

Influence of Age and Gender on the Quantification of Uropathogens Via Streak Plate Technique in Urine Subjects from University of Medical Sciences Teaching Hospital Complex, Akure, Nigeria

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Abstract:- This study was designed to highlight the effects of age and gender on the bacterial count of urine subjects on Cysteine Lactose Electrolyte Deficient and MacConkey agar media using streak plate method as precursor to predict the onset of suspected and unsuspected Urinary Tract Infections (UTIs). Urine samples collection and culture-based enumeration of uropathogenic bacterial count was carried out using standard methods. Data obtained from the enumerated and quantified bacterial counts was analysed using standard techniques. Three age brackets of 21-30, 41-50 and 51-60 yrs in male urine subjects (37.5 %) and five age cohorts of 21-30, 31-40, 41-50 yrs, 51-60 yrs and 61-70 yrs in females (62.5 %) contains bacterial counts higher than the benchmark of 105 Colony forming Units per millilitre (CFU/ml). Male subjects with the age range of 21-30 yrs produced the highest bacterial count (238 CfU/ml) as age range of 1-10 yrs produced the lowest bacterial enumeration (19 CfU/ml). The age

range of 31-40 yrs elicited the highest bacterial enumeration (418 CfU/ml), and age range of 71-80 yrs (15 CfU/ml) among female subjects, with CLED and MAC shunning out varying high bacterial counts for diverse age sets with respect to gender and slight to appreciable discrepancies. Polynomial regression slope also aided the predictability of UTIs onset among the age cohort of 21-30 yrs and lower onset of UTIs among the age set (1-10 yrs) in male subjects and a high onset of UTIs in the age range of 31-40 yrs with least onset of UTIs demonstrated in the age assemblage of 71-80 yrs in female subjects. General inference from this study has revealed the usefulness of specific selective/differential media in the quantitative analysis of urine samples in predicting the onset of UTIs highlighting age and gender.

Keywords:- Age; Cysteine Lactose Electrolyte Deficient agar; Gender, MacConkey agar; Urinary tract infections.

I. INTRODUCTION

Urinary Tract Infections (UTIs) can be branded as either straightforward or complex. Non-symptomatic UTIs characteristically impinge on persons who are else healthy and anatomical/physiological make-up of their urinary tract anomalies are non-existent [9]. UTI is nominally described also as the augmentation of an implicated bacterial organism of closer to 10³ Cfu/ml or more specifically associated with culture-based urine bacterial analysis [22].

Throughout the life span of humans, UTIs arises by 30% in females and by 1% in males. Almost 75% of newborns lower than 3 months old with bacteriuria are males; a rate which skyrockets to 10% amid 12 to 32 weeks of life, and later than 1 year of age, it is experienced only in girls [9]. UTIs severity differs by age and gender, as women and elderly are more vulnerable than men and younger individuals [13, 7]. UTIs are especially widespread uropathogenic disease in women. They frequently crop up amid the ages of 16 and 35 years, with 10% of women getting infected annually and additionally 40% to 60% experiencing it on occasion in their life time at the slightest. Relapses are frequent, with almost not whole experiencing the disease within a year. UTIs take place more frequently in females than males at a ratio of 4:1 [16, 3].

Women could be commonly vulnerable to UTIs due to their urethral aperture's propinquity to the origin of bacteria and the frame of the urethra is petite thus augmenting bacterial route to the bladder owing to the nearness of the urethra and vagina for those of sexually-active age [20]. Women within the reproducing age of 15-50 years are at high risk at a rate of 50-80% of experiencing UTIs (Kibera *et al.*, 2009).

