1
MOLECULAR DETECTION *ctx-M*, *TEM and VIM* in ESBL-producing *E. coli* strains isolated from pregnant women in Osogbo.

2
3
4
5
6

Abstract

7 Urinary tract infection (UTI) is a major bacterial infection causing serious health problem in pregnant
8 women. The physiological and anatomical changes in pregnancy facilitate urinary tract infection
9 (UTI) during pregnancy. Asymptomatic bacteriuria in pregnancy is associated with pyelonephritis,
10 preterm labour and low birth weight infants. The study was designed to characterise phenotypically
11 and genetically the major organism associated with UTI among pregnant women in Osun State. A
12 cross-sectional study design was used to collect mid-stream urine samples between March 2018 to
13 September 2018 from 150 pregnant and 50 non-pregnant women which serve as control. Samples
14 were inoculated into Cysteine Lactose Electrolyte Deficient (CLED) medium, subcultured onto
15 MacConkey and Blood agar plates. A standard agar disc diffusion method was used to determine
16 antimicrobial susceptibility pattern of the isolates and the molecular detection of the antibiotic
17 resistant genes were done. Data were subjected to descriptive statistics. The ages of women enrolled
18 in this study ranges from 22 to 43 years (mean ± standard deviation = 25 ± 4.7 years). The
19 predominant bacteria identified were *E. coli* (34.5%), *S. aureus* (10.3%), coagulase negative
20 Staphylococci [CoNS] (17.2%), *Klebsiella* species (6.9%) and *Enterobacter* species (31.0%).
21 Majority of Gram-negative bacteria isolates were resistant to ampicillin (70%), cefotaxime (62%),
22 while 75–100% of the Gram positive isolates were resistant to ampicillin. Multiple drug resistance
23 was observed, all the *E. coli* isolates were resistant to Cefotaxime, meropenem and ampicillin. Of *E.*
24 *coli* isolates, 4, 3 and 6 were positive for the VIM, *ctx*-M and TEM genes
25 respectively.Similarly, the risk of UTI was higher in those had previous UTI history (OR = 2.29,
26 95% CI = 1.15–4.56, P = 0.019) as compared to those who had no previous history of UTI.

27 Keywords: UTI, pregnant women, *Escherichia coli.*

28
Introduction

29 Urinary tract infection is a common health problem among pregnant women (1). Urinary tract
30 infection (UTI), otherwise known as bladder infection, is a bacterial inflammation in the
31 urinary tract. Pregnant women are at increased risk for UTI's especially during first and
32 second trimesters. It has been observed that pregnant women have a propensity to develop recurrent
33 UTIs (2). Moreover, UTI can be dangerous for both the mother and fetus. Complications that can arise
34 include preterm delivery, premature rupture of membrane and increased incidence of intrauterine

UNDER PEER REVIEW

35 growth restriction. Also, preeclampsia, caesarean delivery, anemia, sepsis, and septic shock may also
36 be associated with UTI in these patients (3). *E. coli* is the most incriminating pathogen causing UTI.
37 The rate of *E. coli* infection among women is alarming, thereby posing a very big health issue among
38 pregnant and non-pregnant women. Treatment of UTI is important in keeping with the goal of
39 safe motherhood initiative; that women safely go through pregnancy and childbirth and
40 produce healthy babies. Antibiotics are prescribed for the treatment of infection. However,
41 antimicrobial resistance to clinically important drugs used for treatment is increasing. Drug resistance
42 in *E. coli* must be promptly addressed before multiple resistant strains start emerging and spreading
43 among the pregnant women.

44 Therefore, the aim of this study was to determine the drug resistance profile and molecular
45 detection of *ctx-M* , VIM and TEM genes in *E. coli* isolated from among pregnant and non-
46 pregnant women with urinary tract infections (UTI) in Osogbo.

47

48 **Materials and Method**

49 **Study area**

50 The study was conducted in the city of Osogbo, Osun State from March 2018 to September 2018.

51 ***Ethics Statement***

52 This work was performed according to University ethics committee code of conduct, verbal

53 informed consent was obtained from all participating subjects.

