing *Escherichia coli* in adults with catheter-associated urinary tract infections". *Antimicrobial Resistance Infectious Control,* 3 **(1)**: 39

Tamalli, M., Sangar, B. and Alghazal, M.A. (2013). Urinary tract infection during pregnancy at Al-khoms, Libya. *International Journal of Medicine and Medical Sciences*;, 3**(5)**: 455-459

Thapa, R., Lamichhane, P., Banjara, M.R and Acharya, G.P. (2015). Prevalence of Extended Spectrum Beta Lactamase Producing Uropathogens in Pregnant Women. *Asian Journal of Pharmaceutical and Clinical Research*, 8**(1):** 207-210

Tijjani, J., Arzai, A. H and Sadiq, W. M. (2012). Antimicrobial Susceptibility pattern of Extended Spectrum Beta-lactamase Producers in Gramnegative Urogenital Isolates in Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences,* 5**(1)**: 20-25.

Umar, M., Maidoki, I. A., Yaya, A. A., Ajiya, G. K., Jakheng, S. P. E., Abdulkarim, I. M and Amuta, I. C. (2018). Antibiotic Susceptibility Profile of *Klebsiella* Species Isolated from Pregnant Women with Dysuria Attending Selected Medical Centre in Northern Nigeria. *Asian Journal of Research and Reports in Urology*, 1**(1)**: 1-8

Vaishali, A and Aarti, B. (2017). Prevalence of the urinary tract infections among pregnant women. *Journal of Applied Medical Sciences,* 5:376-378.

Valentina, Y and Srirangaraj, S. (2016). Pregnancy associated Urinary Tract Infection: Prevalence and Screening. *International Journal of Current Microbiology and Applied Science*, 5**(1)**: 452-460

Zafer, M.M., Al-Agamy, M.H., El-Mahallawy, H.A., Amin, M.A and Ashour, E. M.S. (2014). Antimicrobial Resistance Pattern and their Beta-Lactamase Encoding Genes among *Pseudomonas aeruginosa* Strains Isolated from Cancer Patients. *Biotechnology Medical Research International*, 54-87.