In urine culture, applicable media are meant to augment the cultivation of all urinary pathogens and stall potential contaminating bacteria consortium. Customarily, traditional media like Blood agar (BA) and MacConkey agar (MAC) are being used in conjunction for enumeration of uropathogens specially in the second-world countries for

a while now and Cystine lactose electrolyte-deficient (CLED) agar, a selective culture medium used in characterizing bacteria from urine that sustain the growth of uropathogens and contaminants while thwarting unwarranted swarming increase of *Proteus* spp owing to the absence of electrolytes in the media composition has also come of age [8]. In the same vein, disparity of lactose fermenter and non-fermenter is possible on MAC and CLED agar [17]. Cystine, one of the components of CLED allows the enumeration of coliform bacteria colonies [11]. Hence, the media (CLED and MAC) allows quantification of uropathogens together with *Proteus* which grows on MAC being a selective media with inoculating loop usage enhanced by streaking method. Therefore, the influence of age and gender on the quantification and multiplicity of uropathogens enumerated from urine subjects in the University of Medical Sciences Teaching Hospital Complex, Akure, Nigeria using CLED and MacConkey agar media was juxtaposed in this study.

II. MATERIALS AND METHODS

A. Study design

This study is a hospice-inclined cross-sectional field study of patients or urine subjects with suspected and unsuspected (Urinary Tract Infections) UTIs through quantitative analysis. It is designed to quantify the upshot of age and gender on the bacterial count of urine subjects using selective media (CLED and MAC agar media). Polynomial regression was used to illustrate the spread of UTIs in male and female subjects.

B. Description of the study area

The University of Medical Sciences Teaching Hospital Complex, Akure (UNIMEDTH) formerly State Specialist Hospital, Akure, with coordinates 7.2421° N, 5.1957° E is situated at hospital road, Akure in Akure South Local Government Area of Ondo State (Figure 1). It is a state-run teaching hospice recently merged with other health centres in the state.

