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CHARACTERIZATION OF UROPATHOGENIC *ESCHERICHIA COLI* (UPEC) IN HIV SEROPOSITIVE WOMEN WITH ASYMPTOMATIC BACTERIURIA

BY:

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DECLARATION

I declare that this thesis is the product of research conducted by Harold Kwadzo Amegbletor at the Medical Microbiology Department of the University of Ghana Medical School under the supervision of Professor Japheth A. Opintan and Dr. Appiah-Korang Labi and to the best of my knowledge, has not been conducted nor presented for any other degree nor processional qualification at this or any other institution except as referenced.

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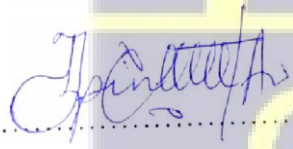
Sign:  Date: 24/08/2022

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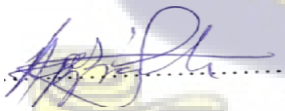
Sign  Date: 24/08/2022

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DEDICATION

I dedicate this work to my father, Rowland Kwaku Amegbletor; my mother, Regina Boateng, and to all loved ones

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ABSTRACT

BACKGROUND: Asymptomatic bacteriuria (ASB), a precursor for Urinary tract infection (UTI), is high among People living with HIV (PLHIV). *E. coli* is the most implicated organism. An understanding of the risk factors for ASB, virulence genes and resistance profile of uropathogens among this population is important to the management and control.

AIM: To characterize uropathogenic *E. coli* (UPEC) in HIV seropositive women with asymptomatic bacteriuria

METHODOLOGY: This cross-sectional study was carried out at St. Martin de Pores Hospital, Eikwe in the Western Region of Ghana. A structured questionnaire was used to extract clinical information from the folders of 400 HIV seropositive women. The information included patient demographics, history of hospitalization, HAART treatment initiation date and WHO disease stage. Urine samples were obtained, cultured and identified with MALDI TOF biotyper. Antibiotic resistance pattern was determined, and genes coding for virulence and integrons were screened for using Multiplex PCR and gel imaging techniques.

RESULTS: From the 400 samples cultured, 21.15% (85/400) were positive. The most prevalent organisms were; *Escherichia coli* 69.4% (59/85), *Enterococcus faecalis* 8.2% (7/85), *Klebsiella pneumoniae* 7.0% (6/85)), *Proteus mirabilis* 7.0% (6/85), *Staphylococcus hemolyticus* 3.5% (3/85). All uropathogenic *E. coli* isolates were resistant to ampicillin and 98.3% (58/59), resistant to trimethoprim sulfamethoxazole, followed by tetracycline 94.9% (56/59), cefuroxime 74.6% (45/59), amoxiclav 49.2% (30/59) and ciprofloxacin 32.2% (19/59). The lowest resistance was recorded to meropenem and

fosfomycin at 1.7% and nitrofurantoin 6.8% (4/59). The commonest virulence genes observed were *ChuA* 66.1% (n=39/59), *PapC* 57.6% (n=34/59), *cnf1* 50.8% (n=30/59), *kpsMTII* 45.8% (n=27/59), *iuAt* 35.6% (n=21/59) and *usp* gene 8.5% (n=5/59). Two isolates (3.4%) harbored all 5 genes (*iuAt*, *cnf1*, *papC*, *chuA*, *kpsMTII*). Five isolates haboured *iuAt*, *cnf1*, *papC* and *chuA* (8.5%, n=5/59). Three genes (*iuAt*, *cnf1*, *papC*) were all observed in 6 isolates (10.2%, n=6/59) whilst 7 isolates (12%, n=7/59) were found to harbor genes *iuAt* and *cnf1*. The commonest integron was *intI* 42% (n=25/59) followed by *intII* 20% (n=12/59). Viral load [(OR=1.000, 95% CI, 1.000-1.000) (*p* = 0.295)], HAART duration [(OR=1.036, 95% CI, 0.287-1.042) (*p*=0.287)], age [(OR=1.020, 95% CI, 0.999-1.042) (p = 0.06)] and WHO disease stage [(OR=1.286, 95% CI, 0.535-1.6) (*p* = 0.885)] showed no significant association with the occurrence of asymptomatic bacteriuria ($p \geq 0.05$). HAART duration was observed to be a predictor of resistance to amoxiclav [(OR=1.329, 95% CI, 1.113-1.588) (*p*= 0.002)].

CONCLUSION:

This study showed that asymptomatic bacteriuria is common among women living with HIV (WLHIV) visiting Eikwe district hospital, with no association with age, viral load, WHO disease stage and HAART duration. High prevalence of multidrug resistant UPEC coupled with the high carriage of virulence genes indicates that WLHIV are at a high risk of developing urinary tract infections with the potential for complications. There is a need for regular monitoring of bacteriuria and antibiotic susceptibility testing among this population.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

HIV	Human Immunodeficiency Virus
AIDS	Acquired Immune Deficiency Syndrome
PLHIV	People Living with HIV
WLHIV	Women Living with HIV
ASB	Asymptomatic Bacteriuria
UTI	Urinary Tract Infection
UPEC	Uropathogenic *Escherichia coli*
ExPEC	Extra Intestinal Pathogenic *Escherichia coli*
UNAIDS	United Nations
CD4	Cluster of Differentiation 4
ART	Anti-Retroviral Therapy
HAART	Highly Active Anti-Retroviral Therapy
UGMS	University of Ghana Medical School
UG	University of Ghana
CFU	Colony Forming Unit
IDSA	Infectious Disease Society of America
PFGE	Pulse Field Gel Electrophoresis
CNF	Cytotoxic Necrotizing Factor
CAUTI	Catheter Associated Urinary Tract Infection
TRAF	Tumor Necrosis Factor
TRL	Toll-like Receptor
NMEC	Neonatal Meningitis Escherichia Coli
ST	Sequence Types
PCR	Polymerase Chain Reaction
PAI	Pathogenicity Island
MSHA	Mannose Sensitive Hemagglutinin

MRHA	Mannose Resistant Hemagglutinin
WHO	World Health Organization
SXT	Trimethoprim Sulfamethoxazole
TET	Tetracycline
CRX	Cefuroxime
CAZ	Ceftazidime
CTX	Cefotaxime
CRO	Ceftriaxone
AMP	Ampicillin
PEN	Penicillin
MEM	Meropenem
CIP	Ciprofloxacin
AMC	Amoxiclav
FOS	Fosfomycin
Nal	Nalidixic Acid
NIT	Nitrofurantoin
AMK	Amikacin
GEN	Gentamicin
FEP	Cefepime
SMDPH	St. Martin De Porres Hospital
CLED	Cysteine Lactose Electrolyte Deficient
EMB	Eosin Methylene Blue Agar
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection

CHAPTER ONE

INTRODUCTION

1.1 Background

Urinary tract infection (UTI) is an infection of the upper and lower urinary tract (Vasudevan, 2014), and is one of the most frequent bacterial infections that constitutes a considerable economic burden to society (Grabe *et al*., 2015). Approximately 150 to 250 million cases of UTIs are reported globally each year (Tabasi *et al*., 2015). In the United States, UTI accounts for 7 million and 1 million office and emergency department visits respectively leading to 100,000 admissions (Foxman, 2010).

A wide range of pathogens are implicated in UTIs, namely; *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus saprophyticus* (Mandracchia *et al*., 2015) with uro-pathogenic *Escherichia coli* (UPEC) accounting for over 80% of the infections (Dielubanza & Schaeffer, 2011). *E. coli* is normal flora in the intestinal tract of warm blooded mammals (Shah *et al*., 2019) but may be pathogenic through the employment of virulence factors which enables an ascending colonization of the urinary tract (Foxman, 2010).

The risk of acquiring UTI is high among females and specific patient groups such as sickle cell disease patients (Bebe *et al*., 2020; Donkor *et al*., 2017), diabetic patients (Mandracchia *et al*., 2015), pregnant women, the elderly, catheterized patients, individuals with urological malformations and people living with Human

Immunodeficiency Virus (PLHIV) (Iweriebor, 2012). Asymptomatic bacteriuria (ASB) is defined as the presence of an established quantitative count of bacteria in a well collected urine specimen from a person with no signs and symptoms ascribable to UTI (Rubin *et al*., 1992). ASB among HIV positive patients was reported at 18% in South Africa (Laker *et al*; 2004), 36.7% in India (Yadhav & Samreen, 2017), 12.3% in Tanzania (Ngowi *et al.*, 2021), 7.0 % in Ethiopia (Fenta *et al*., 2016) and 31.3% amongst HIV pregnant women in Nigeria (Akadri and Odelola, 2020).

In Ghana, there are about 210,000 women aged 15 years and over, living with HIV as of 2019 (UNAIDS, 2019). **While** there have been studies in Ghana on opportunistic infections in PLHIV (Adjei *et al*., 2018; Lartey *et al*., 2015; Opintan *et al*., 2017), the prevalence of ASB and the burden of UTI in WLHIV have not been investigated. The burden of UTI is compounded in Women living with HIV (WLHIV) (Chaula *et al.*, 2017) due to their anatomical predisposition to UTI (Barber *et al*., 2013; Dielubanza & Schaeffer, 2011; Foxman *et al*., 2002) coupled with the distinctive pathogenesis of HIV which causes a decline in the CD4 T cells and makes the body unable to fight foreign invaders such as UPEC. The high prevalence of renal dysfunction among HIV people in Ghana, particularly those on Antiretroviral (ART) drugs (Chadwick *et al*., 2015; Obiri-Yeboah *et al*., 2018) puts WLHIV at risk, since expression of virulence genes can potentiate renal pathology and aid in hematogenous dissemination (Gilks *et al*., 1990; SHRUTHI *et al*., 2012; Taramasso *et al*., 2016).

Uropathogenic (UPEC) *E.coli* is reportedly the predominant uro-pathogen in Ghana among PLHIV (Boaitey *et al*., 2012) and pregnant women which makes it a pathogen of interest to be investigated among this at risk population.

This study determined the prevalence of asymptomatic bacteriuria and its associated risk factors. We determined the antimicrobial susceptibility of UPEC, the distribution of virulence genes and integrons among UPEC.

1.2 Problem Statement

UTI accounts for about 60% of non-AIDS defining illness (Hidron *et al*., 2010), posing clinical burden among PLHIV. Asymptomatic bacteriuria is an important precursor of UTI among PLHIV (Murugesh *et al*; 2014). About 30% of people with asymptomatic bacteriuria develop UTI if not treated (Smaill, 2007).

