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Antibiogram of bacteriuria among pregnant women in urban informal settlements in Port Harcourt municipality

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Abstract
Bacteriuria during pregnancy is a common concern, as it is associated with adverse maternal and fetal outcomes. This study aimed to assess the antibiogram of bacteriuria among pregnant women residing in urban informal settlements within Port Harcourt Municipality, Nigeria. A cross-sectional study was conducted, involving pregnant women attending antenatal health care centers in informal settlements. Midstream urine samples were collected and cultured to identify bacterial pathogens. Antimicrobial susceptibility testing was performed using standard laboratory methods, and antibiograms were constructed to determine the resistance patterns of isolated bacteria. The results of antimicrobial susceptibility testing indicated high levels of resistance of the isolated bacteria such as *Escherichia coli*, *Staphylococcus* sp and *Enterococcus* to commonly prescribed antibiotics, including Ceftazidime, Cefuroxime, and Augmentin, among others. Notably, a considerable proportion of isolates remained susceptible to nitrofurantoin, gentamycin and ciprofloxacin. This study underscores the urgent need for revising empirical antibiotic treatment guidelines for bacteriuria in pregnant women in urban informal settlements. The high levels of antibiotic resistance observed highlight the importance of tailored treatment strategies and antimicrobial stewardship programs to ensure the well-being of both mothers and infants.

Keywords: Urban-informal settlement, bacteriuria, antibiogram

Introduction
Bacteriuria is the presence of bacteria in the urine (Sendi *et al.*, 2017) [24]. Bacteriuria that presents with clinical signs accompanied by symptoms (Symptomatic bacteriuria) is referred as urinary tract infection while that without known symptoms is classified as asymptomatic bacteriuria. Urinary tract infection (UTI) is a common health problem characterized by the presence of microbial pathogens in any part of the urinary tract including the kidneys, ureters, bladder, or urethra. UTI is more common in women due to shorter urethra, closer proximity of the anus to the vagina, as well as easier entry of pathogenic microorganisms by sexual activity (Kalinderi *et al.*, 2018) [14]. In pregnancy, it is considered the most common bacterial infection with increased risks of maternal and neonatal (perinatal) morbidity and mortality (Gilbert *et al.*, 2013) [29].

Although the incidence of bacteriuria in pregnant women is similar to that in non-pregnant women, the incidence of acute pyelonephritis in pregnant women with bacteriuria is significantly increased (Schnarr and Smaill, 2008) [23]. Pregnancy is a unique state with anatomic and physiologic urinary tract changes. While asymptomatic bacteriuria in non-pregnant women is generally benign, pregnant women with bacteriuria have an increased susceptibility to pyelonephritis (Jojan *et al.*, 2017) [13]. The renal pelvis and ureters begin to dilate as early as the eighth week of pregnancy (Jeyabalan and Lain, 2007; Schnarr and Smaill, 2008) [30, 23] and the bladder itself is displaced superiorly and anteriorly. Mechanical compression from the enlarging uterus is the principal cause of hydroureter and hydronephrosis, but smooth muscle relaxation induced by progesterone may also play a role. Smooth muscle relaxation results in decreased peristalsis of the ureters, increased bladder capacity, and urinary stasis. Differences in urine pH and osmolality and pregnancy-induced glycosuria and aminoaciduria may facilitate bacterial growth (Singh *et al.*, 2022) [25]. Urinary tract infections in pregnancy are classified as either asymptomatic or symptomatic. Asymptomatic bacteriuria is defined as the presence of significant bacteriuria without the symptoms of an acute urinary tract infection. Some studies describe it as the isolation of bacteria in at least 1.0 x 10^5 colony-forming units per mL of cultured urine, in the absence of

signs or symptoms of a UTI (Kalinderi *et al.*, 2018) [14]. The impact of antimicrobial overuse on the antimicrobial susceptibility of human pathogens impairs the effectiveness of current and future antimicrobial agents and the emergence of resistant bacterial infections has been increasing (Emiru *et al.*, 2013) [30]. There is paucity of information on the antimicrobial resistance of bacteriuria in pregnant women in informal settlements in Rivers State. Thus, the significant of this study.

Materials and Methods

Sample Collection/ Area

The study's design was cross-sectional and descriptive. Following sufficient counselling and education from the three sampling regions, 200 urine samples from pregnant women were obtained from the participants using a standard procedure, as reported by Chuk Dike *et al*. (2023) [31].

Microbiological Analysis

The samples were inoculated on the respective culture media in accordance with standard microbiological procedure as reported by Chuk Dike *et al.*, (2023) [31]. In this method, aliquot (0.1 ml) of 10^{-1} and 10^{-4} dilutions of the urine specimens were inoculated on surface dried nutrient agar, McConkey agar, Eosin Methylene Blue agar and Mannitol salt agar for isolation of the total heterotrophic bacteria, total coliform, faecal coliform and total staphylococcal counts, respectively. The plates were spread evenly with a flamed bent glass spreader and were incubated at 37 ºC for 24 hours except for the EMB plate that was incubated at 44.5 ºC.