54 **Ethical approval**

55 Ethical approval was obtained from the State Ministry of Health.

56

57 **Study population**

58 The study population were pregnant women attending antenatal clinic (ANC) at Onward specialist
59 hospital, Agunbelewo, Osogbo and Primary health centre, Atelewo, Osogbo during the study period,
60 and some selected non-pregnant women around Osogbo metropolis, those who did not initiate
61 antimicrobial drug therapy for at least 2 weeks prior to sample collection.They were registered in the
62 project register where basic demographic data about the patients including name, age, sex, ward etc.
63 were recorded.

UNDER PEER REVIEW

Sample size calculation was done using Leslie Fisher's formula

Sampling methods

Study participants were selected using simple random sampling technique for the selection of pregnant and the non-pregnant women across Osogbo town.

The calculated sample size was proportionally distributed to Onward hospital (n = 54), Primary Health Centre, Atelewo (n = 96), and non pregnant women (n= 50).

Sample collection

A total of 200 mid-stream urine (MSU) samples were collected from the participants. Ten to fifteen milliliter of freshly voided midstream urine samples were used for microscopic investigation and culture media inoculation. Urine samples were processed within 4 h of collection (4).

In the laboratory, urine samples were centrifuged at 1500 RPM for 5 min. After centrifugation a drop of the sediment was placed on the grease free slide, covered with cover slip and examined under the microscope using the high power objective lens (40X).

Reporting system for microscopic identification was done for pus cells, red blood cells (RBCs), epithelial cells, casts, crystals, yeast cells (5).

Isolation and identification

Standard loop technique was used to place 0.001 ml of urine for inoculation on Cysteine lactose electrolyte deficient (CLED) medium and incubated at 37 °C for 24 h (6). The numbers of colonies were counted to quantify organisms.

Diagnosis of UTI is defined on the basis of significant colony count of ≥105 cfu/ml for Gram-negative and Gram-positive bacteria (7).

Growths on the culture media were identified by using bacterial growth characteristics (morphology), Gram staining and general biochemical tests (8).

Antimicrobial susceptibility testing (AST) of uropathogens.

The antimicrobial susceptibility testing of all isolates was done using commercial disks following the standard disk diffusion method recommended by the National Committee for Clinical Laboratory Standards (NCCLS 2012).

UNDER PEER REVIEW

91 The drugs that tested were Penicillin (PEN, 30 μg), Ampicillin (AMP, 30 μg), Ciprofloxacin (CPR, 5
92 μg), Levofloxacin (LEV, 10 μg), Cefuroxime (CPX, 10 μg), Cefotaxime (CTX, 10 μg), Tetracycline
93 (TET, 300 μg) and Meropenem (MEM, 1.25 μg).

94 All the antimicrobials used for the study were purchased from Oxoid Limited Bashing store, USA.

95

96 Genotypic Identification of *E. coli* Isolates

97 *E. coli* isolates were grown overnight at 37°C on blood agar or for 24 h on LB agar without salt for
98 DNA extraction.

99 DNA Extraction

100 The DNA molecules of the isolates were extracted by suspending bacterial colonies in 500 μl of
101 sterile distilled water in appropriately labelled Eppendorf tubes.

102 The cells were washed three times in sterile distilled water while vortexing and centrifuging at 10, 000
103 rpm. Tubes were covered and sealed with paraffin tape to prevent accidental opening.

104 After the last washing, the bacterial suspensions were boiled at 100 °C for 10 minutes in water bath
105 and cold shocked in ice for 2 minutes. The boiled suspension contained the DNA.

106 PCR Amplification

107 The polymerase chain reaction was set up in a PCR vial, after adding the master mix, the forward and
108 reverse primers and the extracted DNA. A 20μl reaction containing 2μl of 10X buffer, 1μl $Mgcl_2$,
109 0.8μl dNTPs, 0.5μl of forward primer, 0.5μl of reverse primer, 0.2 μl Taq polymerase, 10μl of
110 nuclease free water and 5μl of DNA lysate was used for PCR.

111 Amplification was subjected to initial denaturation at 95C °for 5min, followed by 35 cycles of
112 denaturation at 95°C for 1 min, annealing at 60°C, 56°C, 54°C, 47°C, 52C° for 1 min, for *ctx-M,* VIM
113 and TEM respectively, extension at 72°C for 1 min and final extension procedure was carried out at
114 72°C for 10min.

115 **Gel Electrophoresis**

116 At the completion of the amplification, PCR products were resolved on 1.5% agarose gel prepared by
117 dissolving 1.5g of agarose powder in 100 ml of 1X Tris-borate-EDTA (TBE) buffer solution inside a
118 clean conical flask.

