Evaluation of Standard Wireloop Method in Determination of Significant Bacteriuria among Pregnant Women in Ekpoma

Abstract
This study was carried out to evaluation of standard wireloop method in determination of significant bacteriuria among pregnant women in Ekpoma. A total of one hundred (100) early morning mid-stream urine samples from pregnant women attending antenatal clinics in Ekpoma and environs were collected aseptically using sterile, wide-mouthed, leak-proof universal bottles. Microscopic examination was carried out to detect the presence of pus cells, epithelial cells,red blood cells,yeast cells, crystals, parasites, bacteria and casts. Undiluted urine, 1:10and 1:1000 diluted urine samples were aseptically inoculated into blood agar and MacConkey agar using the Standard Loop method and compared with *Miles* and *Mirsa*method, it was incubated at 37^0C aerobically for 24 hours. After incubation colonies were counted and isolates identified by standard biochemical methods.Colony count of$\geq 10^5$ CFU per ml were considered as significant growth. In vitro antibiotics susceptibility of bacterial isolates was determined using disc diffusion technique. The data generated was analyzed statistically using the chi-squared statistics to ascertain the significance of the results. Out of 100 urine samples collected 39(39%) samples yielded significant bacterial growth, while 61(61%) of the samples had no significant bacterial growth or no growth with higher prevalence in age group <25 years which is a sexually active group than in other age group ≤35 years which had incidence rate of 12 (25%).Standard loop method gave false positive rates of 4/35 (11.4%) and false negative rates of 4/36 (11.1%).The standard lop was found to have a sensitivity of 100%, specificity of 88.9% and identical results in 76.3% of the samples. To reduce bacteriuria and consequently prevent UTIs, it is necessary to take the precautions by doing regular routine check-ups and appropriate treatment. For large scale urine surveys the method chosen should be reasonably accurate, fast and simple to use. The materials required should be minimal, stable and easily transported. It is recommended that the method of choice for most laboratories is the standard loop method.
Keywords: Wireloop, Bacteriuria,Urine, Pregnant,Miles,Mirsa

Introduction

The term "bacteriuria" means the presence of bacteria in urine (Ochei & Kolhatkar, 2000; Smaill & Vazquez, 2019). It may result from contamination during or after collection of urine or it may indicate the presence of bacteria in the bladder urine. And to distinguish among these possibilities led the introduction of the term “significant bacteriuria” which was defined as the occurrence of 10^5 or more bacteria per ml of a voided midstream urine aseptically collected (Hernández-Hernández *et al.*, 2021).Significant bacteriuria is a major indicator of urinary tract infection (Nicolle *et al.*, 2019).

Urinary tract infection is a persistent, actively multiplying bacteria within the urinary tract (Moore *et al.*, 2018). Bacterial infections of urinary tract are one of the most common from urinary tract infections for which antibiotics are prescribed (Colgan *et al.*, 2020). Bacterial infection of the urinary tract can be symptomatic or asymptomatic. The symptomatic urinary tract infection can be uncomplicated or complicated. Uncomplicated urinary tract infection is a symptomatic urinary infection characterized by frequency and urgency in urination, dysuria, or supra-pubic pain in a woman with a normal genitourinary tract (Kranz *et al.*, 2018). Complicated urinary tract infection is also a symptomatic urinary infection,but it is characterized with functional or structural abnormalities of the genitourinary tract which involve either bladder or kidney (Czwikla *et al.*, 2023).

Urinary tract infection is a persistent, actively multiplying bacteria within the urinary tract (Moore *et al.*, 2018). It can occur in both infants and adults without any age specificity and in up to 6% of healthy individuals (Nicolle *et al.*, 2019). It is usually associated with increased risk of intra-uterine growth and low birth weight in infants (Piazzolla *et al.*, 2024) and development of cystitis or pyelonephritis

especially in pregnant women (Grette *et al*., 2020). However, persons with certain condition and within some age groups could be at higher risk like; diabetics, elderly people,pregnant women kidney transplant patients, children with vesico-ureteral reflux and patients with kidney stones (Kim *et al*., 2019). Urinary tract infections occurs reliably more frequently in females as compared with males, to which the apparently higher incidence in females reflects the shorter urethra and lack of a prostrate and the higher incidence in teens and young adults representing urethral trauma due to active sex (Medina & Castillo-Pino, 2019; Agu *et al*., 2020).The prevalence of bacteriuria is 1-2% in school-age girls, 1-3% in pregnant women and 4-7% during pregnancy (Mohammed, 2021). The prevalence of bacteriuria increases with age, and sex ratio of infection becomes nearly equal in elderly (Mohammed, 2021).*Escherichiacoli* is the most common organism isolated from patients with bacteriuria, however other specieslike *Klebsiella* spp, *Proteus* spp, *Pseudomonasaeruginosa, Enterococcus* spp,group B *streptococci* and *Staphylococcus* spp (Hernández-Hernández *et al*., 2021).There are several cultural quantitative techniques for determining bacteriuria but standard wireloop method is the most popular method though relatively simple and cheap (Ochei & Kolhatkar, 2000).