Distinct colonies on respective medium were subculture on sterile nutrient agar plates. The pure bacterial isolates were identified using cultural characteristics, morphology and biochemical tests (Prescott *et al.*, 2011) [22].

Antibiotics Susceptibility Testing

The antibiotic susceptibility patterns of the isolates to common antibiotics were evaluated using the Kirby Bauer disc diffusion technique and 0.5 MacFarland's ($1.5x10^8$ cfu/ml) was employed in inoculum suspensions preparation according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2019). The inoculum for primary sensitivity testing was prepared from a broth that had been incubated for 4 – 18 hours.

The density of the inoculum was adjusted by adding the bacterial suspension to a sterile 4mL saline tube to match the density of the 0.5 McFarland standard. Each isolate was uniformly and aseptically inoculated into a different Mueller-Hinton agar plates by swabbing on the surface of the sterile dried Mueller-Hinton agar plates with the aid of a swab stick. The appropriate antibiotic discs were aseptically placed on the agar using sterile forceps. The plates were then incubated at 37ºC for 24h. Interpretation of results was done using the zones of inhibition sizes as recommended by CLSI and adopted by Ogbonna and Inana (2018) [18].

Statistical analyses

Descriptive statistics on the obtained zone diameter was carried out using the statistical package for social science (SPSS Version 22).

Results

A total of two hundred and forty-eight (248) bacterial isolates consisting of eight genera: *E. coli* (44), *S. aureus* (37), *Enterobacter* spp. (35), *Streptococcus* spp. (16), *Klebsiella* spp. (34), *Bacillus* spp. (12), *Pseudomonas* spp. (33) and *Enterococcus* spp. (37) were isolated. The percentage occurrences of the bacterial isolates were *Escherichia coli* (18%), *Staphylococcus aureus* (15%), *Enterobacter cloacae* (14%), *Enterococcus faecalis* (15%), *Klebsiella pneumoniae* (14%), *Pseudomonas aeruginosa* (13%), *Bacillus cereus* (5%) and *Streptococcus constellatus* (6%). The percentage occurrences of isolated bacteria from each primary health care centers are: Bundu, *Enterococcus faecalis* 17 (22.97%), *Staphylococcus aureus* 14 (18.92%), *Pseudomonas aeruginosa* 13 (17.57%), *E. coli* 12 (16.22%), *Enterobacter cloacae* 9 (12.16%), *Klebsiella pneumoniae* 5 (6.7%), *Streptococcus constellatus* 3 (4.05%) and *Bacillus cereus* 1 (1.35%) respectively; while Churchill, *E. coli* 23 (23.47%), *Klebsiella pneumoniae* 14 (14.29%), *Pseudomonas aeruginosa* 14 (14.29%), *Staphylococcus aureus* 12 (12.24%), *Enterococcus faecalis* 10 (10.20%), *Streptococcus constellatus* 8(8.16%) and *Bacillus cereus* 5 (5.10%) respectively; whereas Potts Johnson, *Enterobacter cloacae* 14 (18.42%), *Staphylococcus aureus* 11 (14.47%), *Enterococcus faecalis* 10 (13.16%), *E. coli* 9 (11.84%), *Pseudomonas aeruginosa* 6(7.89%), *Bacillus cereus* 6 (7.89%) and *Streptococcus constellatus* 5 (6.58%) respectively

Results of the antibiotics resistance showing the percentage resistance of *E. coli* is presented in Fig 1. It showed that *E. coli* from Bundu, Churchill, and Potts Johnson were 100% resistant to Ceftazidime (CAZ) and Cefuroxime (CRX) respectively. Whereas, for Cefixime (CXM), *E. coli* showed the following resistance from Bundu (66%), Churchill (82%) and Potts Johnson (79.2%). On the other hand, for Augmentin, *E. coli* from Bundu and Churchill were both 100% resistant while Potts Johnson was 75% resistant.

Enterobacter spp. from Bundu, Churchill and Potts Johnson were 100% resistant to Ceftazidime (CAZ). It showed various resistance to Cefuroxime (CRX), Cefixime (CXM) and Augmentin (AUG). Meanwhile, for Gentamycin (GEN), only 2.3% of the isolates from Churchill and Potts Johnson (10.5%). Whereas, for Ofloxacin (OFL), only isolates from Bundu were 25.9% resistant (Fig 2).

Klebsiella spp. from Bundu, Churchill, and Potts Johnson were 100% resistant to Ceftazidime. There was various percentage resistance to the following antibiotics: Cefuroxime, Cefixime, Augmentin, Nitrofurantoin, and Ciprofloxacin, of which Nitrofurantoin and Ciprofloxacin showed the lowest percentage resistance. Whereas, only 15.2% of the isolate from Churchill showed resistance to Gentamycin (Fig 3).