UNDER PEER REVIEW

119 The 1.5% agarose solution was heated in a microwave oven for 2-3 minutes and was observed for
120 clarity which was an indication of complete dissolution. The mixture was then allowed to cool to
121 about 50 °C after which 0.5 µl of ethidium bromide was then added. It was allowed to cool further
122 and then poured into a tray sealed at both ends with support to form a mould with special combs
123 placed in it to create wells.

124 The comb was carefully removed after the gel had set and the plate was placed inside the
125 electrophoresis tank which contained 1X TBE solution stained with 1ug/ml of ethidium bromide
126 solution and loaded to the well of the agarose gel. The power supply was adjusted to 100 volts for 25
127 minutes. For each run, a 100 base-pair molecule weight DNA standard (size marker) was used to
128 determine the size of each PCR product.

129 The DNA bands were then visualized with a short wave ultraviolet trans-illuminator and
130 photographed using gene gel bio imaging system. The PCR product was then analyzed.

131 Statistical analysis

132 Data from laboratory investigation and questionnaire survey was entered into Microsoft Excel
133 Spreadsheet. Descriptive statistics was used to summarize the data.

134 Chi-square test was used to assess differences in the proportions of culture positive and negative
135 participants. The prevalence of UTI was calculated. To determine predictors of bacteriuria, odds ratios
136 were calculated using likelihood estimation technique.

137 Independent variables (age, level of education, monthly income, parity, residence, washing habit and
138 previous history of UTI) which are non-collinear and with P-values ≤0.25 in univariable logistic
139 regression analysis were further tested via multivariable logistic regression in order to get adjusted
140 odds ratios and significant predictors of UTI in pregnant women. P-value of <0.05 was considered
141 statistically significant.

142

143 RESULTS

144 There were 200 women enrolled in this study; 150 pregnant women, and 50 non-pregnant women. Of
145 the positive urine samples for UTI, 21 (72.0%) and 8 (28.0%) were pregnant and non-pregnant
146 women respectively (Table 1).The age of pregnant women enrolled in this study ranges from 22 to 43
147 years and non-pregnant women from 25-41 years (Table 2).

148 From 200 urine samples, 29 (14.5%) (95% CI: 14.4–23.54%) were culture positive with colony count
149 of more than 10^5 cfu/ml. Five bacterial species of UTI were isolated in which *E. coli* (n = 10) was the

UNDER PEER REVIEW

150 predominant bacteria followed by *Klebsiella* spp. in 9 cases (31%), *S. saprophyticus* was
151 isolated in 5 cases (17.2%) *S. aureus* in 3 patients (10.3%), and *Enterobacter aerogenes* in
152 two patients (6.9%) (Table 3). The prevalence of symptomatic and asymptomatic UTI was
153 20.4% (95% CI: 13.09–29.46%) and 17.8% (95% CI: 12.70–23.83%) respectively. Of the 29
154 bacterial isolates, 7 (47.6%) were from private hospital and the remaining 14 (52.4%) were
155 from government hospital and 8 from selected people. Monthly income, personal hygienic
156 habits and previous history of UTI are significantly associated with prevalence of UTI ($P <$
157 0.05). One hundred and twenty five (62.5%) of study participants had income level of 20000
158 – 50000 Nigerian naira (21.23–42.37 USD) and seventy five earn above. On the basis of their
159 lifestyle about 115 (57.5%), had a lower level of personal hygiene. About 11 (38%) of
160 positive pregnant women had previous history of UTI.

161

162

163 Table 1: Positive samples of pregnant and non-pregnant women.

164

165 Subjects	No of Samples	Positive culture	Percentage
166 Pregnant women	150	21	57.14
167 168 Non-pregnant women	50	8	42.86

169

170

171

172

173 Table 2: Gestational ages of pregnant women enrolled in this study.

174

Hospital	Patient age range (yr)	Gestational age range (wk)	Total (%)	First trimester	Second trimester	Third trimester
Onward	25-42	10-40	54 (27%)	14	21	9

