STUDIES ON THE ROLE OF *Escherichia coli* IN PREGNANCY RELATED MISCARRIAGES (SPONTANEOUS ABORTIONS) IN UPLAND AREA OF RIVERS STATE OF NIGERIA

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Abstract: *Escherichia coli* infection is a serious health threat to the ordinary people, especially the intending mothers all over the world, but more so in the developing countries. In this cohort study, 300 Endocervical swabs (ECS) and mid-stream urine (MSU) samples were collected within 8 hours to 24 h of miscarriages to determine the prevalence and role of *Escherichia coli* in pregnancy related miscarriages in the Upland Area of Rivers state, in this era of frequent miscarriages amongst Women. The ECS were cultured on chocolate, blood and MacConkey agar while MSU samples were inoculated on MacConkey and CLED media, respectively. The growths were isolated and characterized using biochemical activities including fermentation, Gram reaction, motility, colonial appearance, media selection. ECS samples were also screened for *Chlamydia trachomatis* using Chlamydia Rapid Test Device kit. Of the 130 isolates 54(41.5%) of them were *Escherichia coli* while the proportions of the infected women were highest within the 18-20 years and 21 – 25 years age cohorts ($P<0.05$). The prevalence of *Escherichia coli* showed significant relationship ($p<0.05$) with the illiteracy level of the women, more so, the level of this result statistically ($p<0.05$) associates Escherichia coli with miscarriages in the Upland area of Rivers state of Nigeria, and this calls for mass literacy and awareness among the intending mothers.

Keywords: Miscarriage, *Escherichia coli*, endocervical smear, urine, upland

Introduction

Escherichia coli (*E. coli*) is a normal inhabitant of the human intestinal tract and is mostly harmless, but certain species (such as 0157:H7) can cause serious illnesses of the digestive and genitourinary systems (Phaa, 2015). Urinary Tract Infections (UTIs) caused by *E. coli* may become severe at anytime, but such infections occurring during pregnancy may be dangerous for both the mother and unborn baby (Delzell *et al*., 2000; Smail *et al.*, 2007).

All women and particularly the pregnant ones are at the risk of developing urinary tract infections, and the common causative organism is *E. coli* accounting for 80% to 90% of infections (Phaa, 2015; Delzell *et al*., 2000; McCormick *et al*., 2008). The dangers of *E. coli* infection in pregnant women include spontaneous abortions (miscarriages), premature birth, (premature labor), stillbirth (Toth, 2014; Mills *et al.*, 1988). All these are as a of the production of phospholipase A2 enzyme and it is also believed that human term labor is initiated by amniotic and chorionic phospholipase A2 (Stamm, 1995), and the increased rates of premature labor resulting to miscarriages and premature births have been seen in pregnant women with symptomatic urinary tract infections due to *E. coli* (Stamm, 1995).

Spontaneous abortions and miscarriages are terms that are synonymous. It is the involuntary loss of the products of the conceptions prior to 24 weeks of gestation (Toth, 2014; Shiers, 2001; Nnamdi *et al*., 2012; Joseph, 2014). It has been responsible for the death of many people/women worldwide. *E. coli* has been attributed to 15 to 20% of spontaneous abortions (Phaa, 2015). In Nigeria, it was reported that there were 1,000 maternal deaths per 100,000 live births annually (WHO, 1988; Stanley *et al.*, 1998).

The epidemiological data on miscarriages (spontaneous abortions) in Africa are based on diseases caused by: Chlamydia, Syphilis, Gonorrhoea otherwise referred to as sexually transmitted diseases (Khong *et al*., 1986; Lwin *et al*., 1991; Shiers, 2001). However, these are not only bacteria reported to cause spontaneous abortions (Mirdamadi, 2005), as this was also attributed to the isolation of *E coli, Streptococcus agalectiea* from endocervical smears (ECS), and mid-stream specimen of urine (MSU) samples of Women who suffered miscarriages in Iran. From the result of the works reported, attention should also be be focussed on these other associated bacteria rather than on sexually transmitted diseases only. "A systematic review found that the cumulative risk of miscarriages between 5 and 20 weeks of gestation varied from 11 to 22% in studies assessing miscarriage rates" (Wikipedia, 2014) .

In Nigeria, there has been a paucity of records and reports of the role of *E. coli* infection in pregnancy related miscarriages (spontaneous abortions). Many cases of miscarriages caused by bacterial infections including *E. coli* in Nigeria are not captured because patients may have aborted before they could obtain medical help, partly because of the poor state of some health facilities, ignorance and inaccessibility of most of the people in rural areas (Greenwood, 1999). In a review from Ibadan described by Konge 1986, observed that 119 women with septic/induced abortions were admitted to the University College Hospital (UCH) between 1981 and 1985. And out of these, 82% had had induced/septic abortions. Single girls between the ages of 12 years to 20 years were the largest group (37%) and *E. coli* was the most common causative organism. In a similar report by *Chiwuzie et al*. (1995), working on cases of maternal mortality in a semi-urban Nigeria setting observed that globally, 500,000 women died at pregnancy and child birth yearly , most of whom are from developing countries and that bacterial infections including *E. coli* infection was one of the major causes. In Northern Nigeria, John, 2006 recorded 13.9% *Escherichia coli* in a group of women that suffered miscarriages in Jos, Kano and Bauchi.