Pseudomonas app. from Bundu, Churchill and Potts Johnson were 100% resistant to Ceftazidime. There was various percentage resistance to Cefuroxime, Cefixime, and Augmentin. Meanwhile, for Gentamycin, only 4.3% and 5.9% of the isolates from Churchill and Potts Johnson showed resistance respectively, whereas for Ofloxacin (OFL), only 14.3% of the isolates from Bundu was resistant (Fig 4).

Enterococcus spp. from Bundu, Churchill, and Potts Johnson showed various percentage resistance to Ceftazidime, Cefuroxime, Ceftriaxone, Erythromycin, Cloxacillin and Augmentin. The highest percentage resistance was from Potts Johnson for Cloxacillin (100%). About 3.1% and 28% isolates from Bundu and Churchill

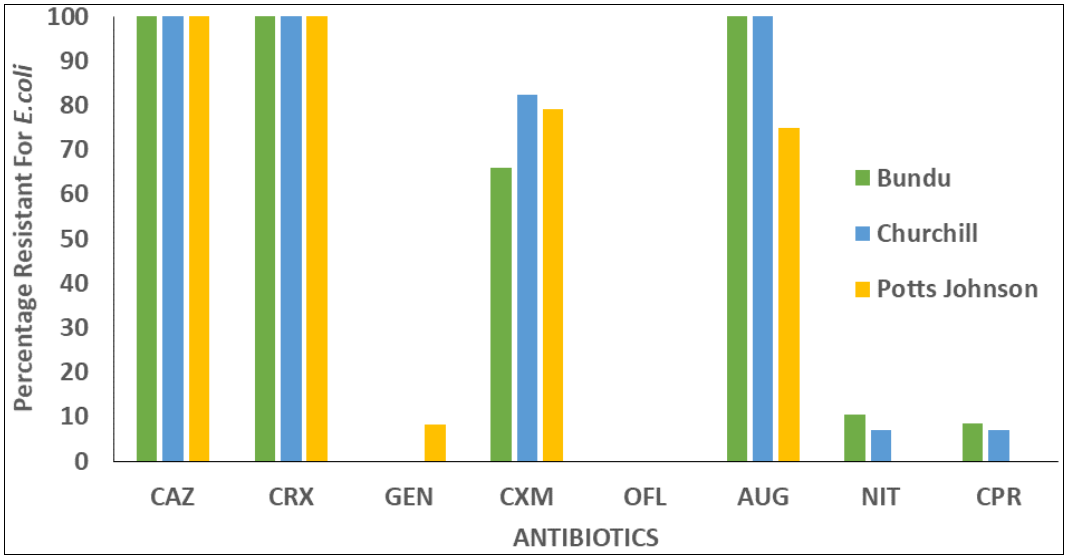
showed resistance respectively to Erythromycin, meanwhile, for Gentamycin, only *E. faecalis* from Bundu was 12.5% resistant (Fig 5).

Bacillus spp. showed various percentage resistance to Ceftazidime, Ceftriaxone, Cloxacillin, and Augmentin of which the highest was 100% from Bundu to the antibiotic Ceftazidime. Meanwhile, for Gentamycin and Erythromycin, only 15% and 10% of *Bacillus* sp from Churchill showed resistance, respectively (Fig 6).

Results of the antibiotic susceptibility testing for *Staphylococcus* spp. is presented in Fig. 7. *S. aureus* from Bundu, Churchill, and Potts Johnson showed 100% resistance to Ceftazidime. There was various percentage resistance to Cefuroxime, Erythromycin, Cloxacillin, and Augmentin of which the highest was 100% from Bundu to the antibiotic Erythromycin. Few *S. aureus* from Potts Johnson were 26.9% resistant to Ceftriaxone.