UNDER PEER REVIEW

Atelewo	22-43	8-39	96 (48%)	31	49	16

175

176

177

178 Table 3: Distribution of the Uropathogens among pregnant and non-pregnant women.

179

Uropathogen	Distribution			
	Onward(Private)	Atelewo(Government)	Nonpregnant	Total
Escherichia coli	3	5	2	10
Staphylococcus aureus	0	1	2	3
Staphylococcus saprophyticus	2	2	1	5
Enterobacter aerogenes	0	2	0	2
Klebsiella pneumonia	2	4	3	9
Total	7 (25%)	14 (49%)	8 (26%)	29

180

181 Table 4: Antibiotic susceptibility pattern of the *E. coli* isolates.

182

Isolates	PEN	AMC	CTX	CPX	TET	LEV	MEM	CIP
Onward	R	R	S	S	R	S	R	S
	S	R	S	S	R	S	R	S
Atelewo	S	R	R	S	R	S	R	S
	R	S	R	S	R	S	R	R
	S	S	S	R	R	S	R	S
	S	R	R	R	S	S	R	R
	S	R	R	R	S	S	R	S
Nonpregnant	R	R	R	R	R	S	R	S
	R	S	R	R	S	S	R	S
	S	R	S	R	S	S	R	R

183

UNDER PEER REVIEW

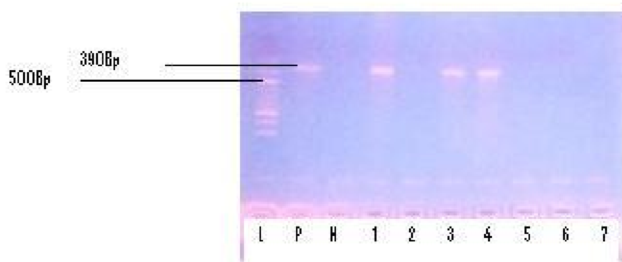
184 Key: PEN- Penicillin, AMC- Ampicillin, CTX- Cefotaxime, CPX- Cefuroxime, CIP- Ciprofloxacin,
185 TET- Tetrecycline, LEV- Levofloxacin, MEM- Meropenem

186 Table 5: Table showing the primers used in the PCR Amplification process.

187

Primer	Sequence 5^1 -3^1	Base pair (bp)	Annealing temp. (ºC)
CTX-M F CTX-M R	CGATGTGCAGTACCAGTAA TTAGTGACCAGAATAAGCGG	585	60
TEM F TEM R	CCCCGAAGAACGTTTTC ATCAGCAATAAACCAGC	517	52
VIM2004A VIM2004A	GTTTGGTCGCATATCGCAAC AATGCGCAGCACCAGGATAG	390	54

188



189

190 Figure 1: Agarose gel electrophoretogram of (VIM) *Escherichia coli* after PCR analysis
191 *Escherichia coli* isolates which bands at 390 bp