In spite of the reports associating *E. coli* and other bacterial infections with cases of miscarriages (spontaneous abortions) in Nigeria and other parts of the world, there are no reports of this condition in the Upland Area of Rivers state in Southern Nigeria. Therefore, in order to determine the prevalence of *E. coli*, its complexities and sequalae among women with reported cases of spontaneous abortion, conducting this study has been considered worthwhile and necessary in the Upland Area of Rivers state. The study would further help to create more awareness, and necessary documentation for future reference for pregnant women and for those planning to

become future mothers, to take adequate precautions against agents of infections that cause miscarriages or spontaneous abortions.

Materials and Methods

Sample size determination

A suitable sample size of pregnant women was selected within the period of this study. The sample size was derived as follows: Expected rate of 27% of pregnant women was used (Naing *et al*., 2006) and a margin of sampling error tolerated was set at 5 , at 95% confidence interval, using the formula for prevalence studies:

$n=N^2P(1-P)/d^2$; where n= sample size; P= Prevalence rate=27%=0.27; d= 5%= 0.05; N= Statistical standard= 1.96.

3.8416 x 0.27x0.73/0.0025=302.87 for convenience=300

Study population

This is a cohort study on Women that suffered miscarriages (spontaneous abortions) whose ages range from 18 years to 30 years from the Upland areas (Ikwerre, Etche, Obio/Akpor, Omuma, Egbema) of Rivers state, who are predominantly farmers and illiterates.

The age range was chosen because according to (Olds *et al*., 2004; Novak and Broom, 1999) ages 40 years and above normally have complications, as well as those below 18 years due to physiological changes and nature.

Collection of mid-stream specimen of urine (mssu) and endocervical smears (ECS)

A total of 300 Endocervical smears (ECS) and Mid-Stream Urine (MSU) samples were collected from women between 8 hours to 24 hours after miscarriages, using speculum, swab sticks for ECS and sterile plastic containers (bottles) for urine samples. These women have earlier been certified healthy by their Doctors in the areas of: hormonal adequacy, uterine and cervical factors, uterine myoma including syphilis screening, before their conceptions.

Ethical clearance

Permission to study was obtained from the ethics and research committee of the hospital where the samples were collected and informed consent was also obtained from the Women who were involved in the study after explaining the purpose and the gain they will derive from the investigation.

Examination of the samples

The actual tests were carried out by adopting the methods of Ochei et al.2008 in which the ECS samples were cultured on chocolate, blood (incubated anaerobically and aerobically), MacConkey while the MSU samples were inoculated on **MacConkey** and CLED media, respectively and incubated aerobically. The inoculated plates were incubated aerobically and anaerobically at 37c for 24 h for growth. The growths (colonies) of the bacterial organisms were isolated using their colonial appearances, gram reactions, sugar fermentation, oxidase reactions, catalase and coagulase reactions as contained in Ochei *et al*. (2008). Chlamydia trachomatis screening was done on the endocervical smear samples using Chlamydia Rapid Test Device (CRTD) kit, which is a qualitative lateral flow immunoassay for the detection of Chlamydia antigen from genital smears and urine samples.

Statistical analysis

The data obtained were subjected to SPSS and T-test to establish relationship between *E. coli* infection and miscarriages (spontaneous abortion) and prevalence, and level of significance was taken as $P<0.05$.

Result and Discussion

The overall prevalence of 43.7% (Table 1) of bacterial infections among the women who suffered miscarriages in the area under consideration is embarassing. Nevertheless, the result is in agreement with the works of Toth (2014); Mirdamadi (2005); John (2006) and Konge (1986) who variously reported the presence of the same bacterial loads in their different studies.

Moreover, the 41.5% prevalence of *E. coli* (Table 2) in this study, is very disturbing and calls for serious attention, moreso, this is a major health threat to the majority of the young intending mothers who are mostly illiterate farmers in this area. This result is too high when compared with the 13.0 % result obtained in Kano, Jos and Bauchi (John, 2006). Nnevertheless, the high prevalence could be as a result of illiteracy, ignorance, environmental condition, unhygienic standard, poor state of health facilities and inaccessibility of most of the rural areas according to (Greenwood, 1999), and disagrees with those of (Verhaegen *et al.*, 2012; Wikipedia, 2014) who had 20.0 and 22.0%, respectively in their surveys. Facilities, manpower and technical knowhow may also play a role in the differences. However, irrespective of the discrepancies in the prevalences, this study and all the previous reports are of the opinion that *E. coli* is highly involved in pregnancy related miscarriages.