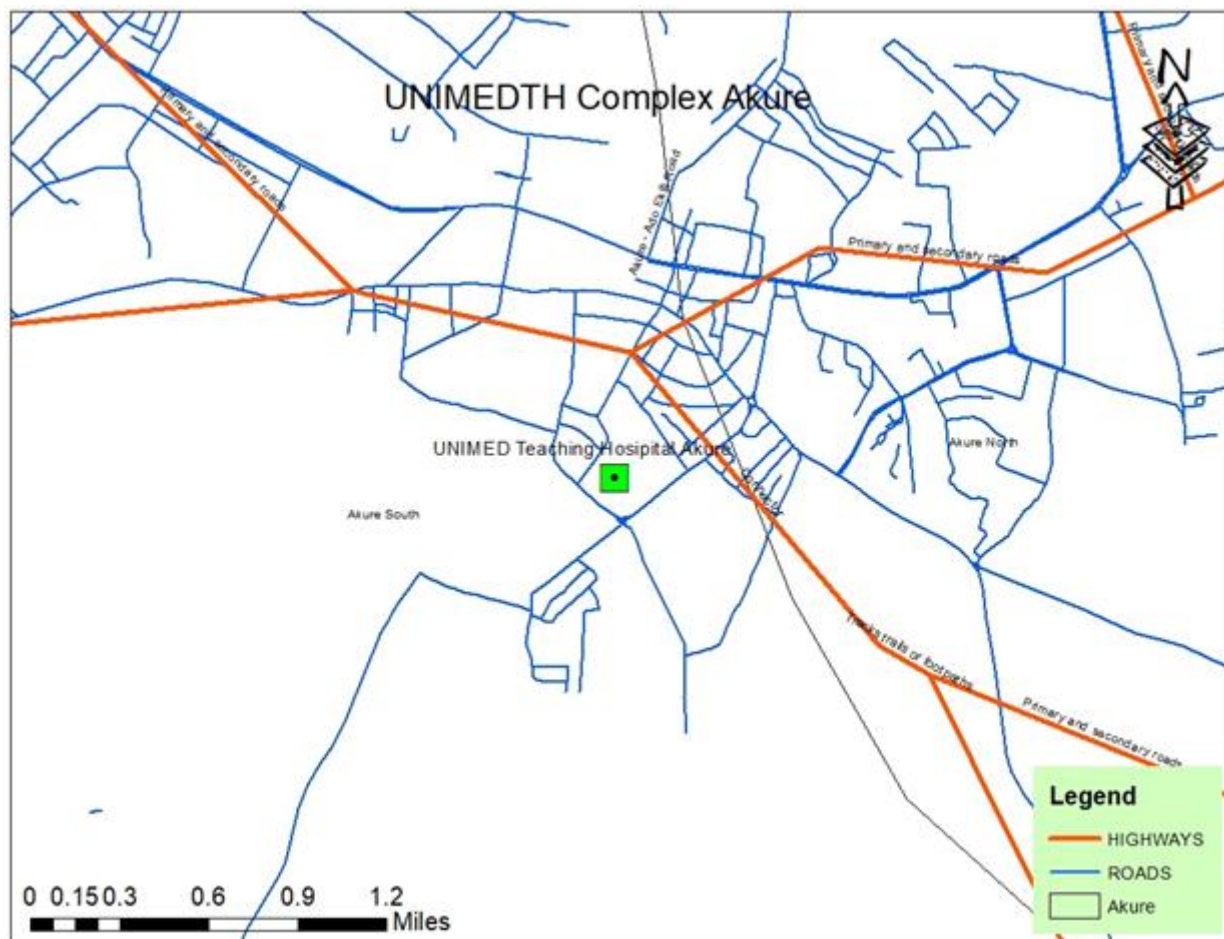


Fig 1:- Geographical Information System (GIS) map of University of Medical Sciences Teaching Hospital, Akure, Akure south local government, Ondo state.

C. Sample collection

A hundred and six (106) urine samples were collected for duration of 3 months from January, 2019 to March, 2019 with the aid of sterile screwed-capped universal bottles with the serial number, age and sex of urine subjects labelled accordingly. The samples were bacteriologically-analysed within 1 hr of collection.

D. Culture-based isolation

Selective media including; Cystine lactose electrolyte deficient (CLED) agar and MacConkey agar (Oxoid, UK) were used for the inoculation of the urine samples using sterile wire loop. The plates were incubated at a temperature of 37 °C for duration of 24 hr and observed for growth. Colony counts were determined/counted on CLED and MAC agar plates at the end of the standard incubation periods for each of the respective media using a colony counter (J-2 Colony Meter PEC MEDICAL, USA). Urine samples from both male and female subjects containing over 105 CfU/ml (colony forming units per millilitre) were noted to be a high-risk factor of predicting or speculating the onset of UTIs in urine subjects as described by Cheesbrough [6].

E. Data analysis

Microsoft Excel 2007 was used to analyze data. ArcGIS (version 10.6) was used for the mapping of the study area. Figures obtained from total bacterial count and age range of urine subjects was analysed by Anaconda version 3.5.2.0 (Spyder-Python 3.6).

III. III.RESULT

Among the 106 urine samples collected from urine subjects in males, 3 (37.5 %) age brackets 21-30 yrs, 41-50 yrs and 51-60 yrs contains colony forming units per millilitre of 238, 182 and 124 CfU/ml respectively, while 5 (62.5 %) age cohorts 21-30 yrs, 31-40 yrs, 41-50 yrs, 51-60 yrs and 61-70 yrs contains 317, 418, 197, 235 and 108 CfU/ml respectively in female urine subjects all of which are greater than the benchmarked 105 CfU/ml which serves as a pointer or a rider to the onset of UTIs. Male subjects with the age range of 21-30 yrs produced the highest enumeration of bacterial count (238 CfU/ml) as age range of 1-10 yrs produced the least bacterial enumeration (19 CfU/ml) as shown in Figure 2. The age range of female subjects between 1-10 produced or elicits no bacterial count, while the age range of 31-40 yrs elicits the highest bacterial enumeration or count (418 cfu/ml), and age range of 71-80 yrs had 15 CfU/ml among female subjects as shown in Figure 3.

The age range of 21-30 yrs showed MAC agar medium had the highest bacterial count in supporting the growth of more uropathogens (122 Cfu/ml) than CLED agar (166 Cfu/ml); while the age range of between 1-10 yrs (least age bracket) elicited the least bacterial count of 13 Cfu/ml on MAC agar medium and 6 Cfu/ml on CLED agar medium as displayed in Figure 4. The age bracket of 21-30 yrs produced the maximum bacterial count of 116 Cfu/ml on CLED agar medium and a minimum bacterial count of 6 Cfu/ml at the age bracket/age group (1-10 yrs). The age cohort of 21-30 also demonstrated the maximum bacterial count of 122 Cfu/ml and the age range of 1-10 yrs shun out the minimum bacterial count of 13 Cfu/ml on MAC agar as also illustrated in Fig 4.