Treatment of UTI in PLHIV with antimicrobial agents is necessary to minimize the risk of renal problems such as HIV associated nephropathy, pyelonephritis, acute and chronic kidney disease (Iweriebor, 2012). High level of opportunistic infections among PLHIV necessitates the intake of antibiotics leading to an increase surge in antibiotic resistant bacteria among this population (Samje *et al*., 2020). Incidence of multidrug-resistant (MDR) bacteria is of public health concern and has made management of UTI very challenging especially in the developing world (Vila & Pal, 2010). High resistance to cotrimoxazole a prophylactic agent, against opportunistic infections in the study population, has been reported in several populations including PLHIV (Adeyemi *et al*.,

2010; Donkor *et al*., 2019; Evans *et al*., 1995; Newman *et al*., 2011; Opintan *et al*., 2015). Drug resistant urinary pathogens leave clinicians with very little treatment options.

1.3 Justification

Among PLHIV, antibiotic resistance is a major concern due to their susceptibility to infections and their tendency to become reservoirs to MDR pathogens from frequent hospital visits and admissions (Olaru *et al*., 2021). This theory was confirmed in Nigeria as it was observed that multidrug resistant pathogens were prevalent in PLHIV which made them a source of MDR pathogens (Kemajou & Ajugwo, 2016).

Ability of an organism to establish UTI depends on the presence of virulence genes (Oelschlaeger *et al*., 2002) and the type of virulence genes present determines the severity of disease. The simultaneous expression of virulence and resistance genes among this population could be very devastating due to the possibility of treatment failure leading to persistence of infection and the possible development of renal complication. This raises concerns as IDSA recommends against screening of ASB (Nicolle *et al*., 2019) despite the high UTI cases and renal problems reported among this population (Chadwick *et al*., 2015; Obiri-Yeboah *et al*., 2018). Furthermore, data on the carriage of virulence genes in MDR uropathogens among this population is scanty in Ghana and the world at large. It is therefore important to identify the virulence gene pool present in resistant isolates to help appreciate the pathogenic potential of these uropathogens so recommendations can be made for screening of bacteriuria among this population.

Also, there is paucity of data on the prevalence of asymptomatic bacteriuria at the study site and its associated risk factors such as viral load, age, WHO disease stage among PLHIV.

This work will fill the knowledge gap on the prevalence of ASB among WLHIV in Ghana. Data is also needed to help clinicians determine if WLHIV having high viral load are potential asymptomatic carriers of bacteriuria so they can treat in time to avoid progression to symptomatic UTI. Antibiotic resistance profiles generated will be useful in the selection of appropriate antibiotics for the treatment of UTIs especially in settings without access to diagnostic microbiology services.

1.4 AIM

To determine the prevalence of asymptomatic bacteriuria and associated virulence factors in Uropathogenic *E. coli* at the St. Martin de Porres Hospital, Eikwe.

1.5 Specific Objectives

The specific objectives are to:

i. determine the prevalence of ASB and its associated risk factors among HIV seropositive women with asymptomatic bacteriuria
ii. determine resistance profile of UPEC and to screen for integrons in UPEC among HIV seropositive women with asymptomatic bacteriuria
iii. **screen for genes encoding adhesins, toxins, siderophores and capsule**

CHAPTER 2

LITERATURE REVIEW

2.1 Urinary Tract Infection

Urinary tract infections may be defined as the presence of significant microbial growth characterized by more than 10^5 colony forming unit (cfu) of the same organism per milliliter of urine with or without the presence of symptoms (Ragnarsdóttir & Svanborg, 2017). Bacteria, fungi and protozoans are possible etiological agents implicated in UTI but bacteria are the most predominant with UPEC being the leading cause of UTI (Flores-mireles *et al*., 2015). Urinary tract infection may affect any part of the urinary tract system; the bladder, kidney, prostate, ureter and the urethra. Due to effective innate immune response and the mechanical action of urine, not all bacterial invasions of the urinary tract usually led to infections. Most UTIs are caused by a single microorganism (monomicrobial) but individuals with structural abnormalities and indwelling catheters may have multiple organisms involved. UTI is more prevalent in young sexually active women and is estimated that 1 in 3 women will develop UTI before reaching 24years (Foxman, 2002).

The possibility of having UTI after sexual intercourse is higher in women due to colonization of the periurethral area and the subsequent movement into the urethra. Infection gradually starts from the lower urinary tract which comprises the urethra and the bladder causing urethritis and cystitis respectively. Its progression to the upper urinary tract can cause infections such as pyelonephritis and may disseminate

hematogenously causing septicemia (Chakupurakal *et al*., 2015). Urinary tract infections have been classified into four main groups; asymptomatic, acute uncomplicated, complicated and recurrent UTIs (Smelov *et al*., 2016).

2.1.1 Asymptomatic bacteriuria

Asymptomatic bacteriuria (ASB) is a very common condition and influenced by variables such as age, sex, sexual activity, pregnancy and genitourinary abnormalities (Colgan *et al*., 2006; Nicolle, 1997; Nicolle, 2003; Zhanel *et al*., 1990). It has been evidenced that, ASB in healthy women increases with age, from 1% in females between five (5) to fourteen (14) years and more than 20% in females above 80 years (Nicolle, 2003). Infecting organisms are diverse and include *Enterococcus species*, *Pseudomonas auroginosa*, Group B *Streptococcus* with UPEC being the most common organism (Mims *et al*., 1990; Warren *et al*., 1982). UPEC may be carried by patients for years and will not induce an immune response. An earlier study postulated that these organisms may not be carrying virulence genes. However, advance molecular epidemiology has explained that these genes are present but has rather failed to express the phenotypes (Plos *et al*., 1990).

In some organisms such as *E. coli* 83972, they have lost the ability to express functional P and type 1 fimbriae rendering it unable to induce an immune response hence has evolved as a commensal in the urinary tract leading to asymptomatic bacteriuria (Klemm *et al*., 2006).

Asymptomatic bacteriuria is usually not considered an infection and not clinically relevant, but for some group of people, it is considered a risk factor for developing symptomatic UTI (Smelov *et al*., 2016). Asymptomatic bacteriuria accounts for 70% of symptomatic UTI cases in unscreened pregnant women (Sibiani, 2010).

The prevalence of ASB among PLHIV has been reportedly high ranging from 4% to 25.3% (Skrzat-Klapaczyńska *et al*., 2018). ASB among HIV positive patients was reported at 18% in South Africa (Laker *et al*; 2004), 36.7% in India (Yadhav & Samreen, 2017), 12.3% in Tanzania (Ngowi *et al.*, 2021), 7.0 % in Ethiopia (Fenta *et al*., 2016) and 31.3% amongst HIV pregnant women in Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria (Akadri and Odelola, 2020).

In Ghana, ASB was reported at 56.1%, 33.3%, 5.5%, and 9.5% among pregnant HIV negative women (Afoakwa *et al*., 2018; Boye *et al*., 2012; Labi *et al.*, 2015; Obirikorang *et al*., 2012) and 7.7% among non-pregnant HIV negative women (Boye *et al.*, 2012). Another study conducted in Ghana by Lutterodt *et al*., (2014), also reported a bacteriuric rate of 31.6% among out and inpatients visiting a hospital on account of UTI.

It has been proposed that spontaneous asymptomatic bacteriuria may offer some form of protection against symptomatic infections caused by other pathogenic organisms and this might be due to competition for space and nutrients (Cai *et al*., 2012).

2.1.1.1 Diagnosis of Asymptomatic Bacteriuria

Asymptomatic bacteriuria is characterized by the presence of one or more species of viable bacteria growing in a properly collected urine specimen from a person with no sign or symptom ascribable to UTI regardless of the presence or absence of pyuria (Nicolle *et al*., 2019). According to the Infectious Disease Society of America (IDSA), the quantitative criteria for defining a significant bacteriuria are; 10^5 CFU/ml in two consecutive samples in women, a single sample in men without symptoms and 10^2CFU/ml may be considered significant in catheterized patients (Nicolle *et al*., 2019). Studies have indicated that a single voided urine specimen has an 80% specificity for diagnosing ASB in women whereas two consecutive specimens have 95% specificity (Kunin *et al*., 1964; Solomkin *et al*., 1992).

According to Deziel *et al*., (2000), the reason for the collection of second urine specimen is to differentiate between true bacteriuria and contamination. They therefore raised questions as to whether it is important to culture second urine specimen and if yes, what time interval is most appropriate. IDSA recommends a minimum of 24hrs time interval but silent on the maximum time interval between the consecutive cultures. Kass, (1952) in his study found a very high reproducibility of significant bacteriuria (66 out 67 women) of the same species from a second urine specimen after 1 to 2 months interval. Similarly, Deziel *et al*., (2000), recorded a high reproducibility of ASB after 2 to 4 months interval of a second specimen collection. Diezel and his colleagues used Pulse Field Gel Electrophoresis (PGFE) technique to differentiate women with reinfection from those with the same strains of organisms that were isolated four months prior. Deziel *et*

al., (2000) suggested that the quantitative criteria of 10^5 cfu/ml should be the discriminatory factor between true bacteriuria and contamination not consecutive cultures as recommended by IDSA. Smaill, (2007) reemphasized that a quantitative value of $>10^5$ cfu/ml in a single mid-stream clean catch specimen is adequate and generally accepted as practical alternative for the diagnosis of ASB.

2.1.1.2 Guideline for the treatment of Asymptomatic bacteriuria

According to the Ghana standard treatment guideline of 2017, oral and IV ciprofloxacin are the first line drugs for the treatment of uncomplicated and complicated UTIs respectively. For asymptomatic people, whether to treat or not to treat bacteriuria is a frequent dilemma in clinical medicine (Colgan *et al*., 2006). Historically, ASB was treated in all population (Dahiya *et al.*, 2018). However, evidence suggests that screening of asymptomatic people for bacteriuria should be done only when factors for poor prognosis are present and antimicrobial therapy can prevent poor outcomes (US Preventive Services Task force, 1996). According to Infectious Disease Society of America (IDSA), treatment of ASB may accelerate antimicrobial resistance and pose needless economic burden on patients. Nonetheless, in some subjects, treatment of asymptomatic bacteriuria can prevent symptomatic urinary tract infection, chronic kidney disease, urinary tract cancer and possibly death *(* Nicolle *et al.*, 2005).