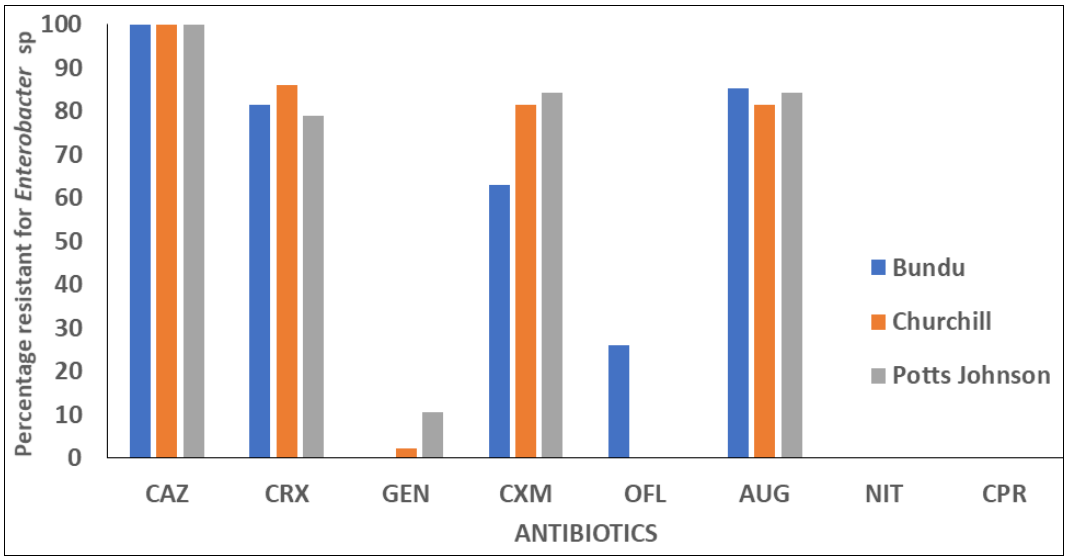
Streptococcus spp. from Bundu, Churchill, and Potts Johnson showed 100% resistance to Ceftazidime (CAZ). There was various percentage resistance to Cefuroxime (CRX), Erythromycin (ERY), Cloxacillin (CRC), and Augmentin (AUG). Meanwhile, for Gentamycin6 and Ofloxacin, only a few *S. constellatus* were 25% and 37.5% resistant respectively (Fig. 8).

Results of the multiple antibiotic resistance indices of the bacteria isolated is presented in Table 1. Generally, 100% of the isolates isolated from the three locations had MAR indices >0.2 except *S. aureus* (97.9%) and *E. cloacae* (97.7%).



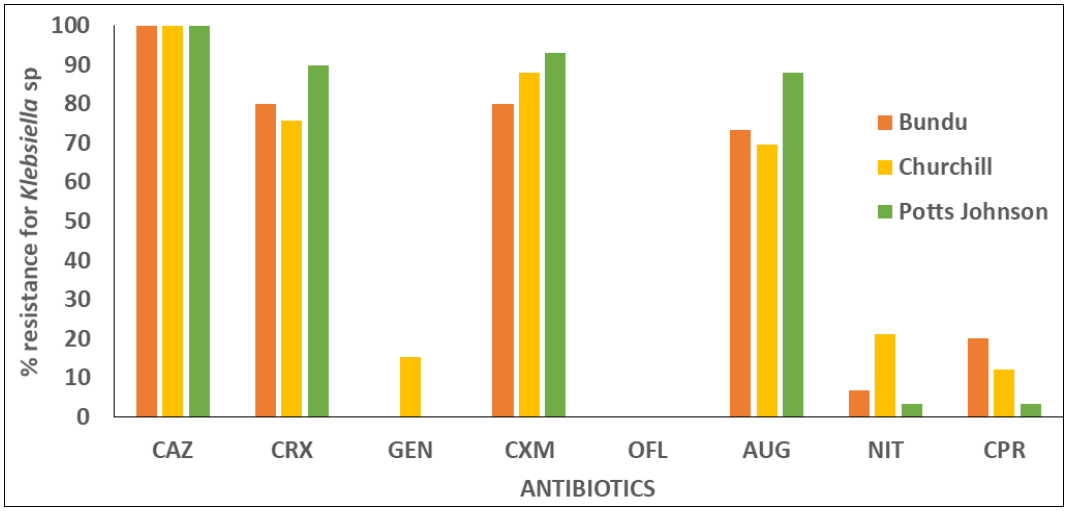
KEY: CAZ= Ceftazidime (30 µg) CRX= Cefuroxime (30 µg), GEN= Gentamycin (10 µg), CXM = Cloxacillin (5 µg), OFL= Ofloxacin (5 µg), AUG= Augmentin (30 µg), CPR= Ceftriaxone (30 µg) NIT= Nitrofurantoin

Fig 1: Percentage (%) Resistant of *Escherichia coli* Isolated from various locations