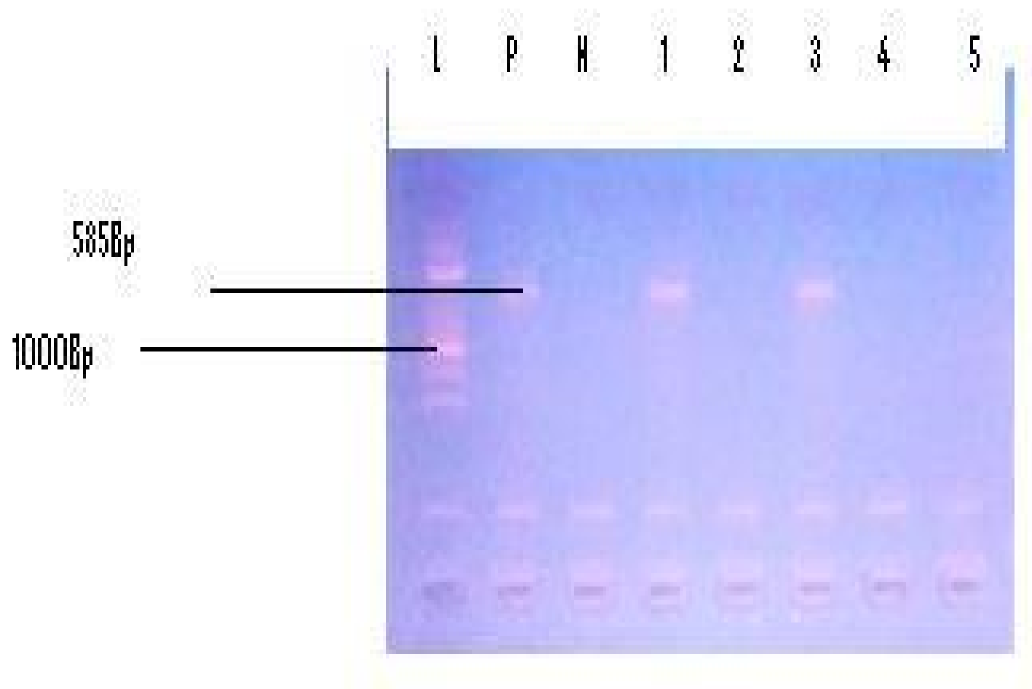
192 **Key:**

193 L (100 bp ladder)

194 P –Positive

195 N-Negative

196

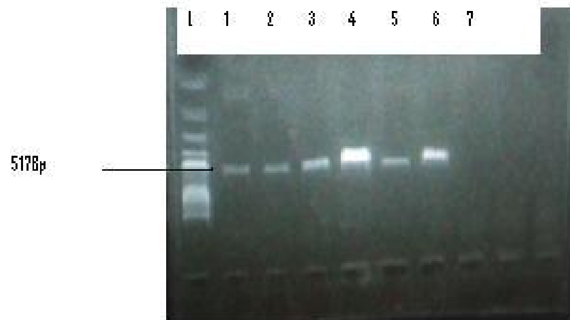


216 Figure 2: Agarose gel electrophoretogram of CTX-M–type β-lactamases (CTX-Ms)
217 *Escherichia coli* after PCR analysis. *Escherichia coli* isolates which bands at 585 bp

218

219

220



221

222 Figure 3: Agarose gel electrophoretogram of TEM *Escherichia coli* after PCR analysis

223

224 which bands at 517 bp

225

226

227 Antimicrobial susceptibility pattern of bacterial uropathogens

228 Bacterial uropathogen isolates from patients with UTIs revealed the presence of high levels
229 of single and multiple antimicrobial resistance against commonly prescribed drugs. Gram-
230 negative isolates showed higher resistance pattern in comparison to Gram-positive for most
231 of commonly prescribed antibiotics. *E. coli*, which is the predominant cause of UTI, showed
232 high percentage of resistance to ampicillin and low resistance to ciprofloxacin and penicillin
233 (Table 4). All the E. coli isolates are sensitive to levofloxacin, and all are resistant to
234 meropenem.

235

UNDER PEER REVIEW

236 Multiple drug resistance patterns of the isolates

237 Multiple drug resistances (MDR) i.e., resistance to two or more antimicrobial drugs, was
238 found in all the *E. coli* isolates (100%). All isolates of Gram-negative and Gram-positive
239 bacteria were resistant to at least two antimicrobials. There was no isolate sensitive to all
240 antibiotics tested (Table 4).

241

242 Associated risk factors

243 Univariable logistic regression analysis showed significant association between prevalence of
244 UTI and income level (P = 0.046), residential place (P = 0.029), personal hygiene (P = 0.04)
245 and previous history of UTI (P = 0.028). Multivariable logistic regression revealed that the
246 odds of acquiring UTI in pregnant women is 4.78 times higher than those of non-pregnant
247 women (95% CI of OR = 1.03–22.21, P = 0.046) . Similarly, the risk of UTI infection is
248 twice and 2.04 times higher in those who had previous history UTI infection (OR = 2.29,
249 95% CI of OR = 1.15–4.56, P = 0.019), as compared to those who had no previous history of
250 UTI.

251

252 Amplification of the resistant genes

253 The primers used for this PCR are *ctx-M*, *TEM*, *VIM*, (Table 5), and the process carried out at
254 normal conditions as described earlier. The *ctx-M* resistant genes were observed at 390bp
255 (Fig 1). Resistant genes *TEM*, *VIM*, were observed at 585bp and 517bp (Fig 2) and (Fig 3).

