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Antibiotic Resistance Pattern of *Escherichia coli* and *Staphylococcus aureus* from Pregnant Women with Urinary Tract Infection (UTI) in Ezza South L.G.A. of Ebonyi State, Nigeria

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Abstract: The study was designed to determine the antibiotic resistance patterns of *Escherichia coli* and *Staphylococcus aureus* isolated from UTI Positive pregnant women from Ezza South L.G.A of Ebonyi State and to determine the location of the resistant genes through plasmid curing. Exactly 106 urine samples were aseptically collected from pregnant women and were analyzed at microbiology laboratory unit of Ebonyi State University using standard procedures. The bacteria organisms isolated were *Escherichia coli* and *Staphylococcus aureus*. It was observed that pregnant women in the age range of 23–28 had the highest (54.4%) cases of UTI while pregnant women in the age range of 17–22 had the least (34.4%) cases of UTI. Pregnant women in their first trimester have the highest prevalence of *S. aureus* 14 (82.3%) while pregnant women in their third trimester had the highest prevalence of *E. coli* 11 (73.3%). Pregnant women with no Formal Education had the highest prevalence of *S. aureus* 100% while the group that had Secondary Education has the lowest prevalence of *S. aureus* 68.8%. The group that had Secondary Education has the highest prevalence of *E. coli* 81.3% while the group that had no Formal Education has the least prevalence of *E. coli* 33.3%. Students and farmers have the highest prevalence (100%) of *S. aureus* while the business women have the least (70.0%) prevalence of *S. aureus.* The civil servants have the highest (100%) prevalence of *E. coli* while the farmers had the least (40.0%) prevalence of *E. coli.* Pearson's chi square revealed that there was no significant difference between the parameters (age, education, occupation and trimester) at $p>0.05$. The result obtained from the antibiotic susceptibility testing showed that the most effective antibiotics against *E. coli* were gentamicin, imipenem, meropenem, ciprofloxacin, ofloxacin and ertapenem. *Staphylococcus aureus* was susceptible to ciprofloxacin, gentamicin, erythromycin, ceftazidime and ofloxacin. The result obtained from multi antibiotics resistant index (MARI) ranged from 0.69 and 0.41 for *Staphylococcus aureus* and *Echerichia coli* respectively. Hence, the need for consistent antimicrobial resistance surveillance for important and commonly isolated clinically significant pathogens to form the basis for developing and implementing measures that can reduce the burden of antimicrobial resistance and prevent a probable impending public health problem.

Key words: Antibiotic Resistance · *Escherichia coli* · *Staphylococcus aureus* · Pregnant Women · Urinary Tract Infection (UTI) and Ezza South L.G.A.

INTRODUCTION

Urinary Tract Infection (UTI) is an infection that is caused by the existence and growth of microorganisms in the urinary tract which occurs in all age groups and in both gender [1]. It is regularly due to bacteria from the digestive tract which may find their way into the opening of the urethra and begin to develop to cause infection [2, 3]. In comparism with men, women are more vulnerable to UTI and this is mostly due to short urethra, nonexistence of prostatic secretion, pregnancy and easy infection of the urinary tract with faecal flora [4].

Urinary tract infection in pregnancy is associated with considerable morbidity for both mother and baby. The mixture of mechanical, hormonal and physiologic changes all through pregnancy contributes to considerable changes in the urinary tract, which has a profound impact on the possession and natural history of

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bacteriuria during pregnancy [5, 6]. Urinary tract infection can be either symptomatic or asymptomatic. Patients with considerable bacteriuria who have symptoms referable to the urinary tract are said to have symptomatic bacteriuria. Asymptomatic bacteriuria (ASB) is an existence of a significant quantity of bacteria in an accurately collected urine specimen from a person without symptoms or signs of UTI. An occurrence of asymptomatic UTI ranging from 2% to 15% was reported compared to symptomatic UTI in pregnant women [7]. *Escherichia coli* are the main etiologic agents in UTI, which accounts for up to 90% of cases. *Staphylococcus aureus* is a relatively rare cause of urinary tract infection in the general population [8]. Nevertheless, isolation of *S. aureus* from urine samples is often secondary to staphylococcal bacteremia (bacteria in the blood) (as the cases of endocarditis) [9], in some patients, *S. aureus* causes ascending urinary tract migration and infection. *Staphylococcus aureus* bacteriuria is connected with a high mortality rate and places a considerable cost and resource load on health care systems. This burden is enlarged by the high likelihood that life threatening complication of *Staphylococcus aureus* bacteriuria will occur, including infective endocarditis and metastatic infections [10].

Proteus mirabilis, *Klebsiella* species, *Pseudomonas aeruginosa* and *Enterobacter* species are fewer repeated offenders. Less commonly, *Enterococci, Gardnerella vaginalis* and *Ureaplasma urealyticum* are also known agents in UTIs. Gram-positive organisms are yet less common in which Group B *Streptococcus, Staphylococcus aureus, S. saprophyticus* and *S. haemolyticus* are known organisms [11].

Nevertheless, as with many community acquired infections, antimicrobial resistance among the pathogens that cause UTI is growing and is a most important health problem in the management of UTI [12]. There is increasing anxiety regarding antimicrobial resistance worldwide, mainly with *E. coli* which is the leading causative agent of UTI in pregnant women [13]. Investigating epidemiology of UTI (prevalence, risk factors and bacterial isolates) and the antibiotics susceptibility pattern of the bacterial isolates during pregnancy is of primary concern for care givers and health planners to guide how they channel the expected interventions.

Aim and Objectives of the Study

Aim of the Study: The aim of this study is to evaluate the antibiotic resistance pattern of *Escherichia coli* and *Staphylococcus aureus* isolated from UTI – positive pregnant women from Ezza South L.G.A of Ebonyi State and to determine the location of the resistance gene by plasmid curing.

Specific Objectives:

- To isolate and identify *E. coli* and *S. aureus* from urine of urinary tract infection positive pregnant women.
- To determine their antibiotics susceptibility pattern to commonly used antibiotics.
- To determine the multidrug resistance index of these isolates.
- To determine the location of antibiotics resistance gene by plasmid curing

MATERIALS AND METHODS

Ethical Clearance: Ethical approval and informed consent of the hospital management, laboratory scientists and ante-natal patients were sought and obtained from the Ethical Committee of General Hospital Onueke, Ezza South L.G.A.

Study Area: Ezza South L.G.A is one of the 13 L.G.As in Ebonyi State created in 1996. It has its headquarters at Onueke. It has boundaries in the North with Abakaliki L.G.A, in the North West with Ezza North L.G.A, in the South with Ikwo L.G.A and in the East with Onicha L.G.A. The L.G.A has 14 political wards and 19 health wards. It has 4 Development centers: Ezza South Development Centre, Ezza East Development Centre, Ezza South East Development Centre and Ezzama Development Centre. The people of Ezza South L.G.A are Igbos and predominantly Christians. The major occupation of the people is farming. The soil is rich and good for agriculture particularly for growing yam, cocoyam, cassava and rice.

Study Population: The study population included one hundred and six (106) pregnant women attending antenatal care in General Hospital Onueke in Ezza South L.G.A of Ebonyi State from March-August 2016.

Collection of Samples: Pregnant women were properly educated on how to catch mid-stream urine samples into the sterile containers after proper cleansing of the external genitalia. The urine samples were labeled and brought to the Applied Microbiology laboratory of Ebonyi State University, Abakaliki in a cold box within 1 hour of collection. A well - structured questionnaire was used to obtain their demographic data like; age, pregnancy gestational age, educational background and occupation.

Sterilization of Glass Wares: All the glass wares were sterilized using an autoclave at 121°C for 15 minutes at 15 p.s.i.

Preparation of Media: The media used in this research was prepared following the instruction of the manufacturers. In the procedure for Nutrient agar; 28 g was dissolved in 1 litre of distilled water; Mac Conkey agar; 50 g was dissolved in 1litre of distilled water; 36 g of Cystein Electrolyte Deficiency Agar (CLED) was dissolved in 1 litre of distilled water; 36 g of Eosine Methylene Blue agar EMB) was dissolved in 1 litre of distilled water; 111 g of Mannitol Salt agar was dissolved in 1 litre of distilled water; 24 g of Simmon Citrate agar was dissolved in 1 litre of distilled water; and 38 g of Mueller Hinton agar was dissolved in 1 litre of distilled water.