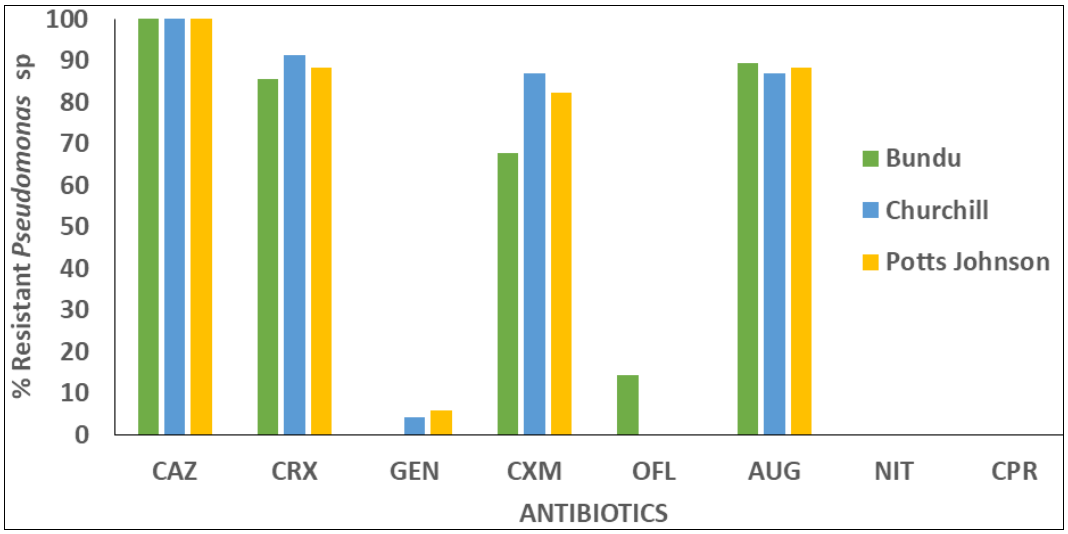
KEY: CAZ= Ceftazidime (30 µg) CRX= Cefuroxime (30 µg), GEN= Gentamycin (10 µg), CXM = Cloxacillin (5 µg), OFL= Ofloxacin (5 µg), AUG= Augmentin (30 µg), CPR= Ceftriaxone (30 µg) NIT= Nitrofurantoin

Fig 2: Percentage (%) Resistant of *Enterobacter* sp isolated from various locations



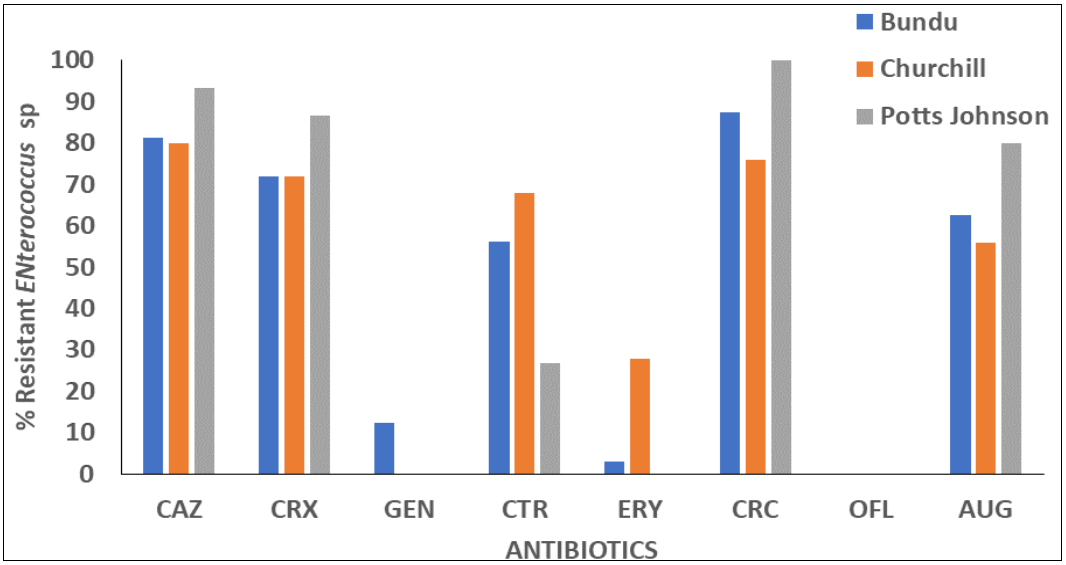
KEY: CAZ= Ceftazidime (30 µg) CRX= Cefuroxime (30 µg), GEN= Gentamycin (10 µg), CXM = Cloxacillin (5 µg), OFL= Ofloxacin (5 µg), AUG= Augmentin (30 µg), CPR= Ceftriaxone (30 µg) NIT= Nitrofurantoin

Fig 3: Percentage (%) Resistant of *Klebsiella* sp isolated from various locations