IDSA recommends against the treatment of asymptomatic bacteriuria in premenopausal non pregnant young women for reasons being that, ASB among this population has not

been linked to any disease with a detrimental outcome such as hypertension, renal failure, or urinary tract cancer. Moreover, study conducted where some asymptomatic women with bacteriuria were given nitrofurantoin and others given placebo revealed that, although women with ASB are more likely to develop symptomatic UTI, treatment of asymptomatic bacteriuria did not decrease the frequency of symptomatic UTI nor prevent bacteriuria within a year after treatment compared to the placebo group (Hooton *et al*., 2000).

Pregnant women with asymptomatic bacteriuria are at a higher risk of delivering low birth weight and premature infants and stand a higher risk of developing pyelonephritis (Hooton *et al*., 2000). A Cochrane systematic review revealed that treatment of ASB in pregnant women decreases the risk of developing pyelonephritis by 75% (Smaill & Vazquez, 2019). Treatment of pregnant women with ASB throughout the course of the pregnancy yields similar benefits as treating them for 14 days with nitrofurantoin and trimethoprim sulfamethoxazole (SXT) (Whalley and Cunningham, 1997). IDSA recommends a 3 to 7 days antimicrobial therapy for pregnant women with asymptomatic bacteriuria and further recommends that these women be screened periodically for the duration of their pregnancy (Nicolle *et al*., 2005).

Among older people, IDSA does not recommend the screening and treatment of asymptomatic bacteriuria (Nicolle *et al*., 2005). Studies conducted revealed that the incidence of asymptomatic bacteriuria among pre and post-menopausal women was independent of age (Evans *et al*., 1900; Bengtsson *et al*., 1998).

In PLHIV, there is no recommendation for the screening and treatment of asymptomatic people with bacteriuria. A study in Kenya reported asymptomatic bacteriuria among female prostitutes at 23%. From the study, no difference was found between proportions of bacteriuria among HIV positive and HIV negative participants and the presence of bacteriuria had no correlation with CD4+ count (Ojoo *et al*., 1996).

De Pinto *et al* found that ASB in HIV men correlates with CD4+ count but no negative clinical outcome has been linked to ASB in this population.

2.1.2 Acute uncomplicated urinary tract infections

Acute uncomplicated urinary tract infection is an infection of the bladder characterized by polyuria, urgency and painful urination in a woman with no abnormality in the genitourinary tract, (Hooton & Stamm, 1997). In the outpatient department, acute uncomplicated urinary tract infection is one of the frequently reported infections and the second most common reason for antibiotic prescription (Wagenlehner *et al*., 2011). This group includes sporadic or recurrent lower (cystitis) or upper (pyelonephritis) UTI in healthy people, especially non-pregnant, premenopausal women with no known comorbidities or abnormalities within their urinary tract (Co-chair *et al*., 2018). In diagnosing uncomplicated cystitis and acute pyelonephritis, urine cultures should yield a bacterial growth of 10^3 CFU/ml and 10^4 CFU/ml of urine respectively.

2.1.3 Complicated urinary tract infections

In complicated urinary tract infections, bacterial counts of 10^5 CFU/ml and 10^4 CFU/ml for women and men respectively, is considered significant. It is more associated with people with a structural or functional impairment of the genitourinary tract or people with underlying conditions that increases the risk of a failing therapy (Co-chair *et al*., 2018). There is a greater diversity of uro-pathogens involved in complicated urinary tract infections and the commonly isolated organism include*; E. coli, Pseudomonas aeruginosa, Klebsiella species, Enterococcus species* among others. The tendency of treatment failure is very high due to the underlying abnormality and the broader range of antimicrobial resistance displayed by the organisms involved (Annual epidemiological report, 2014).

2.1.4 Recurrent urinary tract infections

This type of UTI is more common in healthy young women with functional and normal urinary tracts. Recurrent UTIs are confirmed with at least three interludes of uncomplicated infections with a colony count of 10^3CFU/ml over a period of 12 months (Hooton, 2001). Uro-pathogenic coliforms have the ability to attach to uro-epithelial cells and can therefore easily colonize the vagina of women with recurrent UTI. Antibiotic treatment of recurrent UTIs is dependent on knowledge about the risk factors because treatment is not needed in women in which no risk factor has been identified (Co-chair *et al*., 2018).

2.1.5 Routes of infection

There are three routes by which bacteria can gain access into the human body and colonize the urinary tract; the ascending, hematogenous and lymphatic routes. Hematogenous route is less common in healthy individuals and is associated with blood borne pathogens. In bacteremia and endocarditis, the kidney is the major place of inflammation caused by gram positive organisms such as *Staphylococcus aureus.*

In PLHIV, the cytopathic effect of the virus on renal parenchymal cells leads to disruption of the normal cell activity (Alfano *et al*., 2019) which can potentially cause a breach in the renal parenchyma and enable organisms to gain access to the kidneys in patients with candidemia or staphylococcal bacteremia. Gram negative bacteria are rarely involved in infection of the kidney through hematogenous routes. In retroperitoneal abscesses and severe bowel infections, UTI may result through the lymphatic routes (Davis & Flood, 2011).

In healthy patients, the main route of urinary tract infection is by ascending and this is caused by bacteria from the fecal flora colonizing the vaginal introitus and displacing the normal vaginal flora (Chen *et al*., 2013). After spreading to the perineum and periurethral area, they ascend against mechanical action of the urine in the urethra, by employing various virulence factors such as adhesion, flagella-mediated motility and other mechanisms that defeat the natural antibacterial defense mechanisms against UTI to cause cystitis (Handley *et al*., 2002; Ragnarsdóttir & Svanborg, 2017).

2.1.6 Microbial Spectrum of Urinary Tract Infections

Most UTIs are considered monomicrobial. No much difference has been reported for the microbial spectrum of both uncomplicated upper and lower community acquired UTI. *E. coli* and *Staphylococcus saprophyticus* accounts for about 70 to 95% and 10 to 15% of uncomplicated UTI respectively (Czaja *et al*., 2007; Echols *et al*., 1993). However, complicated UTIs have a wider spectrum with organisms such as *Pseudomonas, Enterococcus, Staphylococcus, Serratia, Providencia* and fungi involved (Nicolle *et al*., 2005).

In Ghana, a wide range of uropathogens have been reported but *E. coli* constitutes a significant proportion. Varying proportions of *E. coli* dominated bacteriuria cases ranging from 36.0% to 62.5% were reported among different populations (Acheampong *et al*., 2018; Adjei & Opoku, 2004; Afoakwa *et al*., 2018; Afriyie *et al*., 2018; Gambrah *et al*., 2021; Lutterodt *et al*., 2014; Obirikorang *et al*., 2012; Turpin *et al*., 2007; Yenli *et al*., 2019). Also, other studies have reported *Pseudomonas, Klebsiella, Enterococcus* and *Staphylococcus species* as predominant uropathogens among different populations (Ambrose *et al*., 2009; Donkor *et al*., 2017; Donkor *et al*., 2019; Dzifa *et al*., 2021; Karikari *et al*., 2020; Labi *et al*., 2015; Omoregie & Eghafona, 2016; Samje *et al*., 2020).

In children, *E. coli* constitutes about 80% to 90% of the community-acquired acute upper urinary tract infections (pyelonephritis) (Oelschlaeger *et al*., 2002). They possess chromosomal virulence genes forming the "pathogenicity island" which are expressed to enhance the survival and establishment of the bacteria in the urethra (Oelschlaeger *et al*.,

2002). Patient's age has an influence on the type of infective agent as *Staphylococcus saprophyticus* is reported to account for 10% of UTIs in young females compared to less than 1% in elderly females (Davis & Flood, 2011).

2.1.7 Pre disposing factors to Bacteriuria in females

Many factors can account for the high prevalence of bacteriuria in females including sexual activity, menopause, genital prolapse, immunosuppression and antimicrobial use

Sexually active women, especially those that have coitus three (3) or more times a week have a 2.6-fold increased risk of UTI compared to their counterparts who do not engage in sexual activities (Hooton *et al*., 1996). There is a likelihood that uro-pathogens will be mechanically introduced into the bladder during coitus and this coupled with injury to the vaginal introitus during sexual intercourse, most likely puts sexually active women at an increased risk of developing UTI (Foxman *et al*., 1997; Hooton, Hillier, *et al.*, 1991). Additionally, behavioral factors such as vaginal douching, use of hot tubs, tightening underwear, personal hygiene, change of sexual partner within a year, and circumcision status of male partners have been suggested to increase females susceptibility to UTI (Hooton *et al*., 1996; Scholes *et al*., 2000). This is evidenced in a study conducted in Eastern Ethiopia, where 61.4% of married and sexually active HIV positive participants were reported to have bacteriuria (Marami *et al.*, 2019). Also unprotected sexual activity in marriage is almost inevitable because of cultural beliefs and this might account for the high level of ASB, 83.5%, reported from a study conducted amongst married and cohabitating women in Ghana (Afoakwa *et al*., 2018). Similarly, another study revealed that the prevalence of UTI among young college women was about 48% few days after

sexual intercourse, 25% within 2 weeks and this decreasing trend was observed with increasing days of abstinence (Vincent *et al*., 2013).

Frequent use of spermicides among sexually active women has also been linked to UTI. The active ingredient used in spermicidal compounds in the united states is Nonoxynol-9, a nonionic surfactant which has been in use for several decades for family planning (Schreiber *et al*., 2006). An intact vaginal flora ecosystem is of a high importance in the prevention of urogenital infections and some researchers have tried to investigate the impact of nonoxymol-9 on vaginal ecosystem (Schreiber *et al*., 2006). Some in-vitro studies have suggested that the use of nonoxynol-9 containing spermicides decreases *Lactobacillus* colonization in the vagina (Mcgroarty *et al*., 1992) especially the hydrogen peroxide producing ones (Hooton, *et al*., 1991).

Whiles studies have demonstrated the role of hydrogen peroxide producing *Lactobacillus* in preventing bacterial vaginosis and candidiasis (Hawes *et al*., 1996; Hillier *et al*., 1992), a case control study has also established an increase in vaginal *E. coli* colonization in females without the hydrogen peroxide producing *Lactobacillus* strains (Gupta *et al*., 1998) which predisposes them to urinary tract infections. The findings of Gupta and his colleagues have been highlighted in a study by Rosenstein and his colleagues when they found out that introduction of nonoxymol-9 into the vagina in the absence of sexual intercourse caused a decrease in hydrogen peroxide producing lactobacillus and an increase *E. coli* colonization (Rosenstein *et al*., 1998).