Urinary tract infections (UTIs) are caused by the presence and growth of micro-organisms anywhere in the urinary tract and are perhaps the single commonest bacterial of mankind (Ajani *et al*., 2018; Averbeck *et al*., 2018). It is one of the most common causes of hospitalization and referral among pregnant women,having an estimated figure of 150 million per annum worldwide (Chockalingam *et al*., 2019; Kurt Şükür *et al*., 2020). The leading causes of UTIs have beenreportedtobedue to *Escherichia coli, Klebsiella* spp, *Proteus* spp,*Pseudomonas aeruginosa, Enterococcus* sppand *Staphylococcus aureus* (Akortha *et al*., 2014; Mohapatra *et al*., 2022). Standard wireloop method is a semi quantitative method(Ochei & Kolhatkar, 2000).Urinary tract infection is one of the recurrent infection among teenager,adult and pregnant women who are sexually active. Screening subject for bacteriuria is therefore necessary as bacteriuria has adverse outcomes that can be prevented by antimicrobial therapy (Wingert *et al*.,2019). Apart from that, there is need to evaluate the effect of wireloop in determining bacteriuria.The aim of the research work is to determine the prevalence urinary tract infection (significant bacteriuria) among pregnant women in Ekpoma and also evaluate the effect of wireloop on significant bacteriuria.

Materials and Method

Area of Study

This study was carried out in Ekpoma. The administrative headquarter of Esan West Local Government Area of Edo State which lies between latitude 6.45'N to 6.75'N of the Equator and longitude 6.08'E to 6.13'E of the Greenwich Meridian with altitude of about 332m above sea level (Aziegbe, 2006).It is made up of quarters including Eguare, Iruekpen, Emaudo, Ujoelen, Ihumudumu,Illeh,Uke, Uhiele, Ujemen,Ukpenu, Idua,Ukhur, Egoro,Emehi, Igor and Idumebo(Aziegbe, 2006). Ekpoma has a population of 89,628 in 1991 and 127,718 in 2006, majority of which are civil servants, traders, business men/women, transporters, farmers, teachers/lecturers and students by occupation.A university (Ambrose Alli University) is situated in this town. The main sources of water in the locality are rainfall and wells. It is has 2 distinct seasons, wet and dry seasons. The wet season occurs between April and October with peck in August, average rainfall ranging 150cm to 250cm. The dry season occurs between November and March with cold harmattan between December and January,average temperature of about 25^0C (Edo state of Nigeria,1992).

Study Population

The study population of this research work comprise of pregnant women (n=100)within age of 20-45years in Ekpoma.

Ethical Approval and Informed Consent

Ethical approval for the collection of samples was obtained from the Ministry of Health, Benin City, and from Ambrose Alli University, Ekpoma, both in Edo State. Informed consent was also obtained from each subject who participated in the study before the collection of blood sample.

Collection of Samples

A total of 100 (hundred) early morning mid-stream urine samples were aseptically collected using sterile, wide-necked, leak-proof universal container from pregnant women (aged 20 to 45 years) in Ekpoma after giving their informed consent. The ages as well as the history of urinary tract infections (UTIs) were obtained from the subject before collection of samples. Samples wore not be collected from subject are on antimicrobial therapy at time of the sample collection or who had been on antimicrobial therapy within 2(two) weeks prior to sample collection.After collection, the samples were immediately sent to the laboratory for analysis.

Sterilization of Materials

The steps involve in the sterilization process are:

a. The entire work bench was disinfected with 0.5 hypochlorite solution.
b. All glass wares (i.e pipette, Durham tubes, bijou bottles, universal bottles beakers) were washed with detergent and sterilized usinghot air oven at 160□for 1 hour.
c. Standard wireloop was flamed red-hot to sterilized.