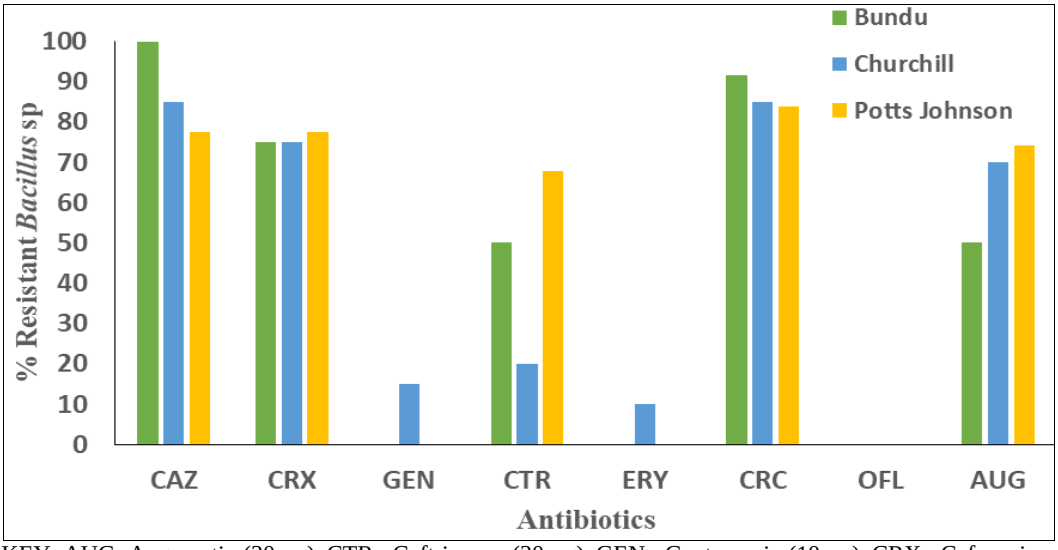
KEY: CAZ= Ceftazidime (30 µg) CRX= Cefuroxime (30 µg), GEN= Gentamycin (10 µg), CXM = Cloxacillin (5 µg), OFL= Ofloxacin (5 µg), AUG= Augmentin (30 µg), CPR= Ceftriaxone (30 µg) NIT= Nitrofurantoin

Fig 4: Percentage (%) Resistant of *Pseudomonas* sp isolated from various locations



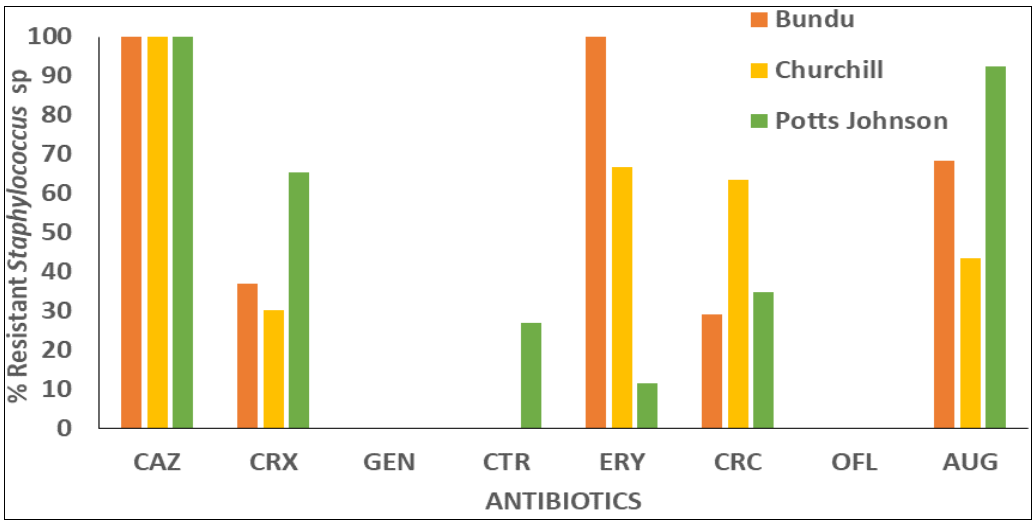
KEY: AUG=Augmentin (30 µg), CTR= Ceftriaxone (30 µg), GEN= Gentamycin (10 µg), CRX= Cefuroxime (30 µg), OFL= Ofloxacin (5 µg), ERY= Erythromycin (5 µg), CRC= Cloxacillin (30 µg), CAZ= Ceftazidime (30 µg)

Fig 5: Percentage (%) Resistant of *Enterococcus* sp. Isolated from various locations



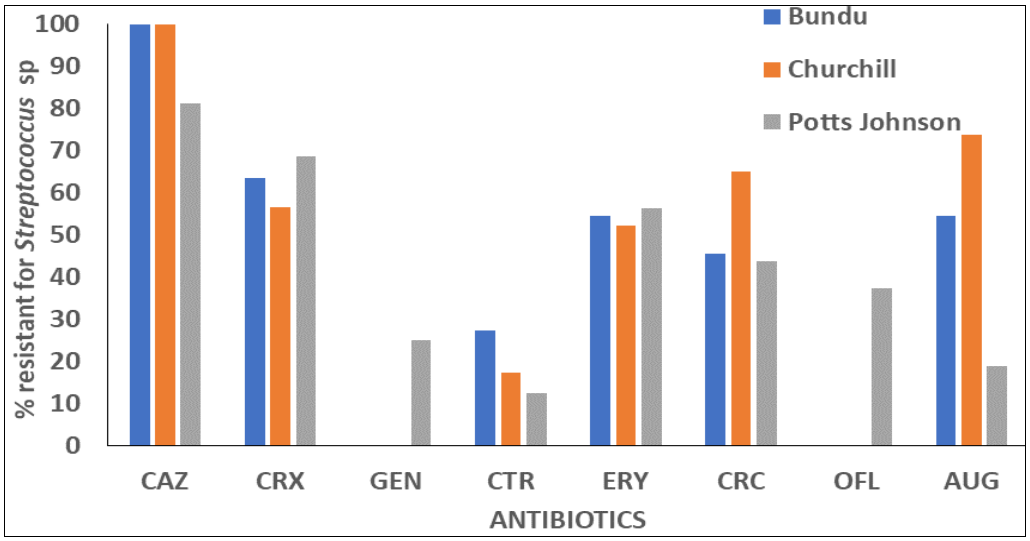
KEY: AUG=Augmentin (30 µg), CTR= Ceftriaxone (30 µg), GEN= Gentamycin (10 µg), CRX= Cefuroxime (30µg), OFL= Ofloxacin (5 µg), ERY= Erythromycin (5 µg), CRC= Cloxacillin (30 µg), CAZ= Ceftazidime (30µg)