Even though some studies have suggested otherwise, (Moncla & Hillier, 2005; Schreiber *et al*., 2006), the above discussions indicate that the antimicrobial potential of nonoxynol -9 can cause changes in the normal vaginal flora lead to the proliferation of UPEC.

Vulvovaginal atrophy is a common condition characterized by vaginal dryness, irritation, postcoital bleeding and soreness with a reported prevalence rate of 47% among post-menopausal women (Bride *et al*., 2010). It is the result of hypoestrogenism and has been reported together with reduced glycogen production and depletion of *Lactobacilli* in the vagina as risk factors for UTI among post-menopausal women. In Pre-menopausal women, the vagina *Lactobacilli* utilizes lactose and produces lactic acid which decreases the vaginal pH, preventing colonization by uro-pathogens (Raz, 2001). However, this mechanism is reduced or absent in post-menopausal women making them prone to UTI. A study by Raz and Stamm has re-emphasized the role of hypoestrogenism in UTI as they have proven that topical application of estrogen cream in post-menopausal women exponentially restored *Lactobacilli* which translated into reduced vaginal colonization of *E. coli* by half as a result of reduced vaginal pH caused by the production of lactic acid (Raz & Stamm, 1993).

Pelvic organ prolapse is the fall of the uterus and the vaginal walls through the vaginal canal (Aytan *et al*., 2014). It is a pelvic floor dysfunction in which pelvic organs descend due to anatomical or functional abnormalities of the tissues that give the pelvic organs support (Aytan *et al*., 2014). This is reportedly prevalent in elderly women (Storme *et al*., 2019). Emptying the bladder is a natural mechanical defense against UTI. However, this is compromised in patients with genital prolapse as voiding dysfunction has been

reported as a very common condition among these patients, prompting an increased risk of UTI (Aytan *et al.*, 2014). Voiding dysfunction has also been reported among PLWHIV, making pathogen clearance from the urinary tract inefficient and promoting the development of UTI (Lebovitch & Mydlo, 2008). Hamid and Losco have proposed that residual urine volume as low as 30mls can increase the risk of UTI.

Amongst the types of prolapse, anterior prolapse or cystocele (the drop of the bladder into the vagina), is most significantly associated with UTI (Aytan *et al.*, 2014). Nonetheless, posterior prolapse or rectocele can also exert pressure on the urethra causing voiding dysfunction which accelerates the development of UTI. Despite the high occurrence of voiding dysfunction in women with genital prolapse, the occurrence of prolapse does not automatically translate to presence of voiding dysfunction hence it is needless to treat patients with asymptomatic bacteriuria with good voiding (Hamid & Losco, 2014) except for pregnant women and patients going for urinary tract surgery (Aytan *et al*., 2014).

Genetical predisposition to UTI has been proposed by some researchers, as they observed that having a mother or a sister with a history of UTI is another risk factor for developing UTI. Recurrent UTI has been shown to be four times higher in women who are non-secretors of the ABO antigen, chiefly because UPEC has a better adherence to uro-epithelial cells in these women thereby compounding the risk of UTI in this population (Stapleton *et al*., 1992). ABO blood group antigens are primarily found on red blood cells but some levels of these antigens are secreted into body fluids such as saliva, sweat, tears, semen and the plasma. Whiles some are able to secrete the ABO antigens in their body

fluids, others are not able and they are called the non-secretors. These non-secretors have inherited the recessive form of the gene, se se, making them unable to secrete these proteins into the body fluid (Adamo & Kelly, 2014; Metgud *et al*., 2016). These genes can be inherited from parents and this forms the basis for genetic susceptibility.

Neutrophils play key role in phagocytosis by moving across urothelial cells in response to interlukin-8, an inflammatory cytokine (Godaly *et al*., 1998; Godaly *et al*., 1997). Absence of the receptor for this cytokine, CXCR1, will inhibit the migration of neutrophils across urothelial cells to perform phagocytic functions. This has been proposed to be the cause of recurrent pyelonephritis in pediatric patients who demonstrated a defect in the CXCR1 receptor (Frendéus *et al*., 2000).

Catheter associated urinary tract infection (CAUTI) is the major cause of UTI in hospitalized patients and accounts for a significant number of hospital acquired infections (Asafo-Adjei *et al*., 2018; Nandini & Madhusudan, 2016; Ray *et al*., 2015). CAUTI is the result of non-adherence to infection prevention and control protocols, poor aseptic techniques and poor catheter insertions (Storme *et al*., 2019). A survey in 66 hospitals in Europe and 183 hospitals in USA revealed that 17.5% (Nandini & Madhusudan, 2016) and 23.6% (Nandini & Madhusudan, 2016; Ray *et al*., 2015) respectively, of patients on admission are catheterized.

The most important determinant of bacteriuria in catheterized patients is the length of catheterization (Hooton *et al*., 2010) as CAUTI develops in 100% of patients that have been catheterized for more 30 days and present with clinical signs such as hematuria

fever and loin pain (Parida & Mishra, 2013). Bacterial colonization within the lumen of the human catheter occurs within 48hrs whiles the colonization of the external wall of the catheter occurs within 72 to 168hrs (Olmsted *et al.*, 2013) with a daily risk of acquisition of bacteriuria reported at 3-7% (Hooton *et al.*, 2010). Prolonged catheterization can compromise the natural defense of the host and create a niche for bacteria through biofilm formation (Storme *et al.*, 2019). Long term catheterization coupled with other host factors such as local trauma, anatomical or functional deformities of the urinary tract, immunosuppression and diabetes mellitus increases the risk of catheter associated UTI (Storme *et al.*, 2019).

Within 24hrs of the birth of a female child, the vagina is colonized by certain microorganisms and this creates an ecosystem that provide several health benefits to the female throughout their life time. Studies, both in human and animals, have shown that antimicrobial agents alter this ecosystem, predisposing females to UTIs (Hertbelius-elman *et al.*, 1992; Winberg *et al.*, 1993). Increased level of amoxicillin administration in apes have been observed to cause a high colonization of the vagina with *E. coli* (Hertbelius-elman *et al.*, 1992). This study was replicated with nitrofurantoin and trimethoprim in monkeys but no significant increment was found in the vaginal colonization of *E. coli* and this suggest that beta lactam antibiotics may be more associated with vaginal dysbiosis in females. Cotrimoxazole and fluoroquinolones have also been associated with vaginal colonization of uro-pathogens thereby increasing the risk for UTI. Even though this is to a lesser extent, it calls for concern as cotrimoxazole is a prophylactic drug commonly used in WLHIV and prolonged usage may predispose them to vaginal dysbiosis and subsequently lead to UTI.

Antecedent antimicrobial use has been found to increase the risk of cystitis among women as a prospective study demonstrated that females using antimicrobial agents 15 to 28 days prior, have an increased risk of developing uncomplicated cystitis (Smith *et al*., 1997)

The risk of acquiring UTI is increased in specific groups of people including sickle cell disease patients (Bebe *et al*., 2020; Donkor *et al*., 2017), diabetic patients (Mandracchia *et al*., 2015), pregnant women, the elderly, catheterized patients, people diagnosed with urological malformations and PLHIV (Iweriebor, 2012a) . The incidence rate of UTI in HIV/AIDS patients has been reported at 7 to 50% (Maria *et al*., 1994; Obulesu *et al*., 2017) and this is the result of increased likelihood of opportunistic infections in PLHIV (Kuipers & Zamparutti, 2014). Opportunistic infections account for 90% of HIV-related morbidity and mortality whereas opportunistic cancers and other causes account for 7% and 3% respectively (Staine, 2008). Higher prevalence of asymptomatic bacteriuria (ASB) has also been reported among women living with HIV compared to non-HIV pregnant women (Chaula *et al*., 2017) putting them at a higher risks of developing UTI.

2.1.8 Molecular Pathogenesis of UTI

Colonization of the bladder by UPEC is contingent on its ability to overcome the body's formidable natural defense to infection, the mucosal barrier (Hannan *et al*., 2013). UPEC usually migrate from the gastrointestinal tract into the periurethral space, up the urethra and into the bladder (Hooton, 2001). When UPEC reaches the bladder, it attaches to the umbrella or facet cells using adhesin FimH on the tip of the type 1 pilli (Hannan *et*

al., 2013). Umbrella cells are mono layer of a highly differentiated and polarized cells that have clear apical and basolateral membrane regions defined by tight junctions (Varley *et al.*, 2007). This colonization event activates inflammatory response in the bladder epithelium. Bladder cells recognize UPEC Lipopolysaccharide (LPS) through its toll-like receptor 4 (TLR-4)-CD14 pathway which results in the generation of IL8, a strong chemoattractant for neutrophils. A cycling of the apical membrane of the facet cells is a normal process (Bishop *et al.*, 2007) which allows for internalization of the attached UPEC (Martinez *et al.*, 2000; Schwartz *et al.*, 2011). To mitigate this pathogenic activity, the bladder cell undertakes active expulsion of the endocytosed UPEC. UPEC has the ability to neutralize lysosomes and this neutralization process is detected by lysosomal membrane protein which in turn activate pathways that direct the exocytosis of the UPEC-containing lysosomes (Miao *et al.*, 2016). This is further enhanced by the activation of Toll-like receptor 4 (TLR4) by the UPEC leading to ubiquitylation of Tumor Necrosis Factor 3 (TRAF3) which facilitates communication with a guanine-nucleotide exchange factor that directs assembly of exocyst complex, thereby accomplishing expulsion of intracellular bacteria (Miao *et al.*, 2017).

Despite these innate mechanisms available for the expulsion of UPEC from the cells, UPEC is able to employ a by-pass mechanism to gain access into the cytoplasm of bladder cells and develop clonal biofilm-like masses called intracellular bacterial communities (IBCs) (Anderson *et al.*, 2014; Schwartz *et al.*, 2011). The host responds to this by exfoliating the infected facet cells (Roth *et al.*, 1998) and discharging the IBCs into the urine. Molecular mechanisms transduce signals that lead to differentiation and

proliferation of the underlying transitional cells to restore the shed superficial cells. Albeit the robust inflammatory response and exfoliation of infected cells, UPEC is able to maintain high titers in the bladder for several days.