The age range of between 31-40 yrs showed CLED agar medium had the highest bacterial count in augmenting the growth of more uropathogens (228 Cfu/ml) than MAC agar (190 Cfu/ml); while the age range of 71-80 yrs (highest age bracket) brought about the least bacterial count

of 7 Cfu/ml on CLED agar medium and 8 Cfu/ml on MAC agar medium as displayed in Figure 5. The age bracket of between 31-40 yrs produced the most bacterial count of 228 Cfu/ml on CLED agar medium and a minimum bacterial count of 7 Cfu/ml at the age range of 71-80 yrs. The age group of 51-60 yrs demonstrated the maximum bacterial count of 150 Cfu/ml whereas the minimum bacterial count of 8 Cfu/ml from age group 71-80 yrs on MAC agar medium as also shown in Figure 5.

Polynomial regression analysis of total bacterial count against age range of male urine subjects showed a high onset of UTI disease among the age cohort amid 21-30 yrs and lower onset of UTI disease among the age set 1-10 yrs. High onset of UTI disease was also exhibited in the age range of 31-40 yrs with least onset of UTI disease juxtaposed in the age assemblage of 71-80 yrs as the age cohort of between 1-10 yrs displayed no onset of UTI disease in female subjects.

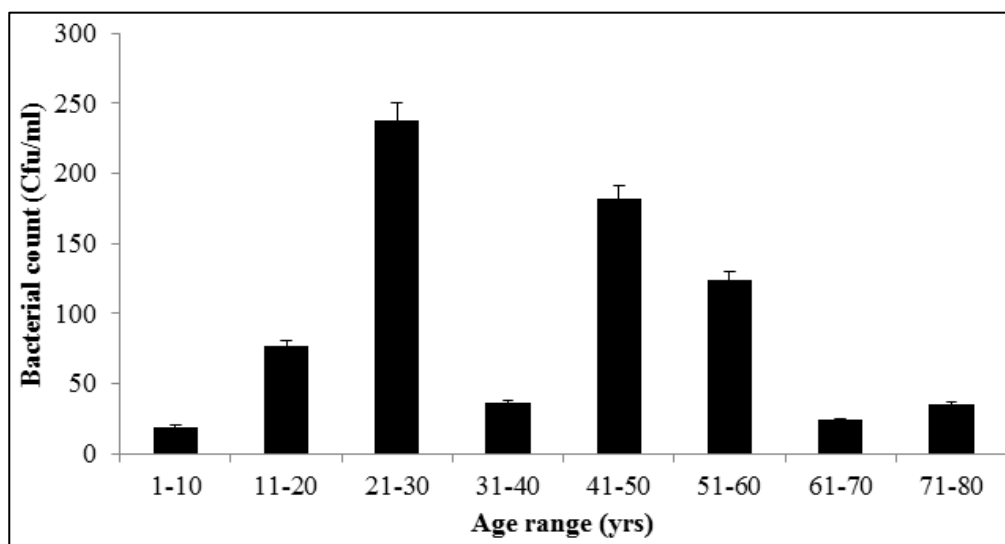


Fig 2:- Frequency of age range and bacterial count of urine samples from male subjects