The media were sterilized in the autoclave at 121°C for 15 minutes in a corked conical flask. The sterilized media were allowed to cool to 45°C before they were dispensed aseptically into the petri dishes in 20ml volumes and then allowed to solidify at room temperature. For agar slants, the media were dissolved and first heated in a Bunsen burner to properly dissolve. The well dissolved media were dispensed in 10 ml volume into the Bijou bottles and sterilized. They were then kept in a slanting position to solidify.

Sample Processing: Nutrient broth was prepared by dissolving 28 g in 1 litre of water and after heating in a Bunsen burner, 5 ml was added into the test tube before it was autoclaved. After autoclaving it was allowed to cool at room temperature before the inoculation. A loopful was used to inoculate the urine into the nutrient broth; this was to get more growth as the organism is in liquid phase [14]. The inoculated test tubes were covered with cotton wool and left on the bench for 24hours. Three test tubes were left and not inoculated. The inoculated test tubes were then cultured on the prepared media of Nutrient agar and MacConkey agar plates. This was further sub-cultured on cystein electrolyte deficiency agar (CLED) and eosin methylene blue agar for *E. coli* and mannitol salt agar for *S. aureus* by the streak method technique. Inoculated plates were incubated at 37°C aerobically overnight [14]. Then the bacterial growths were further sub-cultured on Nutrient agar plates to obtain pure isolates for further biochemical tests and also on Nutrient agar slants for storage of the isolates for further use. These isolates were further identified based on the morphological characteristics and biochemical tests.

Bacterial Identification

Gram Staining: Gram Staining was carried out as described by [14].

Biochemical Tests: The isolates were subjected to the following biochemical tests:

Citrate Test: Citrate Test was done according to the method described by [14].

Indole Test: Indole Test was done according to the method described by [14].

Methyl Red Test: Methyl Red Test was done according to the method described by [14].

Catalase Test: Catalase Test was done according to the method described by [14].

Coagulase Test: Coagulase Test was done according to the method described by [14].

Voges Prokauer (VP) Test: Voges Prokauer (VP) Test was done according to the method described by [14].

Oxidase Test: This was done as described by [14].

Sugar Fermentation Test: The sugars that were used include sucrose, glucose and lactose. The respective isolates were inoculated in 10 ml of sterilized 1 % sugar solution in bijou bottles with 3 drops of phenol red and Durham tubes. They were then incubated at 37 °C for 48 hours and observed for the presence of colour change from red to yellow showing acid and gas production in the Durham tubes.

Antibiotics Susceptibility Test: Individual identified isolates from the freshly prepared Nutrient agar plates were suspended in normal saline in a sterile test tube. The prepared McFarland standards were used as a standard to compare with the turbidity of test organism that was prepared.

McFarland Turbidity Standard: This was done according to methods described by [14].

Bacterial Culture: Using a sterile swab stick, the suspensions were soaked and squeezed by the side of the tube to lose some of the fluid using the method of [15].

The zones of inhibition were measured using a meter rule in milliliter and was interpreted according to Clinical Laboratory Standard Institution [15]. The results were interpreted as susceptibility or resistant.

Plasmid Curing: Curing experiment was carried out in line with previously described methods [16].

RESULTS

Table 1 represents the identification of the isolates. It gives a summary of the morphological, microscopic and biochemical characteristics of the bacteria isolated from UTI positive pregnant women from Ezza South L.G.A of Ebonyi State. According to the different tests carried out, *Escherichia coli* and *Staphylococcus aureus* were identified.

Percentage occurance of the UTI infection among the pregnant women according to their Age Brackets is shown in table 2. It shows that out of the one hundred and six (106) urine samples tested, forty seven (44.3%) samples had growth on cultured media. Pregnant women in the age range of 23 – 28 had the highest occurrence of UTI (54.4%) while 17 – 22 had the least occurrence of UTI (34.4%). Pearson's chi square revealed that there was no significant difference (P > 0.05) between the age groups.

Percentage occurance of the UTI infection among the pregnant women according to their Age Brackets is shown in table 3. It shows that the prevalence of *E. coli* is high among the women between the age group of 17-22yrs, while the prevalence is low among the pregnant women of 23 - 28 years age bracket. The prevalence of *S. aureus* was observed to be high among the age range of 23 - 28 years while it is low among 17-22 years age range.

Percentage occurence of the UTI infection among the pregnant women according to their gestational period are shown in table 4. The pregnant women in their first trimester had the highest prevalence of *S. aureus* 14(82.3%) while pregnant women in their third trimester had the highest prevalence of *E. coli* 11 (73.3%). Pregnant women in their first trimester had the lowest prevalence of *E. coli* 9(52.9%) while pregnant women in their second and third trimesters had the lowest prevalence of *S. aureus* 11(73.3%).

Percentage occurance of the UTI infection among the pregnant women according to their Educational Background are shown in table 5, it can be seen that the group with no Formal Education had the highest prevalence of *S. aureus* 3(100%) while the group that had Secondary Education had the lowest prevalence of *S. aureus* 11(68.8%). Also the group that had Secondary Education had the highest prevalence of *E. coli* 13(81.3%) while the group that had no Formal Education had the least prevalence of *E. coli* 1(33.3%).

Percentage occurance of the UTI infection among the pregnant women according to their Occupation is shown in table 6. It shows that the students and farmers had the highest prevalence 5(100%) of *S. aureus* while the business women had the least 20(70.0%) prevalence of *S. aureus*. The civil servants had the highest 4(100%) prevalence of *E. coli* while the farmers had the least 2(40.0%) prevalence of *E. coli*.

The obtained results from the antibiotic susceptibility testing for the bacteria isolated from pregnant women by disc diffusion method are presented in Figures 1 - 8. They represent the antibiotic Susceptibility and Resistance pattern of *E. coli* isolated from pregnant women according to their different categories.

Table 1: Percentage Occurrence of UTI Infections among the Pregnant Women According to Their Age Brackets

Age range (years)	Total No of Samples Tested	Cases with UTI	Percentage Occurrence (%)
17 – 22	32	11	34.4
23 – 28	44	24	54.4
≥ 29	30	12	40.0
Total	106	47	

Key: NO = Number, % = percent, ≥ = greater than, UTI = Urinary Tract Infection, *S. aureus* = *Staphylococcus aureus, E. coli* = *Escherichia coli*, yrs = years

Table 2: Percentage Distribution of *E. coli* and *S. aureus* among Pregnant Women with UTI According to Their Age Brackets

Age(yrs)	No with UTI	No with *E. coli*	No with *S. aureus*	No with both *E. coli* and *S. aureus*
17-22	11	9(81.8%)	8(72.7%)	6(54.5%)
23-28	24	13(54.2%)	19(79.2%)	8(33.3%)
≥ 29	12	7(58.3%)	9(75.0%)	4(33.3%)
Total	47	29	36	18

Key: NO = Number, % = percent, ≥ = greater than, UTI = Urinary Tract Infection, *S. aureus* = *Staphylococcus aureus, E. coli* = *Escherichia coli*, yrs = years

Table 3: Percentage Distribution of *E. coli* and *S. aureus* among Pregnant Women with UTI According to Their Gestational Period

Gestational age	No Examined	No with UTI	No with *E. coli*	No with *S. aureus*	No with both *E. coli* and *S. aureus*
First	33	17(51.5%)	9(52.9%)	14(82.3%)	6(35.3%)
Second	40	15(37.5%)	9(60%)	11(73.3%)	5(33.3%)
Third	33	15(48.4%)	11(73.3%)	11(73.3%)	7(46.7%)
Total	106	47	29	36	18

Key: NO = Number, % = percent, = = greater than, UTI = Urinary Tract Infection, *S. aureus* = *Staphylococcus aureus, E. coli* = *Escherichia coli*, yrs = years

Table 4: Percentage Distribution of *E. coli* and *S. aureus* among Pregnant Women with UTI According to their Educational Background