Fig 6: Percentage (%) Resistant of *Bacillus* sp Isolated from various locations



KEY: AUG=Augmentin (30 µg), CTR= Ceftriaxone (30 µg), GEN= Gentamycin (10 µg), CRX= Cefuroxime (30 µg), OFL= Ofloxacin (5 µg), ERY= Erythromycin (5 µg), CRC= Cloxacillin (30 µg), CAZ= Ceftazidime (30 µg)

Fig 7: Percentage (%) of Resistant *Staphylococcus* sp. Isolated from various locations



KEY: AUG=Augmentin (30 µg), CTR= Ceftriaxone (30 µg), GEN= Gentamycin (10 µg), CRX= Cefuroxime (30 µg), OFL= Ofloxacin (5 µg), ERY= Erythromycin (5 µg), CRC= Cloxacillin (30 µg), CAZ= Ceftazidime (30 µg)

Fig 8: Percentage (%) Resistance of *Streptococcus* sp. Isolated from various location

Table 1: MAR Indices of the Isolated Bacteria

Mar index	Isolates and percentage							
	E. coli **(n=44)**	***S. aureus*** **(n=37)**	***Enterobacter*** **sp. (n=** ***35*****)**	***Streptococcus*** **sp. (n=16)**	***Klebsiella*** **sp (n=34)**	***Bacillus*** **sp. (n=12)**	***Pseudomonas*** **sp. (n=33)**	***Enterococcus*** **sp. (n=44)**
0.1	0(0)	0(0)	3(8)	0(0)	0(0)	0(0)	0(0)	0(0)
0.2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
0.3	0(0)	5(13.5)	4(11.4)	2(12.16)	3(8.8)	1(8.3)	5(15)	3(6.8)
0.4	12(27.2)	15(40.5)	10(28.5)	4(25.0)	10(29.4)	3(25)	8(24.2)	10(22.7)
0.5	25(56.8)	13(35.1)	17(48.5)	9(56.25)	15(44.1)	7(58.3)	15(45.4)	24(54.5)
0.6	7(15.9)	2(5.4)	1(2.85)	1(6.25)	6(17.6)	1(8.3)	5(15.1)	5(11.3)
0.7	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
0.8	0(0)	2(5.4)	0(0)	0(0)	0(0)	0(0)	0(0)	2(4.5)

Discussion

In the developing world, where there is a high level of poverty, ignorance, and poor hygiene habits, as well as a significant incidence of fake and spurious medications of uncertain quality in circulation, the rise of antibiotic resistance in UTIs is a severe public health concern (Abubakar, 2009) [2]. The antibiotics susceptibility pattern of the bacterial isolates in the present study varied with respect to the location. For instance, 75% of *E. coli* isolates in Pott Johnson showed resistance to Augmentin while all *E. coli* (100%) from Bundu and Churchill were completely resistant to same antibiotics (Augmentin). This was also observed in other antibiotics on other bacterial isolates. This observation agreed with previous reports which stated that regional and geographic differences affect the antibiotic susceptibility patterns of UTI (Hani *et al.*, 2013; Kibret and Abera, 2014) [12, 15].