256 Discussion

257 The study was undertaken to determine the occurence of urinary tract infection caused by *E.*
258 *coli* and other uropathogens among pregnant and non-pregnant women and also to analyse
259 the risk factors for predisposition to UTI, and resistance patterns. The culture positive urine
260 samples belong to Gram-negative and Gram-positive bacteria. The low incidence of urinary
261 tract infection reported in the private hospital (Onward) may be attributed to the extensive
262 health care talk given regularly by the staff of the hospital's ante-natal section, higher level of
263 education and exposure, higher standard of living, among others. This study shows a higher
264 incidence of urinary tract infection among pregnant women than non-pregnant women. A

UNDER PEER REVIEW

265 higher percentage of the organisms found in this study were isolated mainly from pregnant
266 women. It is commonly accepted that a high frequency of UTI during pregnancy is due to
267 physiological changes that the human body undergoes in the pregnant condition (10). The
268 higher incidence of urinary tract infections in pregnant women might be as a result of a
269 variety of factors, such as more open and exposed uterus and bladder due to distended
270 stomach (9) and incomplete and in coordinate voiding of urine in pregnant women and
271 encourages infection of the urinary tract (9). A total number of 29 isolates were obtained
272 from the 29 women with positive cultures, that is only one bacterium was isolated from each
273 patient, suggesting a mono-microbial nature of infection in the study population. The pattern
274 and frequency of occurrence of the bacterial isolates found in this study is similar to those
275 reported by other workers. Lavigne *et al*. 2011 reported in their study that *E. coli* was the
276 most commonly isolated pathogen in significant bacteriuria (11). The result of this study
277 shows that 100% of the *E. coli* isolates were sensitive to Levofloxacin, 33.3% to ampicilin,
278 55% to penicillin 36% to cefotaxime, 39% to cefuroxime, 77.8% to ciprofloxacin and 0% to
279 meropenem.. The antibiotic sensitivity test of this study shows that Levofloxacin was the
280 most effective antibiotic in *in vitro* testing against *E. coli* isolates followed by ciproflaxin
281 which was effective against 77.8% of the isolates. A reduced sensitivity of *E. coli* to
282 nitrofuratoin was observed in this study as only 45% of the *E. coli* was sensitive to the
283 antibiotics as opposed to the findings of Goldraichi and Manfrori (12), who reported a higher
284 efficacy of the drug against *E. coli in vitro*. They reported a sensitivity of *E. coli* to
285 nitrofuratoin of 92, 95 and 94%, respectively over a three-year period. Olowu and Oyetunji
286 reported a 57.9% sensitivity of pathogens towards nitrofuratoin(13). In this study,
287 Meropenem was the most ineffective antibiotic in *in vitro* testing, since 100% of the
288 pathogens were resistant to it. Resistance of *E. coli* to cefuroxime was 40% and is in contrast
289 to results obtained elsewhere. Christiaen et al. (1998) reported a resistance of 17% to
290 cotrimoxazole and a similar result was reported for resistance to quinolones. This study
291 shows a high level of resistance to cefuroxime, ampicillin and tetracyclin as more than 60%
292 of the isolates were resistant to them *in vitro* and, as such, these antimicrobials may not be
293 suitable for treating case of UTI caused by *E. coli* in Osogbo. Multiple drug resistance was
294 observed among *E. coli,* of *E. coli* isolates, 4, 3 and 6 were positive for the VIM, *ctx-M* and
295 TEM genes respectively. Edelstein and colleagues reported that *ctx-M* beta lactamases have a
296 destructive effect on Cefuroxime. The detection of VIM suggested the presence of
297 carbapenem-resistant gene and TEM the production of beta-lactamases.

UNDER PEER REVIEW

298

299 Conclusion

300 The predominant bacteria identified were *E. coli*, majority of Gram-negative bacteria isolates were
301 resistant to ampicillin , cefotaxime and meropenem while Gram positive isolates were resistant to
302 ampicillin. Multiple drug resistance was observed, all the *E. coli* isolates were resistant to Cefotaxime,
303 ampicillin and meropenem. Some of the *E. coli* isolates were positive for the VIM, *ctx-M* and
304 TEM resistant genes.

305 Recommendation

306 Health education, continuous and collaborative surveillance of UTI and antimicrobial
307 resistance pattern are essential to reduce the consequence of symptomatic and asymptomatic
308 bacteriuria and multi-drug resistant bacteria in pregnant women. Enlightenment programs
309 informing the general public on the importance of good personal hygiene and the
310 implications if neglected should be encouraged. This will not only reduce the risk of UTIs but
311 other infections as well. Likewise, there should be continuous education for pregnant women
312 on the need to maintain a high level of personal hygiene during pregnancy as they are at high
313 risk for the infection. They should also be educated on the importance of routine medical
314 check-up during the period of pregnancy.