Level of Education	No Examined	No positive For UTI	No with *E. coli*	No with *S. aureus*	No with both *E. coli* and *S. aureus*
No formal Education	4	3(75%)	1(33.3%)	3(100%)	1(33.3%)
Primary	50	23(46.0%)	12(52.2%)	18(78.3%)	8(37.8%)
Secondary	43	16(37.2%)	13(81.3%)	11(68.8%)	7(43.8%)
Tertiary	9	5(55.6%)	3(60.0%)	4(80.0%)	2(40.0%)
Total	106	47	29	36	18

Key: NO = Number, % = percent, = = greater than, UTI = Urinary Tract Infection, *S. aureus* = *Staphylococcus aureus, E. coli* = *Escherichia coli*, yrs = years

Table 5: Percentage Distribution of *E. coli* and *S. aureus* among Women with UTI According to Their Occupation

Occupation	No Examined	Cases with UTI	No with *E. coli*	No with *S. aureus*	No with both *E. coli* & *S. aureus*
Students	11	5(45.5%)	3(60.0%)	5(100%)	3(60.0%)
House wives	5	4(80.0%)	2(50.0%)	3(75.0%)	1(25.0%)
Business women	69	29(42.0%)	18(62.1%)	20(70.0%)	9(31.0%)
Farmers	10	5(50.0%)	2(40.0%)	5(100%)	2(40.0%)
Civil servants	11	4(36.4%)	4(100%)	3(75.0%)	3(75.0%)
Total	106	47	29	36	18

Key: NO = Number, % = percent, = = greater than, UTI = Urinary Tract Infection, *S. aureus* = *Staphylococcus aureus, E. coli* = *Escherichia coli*, yrs = years

Figure 1-3 shows antibiotics susceptibility pattern of *Staphylococcus aureus* and *E.coli* isolated from UTI patients with respect to age. Results obtained revealed that the *E. coli* isolated were resistant to tobramycin, amoxillin, cefuroxime and ceftazidime (66%). While *S. aureus* 99 % resistant to penicillin, erythromycin, oxacilin and amoxicillin/clavulanic acids.

Fig. 4 – 6 shows the antibiotics susceptibility studies of *E. coli* and *S. aureus* isolated from UTI patients according to their gestation period. Results obtained showed that *S. aureus* express remarkable resistant genes against penicillin, Erythromycin, clindamycin, oxacillin and amoxicillin/clavulanic while *E. coli* had resistant ampicillin, tobramycin, ciprofloxacin, cefuroxime. These result indicated that these test organisms harbor resistant genes and which could be transferred to other organisms.

Figure 7-9 shows antibiotics susceptibility profile of *E. coli* and *S. aureus* isolated from UTI patients according to educational background. The result obtained showed that *E.coli* isolated were mostly resistant to amoxicillin, tobramycin, cefuroxime and ceftazidime while *S. aureus* isolated were resistant to oxacillin, amoxicillin/clavulanic acid, ciprofloxacin, cefuroxime and ceftriazone.

Figure 10-15 shows antibiotics susceptibility profile of *E. coli* and *S. aureus* isolated from UTI patients according to their occupation. The result obtained showed that *E. coil* had significance resistant profile against the following antibiotics amoxicillin/clavulanic acid, tobramycin, ceftazidime while *S. aureus* also showed remarkable resistant profile against penicillin, erythromycin, oxacillin, gentamycin and clindamycin.

The Antibiotic Susceptibility and Resistance Pattern of *E. coli* and *S. aureus* isolated from UTI positive women according to their Age brackets, is presented in Figures 1-3.

The Antibiotics susceptibility and resistance pattern of *E. coli* and *S. aureus* from UTI positive women according to their gestation period are presented in Figures 4-6.

The Antibiotic susceptibility and resistance patterns of *E. coli* and *S. aureus* from UTI positive women according to their Educational background are presented in Figures 7-9.

Antibiotic susceptibility and resistance of *E. coli* and *S. aureus* from UTI positive women according to their occupation are presented Figures 10-14.

Table 6 shows the number of occurance of each MARI values and the average of *S. aureus* and *E. coli*. It was revealed that the isolates had an average MARI of 0.69 and 0.41 for *Staphylococcus aureus* and *Escherichia coli* respectively.

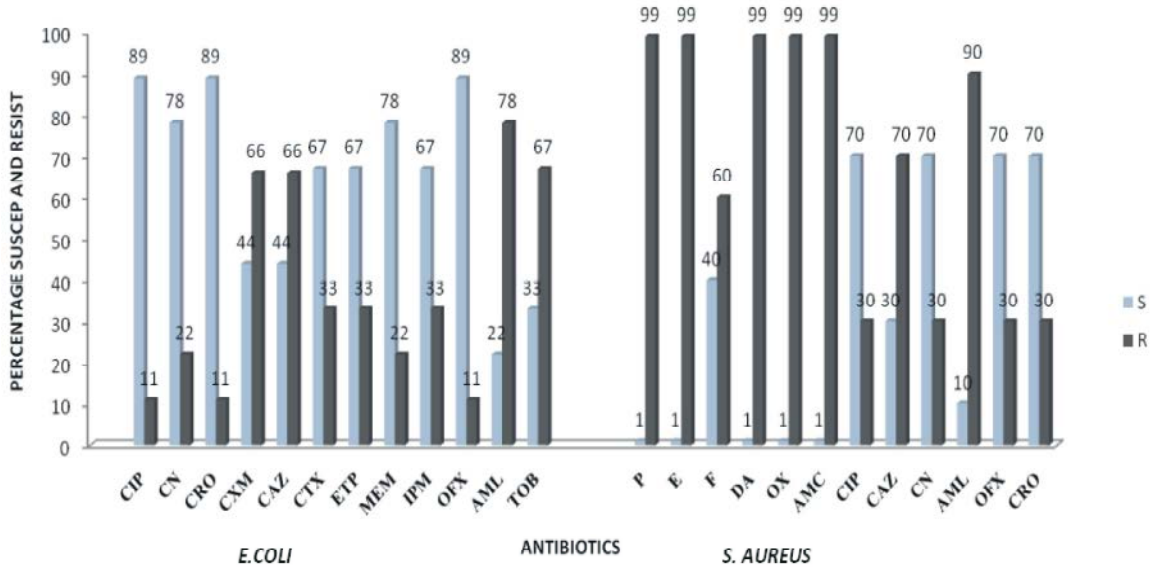

Fig. 1: Antibiotics Susceptibility and Resistance Patterns of *E. coli* and *S. aureus* Isolated from Pregnant Women of Age 17 – 22 yrs

Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imerepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

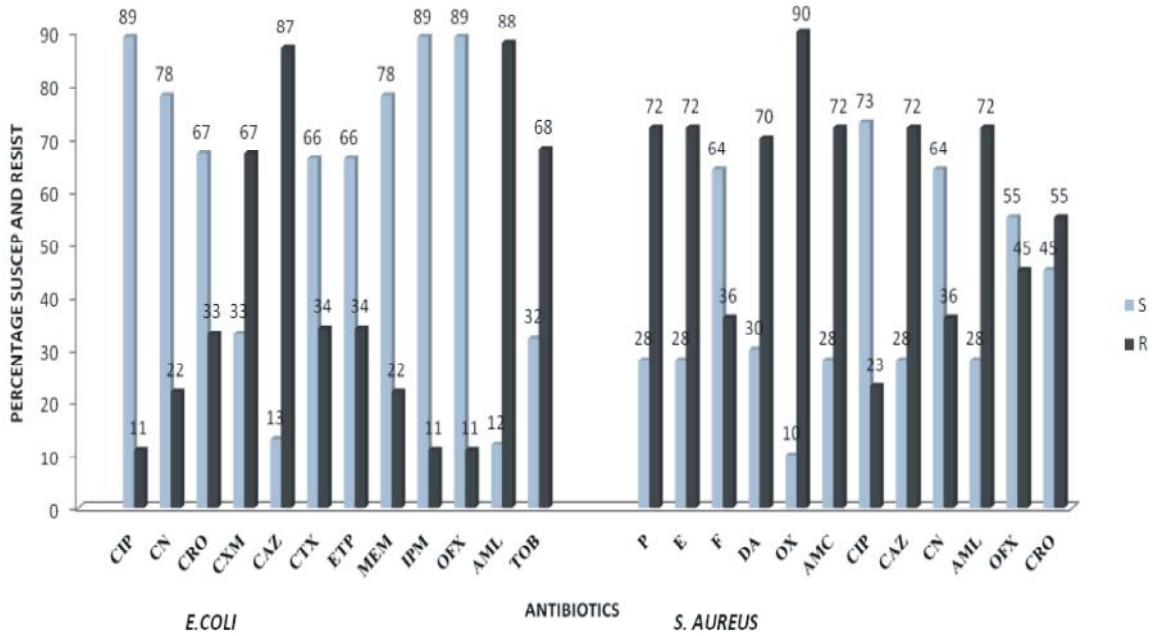

Fig. 2: Antibiotic susceptibility and resistance patterns of *E. coli* and *S. aureus* isolated from pregnant women of Age 23 – 28 yrs

Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imerepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

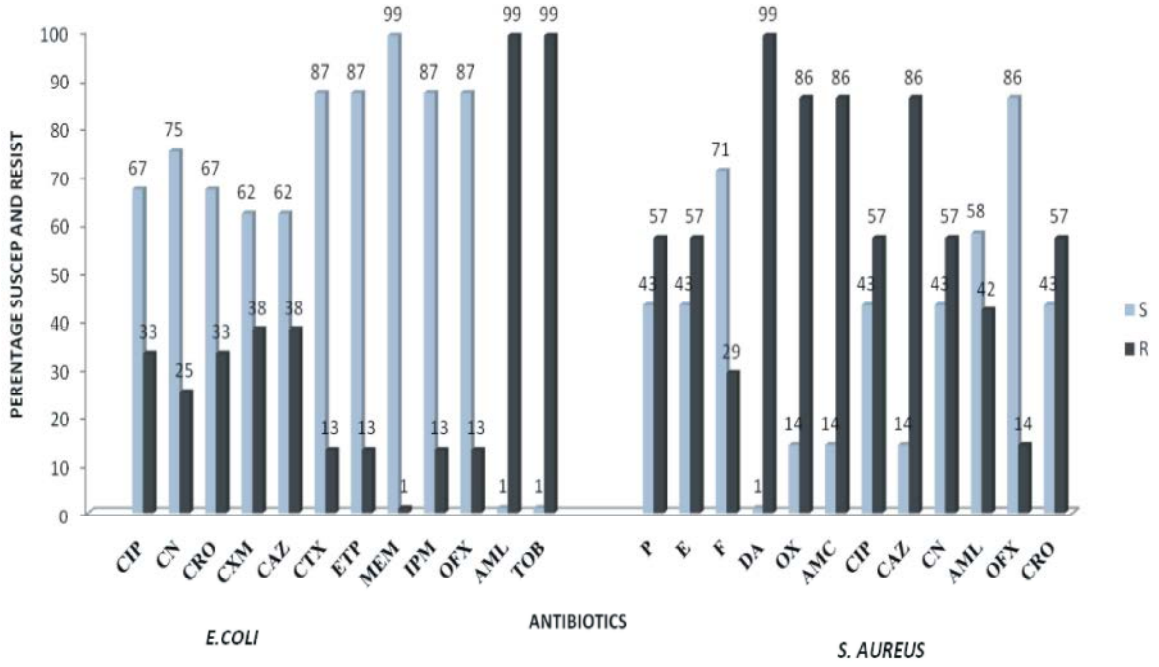

Fig. 3: Antibiotics susceptibility and resistance pattern of *E. coli* and *S. aureus* isolated from pregnant women of Age ≥ 29 yrs.

Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imerepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

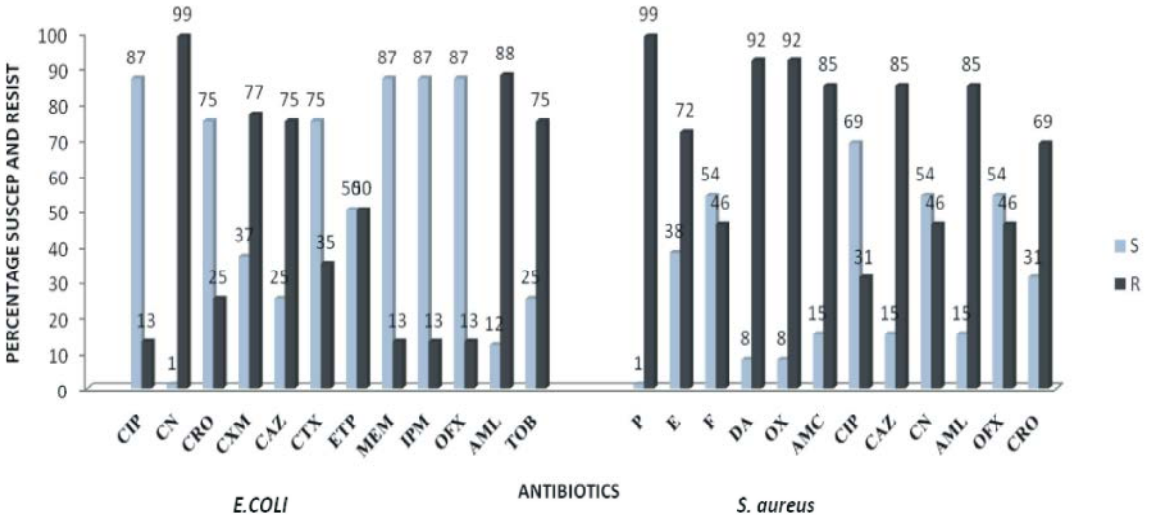

Fig. 4: Antibiotics susceptibility and resistance patterns of *E. coli* and *S. aureus* from pregnant women in their first trimester

Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imerepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

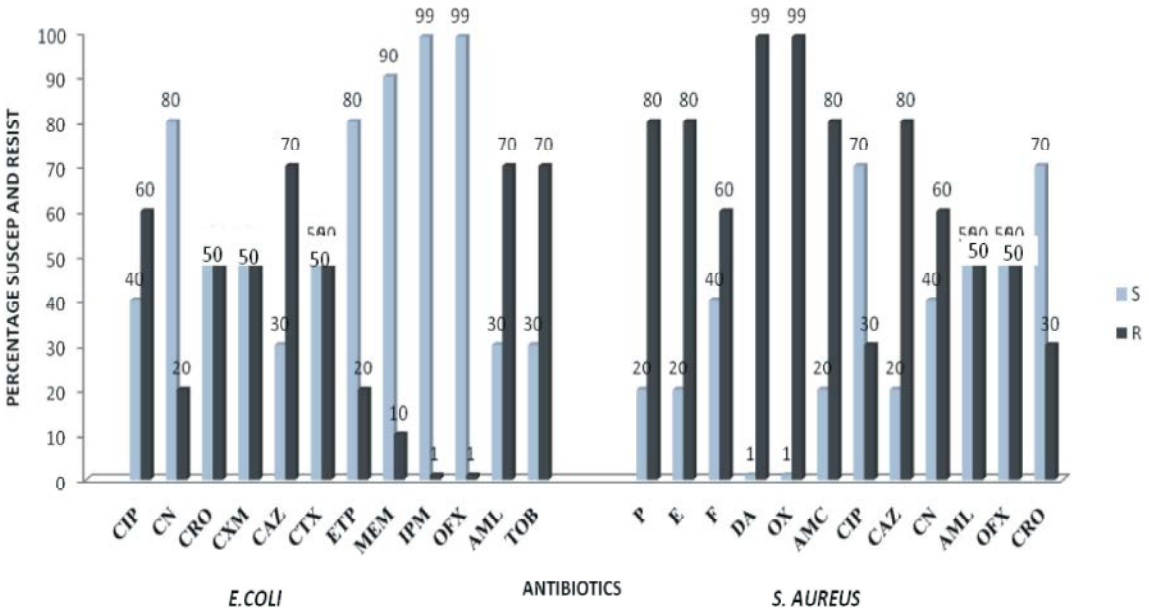

Fig. 5: Antibiotics susceptibility and resistance patterns of *E. coli* and *S. aureus* from pregnant women in their second trimester.

Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imerepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

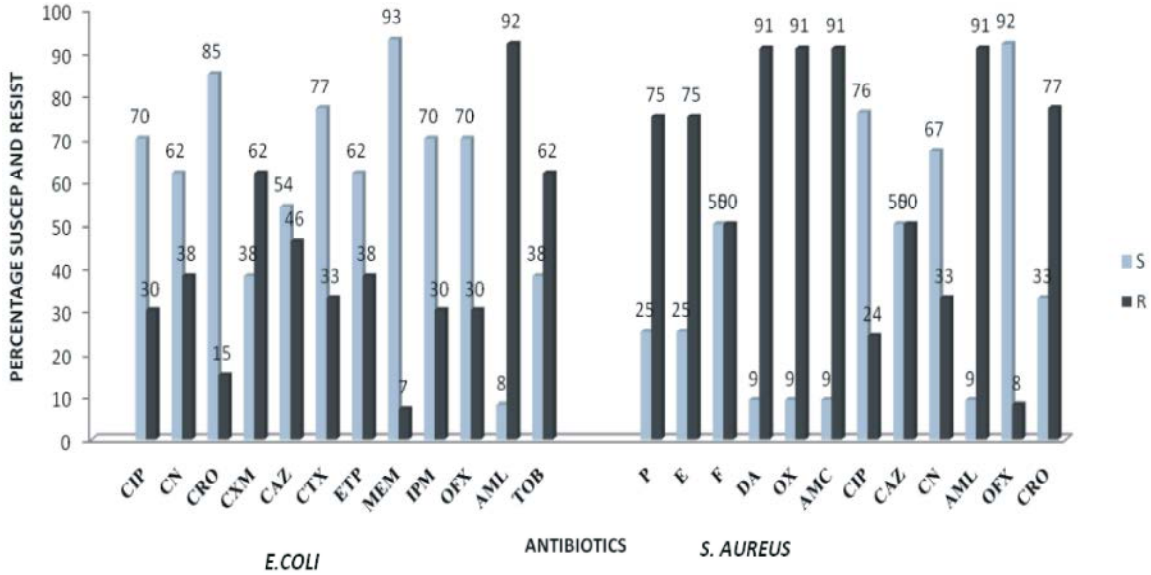

Fig. 6: Antibiotics susceptibility and resistance patterns of *E. coli* and *S. aureus* from pregnant women in their third trimester

Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imerepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

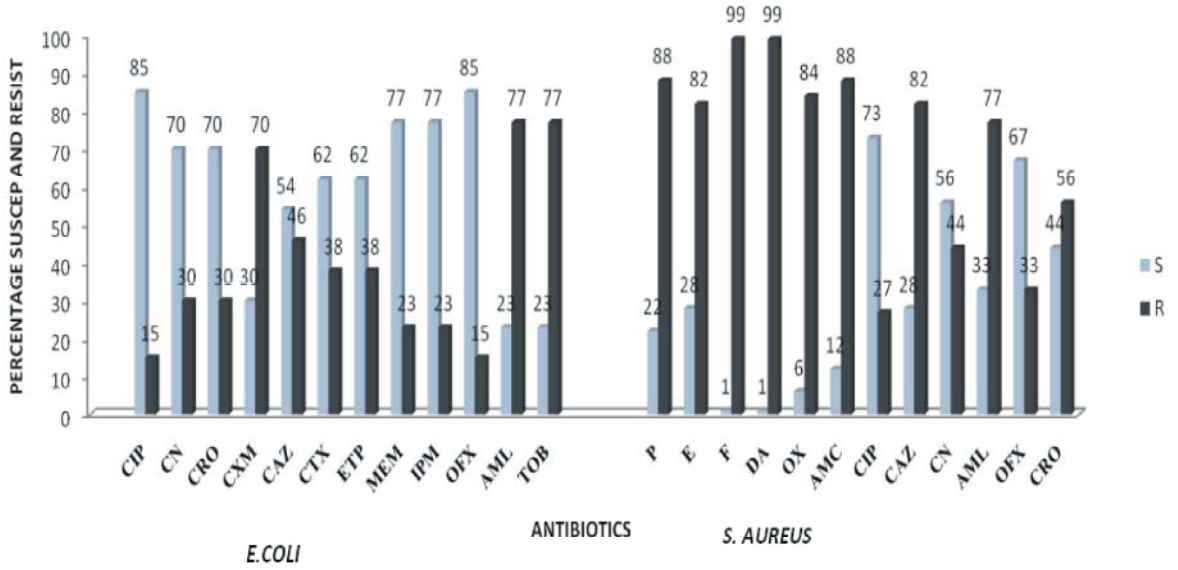

Fig. 7: Antibiotics susceptibility and resistance patterns of *E. coli* and *S. aureus* from pregnant women of primary school level

Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

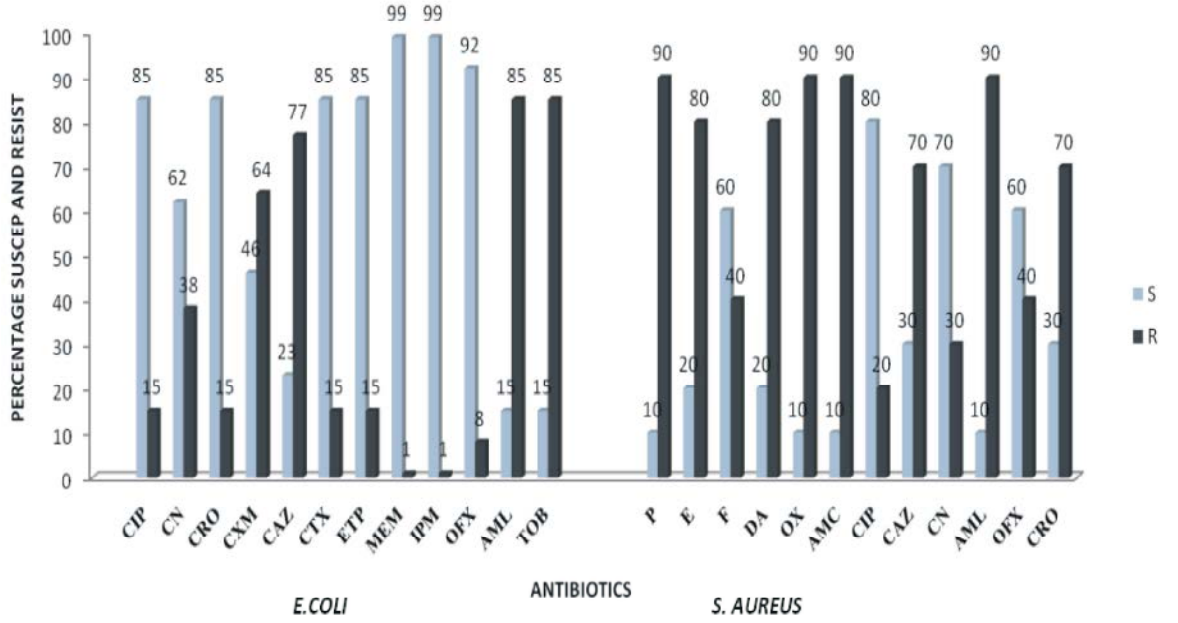

Fig. 8: Antibiotic susceptibility and resistance patterns of *E. coli* and *S. aureus* from pregnant women of secondary school level

Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

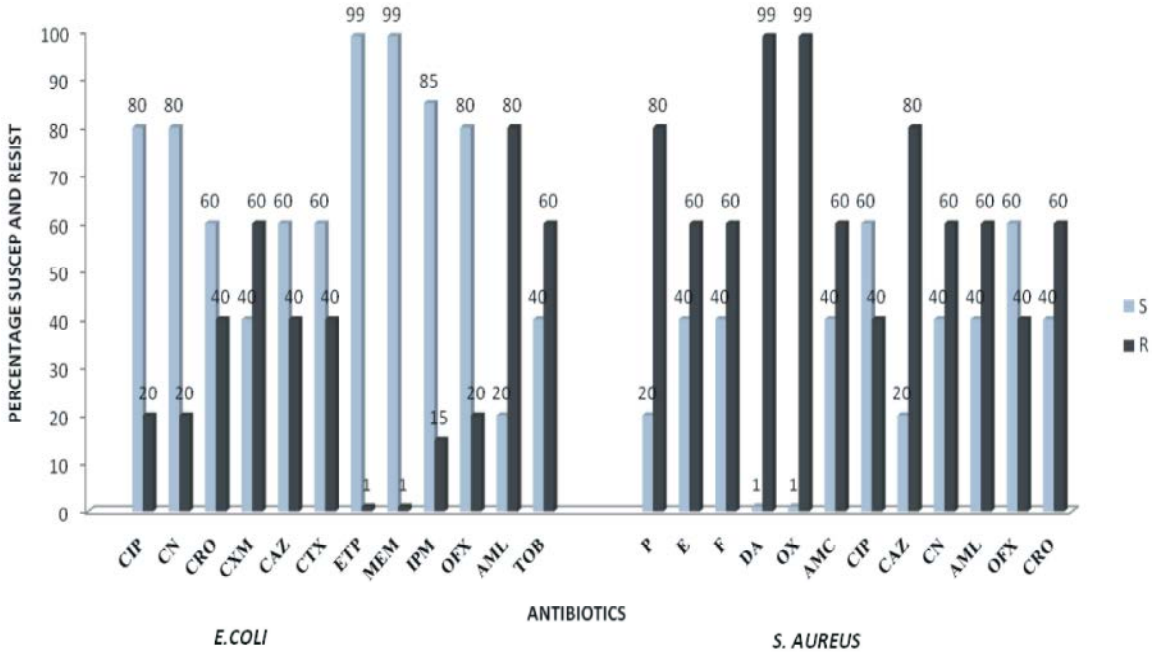

Fig. 9: Antibiotic susceptibility and resistance pattern of *E. coli* and *S. aureus* from pregnant women of tertiary level. CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imerepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

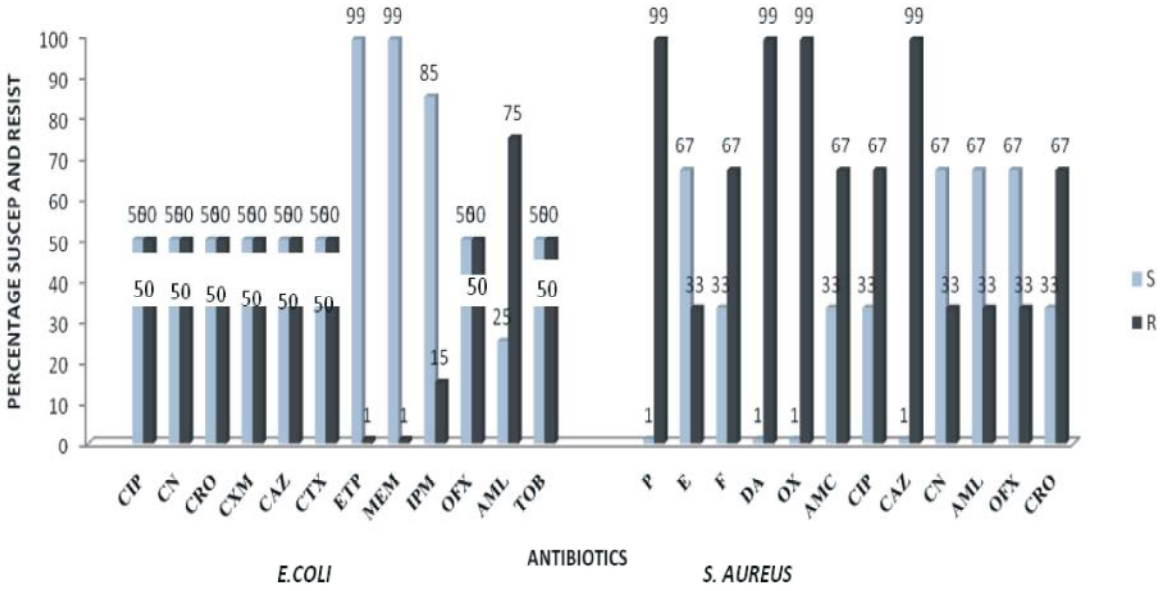

Fig. 10: Antibiotic susceptibility and resistance pattern of *E. coli* and *S. aureus* from pregnant women who are civil servants.

Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

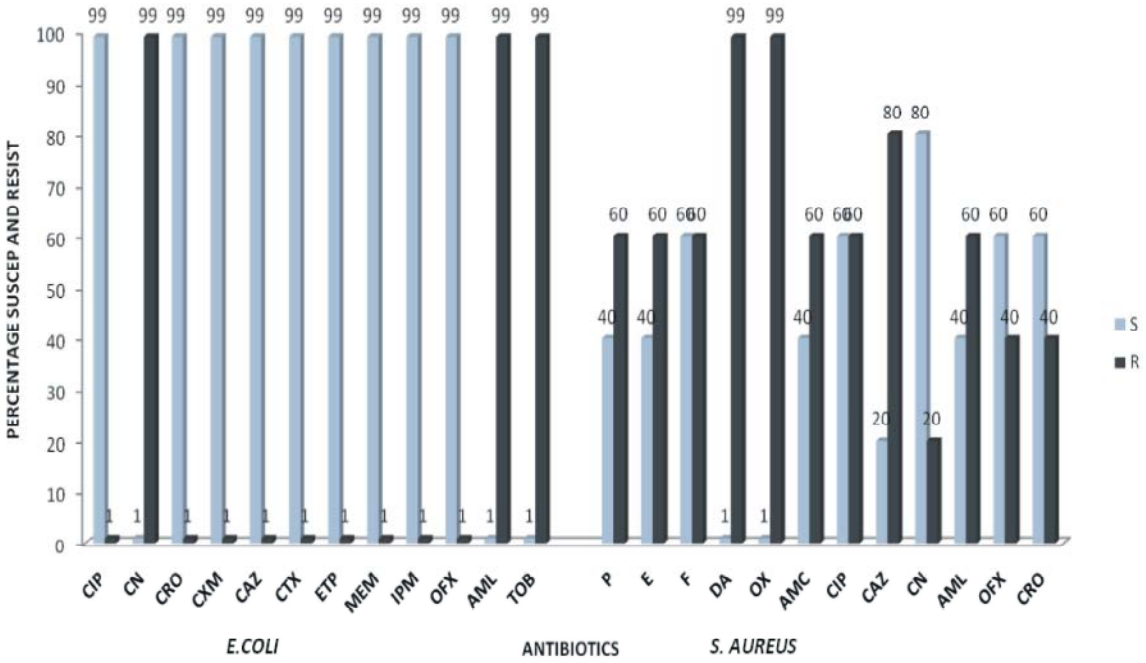

Fig. 11: Antibiotic susceptibility and resistance pattern of *E. coli* and *S. aureus* from pregnant women who are farmers.
Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid

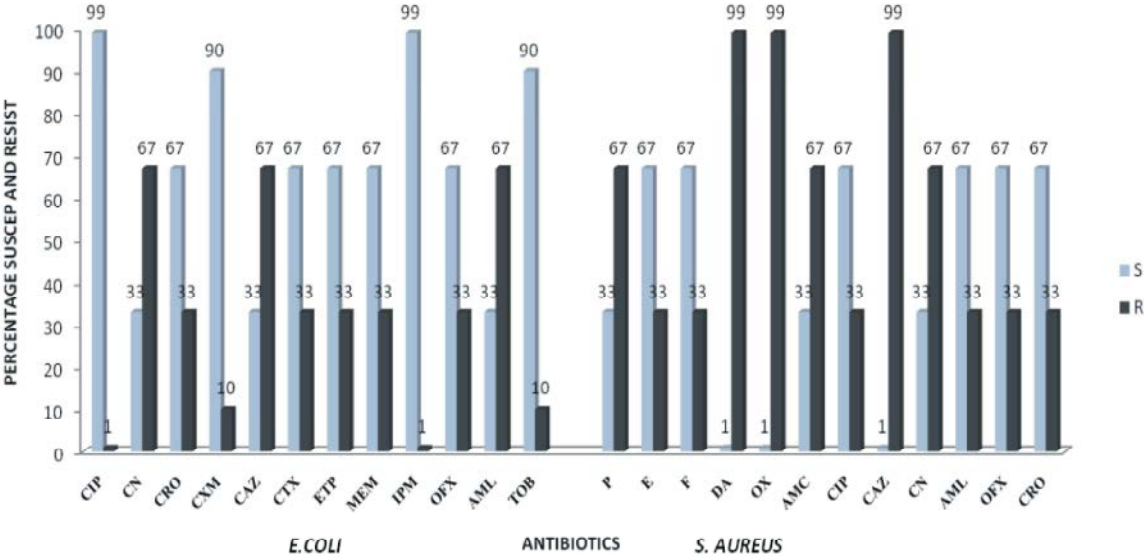

Fig. 12: Antibiotic susceptibility and resistance pattern of *E. coli* and *S. aureus* from pregnant women who are house wives
Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