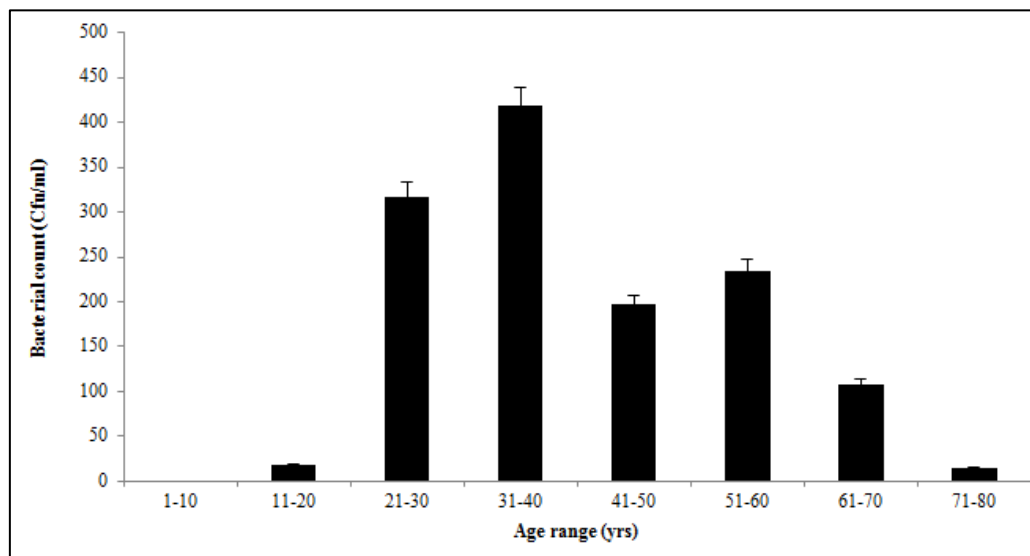


Fig 3:- Frequency of age range and bacterial count of urine samples from female subjects

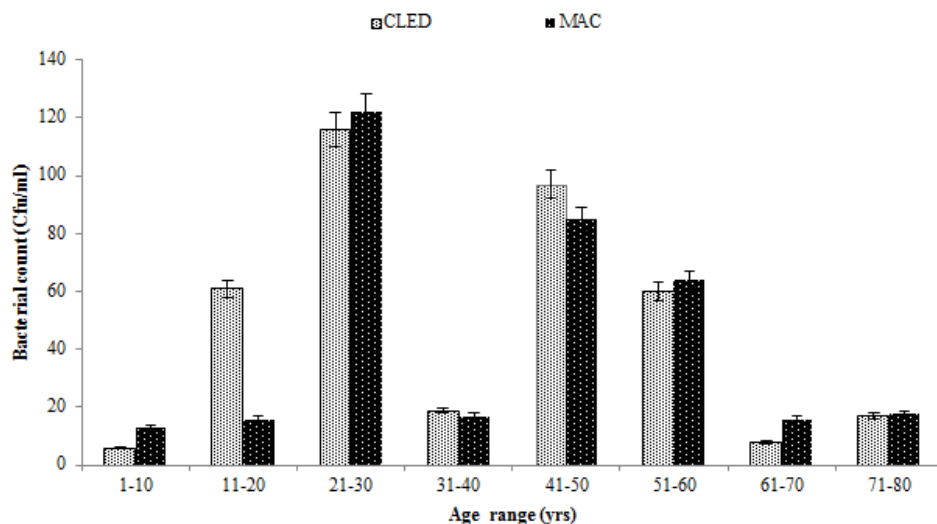


Fig 4:- Age and gender-related bacterial count of male urine subjects on CLED and MAC agar medium
Keys: CLED-Cysteine Lactose Electrolyte Deficient agar; MAC-MacConkey agar