315 **Conflict of Interest:**

316 There is no conflict of interest to be declared

317 **References**
318

319 1. Thomas M, Hooton MD. Uncomplicated urinary tract infection. N Engl J Med. 2012;
320 366:1028–37. doi: 10.1056/NEJMcp1104429.
321 2. Mittal P, Wing DA: Urinary tract infections in pregnancy. Clin Perinatol 2005,
322 32:749-7641. PubMed Abstract | Publisher Full Text
323 3. Nowicki B. 2002. Urinary tract infection in pregnant women: old dogmas and current
324 concepts regarding pathogenesis. Curr. Infect. Dis. Rep. 4:529 –535.
325 4. Mgbakogu RA, Eledo BO. Polymicrobial agents and antibiotic profile of urinary tract
326 infections among pregnant women in Anambra state, Nigeria. Advances in Life Sci
327 and Tech. 2015;35(38):224–36.
328 5. Alemu A, Moges F, Shiferaw Y, Tafesse K, Kassu A, Anagaw B, Agegn A. Bacterial
329 profile and drug susceptibility pattern of UTI in pregnant women at University of

UNDER PEER REVIEW

330 Gondar teaching hospital. Ethiop J Health Sci. 2012;25(5): 197. doi: 10.1186/1756-
331 0500-5-197.

332 6. Alfred AO, Chiedozie I, Martin DU. Pattern of asymptomatic bacteriuria among
333 pregnant women attending an antenatal clinic at a private health facility in Benin,
334 South Nigeria. Ann Afr Med. 2013;12(3):160–4.

335 7. Tadesse E, Teshome M, Merid Y, Kibret B, Shimelis T. Asymptomatic urinary tract
336 infection among pregnant women attending the antenatal clinic of Hawassa referral
337 hospital, southern Ethiopia. BMC Res Notes. 2014;7:155. doi: 10.1186/1756-0500-7-
338 155.

339 8. Assefa A., Asrat D., Woldeamanuel Y., G/Hiwot Y., Abdella A., and Melesse T.
340 (2008). Bacterial profile and drug susceptibility pattern of UTI in pregnant women at
341 Tikur Anbessa specialized hospital. Ethiop Med J, 46 (3), 227-235.

342 9. Norinder BS, Koves B, Yadav M, Brauner A, Svanborg C. 2011. Do *Escherichia coli*
343 strains causing acute cystitis have a distinct virulence repertoire? Microb. Pathog.
344 52:10 –16.

345 10. Dormingy JA, Nabeth P, Juergens-Behr A, Perrier-Gros-Claude JD. Risk factors for
346 antibiotic resistance Escherchia coli isolated from community–aquired urinary tract
347 infections in Dakar, Senegal. J Antimicrob Chemother. 2005;56(1):236–9. doi:
348 10.1093/jac/dki158.
349

350 11. Jolley JA, Wing DA. 2010. Pyelonephritis in pregnancy: an update on treatment options for
351 optimal outcomes. Drugs 70:1643–1655. Jolley JA, Wing DA. 2010. Pyelonephritis in
352 pregnancy: an update on treatment options for optimal outcomes. Drugs 70:1643–1655.

353 12. Lavigne JP, Boutet-Dubois A, Laouini D, Combescure C, Bouziges N, Mares P, And Sotto A.
354 2011.Virulence potential of E. coli strains causing asymptomatic bacteriuria during pregnancy. J
355 Clin Microbiol,;49(11):3950–3.

356 13.Goldraichi NP, Manfroi A (2003). Febrile urinary infection. Escherichia coli susceptibly to
357 oral antimicrobials Paediatr. Nephrol 17 (3): 173- 176.

358 14. Olowu WA, Oyetunji TG (2003). Nosocomial significant bacteriuria prevalence and pattern of
359 bacterial pathogens among children hospitalized for non-infective urinary tract disorders. West
360 Afr. J. Med. 22 (1): 72-75.

361 15. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchoun Sh. Prevalence and molecular
362 epidemiology of CTXM extended–Spectrum B-lactamase-producing Escherchia coli and
UNDER PEER REVIEW

363 Klebsiellapneumoniae in Russian hospitals. Antimicrob Agents Chemother. 2003; 47(12):3724–
364 32. doi: 10.1128/AAC.47.12.3724-3732.2003.

365

366

367

368

369

370

UNDER PEER REVIEW