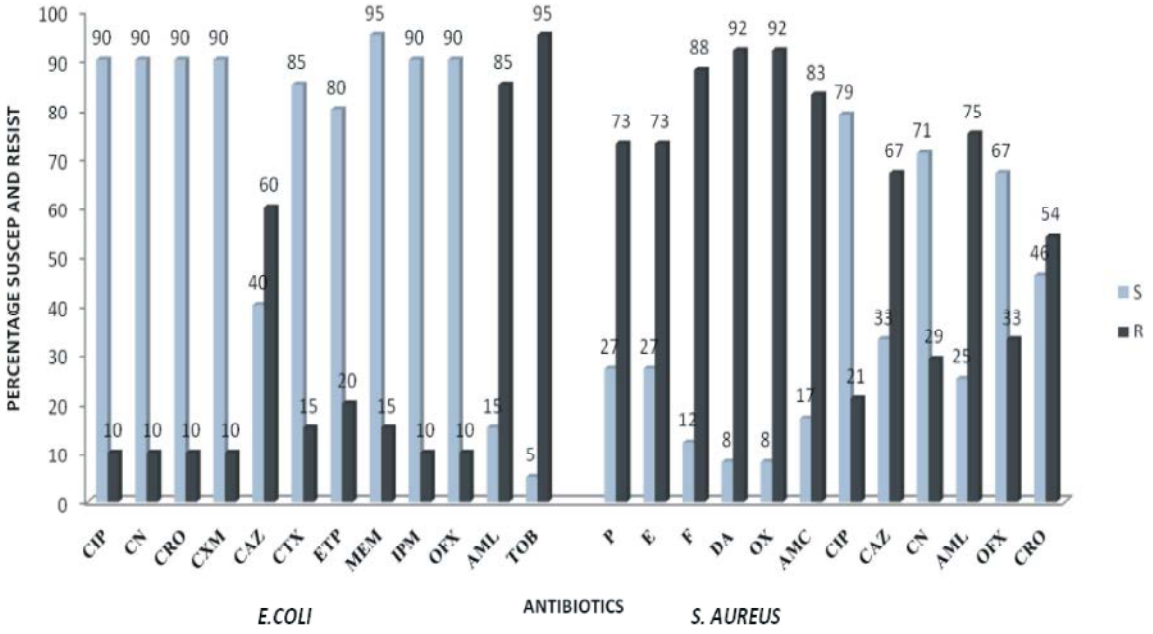

Fig. 13: Antibiotic susceptibility and resistance pattern of *E. coli* and *S. aureus* from pregnant women who are traders. Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

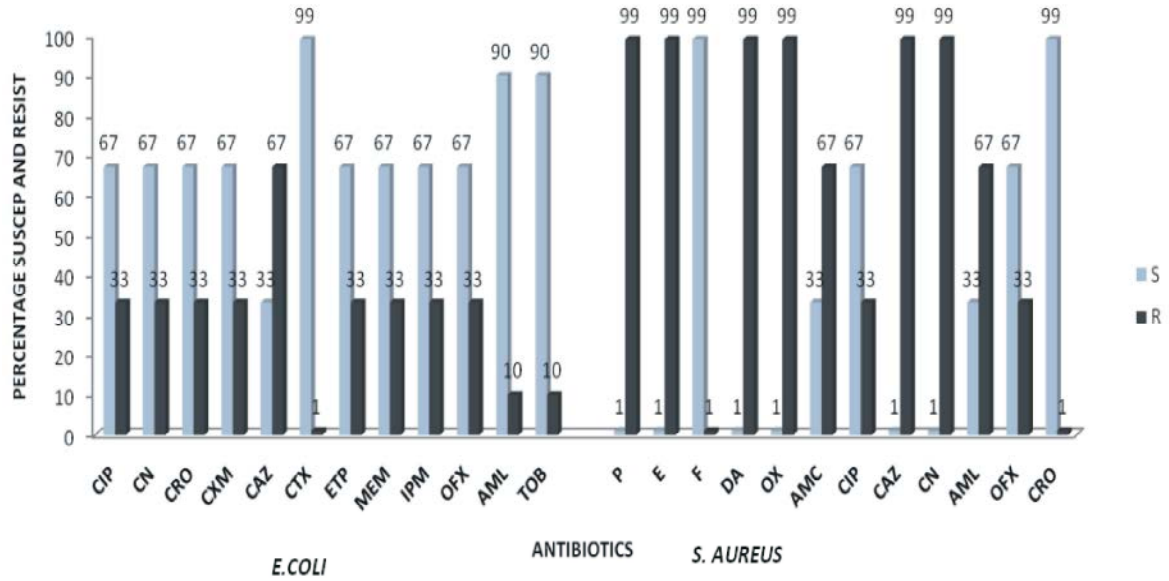

Fig. 14: Antibiotic susceptibility and resistance patterns of *E. coli* and *S. aureus* from pregnant women who are students

Keys: CIP = Ciprofloxacin, CN = gentamicin, CRO = ceftriaxone, CXM = cefuroxime, CAZ = ceftazidime, CTX = cefotaxime, ETP = ertapenem, MEM = meropenem, IPM = imepenem, OFX = ofloxacin, AML = amoxacillin, TOB = tobromycin, P = penicillin, E = erythromycin, F = nitrofurantoin, DA = clindamycin, OX = oxacillin, AMC = amoxacillin- clavulanic acid.

Table 6: Total and Average Multiple Antibiotics Resistance (MAR) Index for the Bacteria Isolates.

MARI FOR *S.AUREUS*	No. of Occurance
0.42	1
0.75	9
0.83	7
0.67	7
0.50	4
0.58	6
0.92	2
MARI FOR *E. COLI*	No. of Occurance
0.50	3
0.17	5
0.42	7
0.25	5
0.08	1
0.58	5
0.83	1
0.67	1
0.92	1
Mean for *S. aureus*: 24.99/36 =0.69	
Mean for *E. coli:* 11.94/29= 0.41	

Key: NO = Number, UTI = Urinary Tract Infection, *S. aureus* = *Staphylococcus aureus, E. coli* = *Escherichia coli*, MAR = Multi-drug resistant

Figure 15-19 shows the results obtained from the antibiotic susceptibility pattern of plasmid curing (CURED and UNCURED) of *E. coli* and *S. aureus* from UTI positive women. The result obtained shows that the organisms lose its resistant genes as the concentration of the acridine orange. The organisms were found to be susceptible to those antibiotics they were resistant to before. This indicates that the resistant genes are plasmid mediated.

DISCUSSION

In this study, *Escherichia coli* and *Staphylococcus aureus* were isolated as major occurring organisms from UTI positive pregnant women from Ezza South L.G.A of Ebonyi State. This result correlates with the report obtained in a study by [17], among pregnant women attending Jinnah University for Women (JUW), Pakistan. They reported that *Escherichia coli, Staphylococcus* species, *Enterococcus* species, *Klebsiella* species, *Streptococcus* species, *Citrobacter* species, *Acinobacter* species and *Pseudomonas* species were among the microorganisms implicated in UTI during pregnancy. In a similar study, Onwuezobe and Orok, (2015), [18], reported *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Citrobacter* spp., *Proteus mirabilis, Enterobacter spp.* and *Acinetobacter baumanii* as a cause of Urinary tract infection (UTI) in pregnant women attending a specialist hospital in Uyo, Nigeria. This further confirms the study of Guerra *et al.,* (2012), [19], which reported that the organisms causing UTI in pregnant women include *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp. *Staphylococcus* spp. and *Streptococci* spp.