2.1.9 Epidemiology of Extra Intestinal-Pathogenic *E. coli* (ExPEC)

Escherichia coli is a genetically diverse facultative gram-negative bacillus, comprising nonpathogenic strains naturally found in the fecal flora of warm-blooded animals and other strains responsible for intestinal and extra intestinal diseases (Dale & Woodford, 2015). ExPEC can molecularly be defined as *E. coli* isolates with at least two of the following virulent factor genes within their genome; papA and/or papC, sfa/foc, afa/draBC, kpsM II and iutA (Peirano *et al*., 2013). In the past, ExPEC isolates were grouped based on the disease they are associated with namely; Uropathogenic *E. coli* (UPEC), neonatal meningitis-associated *E. coli* (NMEC), and sepsis-causing *E. coli* (SEPEC). However, it was later observed that these organisms can cause disease at multiple anatomical sites (Dale & Woodford, 2015).

Based on genetic, clinical considerations and significance to humans, *E. coli* was later classified into three groups namely; commensal strains, intestinal strains and extraintestinal pathogenic strains (ExPEC) where ExPEC comprises all non-commensals capable of causing disease at sites other than the intestine (Russo & Johnson, 2000). Extra intestinal infections can occur in all age groups and at any anatomical site (Russo & Johnson, 2000). However, majority of ExPEC are uro pathogenic (UPEC) and constitutes about 90% of community and hospital acquired urinary tract infections (Talan *et al*.,

2015). Comparably, the genomes of UPEC are larger than those of commensal *E. coli* strain because of their continuous acquisition and occasional loss of genetic materials (Ahmed *et al*., 2008; Hacker *et al*., 1997; Touchon *et al*., 2009). This difference in genome size is observed in three typical UPEC strains; CFT073, 536 and UTI89 when compared to the genome of a commensal reference strain MG1655 (Brzuszkiewicz *et al.*, 2006; Chen *et al*., 2006; Welch *et al*., 2002).

Clemont and his colleagues have grouped *E. coli* phylogenetically into 4 groups; A, B1, B2, and D using a triplex polymerase chain reaction method (Bonacorsi ***et al.***, 2000). The virulent ExPEC belongs mainly to the group B2 and marginally group D whereas majority of the intestinal commensals belong to group A (Bonacorsi et al., 2000). With advancement in molecular epidemiology, Multilocus Sequence Typing (MLST) has enabled an in depth analysis of the ExPEC where isolates were grouped into distinct sequence types (STs) which are based on identical allelic profiles (Maiden *et al*., 1998; Wirth *et al*., 2006; Yun *et al*., 2014). These STs include ST131, ST73, ST95, ST127 to which all B2 belong and ST69 to which group D belongs (Banerjee *et al.*, 2013; Siu *et al*., 2008). They are further put into broader clonal complexes which are described as a group of at least three STs each, which differ from each other by not more than 1 of 7 alleles (Salvador *et al.*, 2012). Some of these clones are able to escape antimicrobial effects by displaying resistance to certain classes of antibiotics. For instance, ST131-O25b clone has been associated with high resistance to fluoroquinolones and cephalosporins whiles ST69 shows high level of resistance to trimethoprim-sulfamethoxazole (Johnson *et al.*, 2011; Nicolas-Chanoine *et al*., 2008).

2.1.10 Identification of Uro-pathogenic *E. coli* (UPEC)

Identification is the use of established criteria to differentiate, isolate and identify organisms that cause disease. Identification is done routinely by morphological and biochemical characteristics and less frequently, by serological tests. Advancement of molecular works, has paved way for identification of an organism to the genomic level, making it easier and faster (Barron, 1996).

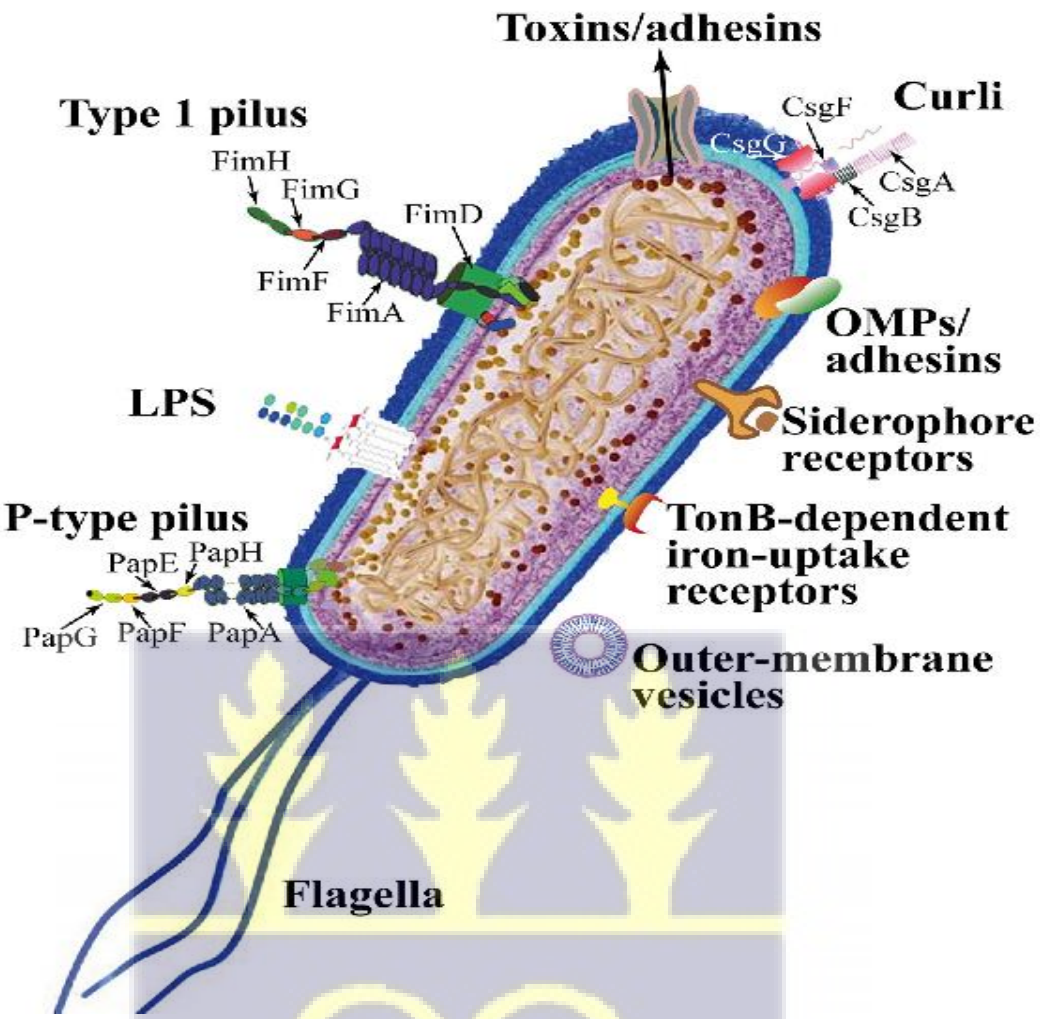
According to Johnson and Stell, (2000), UPEC can be identified by the presence of two or more virulent factor genes. However, this hypothesis suffers some drawbacks because these genes are not UPEC-specific. Also, the PCR methods and approaches available for the identification of UPECs as proposed by some studies have limitations. In that, the genes identified are not specific to UPEC but are also harbored by commensal *E. coli*. For instance, fimH as recommended by Johnson and Stell, (2000), rfaH by Van Der Zee *et al*., (2016), and ecp, fyuA, sfa/ focDE by Blackburn *et al*., (2009) and López-banda *et al.*, (2014) are not exclusively found in UPEC but also in commensal strains. Brons *et al.*, (2020) developed a PCR assay to molecularly determine UPEC using three selected UPEC-specific genes involved in virulence as targets. These include *c3509, c3686* (*yrbH*) and *chuA*. A combination of these genes can be used in the detection of UPEC.

2.1.11 Virulence factors for Uro-Pathogenic *E. coli* (UPEC)

It has been reported that not all *E. coli* found in the fecal flora can cause a urinary tract infection and the ability of an organism to establish UTI depends on the presence of virulence genes (Oelschlaeger *et al*., 2002). The type of virulence genes present

determines the type and severity of the disease and these genes can be transferred horizontally between bacterial populations because of their location on mobile genetic elements such as pathogenicity island (PAI).

Virulence factors are encoded by specific genes and the expression of those genes enables pathogenic organisms to establish an infection (Pitout, 2012). Establishment of an infection depends on the expression of more than one gene as a single factor rarely causes infection and the level of expression is also of importance because higher levels increase the chances of infection (Dobrindt, 2005) This has been demonstrated in several studies where it was observed that more virulent factors are needed to invade immunocompetent people than the immunosuppressed (Maslow *et al*., 1993; Otto *et al*., 2001; Rijavec et al., 2008). Expression of genes can be regulated or influenced by environmental factors such as temperature, nutrient and iron deprivation (Nagy & Kere, 2003). For example, siderophores are expressed when there is iron deprivation whereas Type 1, P and S fimbriae are expressed at the normal body temperature (Collinson *et al*., 1992).



(Terlizzi *et al*., 2017)

Figure 1. Diagram of UPEC virulent factors

2.1.11.1 Adhesins

The first step to colonization is adhesion. This is mediated by bacterial surface fimbriae and other structures such as Dr adhesins which recognize host cell surface receptors on other uroepithelial cells. Fimbriae are crowned with different adhesins which referees tropism in the upper or lower urinary tract by discerning receptors on the bladder or the kidney epithelium (Wright & Hultgren, 2006). Initially, fimbriae were thought to

augment bacterial virulence by simply attaching bacteria to the cells (Lelffler & Svanborg-edrnn, 1980). However, a study has shown that fimbriated bacteria activate specific transmembrane signaling pathway in the uroepithelial cells (Hedlund *et al.*, 1996). These cytokines recruit inflammatory cells such as neutrophils from the blood stream, across epithelial barrier in the lumen. Both P and type 1 fimbriae have been shown to stimulate cytokine production in epithelial cells (Godaly *et al*., 1998).