The gram-negative bacterial isolates: *E. coli, P. aeruginosa, Klebsiella pneumoniae* and *Enterobacter cloacae* which were completely resistant to cefixime, ceftazidime and Augmentin were susceptible to ofloxacin, nitrofurantoin, ciprofloxacin and gentamycin. Resistance of gram-negative isolates from UTIs to cefixime, ceftazidime and Augmentin has been reported in previous a study (Taye *et al.*, 2018) [27] which is in line with the present study. All the *E. coli* isolates from the three locations were completely susceptible to ofloxacin while susceptibility range of the isolates to nitrofurantoin, ciprofloxacin and gentamycin was 89.4-100%, 91-100 and 66.7-73.7%, respectively. This agreed with Yasin *et al*. (2020) [28] who reported that all the *E. coli* isolated from asymptomatic bacteriuria were susceptible to gentamycin, ciprofloxacin and nitrofurantoin. Susceptibility of *E. coli* isolates to gentamycin was also reported by Taye *et al.* (2018) [27]. Although they reported resistance of *E. coli* to nitrofurantoin which agreed with the observed resistance in the present study only that the resistance to nitrofurantoin reported in their study was higher than the present study. Okorondu *et al*. (2013) [21] in a previous study reported higher sensitivity of *E. coli* isolates to gentamycin, ciprofloxacin and ofloxacin which agreed with the present study except that the level of susceptibility in the present study was higher than the percentage reported in their study. The difference in percentages could be attributed to the low number of isolates of *E. coli* in their study compared to the present study. The percentage susceptibility of *E. coli* isolates in the present study to gentamycin are lower than the 75% reported by Mwei *et al*. (2018) [32]. Ofloxacin and ciprofloxacin are quinolone while gentamycin is an aminoglycoside antibiotic. In Nigeria, several studies have reported varying level of resistance and susceptibility of UTI isolates to this class of antibiotics. Quinolone and aminoglycoside resistance was reported by Ezeh *et al*. (2016) [11] in Zaria, Nigeria (12% and 3%, respectively). The findings of Okon *et al*. (2012) [20] in Maiduguri, Nigeria, and those of Ezeh *et al*. (2016) [11] all indicated a decrease in quinolone antibiotic resistance. As with Okon *et al*. (2012) [20], the observed discrepancy may be due to variations in drug usage and study locations. In this case, the bacteria must have developed resistance to quinolones in the present study due to indiscriminate use of these drugs in the study region, or the organisms must have developed resistance due to the use of the drugs at their sub-MIC in animal feeds (Oko *et al*., 2017) [19]. The plasmid profile of the isolates showed the presence of quinolone and aminoglycoside resistant genes. Thus, this could also be the reason for the observed resistance to these antibiotics. Gentamycin a nephrotic antibiotic (Amala *et al*., 2015) [5] is cheap and effective but requires parenteral administration (Okorondu *et al.*, 2013) [21]. Thus, this could be the reason why resistance to the antibiotics is not very high as to those of the penicillin antibiotics. Susceptibility of *K. pneumoniae* to nitrofurantoin was reported in previous study (Yasin *et al.*, 2020) [28] even though the percentage susceptibility in their study was higher than the range reported in the present study. In a recent study, susceptibility of some gram-negative bacterial isolates such as *K. pneumoniae* and *P. aeruginosa* was gentamycin (75.5%), nitrofurantoin (75.5%) and ciprofloxacin (71.4%) (Ali *et al.*, 2022) [1]. Although both their study and the present study agreed that these isolates are still susceptible to gentamycin, nitrofurantoin and ciprofloxacin, they differ in the observed percentage susceptibility. More so, susceptibility of *K. pneumoniae* and *P. aeruginosa* to gentamycin was53–100%, and ciprofloxacin between 57–75% (Akpan, 2019) [3].

The gram-positive bacterial isolates: *S. aureus, Enterococcus faecalis, Streptococcus constellatus* and *Bacillus cereus* were highly susceptible to gentamycin, erythromycin, ofloxacin. *Bacillus cereus* in addition to being susceptible to the afore-mentioned antibiotics were also susceptible to ceftriaxone. Susceptibility of gram-positive bacterial isolates to erythromycin, cefoxitin, ceftriaxone, gentamycin and ciprofloxacin has been reported (Ali *et al.*, 2022) [1]. Similar findings had been made in the Ivory Coast, Dire Dawa, Eastern Ethiopia, and Gonder Ethiopia (Derese *et al*., 2016; Bitew *et al*., 2019; Koffi *et al*., 2020) [9, 7, 16]. According to a study report from Southern Ethiopia, ceftriaxone was 100% ineffective against gram-positive bacteria which is slightly contrary to the present study. More so, previous study has reported the susceptibility of *E. faecalis* to gentamycin and ciprofloxacin (Yasin *et al.*, 2020) [28] which is in agreement with the

present study. Drug misuse is not the only reason that some isolates are resistant to certain antibiotics; another factor could be that some isolates have a porous cell wall that inhibits drugs from penetrating them (Okorondu *et al.*, 2013) [2] as well as the possession of antibiotic resistant genes. More so, all the bacterial isolates were multi-drug resistant isolates showing resistance in more than two types of antibiotics. This is reflected in the MAR index which was greater than 0.2. Previous studies have reported multi-drug resistance in bacterial isolates similar to the present study (Behailu *et al*., 2016; Mwei *et al.*, 2018; Okorondu *et al.*, 2013; Tadesse *et al.*, 2018) [6, 32, 21, 26].

Conclusion
The antibiogram of bacteriuria in pregnant women living in urban slums in Port Harcourt Municipality, highlights the difficulties that antibiotic resistance poses in this vulnerable population. To reduce the negative effects of bacterial infections during pregnancy in these underprivileged regions, targeted interventions, better knowledge, and improved access to adequate treatment are crucial.

Conflict of Interest
Not available

Financial Support
Not available

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