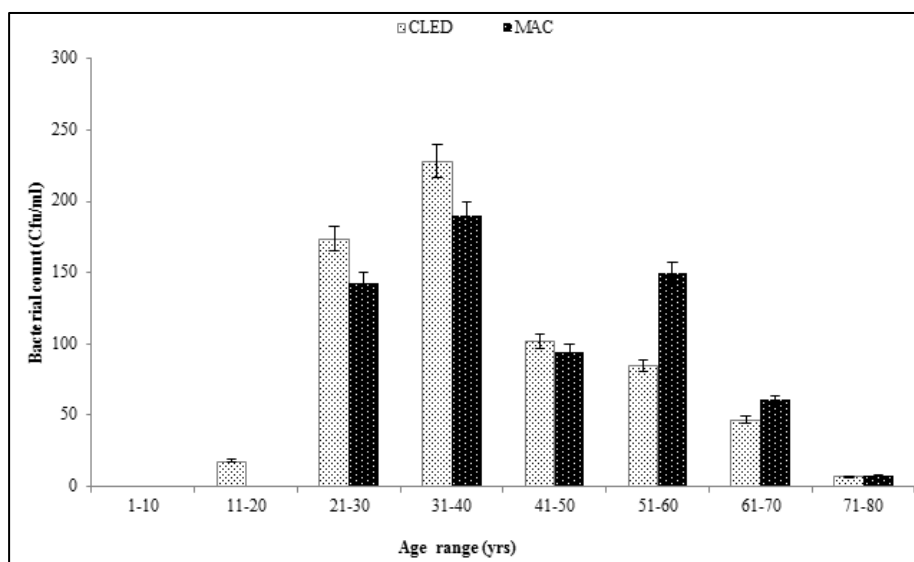


Fig 5:- Age and gender-related bacterial count of female urine subjects on CLED and MAC agar medium
Keys: CLED-Cysteine Lactose Electrolyte Deficient agar; MAC-MacConkey agar

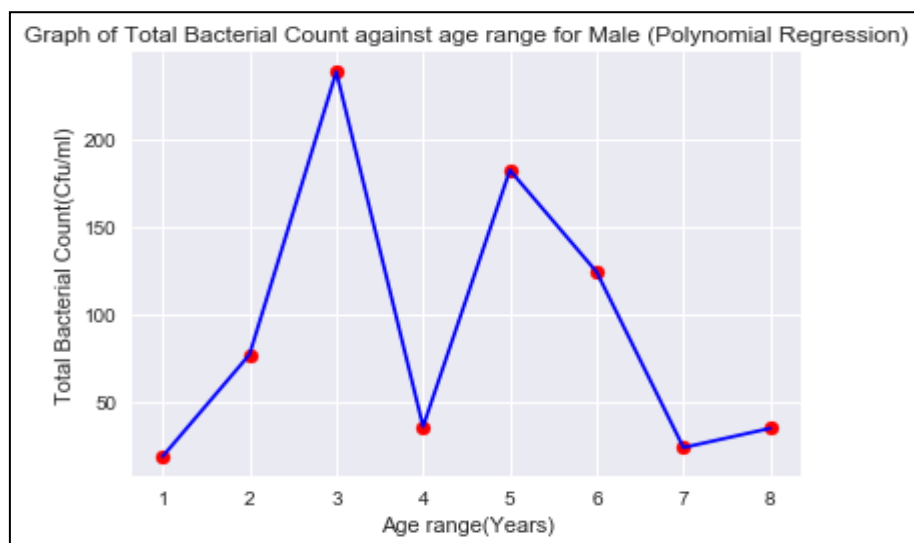


Fig 6:- Polynomial regression of total bacterial count against age range of male urine subjects
Keys: 1=1-10 yrs; 2=11-20 yrs; 3=21-30 yrs; 4=31-40 yrs; 5=41-50 yrs; 6=51-60 yrs; 7=61-70 yrs; 8=71-80 yrs

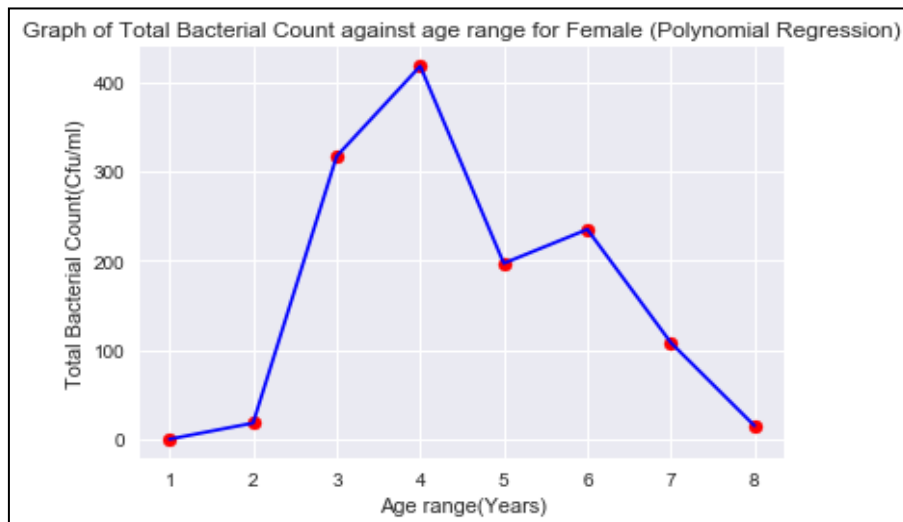


Fig 7:- Polynomial regression of total bacterial count against age range of female urine subjects