Type 1 fimbriae Mannose sensitive Hemagglutinin (MSHA) is crowned with *FimH* adhesins (Hannan *et al*., 2013) which attaches to mannose rich epitopes located in the Tamm-Horsfall glycoprotein, secretory IgA or bladder cell uroplakin (Eto *et al*., 2007). Type 1 fimbriae do not only aid in attachment, it also plays a role in invasion. It uses uroplakin Ia and Ib receptors for internalization (Martinez *et al.*, 2000). S fimbriae binds to sialic acid epitopes present in sialylated glycoproteins and glycolipids. P fimbriae Mannose Resistant Hemagglutinin (MRHA) is tipped with *PapG* adhesin which recognize Galα1-4Galβ- epitopes in the globoseries-containing glycolipids (Wright & Hultgren, 2006).

P fimbriae has a higher virulence and is being expressed in about 80% of bacterial strains that causes uncomplicated acute pyelonephritis and less than 20% of strains causing asymptomatic UTI (Leffler & Svanborg-edrnn, 1981; Marklund *et al*., 1995). *PapG* adhesins compromises immunoglobulin A transport into the urinary space and this enables UPEC to evade an important protective mechanism permitting it to initiate an ascending infection (Rice *et al*., 2005; Ashkar *et al*., 2008). It has been hypothesized that

after invasion into the bladder, *FimH* adhesins are expressed which promotes attachment to the bladder epithelium. Howbeit, when the bacteria ascend the renal parenchyma, phase variation occurs which suppresses the *FimH* adhesins and expresses the *PapG* adhesins enabling attachment to renal parenchymal cells.

2.1.11.2 Distribution and role of Toxin in renal pathology

UPEC has several genes that encode toxins that cause pathology in patients (cnf1, hlyA, set, astA, vat, usp, and cva/cvi) (Abe *et al*., 2008; Chiou *et al*., 2010). The cytotoxic necrotizing factor type 1 (*cnf1*) has been shown to induce apoptosis in bladder cells lines of human in vitro (Mills *et al*., 2000) and has also been shown to increase F-actin, superoxidase generation and also decrease the phagocytic function of polymorphonuclear neutrophils (Hofman *et al*., 2000). About 31% to 44% and 36% to 48% of UPEC isolates are associated with cystitis and pyelonephritis respectively (Andreu *et al*., 1997; Mitsumori *et al*., 1999; Yamamoto *et al*., 1995). A study by Horcajada *et al*., (2005) revealed that *cnf1* is a good promoter of bacteremia emanating from urinary tract infections. (Rijavec *et al*., 2008) reported that 10% and 24% of UPEC isolated from immunosuppressed and immunocompetent patients respectively, had *cnf1* gene. Similarly, a study conducted by Rezatofighi *et al*., (2021) where the distribution of virulent factor genes was investigated revealed that 12.3% of UPEC isolated from patients with urinary tract infection had *cnf1* gene whiles commensal *E. coli* had 3.3%. Additionally, an earlier work performed by Tarchouna *et al*., (2013) on 96 UPEC strains revealed that 3% of the isolates associated with cystitis were carrying *cnf1* gene but none was detected among isolates associated with pyelonephritis. Opposed to the findings of

Tarchouna and his colleagues, 63.6% of UPEC from patients with pyelonephritis were found to be carrying the *cnf1* gene whereas 34% and 25.8% carriage, was recorded in patients with cystitis and ASB respectively (Tabasi *et al*., 2016).

Uropathogenic specific protein (USP) gene encodes a 346 amino acid protein known as the uropathogenic specific protein. Reportedly, this gene is more associated with UPEC than fecal *E. coli* isolates. It was first discovered in the UPEC strain Z42 isolated from a patient with prostatitis. Yamamoto and his colleagues found *usp* gene in about 93.4% and 88% of UPEC isolated from patients with pyelonephritis and prostatitis respectively (Yamamoto *et al*., 2001). Also, 30% and 59% of this gene was found in UPEC isolates respectively, from immunosuppressed and immunocompetent patients (Rijavec *et al*., 2008). In South Korea, Yun *et al*., (2014) isolated UPEC from children with ASB and symptomatic UTI had 10% and 4% carriage of *usp* gene respectively.

A genotypic examination of the relationship between virulent factors and different clinical symptoms revealed that 52.4%, 72.7% and 22.6% of UPEC from patients with cystitis, pyelonephritis and ASB respectively, carried the *usp* gene (Tabasi *et al*., 2016). Based on sequence homology analysis, the possibility of *usp* being a bacteriocin was proposed. A bacteriocin is a peptidic toxin or an antibiotic produced by some strains of bacteria to inhibit the growth of closely related strains (Leroy & De Vuyst, 2010). Bacteria that produce bacteriocin co-synthesizes immunity proteins that protects it from the harmful effect of its own toxin. Detailed analysis has shown that close to half of the nucleotide sequence of the *usp* C-terminal shares the same identity with the *E. coli* bacteriocin colicin E7 which has a nuclease activity whiles the N-terminal shares some

similarity with the type VI protein secretion system component (Nakano *et al*., 2001; Papadakos *et al*., 2012). Most bacteriocins with nuclease activity have three functional domains that are responsible for recognition of specific receptor protein on cell membrane of the target organism, translocation of protein into the target cell and the eventual destruction of genetic material of target cell (Cascales *et al*., 2007).

2.1.11.3 Capsular polysaccharide

These are products of oligosaccharide polymers with unique antigenic epitopes. They serve as a shield surrounding the bacteria and offers resistance to phagocytosis, which makes the bacteria able to survive in tissues and circulation (Horwitz & Silvertein., 1980). Capsule also confers serum resistance by offering protection against complement mediated killing (Rasko *et al*., 2013). Examples of capsule antigens include *kpsMT* I *kpsMT* II, *kpsMT* III K1 and K5 (Pitout, 2012). In a research conducted by Yun *et al*., (2014),

40% and 14% of UPEC isolates from children in South Korea with ASB and UTI respectively, carried *kpsMTII.* Also, Rezatofighi *et al*., (2021) reported that *kpsMTII* carriage among UPEC and commensal *E. coli* strains were 32.6% and 30% respectively. Additionally, Taylor *et al*., (2015) revealed that 20% of UPEC isolates from patients with UTI were found to be carrying the *kpsMTII* gene and 64% mortality rate was recorded among these patients. Johnson & Stell, (2000) rather recorded a higher prevalence (63%) of *kpsMTII* gene among UPEC isolates.

2.1.11.4 Siderophores

These are specialized structures used by bacteria to compete with host for nutrients such as iron. All uropathogen bacteria express some form of iron intake system. Enterobactin is expressed by all ***E. coli*** strains but most strains correlated with acute pyelonephritis produces high affinity iron binding protein called aerobactin (*iutA*), yersinibactin (*fyuA*) and *ChuA* (Garcia *et al*., 2011). These virulent genes are usually expressed in response to iron deprivation (Yun *et al*., 2014). Varying prevalence (80%, 19%,) of *iutA* has been recorded among UPEC isolates by different researchers (Johnson & Stell, 2000; Taylor *et al*., 2015).

2.2 Human Immunodeficiency Virus / AIDS

Human Immunodeficiency virus (HIV) belongs to the lentivirus group within the family of retroviridae. It has been classified into two, HIV-1 and HIV-2, based on the genetic characteristics and some differences in the viral antigens. The core of the virus comprises two identical single-stranded RNA molecules which are converted by reverse transcription, into DNA (C. Lee et al., 2000). HIV-1 has been proven to have evolved from central African Chimpanzees whereas HIV 2 to have evolved from sooty Monga beys (Sharp, 2014) with HIV 1 being highly pathogenic compared to HIV 2 (Advisory *et al*., 2016).

2.2.1 Pathophysiology of HIV/AIDS

Infection is preceded by attachment of the virus to the host cells through a series of interactions between viral structural proteins and the host cell receptors. The virus has a

surface glycoprotein called gp120 with which it attaches to the CD4 receptor on the host cells such as CD4 T lymphocytes, macrophages and dendritic cells (Feng *et al*., 2012). Immunosuppression, which is a major feature of HIV infection, results from lysis of the infected CD4 cells and the inhibition of the production of CD4 T lymphocytes which leads to a steady reduction in T helper cells (Sharp, 2014; Zhang *et al*., 1997).

2.2.2 Opportunistic Infections

The advancement of immunosuppression leads to a depletion of the CD4 cell to a level where immune response is impaired such that opportunistic infections and neoplasm begin to develop (Medina-ramı *et al*., 2017). Opportunistic infections are defined as infections caused by non-pathogenic organisms which become pathogenic when the immune system is compromised (Faria *et al*., 2015). Common opportunistic pathogens usually associated with immunosuppression includes *Toxoplasma gondii*, *Cryptosporidium parvum*, *Pneumocystis jirovecii*, *Mycobacterium tuberculosis* amongst others (Advisory *et al*., 2016).

2.2.3 WHO clinical stages of HIV/AIDS for Adults

The WHO clinical stages for HIV/AIDS classification employs the use of clinical parameters to guide clinicians in making decisions, especially those in resource limited countries where access to laboratory services is not readily accessible (WHO, 2006). This system groups patients into four hierarchical clinical stages from asymptomatic patients (stage 1) to patients with AIDS (stage4). Assignment of patients to the groups depends on the presentation of one or more symptoms from that group. This system has been shown

to be an adequate and a practical way of managing HIV seropositive people because of the positive correlation between the clinical signs listed and the CD4+ cell count (Hunter & Cyr, 2006; Kassa *et al*., 1999; Lynen *et al*., 2006; Malamba *et al*., 1999).

Very few works have looked into the association between ASB and WHO clinical stages. In Nairobi, a study reported a bacteriuric rate of 23% among asymptomatic HIV seropositive commercial sex workers assigned to WHO clinical stage 1 (Ojoo *et al*., 1996).