Microscopic Examination

Two (2) loopful of uniformly mixed uncentrifuged urine samples was placed on a grease-free slide and covered with coverslip. It was examined microscopically to detect the presence of pus cells, epithelial cells, red blood cells, yeast cells,crystals, casts,parasites and bacteria using x10 and x40 objectives with condenser iris closed sufficiently to give good contrast (Cheesbrough,2005).

Preparation of wire-loop

A wire loop was be made and labeled. 0.1ml of water was pipetted into a clean test tube. A wireloop of 4mm in diameter was used remove loopful from the test tube and placed on a filter paper to determine the number of loopful per 0.1ml (Ochei & Kolhatkar, 2000;Cheesbrough, 2005). It was performed thrice with 18,18 and 15 loopful respectively, and average taken.

$$\frac{18 + 18 + 15}{3}$$

=17 loopful

If 17 loopful is present in 0.1ml

1 loopful will contain X

$X = \frac{0.1 \times 1}{17}$ =0.006ml

Standard Wireloop Method

The calibrated sterile wireloop (flamed red hot) was held vertically and the loop into a well mixed uncentrifuged urine. A loopful of urine was inoculated into prepared blood agar and MacConkey agar, and incubated at 37°C aerobically for 24hours. After incubating the cultures, count bacterial colonies. A single colony is accepted to represent one colony forming unit (CFU) (Ochei & Kolhatkar, 2000;Cheesbrough, 2005). Bacterial count/ml is obtained by first principle (bacterial count divided by volume wireloop) (Ochei & Kolhatkar, 2000).

0.006ml of urine will contain X

$X = \frac{100000 \times 0.006}{1}$ =600CFU

This implies that using 0.006 ml wireloop with diameter of 4mm, 600colony forming unit (CFU) is equivalent to 10 CFU/ml (significant bacteriuria).

The Miles and Misra Surface Viable Count

10-fold dilutions of each urine were made using normal saline as the diluents. 0.04ml volume of the undiluted and of each dilution from 10-' to10-3 were dropped onto labelled sectors on the surface of well dried MacConkey agar and Blood agar plates using a calibrated dropper pipette.

If 1 ml of urine contains 100,000 CFU

0.04ml of urine will contain X

$$X = 4000 \text{ CFU} = \frac{100000 \times 0.04}{1}$$

This implies that using 0.04 ml calibrated dropper pipette, 4000 colony forming unit (CFU) is equivalent to 105 CFU/ml (significant bacteriuria).

Statistical Analysis
Percentage and chi-square test were used to analyze the data obtained from this study.

Results
Out of 100 urine samples collected, 26 of the urine samples did not show any growth at all, 16 urine samples showed there was growth of a single bacterial specie, 55 urine samples showed mixed growth of 2 or 3 species with 1 specie clearly predominating in 23 samples and 3 samples showed growth that was too mixed (>3 types) for accurate counts to be made these were eliminated from this study.

Table 1 shows that of the 100 urine samples examined; for the standard loop method revealed 26 (26%) had no growth, 32 (32%)had non significant growth, 39 (39%) had significant growth and 3 (3%) had mixed growth as compared with Miles and *Misra* method which revealed 26 (26%) had no growth,36 (36%) had non significant growth, 35 (35%) had significant growth and 3(3%) had mixed growth. Although the Miles and Misra method had 35(35%)significant growth while the Standard loop method had 39 (39%) significant growth,but the difference was not statistically significant.

Table 2shows the age distribution of some abnormal parameters found in urine samples as 22(22%), 19(19%),9(9%), 4(4%),17(17%) and 21(21%) had epithelial cells, pus cells, yeast cells, crystals, casts and red blood cell respectively.The result shows that aged group>35 years had the highest number of epithelial cells;while the highest yeast cells were found in aged group <35 years.

Table 3 shows the age distribution of some abnormal parameters in urine samples examined, it revealed a higher occurrence in age group ≤35 years which had incidence rate of 12 (25%) and age group ≥35 years which had incidence rate of 6 (11.5%). The difference was not statistically significant.

Table 4 summarises the result of the viable bacterial count of standard loop method to the reference method (Miles and Mizra). Standard loop method gave false positive rates of 4/35 (11.4%) and false negative rates of 4/36 (11.1%).The standard lop was found to have a sensitivity of 100%, specificity of 88.9% and identical results in 76.3% of the samples.

Table 1: Growth pattern of all samples examined using Standard loop method as compared with Miles and *Misra* surface count method

	Number of sample examined	No growth	Insignificant growth($<10^5$)	Significant growth ($<10^5$)	Mixed growth
Miles and Misrasurface countmethod	100	26	36	35	3
Standard loop method	100	26	32	39	3

$X^2 = 0.343$ p=0.558

Table 2: Age distribution of some abnormal parameters in urine samples examined.