The results presented in Table 1 represent the result of the percentage occurrence of UTI infections among the pregnant women according to their age bracket. It was observed that pregnant women in the age range of 23 – 28yrs had the highest (54.4%) cases of UTI while pregnant women in the age range of 17 – 22yrs had the least (34.4%) cases of UTI. This may be attributed to the fact that most women at this age are likely to have gotten married and would want to have children as revealed by their more participation in this study also they age bracket are known to be sexually active stage. The result of the percentage occurrence of UTI infections among the pregnant women according to their age bracket in this study is in agreement with the result obtained by Onwuezobe and Orok (2015), [18], which indicated that the highest percentage of UTI infections (56.3%) was obtained among women of age bracket 25 - 34 years. The high prevalence of UTI cases between the age ranges of 23 – 28 years supports the result obtained by Asad and Mohd, (2006), [20], which showed that the highest percentage of UTI (53%) was obtained among pregnant women of age bracket 20-30 years. UTI has been reported in 20% cases of pregnant women and the most common cause of admission in obstetrics wards [21].

The results presented in table 2 represent the result of the percentage distribution of *E. coli* and *S. aureus* among pregnant women with UTI according to their age brackets. It shows that the prevalence of *E. coli* is high among the women between the age group of 17-22yrs. This may be due to the fact that at this age personal hygiene is probably not achieved. Similarly, the prevalence is low among the pregnant women of 23 - 28 years age bracket. Asad and Mohd, (2006), [20], reported that the number of UTI samples identified as *E. coli* was higher (61%) among pregnant women at a lower age range (20-30 years) than 23% obtained from women between the age group of 31-45. Ayub *et al.* (2016), [17], reported that *E. coli* had a 28% while *Staphylococcus aureus* 8% in their study. Other studies of UTIs in outpatients have shown *E. coli* to be the most common etiological agent (>80%), with a preponderance in young females [22].

Table 3 represents the result of the percentage distribution of *E. coli* and *S. aureus* among pregnant women with UTI according to their gestational period. It shows that the pregnant women in their first trimester have the highest prevalence of *S. aureus* 14(82.3%) while pregnant women in their third trimester have the highest prevalence of *E. coli* 11 (73.3%). Pregnant women in their first trimester have the lowest prevalence of *E. coli* 9(52.9%) while pregnant women in their second and third trimesters had lowest prevalence of *S. aureus* 11(73.3%). In a similar study, Asad and Mohd, (2006), [20], reported that out of 59.8% (61/102) infected young women, 86.8% (52/61) were found in their early or late phases of pregnancy.

Table 4 represents the result of the percentage distribution of *E. coli* and *S. aureus* among pregnant women with UTI according to their educational background. It could be seen that the group with no Formal Education had the highest prevalence of *S. aureus* 100% while the group that stopped at Secondary Education had the lowest prevalence of *S. aureus* 68.8%. Also the group that stopped at Secondary Education had the highest prevalence of *E. coli* 81.3% while the group that had no Formal Education had the prevalence of *E. coli* 33.3%.

Table 5 represents the result of the percentage distribution of *E. coli* and *S. aureus* among pregnant women with UTI according to their occupation. It shows that the students and farmers have the highest prevalence (100%) of *S. aureus* while the business women have the least (70.0%) prevalence of *S. aureus.* The civil servants have the highest (100%) prevalence of *E. coli* while the farmers have the least (40.0%) prevalence of *E. coli*.

The discrepancies and variations in Table 5 and 6 could be attributed to differences in educational levels, religious observations, social lifestyles, environmental conditions and personal hygiene. Atlas (2004), [23], reported that rural dwelling, socio-economic status, sex habit and personal hygiene do affect the prevalence of significant bacteriuria. Certain behaviors that can influence the contraction of UTI include: Wiping back to front after defecating or urinating, not washing hands before and after using the toilet, not cleaning the urethral meatus first when bathing.

The result obtained from the antibiotic susceptibility testing for the bacteria isolated from pregnant women is shown in Figure 2 - 15. The most effective antibiotics in this study against *E. coli* were found to be gentamycin, imepenem, meropenem, ciprofloxacin, ofloxacin and ertapenem (Carbapenems and 2nd Generation Quinolones) while the isolates showed resistance to the following drugs Tobramycin, Cefuroxime, Cefotaxime, Amoxycillin, Ceftazidime and Ceftriaxone. In a similar study, Akingbade *et al.* [24], reported that *Escherichia coli* isolates obtained from pregnant women with UTI showed high resistance to Cloxacillin, Amoxicillin, Ampicillin, Erythromycin, Cotrimoxazole, Streptomycin and Tetracycline but were susceptible to Gentamycin and Ceftazidime. In a study carried out by Iqbal *et al.* [25], in Islamabad, *Escherichia coli* recorded high resistance to third generation Cephalosporins. In previous years, *E. coli* was 100% susceptible to the fluoroquinolones. Similar high resistance of *E. coli* to ofloxacin has also been documented by Alex *et al*. [26]; they observed that 24% of 189 *E. coli* isolates were resistant to Ofloxacin. [26] also reported very high resistance levels (>75%) against Tetracycline, Augmentin and Amoxicillin while Nitrofurantoin and Ofloxacin recorded the least resistance levels of 6.0% and 19.0% respectively among the *E. coli* isolates. The resistance of *E. coli* to Tobramycin, Cefuroxime, Cefotaxime, Amoxycillin, Ceftazidime and Ceftriaxone could be as a result of production of β-lactamse enzyme which has the ability to deactivate the efficacy of these â-lactam drugs as reported by [27].

Staphylococcus aureus was susceptible to ciprofloxacin, gentamicin and ofloxacin while the isolates showed resistance to the following drugs; Nitrofurantoin, Amoxycillin, Clindamycin, Oxacillin, Penicillin, Amoxycillin/clavulanic acid, erythromycin, ceftazidime and Ceftriaxone. Resistance against these antibiotics revealed that the resistance is purely plasmid based since β-lactamase production is plasmid based [28]. *Staphylococcus aureus* resistance to antibiotics is a serious medical problem worldwide [29]. The difference in susceptibility or resistance pattern demonstrated in different geographic locations may be attributable to factors like exposure to antibiotics. From the results of this study, Gentamycin may be considered as empirical therapy of first choice for *Escherichia coli* / *Staphylococcus aureus* urinary tract infections, followed by Ciprofloxacin, Ofloxacin and Imerepenem.

The result presented in table 6 below shows the multiple antibiotics resistance (MAR) index for the bacteria isolates. It was revealed that the isolates had an average MARI of 0.69 and 0.41 for *Staphylococcus aureus* and *Escherichia coli* respectively. The MAR indices in this study were greater than 0.20, this confirms the report of Olayinka *et al.* [30], that the MAR index

greater than 0.20 indicates that the organisms must have originated from an environment where antibiotics are often used [31]. Thus, from the result of the multiple antibiotic index in this work it could be reported that these pathogens might have originated from where these antibiotics are used.

Summary: Urinary tract infections are the most frequent bacterial infection in women [32]. They occur most frequently between the ages of 16 and 35 years, with 10% of women getting an infection yearly and more than 40–60% having an infection at some point in their lives [33]. Recurrences are common, with nearly half of people getting a second infection within a year. Thus results obtained from this study showed that test organisms (*E. coli* and *S. aureus*) harbour antibiotics resistant genes and rate of bacteria isolation is high across all the demographic data (Age, Occupation, Education background, Gestation period).

CONCLUSION

The high prevalence of multiple antibiotics resistant *Escherichia coli* and *Staphylococcus aureus* among the patients in this study raises the important issues for infection control in this environment. There is also the need for consistent antimicrobial resistance surveillance for important and commonly isolated clinically significant pathogens of *Escherichia coli* and *Staphylococcal* species to form the basis for developing and implementing measures that can reduce the burden of antimicrobial resistance and prevent a probable impending public health problem. Plasmid curing analysis has been shown to be a good epidemiological tool in investigating epidemics or outbreaks of bacteria diseases. Lastly, research should be intensified in the area of chemotherapeutic agents; more effective antibiotic should be discovered that can be effective against organisms with resistant plasmid.

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