Table 2.1 WHO disease stage classification

Clinical Stage	**Description**
1	Asymptomatic Persistent generalized lymphadenopathy
2	Unexplained moderate weight loss (<10% of presumed or measured body weight), Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media and pharyngitis), Herpes zoster, Angular cheilitis, Recurrent oral ulceration, Papular pruritic eruptions, Seborrhoeic dermatitis, Fungal nail infections
3	Weight loss of greater than 10 percent of total body weight, prolonged (more than 1 month) unexplained diarrhea, pulmonary tuberculosis, and severe systemic bacterial infections including pneumonia, pyelonephritis, empyema, pyomyositis, meningitis, bone and joint infections, and bacteremia. Mucocutaneous conditions, including recurrent oral candidiasis, oral hairy leukoplakia, and acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis
4	HIV wasting syndrome, *Pneumocystis pneumonia* (PCP), recurrent severe or radiological bacterial pneumonia, extrapulmonary tuberculosis, HIV encephalopathy, CNS toxoplasmosis, chronic (more than 1 month) or orolabial herpes simplex infection, esophageal candidiasis, and Kaposi's sarcoma

2.3 Mechanism of action and Antibiotic resistance

Antimicrobial agents have been grouped based on their mechanism or site of action. They've been grouped into cell wall synthesis inhibitors (β lactams, cephalosporins) nucleic acid synthesis inhibitors (fluroquinolones and others), inhibition of ribosome function (aminoglycosides, macrolides among others), inhibitors of cell membrane function (polymyxins among others) and inhibitors of folate metabolism (Dowling *et al*., 2017). Several mechanisms have been employed by bacteria to directly inactivate antimicrobial molecules, modification of target sites or reduction of antibiotic uptake. Resistance can be intrinsic, acquired or adaptive (Munita *et al*., 2016). Acquired resistance can occur through horizontal transfer of resistant genes usually by mobile genetic elements and by mutation (Breidenstein *et al*., 2011).

2.3.1 Epidemiology of Antibiotic resistance in UPEC

The Ghana Standard Treatment guideline, (2017) recommends the use of ciprofloxacin, cefuroxime, Amoxiclav, Ceftriaxone and Gentamicin for the management of UTI. However, uropathogens especially UPEC, are increasingly developing resistance to these commonly prescribed antibiotics and this is worryingly limiting the treatment options available for UTI (Al Sweih *et al*., 2005). In Nigeria, these multidrug resistant pathogens are constantly multiplying especially in PLHIV making them a source of MDR pathogens (Kemajou & Ajugwo, 2016). In Tanzania, 75% of uropathogens isolated from PLHIV were resistant to Trimethoprim Sulfamethoxazole (SXT) (Marwa *et al*., 2015). A similar study in Tanzania reported that 90%, 16.7%, 10% and 13.3% of UPEC isolated from PLHIV were resistant to SXT, nitrofurantoin, gentamicin and ceftriaxone,

respectively (Chaula *et al.*, 2017). A study conducted on ASB among women of reproductive age in Ghana by Afoakwa *et al.*, (2018) revealed that 98.25%, 96.49%, 94.73%, 5.26%, 8.77% and 17. 54% of UPEC were resistant to Ampicillin, SXT, Tetracycline, Cefotaxime, Ciprofloxacin and Cefuroxime respectively. The study however reported very low resistance (5.6%) to Gentamicin and no resistance to Amikacin.

In a study conducted among PLHIV by Kemajou & Ajugwo, (2016) in Nigeria, it was observed that 88.0%, 16.7%, 42.9%, 69.0% and 7.1% of UPEC were resistant to Ampicillin, Ciprofloxacin, trimethoprim-sulfamethoxazole, Ceftriaxone and Gentamicin respectively. In Ethiopia, Fenta *et al.*, (2016) also found UPEC isolates among patients on HAART to be resistant to Ampicillin, Amoxiclav, trimethoprim-sulfamethoxazole, gentamicin, ciprofloxacin, cefotaxime and amikacin in the proportions of 86.4%, 59.1%, 72.7%, 0%, 22.7%, 18.2% and 0% respectively. **This high sensitivity of UPEC to aminoglycosides is consistent with the findings of other researchers in Ghana who worked on non-HIV participants (O. Adjei & Opoku, 2004; Afoakwa *et al.*, 2018; Asafo-Adjei *et al.*, 2018; Dzifa *et al.*, 2021; Lutterodt *et al.*, 2014a)**

Contrary to the findings of Kemajou & Ajugwo, (2016) and Fenta *et al.*, (2016), 60% resistance to Gentamicin was recorded by Zakka *et al.*, (2018) for UPEC isolated from PLHIV in Jos Metropolis, Nigeria. The challenge of administering aminoglycosides is that it has nephrotoxicity potentials and should be used only when all treatment options are exhausted (Martínez-Salgado *et al.*, 2007).

2.3.2 Association between Antibiotic resistance and Virulent factors in UPEC.

Mobile genetic elements such as plasmids, transposons and integrons carry and facilitate the spread of antibiotic resistant and virulent factor genes which provide survival advantage to the microorganisms (Silva & Mendonça, 2012).

The susceptibility pattern of *E. coli* has changed since the introduction of third generation cephalosporins and subsequent development of extended spectrum beta lactamases (ESBL) (Silva & Mendonça, 2012). Lee *et al*., (2010), in their investigation of nine virulent factors have found out that aerobactin receptor (*iuAt*) was significantly common in ESBL producing *E. coli* carrying the $^{bla}_{\text{CTX-M-1}}$ whereas serum resistance associated outer membrane protein (*traT*) was more common in isolates with $^{bla}_{\text{CTX-M-9}}$. Another study has indicated that CTX-M producing ESBL *E. coli*, though with a comparably lower prevalence of *iuAt* and *traT*, carried more virulent factors than CTX-M producers (Pitout *et al*., 2005).

A study conducted in Iran revealed a significant association between antibiotic resistance and virulent factors in UPEC, particularly among biofilm formers (Shah *et al.*, 2019). Similarly, Tabasi *et al*., (2015) reported a strong correlation among UPEC biofilm formers and resistance to ampicillin, norfloxacin and trimethoprim-sulfamethoxazole. However, hemolysin (hlyA) producing isolates showed a significantly lesser resistance to tetracycline, nalidixic acid and trimethoprim-sulfamethoxazole compared to non-hemolysin producing isolates.

In Ghana, Forson *et al*., (2019), reported on UPEC associated ASB in pregnant women and found a significant association between antibiotic resistant isolates and virulence. They also observed that ampicillin resistant UPEC isolates harboring the ^{Bla}TEM gene had a higher number of virulent factors compared to isolates resistant to tetracycline or gentamicin.

An understanding on the interplay between virulent factors and antibiotic resistance is very crucial in era where available treatment options are failing and world is speedily transitioning into the post antibiotic era.

2.3.3 Role of Integrons in Antimicrobial resistance

Resistance genes are usually carried on integrons, bacteriophages and mobile genetic elements such as transposons and plasmids and transferred horizontally (Xu *et al*., 2011). Integrons are site-specific recombination systems that arrest mobile gene cassettes encoding antibiotic resistance and has the potential of expressing these gene cassettes (Butzin *et al*., 2009).

A functional integron is characterized by the presence of an integrase gene (intI), a recombination or attachment site (attI) for insertion of DNA sequences and a promoter gene (Pc) (Butzin *et al*., 2009). Integrons are generally classified into 4 (intI1, int12 intI3 and intI4) based on the differences in the amino acid sequences of the intI integrases (Deng *et al*., 2015). Amongst the classes of integrons associated with antibiotic resistance, *intI* is the most prevalent and most clinically relevant because of their wide distribution in 22-59% of gram negative bacteria (Butzin *et al*., 2009). Their immobility

makes them closely associated with mobile elements such as transposons and conjugative plasmids and this is what accounts for their wide distribution (Butzin *et al*., 2009).

A study conducted in Ghana on non-HIV pregnant women revealed that 12.2% and 2.4% of UPEC isolates were carrying *intI* and *intII* genes respectively (Forson *et al*., 2019). In Syria, a rather high prevalence (54.66%) of class I integrons was found in Trimethoprim resistant UPEC with a significant association with multidrug resistance and ESBL production (Al-assil *et al.*, 2013). In Pakistan, Khan *et al*., (2018) reported ESBL producing UPEC as an emerging health issue as they found class 1 integrons in about 79% MDR UPEC isolates carrying CTX-M1, TEM and SHV genes.

A comprehensive study conducted in European countries and Korea revealed a slightly higher prevalence of class 1 integrons in 57.6% and 69% of trimethoprim resistant UPEC isolates respectively (Pondé, 2013; Yu *et al*., 2004). Solberg *et al*., (2006) highlighted the role of integrons in antibiotic resistance as they reported that about 71.6% of trimethoprim-sulfamethoxazole resistant UPEC isolates carried the class 1 integron whereas 28.4% of the trimethoprim-sulfamethoxazole resistant isolates carried class 2 integrons. This finding reenforces our knowledge on the role of integrons, especially *intI*, in antibiotic resist

CHAPTER THREE

METHODOLOGY

3.0 Study site and design

Women living with HIV (WLHIV) and attending the Antiretroviral treatment (ART) clinic at the St. Martin de Porres Hospital (SMDPH), Eikwe in the Western Region of Ghana, were sampled. St. Martin de Porres Hospital is a faith-based institution managed by the Catholic Diocese of Sekondi-Takoradi and a member of the Christian Health Association, Ghana (CHAG). It is a rural facility and a district hospital within Ellembelle which serves as a major referral center for the people of Nzema and neighboring towns in Ivory Coast. SMDPH has about 200 bed capacity, annual OPD attendance of over 80, 000 patients and over 14,000 admissions. The facility is well known for its maternal and child health services and has an annual maternal delivery of about 2,800. It has an emergency ward, infectious disease unit, pediatric ward, neonatal intensive care unit (NICU), general adult ward and a high dependency unit with an outpatient department (OPD). Its ART Clinic serves about 1900 active clients with an average monthly attendance of about 150 clients.

3.1 Study Participants

HIV sero-positive women (15 years and above) with no complaints suggestive of UTI, reporting to the ART Clinic between March and June 2021 were enrolled. Blood and urine samples, and clinical information were taken from consenting participants.

3.2 Inclusion and Exclusion criteria

The inclusion criteria for this study were females above 15years on Antiretroviral therapy, taking cotrimoxazole prophylaxis.

The exclusion criteria comprise treatment naïve females and females taking antibiotics other than cotrimoxazole were excluded from the study.

3.3 Sample Size (n) Determination

The minimum sample size for this study was determined with the Yamane (1967:886) formula, $n = \frac{N}{1+N(e^2)}$, using a 95% confidence interval, 0.5 standard deviation and a ±5% precision. Where n = sample size, N = Population and e = allowable error.

N = 210,000 (Women above 15yrs living with HIV in Ghana (UNAIDS, 2019)

e = 5% (0.05)

$n = \frac{210000}{1+210000(0.05^2)}$ = 399

The minimum sample size (n) for this study was 399. However, 400 HIV seropositive women were enrolled.

3.4 Participant Information

A structured questionnaire was used to extract clinical information from the patient and the patient folders. These included patient demographics, history of hospitalization within the past three months, HAART treatment initiation date and WHO disease stage.