Age (years)	Samplesexamined	Epithelialcells	Puscells	Yeastcells	Crystals	Casts	Redbloodcells
≤35	48	10	10	5	2	7	8

≥35	52	12	9	4	2	10	11
Total	**100**	**22**	**19**	**9**	**4**	**17**	**21**

Table 3: Age distribution of significant bacteriuria in samples examined.

Age(years)	Number Examined	Number Infected	Prevalence (%)
≤35	48	12	25
≥35	52	6	11.5

$X^2 = 3.064$ p=0.080

Table 4: Results of viable bacterial count by the method.

Organisms/ml	Miles and Misra surface count method	Standard loop method
$>10^5$	35	39
$<10^5$	36	32
No growth	26	26
Total no of specimens	97	97
False positive number and rate	-	4/35(11.4%)
False negative number and rate	-	4/36(11.1%)
Identical result	-	77/97(76.3%)

Sensitivity=$\frac{\text{No of specimens}>10^5 \text{ org./ml by test method}}{\text{No. of specimens}>10^5 \text{ org./ml by Miles \& Misra}}$ x100

Specificity = $\frac{\text{No of specimens }>10^3 \text{ org./ml by test method}}{\text{No. of specimens }>10^3 \text{ org./ml by Miles \& Misra}}$ x100

Discussion

The susceptibility of urinary tract infections pregnant women among is a major concern to health due to high mortality and morbidity, and the standard wireloop method is the most popular semi-quantitative culture method for diagnosis and evaluation of urinary tract infections. It is cheap, simple to perform, and provides individual colonies that are easier to identify and remove for antimicrobial susceptibility testing.This is a very small study undertaken to compare the relative merits of standard loop method which is a semiquantitative culture test that makes use of a calibrated loop.

In this study, 100 mid stream urine samples of pregnant women were examined for evidence of urinary tract infections (significant bacteriuria) and evaluation of standard wireloop method. Out of the 100 urine samples examined 39(39%) showed significant bacteriuria. This result is in agreement with the study of Ezugwu et al, (2021) who reported a prevalence of 37% of significant bacteriuria among pregnant women in Enugu, Nigeria. However, this result is in disagreement with previous studies which reported lower prevalence of 4.8%of among students of Ambrose Alli University, Ekpoma (Iyevhobu *et al.*, 2020). It was also lower than 5%incidence among students of secondary school as reported by Agu et al, (2020).

The difference in prevalence rate could be as a result of endogenous infection or mere commensals leading to opportunistic infection (Grette *et al.*, 2020).Pregnant women are also prone to urinary tract infections due to the short ureter of women and lower immunity of women during pregnancy.Microscopic examinations of urine sample revealed the presence of epithelial cells, crystals, pus cells cast cells yeast cells and red blood cells (Kim *et al*., 2021).

Furthermore, the highest significant bacteriuria was found among subjects of the age group <35 years and this is in agreement with the study of Shaikh et al, (2020). They reported a high a prevalence of bacteriuria within the age range of <35 years. However, this result is contrary to the findings of Obeagu et al, (2023) whose highest significant bacteriuria was reported in the age range>35 years. It has often been postulated that men and women in this age range of 16-25 years are sexually active and this may predispose them to UTI (Cheesbrough,2005).

The standard lop was found to have a sensitivity of 100%, specificity of 95.5% and identical results in 76.3% of the samples. This result is in agreement with the study of Thass et al, (2019) who reported a sensitivity of 100%,specificity of 97.5% and identical results in 74.7%.

Conclusion

An ideal method for detecting significant bacteriuria is one that is simple,inexpensive, accurate and convenient its use. It should also not require too much skill.Standard loop require the use of culture media with may or may not be readily available to private clinics/laboratories in this country. Most strips are affected by antimicrobial agents and fails to detect yeast and slow growing streptococci, thereby restricting it usefulness. This problem can be avoided if a l/4orl/2 plate of blood agar is streaked with loopful of undiluted urine. Also, most strip are slightly more difficult to interprete when compared with the Standard loop,it was found to be simple, fast, inexpensive and gave acceptable sensitivities and specificities. Other medical personnel reported similar findings.

For large scale urine surveys the method chosen should be reasonably accurate, fast and simple to use. The materials required should be minimal, stable and easily transported. Therefore, as recommended the method of choice most laboratories and most suitable method for this purpose will be Standard loop method.

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