Keys: 1=1-10 yrs; 2=11-20 yrs; 3=21-30 yrs; 4=31-40 yrs; 5=41-50 yrs; 6=51-60 yrs; 7=61-70 yrs; 8=71-80 yrs

IV. DISCUSSION

The tendency of the incidence of UTIs in males at 37.5% is parallel with 32% detailed by Mane [12] and Preethshree [15] with a slender disparity of just 5%. The rationale for this might be due to a conventional practise of masculine circumcision at birth which has a tendency to potentially lessen the predisposed risk factors of UTIs to a large extent as supported by Singh-Grewal [19]. The fathomed occurrence of UTIs in childhood is of elevated peril in non-surgically-excised males than in circumcised males as opined by Schone [18]. The 67.2% juxtaposed by Abubakar [1], and Yasmeen [21] and 62.0% demonstrated by Anejo-Okopi [4] for frequency of UTIs in female urine subjects aligns with 62.5% across 5 age sets observed in this study which may perhaps is owed to their urethral aperture's nearness to the origin of bacteria (causal agent) hence facilitating bacterial passage to the bladder owing to the proximity of the urethra and vagina due to their sexually-active habits as stated by Tambekar [20].

Bashir [5] in his work on the diversity of urinary tract pathogens in dissimilar age and gender groups of Pakistanis observed the emergence of high tendency of (UTIs) in male subjects than in female subjects of less than ten years of age which bears compatibility to the observations of this study as no bacterial count was noticed in the 1-10 yrs age bracket for female subjects which could owed to the high probability of UTIs cropping up early at teenage and youthful years of between 16-35 yrs in females in equivalence with Sakamoto et al. [16] and Alperin [3]. Bashir [5] observed the age cohort of 20 years and above among female subjects displayed two folds of UTIs tendency in male subjects which bears resemblance with the respective higher bacterial count in 21-30 and 31-40 age sets displaying skyrocketing figures in female subjects. As observed by Bashir [5], the age assemblage of 40 yrs+ and 60 yrs+ in female subjects showed higher tendency of the UTI disease was demonstrated respectively than the age-matching male subjects also in alignment with the result of this study. The 70 years and above age range in male

subjects showcased double figures in comparison with the lower figures with the same female age cluster recorded by Bashir [5] which is also analogous to the proceeds of this study.

The higher yield of bacterial count noticed in MacConkey agar medium could be due to it being useful for the enumeration of the bacteria family of *Enterobacteriaceae* which includes predominantly total coliforms, with lactose and non-lactose fermenters in male urine subjects which concurs with Akter [2]. Similarly, somewhat inferior-yielding rate on MAC agar can be clarified by its drawback of prohibiting all organisms associated with UTIs like *Staphylococcus saprophyticus* and *Enterococcus faecalis* from not growing in as much it is a selective medium for members of *Enterobacteriaceae* bacteria group, a notion supported by Akter [2] and Sekikawa [17]. The higher yielding-rate of bacterial count observed in CLED agar medium was due to its peculiarity for the quantification of uropathogenic bacterial consortium and its ability to distinguish between lactose and non lactose fermenters like MAC agar medium also observed by Akter [2] and Sekikawa [17]. Consequently, MAC prohibits the growth of gram positive organisms and haemolytic organisms, which is a major defect and hence a criterion for its lower yield of bacterial count.

Findings from the polynomial regression analysis of predicting onset of UTIs bears some semblance with the observations of Nakamura [14] who predicted uropathogens in urine specimens by flow cytometry and dip-stick test results via multivariate logistic regression analysis.

V. CONCLUSION

General inference from this study has revealed the usefulness of specific selective/differential media in the quantitative analysis of urine samples in predicting the onset of UTIs highlighting age and gender using the customary streak plate method.

ETHICAL CONSIDERATION

Ethical approval was obtained from the Ondo state health research ethics committee (OSHREC), ministry of health, Akure, Ondo State with a protocol number of OSHREC/16/09/2019/245.

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