Table 1: Age related prevalence of bacterial organisms amongst the women

Ages (years)	No screened (%)	No negative (%)	No positive (%)
18 – 20	100 (33.3)	50(50.0)	50 (50.0)
21 – 25	100(33.3)	54 (54.0)	46 (46.0)
26 – 30	100(33.3)	66 (66.6)	34 (34.0)
TOTAL	300 (100.0)	170 (56.7)	130 (43.7)

P>0.05

Table 2: The Prevalence of Different Pathogens Isolated From ECS and MSSU Samples

Pathogens	ECS	MSSU	Frequency (%)
Neisseria gonorrhea	16	-	16(12.3)
Chlamydia trachomatis	11	4	15(11.5)
Escherichia coli	20	34	54(41.5)
Proteus mirabilis	3	8	11(8.5)
Staphylococcus saprophyticus	-	12	12(9.2)
Serratia species	-	6	6(4.6)
Klebsiella species	4	7	11(8.5)
Enterobacter species	-	5	5 (3.8)
Total	54	76	130(100.0)

P>0.05

Table 1 is the age-relate prevalence of bacterial organisms. 50% of the 18 – 20 years cohort were infected with different types of bacterial organisms while 21-25 years and 26-30 years had 46.0 and 34.0%, respectively. The high prevalence of infection amongst the 18-20 years was credited to high illiteracy; unhygienic living coupled with high level of promiscuity within the age cohort. Table 2 is the prevalence of different pathogens isolated from ECS and MSU samples. It shows the combined prevalence of various pathogens isolated. *E. coli* was the most prevalent. The involvement of *E. coli* infections in miscarriages (spontaneous abortions) due to production of phospholipase A2 enzyme had earlier been established by (Stamm, 1995; David *et al.*, 2010), in clearing the phenomenom,they maintained that the womb amniotic fluid and the environment in which the baby develops is normally sterile, however, sometimes microorganisms such as *E. coli* find their way into this environment causing the fetal membranes to become inflamed and infected, this they say is Chorioamnionitis which in itself triggers preterm contractions leading to miscarriages or preterm births.

Table 3: Prevalence of pathogens causing miscarriages amongst various age groups

Pathogens	18-20	21-25	26-30	Total
Neisseria gonorrhea	8	6	2	16
Chlamydia trachomatis	7	5	3	15
Escherichia coli	30	15	9	54
Proteus mirabilis	5	4	2	11
Staphylococcus saprophyticus	2	6	4	12
Serratia species	2	3	1	6
Klebsiella species	8	-	3	11
Enterobacter species	3	1	1	5
Total	**65**	**40**	**25**	**130**

P<0.05

Table 3 shows the prevalence of various pathogens encountered amongst various age groups. *E. coli* was the most prevalent among the age group 18-20 years and significantly associated with miscarriages amongst the group (P<0.05). This study has proved that *E. coli* infection is mostly in the age cohort, 18-20 years (Table 3). This could be attributed to the high promiscuity, illiteracy and inaccessibility to few available healthcare facilities,among the single and married women. This result corroborates the statement that E.coli was the most causative organism in the septic/induced abortions amongst single girls between the age cohorts of 12 years to 20 years (Konge, 1986) and buttressed the World bank's position that third world women in the most sexually active years are most vulnerable . It concluded that these age groups are persons with greatest sexual activity, and that incidence decreases with age (WHO, 2006).

The discovery of other bacterial organisms (Tables 3, 4 and 5) in this study is in line with the work of Chiwuzie *et al*. (1995), who stated that the knowledge of the involvement of other bacteria is useful in the correlation of instances involving *E. coli*, in order to obtain an overall picture of the role of this organism (*E. coli*) in spontaneous abortions (miscarriages). Despite the findings of the traditional bacterial organisms such as Neisseria gonorrhea and Chlamydia trachomatis known to be causative agents of spontaneous abortions (miscarriages), the high prevalence of the *E. coli* amongst this group of women suggests seriously that this bacterial organism plays a prominent role in miscarriages. This should refocus concern and to my mind more emphasis should be laid on it than the older ones that are known to be culprits.

Table 4: The prevalence of bacterial organisms isolated from ECS of women

Bacterial organisms	Frequency (%)
Neisseria gonorrhea	16(27.6)
Escherichia coli	20 (34.5)
Chlamydia trachomatis	15 (25.9)
Klebsiella species	4 (6.9)
Proteus mirabilis	3(5.9)
Total	**58 (100.0)**

Table 5: prevalence of pathogens isolated from MSSU samples of the women

Bacterial organisms	Frequency(%)
Escherichia coli	34 (47.2)
Staphylococcus saprophyticus	12 (16.7)
Proteus mirabilis	8 (11.1)
Klebsiella species	7 (9.7)
Enterobacter species	5 (6.9)
Serratia species	6 (8.3)
Total	**72 (100.0)**

In conclusion, *E. coli* infection is a serious health threat to the ordinary people of the area especially those who are pregnant, moreso, those planning to be future mothers. Therefore , there should be adequate enlightenment, in order to create more awareness about this silent but very disastrous infection in Upland area of Rivers state, and Nigeria as a whole. Health education of the people of this area is very important to promote among other things; cleanliness, good hygienic standard, quality and affordable education, and consciousness among the indigenes. It is also a wakeup call to the developing countries including Nigeria that *E. coli* infection is not an infection that should be ignored as an ordinary commensal, but should be taken seriously, thereby, preventing its devastating effects on pregnancy, women and unborn babies.

Conflict of Interest

No conflict of interest.

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