3.5 Specimen Collection and Processing

Participants were directed on proper specimen collection procedures and were given 50mls properly labelled sterile urine containers to collect 20mls of mid-stream urine. 5ml of venous blood was collected from the antecubital fossa of each patient into ethylenediaminetetraacetic acid (EDTA) vacutainer using precision needles.

3.6 Laboratory Investigation

Laboratory investigation occurred in two (2) phases at three different laboratories

PHASE 1 –Culturing of urine samples, bacteria identification, antibiotic susceptibility test and stocking of uro-pathogens (UPEC) at the Bacteriology Department of St. Martin de Porres Hospital

PHASE II – Molecular characterization of UPEC (Screening for integrons and virulent factor genes) at the Bacteriology Department, Noguchi Memorial Institute of Medical Research.

3.6.1 Phase I

Urine samples were aseptically streaked on Cysteine Lactose Electrolyte Deficient (CLED) **agar using a 10µl loop and incubated at 37^0C. Plates were inspected after 18 to 24 hours and significance was given to plates yielding bacterial count of 1 x 10^5 CFU/ml** (Chesbrough, 1999).

3.6.1.1 Identification of organisms

Standard microbiological procedures were followed. Lactose fermenting (yellow) discrete colonies were sub-cultured onto Eosin Methylene Blue (EMB) agar (Techno PharmaChem, India) and incubated aerobically for 10hrs (Leininger *et al*., 2001). UPEC was differentiated from other lactose fermenters of the Enterobacterales order by the production of greenish metallic sheen on EMB agar (Leininger *et al*., 2001). Identity of the isolates were confirmed using Maldi-Biotyper version, 4.1.100.(PYTH) 1742019-06-158_01-16-09.

3.6.1.2 Antimicrobial susceptibility test (AST)

AST was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2020 with the following antibiotics; trimethoprim-sulfamethoxazole (Cotrimoxazole) (25µg), nitrofurantoin (300µg), Nalidixic acid (30µg), Cefuroxime (30µg), ampicillin (10µg), Fosfomycin (200µg), Tetracycline (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Cefepime (30µg) and Amikacin (30µg), Meropenem (30µg), Ceftriaxone (30µg), Amoxiclav (30µg) (Oxoid ltd Basingstoke, UK). This was done using Kirby Bauer's disc diffusion method on Muller-Hinton agar (Oxoid Ltd) as follows;

- Three to five well isolated colonies of the same morphology were picked with a sterile loop and transferred into 4 to 5 ml of sterile broth medium.
- This was emulsified and incubated at 35 ± 2º C until a 0.5 McFarland standard is achieved.
- Within 15 minutes of attaining the 0.5 McFarland standard, a loopful of the

organism was transferred on to Mueller-Hinton agar plate and a sterile cotton swab was used to streak the entire surface of the agar.

- Within 15 minutes of streaking, sterile forceps was used to pick **antibiotic discs** unto the agar plate and incubated at 37^{o}C for 18 to 24hrs.

  Zones of inhibition were measured using transparent ruler and **interpreted** according to CLSI break point systems.

The efficacy of the antibiotic discs and growth promotion potential of the media were ascertained using American Type Culture Collection (ATCC) control strains (*E. coli* ATCC 25922) and the results were interpreted using CLSI 2020 guideline.

3.6.1.3 Stocking of Isolates

Sterile plastic loops were used to transfer pure colonies into Skim milk, Tryptone, Glucose and Glycerol (STGG) stocking media and stored under -20^{0}C until further processing.

3.6.2 Phase II

3.6.2.1 Extraction of DNA for Virulent Factor Genes and Integrons in UPEC

DNA was extracted from 59 UPEC isolates using fresh overnight cultures plated on nutrient agar. This was done according to the boiling lysis method described by Ribeiro *et al*, (2016) using 200µl of double distilled water against 3 well isolated colonies, heated at 98^{o}C for 10minutes, refrigerated for 10minutes in -20^{o}C and centrifuged at 1,350rpm for 5minutes. 150µl of the supernatant (DNA template) was pipetted into 2ml Eppendorf tubes and stored in -20^{o}C until further works.

3.6.2.2 Screening for Virulence and Integron genes using Multiplex PCR

Multiplex PCR was performed **to determine** the following genes; iron capture systems (*iutA, chuA),* adhesin *(PapC),* capsule (*kpsMTII*), toxin (*cnf1*, *usp*) and integrons (*intI* and *intII*) using primers in Table 3.1. The PCR was run in two primer sets: Set 1; (*iutA, kpsMTII, USP, cnf1*) Set 2; (*PapC, intI, intII, chuA*). Each PCR reaction had 1µl of 25 Pmol primer, 6.25µl of One taq Quick-Load 2× Master Mix with Standard Buffer, 3.25µl of nuclease free water and 1.5µl of DNA template giving a final reaction volume of 15µl.

Amplification was performed using applied Biosystems Thermal cycler (Thermo Fisher Scientific, USA). Cycling conditions were as follows; initial denaturation at 95^{o}C for 5 min for 30cycles, followed by a final denaturation (94^{o}C, 30 s), annealing (63^{o}C, 30 s), extension (72^{o}C, 5 min) and final extension (72^{o}C, 4 min). The amplicons were loaded onto a 2% agarose gel containing sybr red and electrophoresed for one hour. The gel was observed under in a gel doc under UV light. The band sizes were compared to a 100 bp DNA ladder.

Table 3.0 Primer sequences and Amplicon sizes for Virulent and Integron genes

Gene	Primer Sequences (5' – 3')	Amplicon length (bp)	Reference
Virulent Genes			
kpsMT II	F: GCGCATTTGCTGATACTGTTG R: CATCCAGACGATAAGCATGAGCA	272	(Johnson & Stell, 2000)
cnf1	F: AAG ATG GAG TTT CCT ATG CAG GAG R: CAT TCA GAG TCC TGC CCT CAT TAT T	498	(López-banda *et al.*, 2014)
PapC	F: GTGGCAGTATGAGTAATGACCGTTA R: ATATCCTTTCTGAGGGATGCAATA	200	(López-banda *et al.*, 2014)
USP	F: ACATTCACGGCAAGCCTCAG R: AGCGAGTTCCTGGTGAAAGC	440	(Bauer *et al.*, 2002; Kurazono *et al.*, 2000)
iutA	F: GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	300	(Johnson & Stell, 2000)
chuA	F: GACGAACCAACGGTCAGGAT R: TGCCGCCAGTACCAAAGACA	279	(Johnson & Stell, 2000)
Integron Genes			
Int I	F: GGGTCAAGGATCTGGATTTCG R: ACATGGGTGTAAATCATCGTC	483	(Lucey *et al.*, 2000)
IntII	F: CACGGATATGCGACAAAAAGGT R: GTAGCAAACGAGTGACGAAATG	788	(Lucey *et al.*, 2000)

3.7 Ethical considerations

This study was given ethical approval (CHS-Et/m.4.5.8/2020-21) by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana and the Management of St. Martin de Porres Hospital, Eikwe. Written Informed consents were sought from participants seeking care at the ART clinic of St. Martin de Porres, Eikwe.

3.8 Data analysis

Primary data extracted using questionnaires were keyed into Microsoft Excel for preliminary analysis. The data from excel spreadsheet was transferred to Statistical Package for Social Sciences (SPSS) (Version 20) for further analysis. Descriptive statistics was used to determine percentages and mean. The chi square test and Logistic regression analysis was used to investigate association between dependent and independent variables. And a p value<0.05 was considered significant.

CHAPTER FOUR

RESULTS

4.1 DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

A total of 400 HIV seropositive women were enrolled in the study. Participants demographic characteristics, viral load and HAART duration are summarized in Table 4.1. The mean age of the participants was 43.54 years ± 12.027. Majority of the participants were between the age range of 36 to 45 years. The overall prevalence of uropathogen amongst the study population was 21.25% (85/400). UPEC accounted for about 69.4% (59/85) of the uropathogens isolated.

4.1.1 Distribution of uropathogens by viral load

Viral load was grouped according to WHO, (2013) criteria for viral suppression; < 1000 copies/ml and ≥1000 copies/ml. Majority of the clients had viral loads less than 1000 copies/ml, 78.5% (314/400). These are presented in Tables 4.1 and 4.2

4.1.2 Distribution of uropathogens by duration of HAART therapy

Majority of the participants had been on HAART treatment for a period of 1 to 5 years accounting for about 54.9% (225/400) of the total participants. Participants with a HAART duration **of** less than one year had the highest positive microbial culture whereas those within the range of 1 to 5 years had the least positive microbial culture. However, no significant association was found between positive microbial culture and duration of HAART ($p>0.05$).

Table 4.1 Distribution of risk factors among study participants

Variables	Frequency	Percentage (%)
Age groups (years)		
15 to 25	19	4.8
26 to 35	79	19.8
36 to 45	135	33.8
46 to 55	114	28.5
56 to 65	41	10.3
>65	12	3.0
WHO Disease stage		
Stage 1	38	9.5
Stage 2	76	19.0
Stage 3	250	62.5
Stage 4	36	9.0
Duration of HAART (years)		
< 1	17	4.1
1 to 5	225	54.9
6 to 10	110	26.8
>10	58	14.1
Viral load (copies/ml)		
< 1000	314	78.5
≥1000	86	21.5

< 1000 copies/ml indicates viral suppression (WHO, 2013).

Table 4.2: Risk factor association to bacteriuria

Variables	Positive Microbial culture (85) n (%)	Negative Microbial culture (315) n (%)	P-value
Age Group			**0.202**
15 to 25	6 (33.3)	12 (66.7)	
26 to 35	10 (11.8)	75 (88.2)	
36 to 45	30 (21.9)	107 (78.1)	
46 to 55	32 (26.9)	87 (73.1)	
56 to 65	12(28.6)	42 (76.2)	
>65	2 (22.2)	7 (77.8)	
WHO Disease Stage			**0.884**
Stage 1	9 (23.7)	29 (76.3)	
Stage 2	14 (18.4)	62(81.6)	
Stage 3	55(22)	184 (78)	
Stage 4	7 (19.4)	29 (80.6)	
Duration of HAART			**0.212**
< 1	8(47.1)	9 (52.9)	
1 to 5	38(16.9)	187(83.1)	
6 to 10	27(24.5)	83(75.5)	
>10	15(25.9)	43(74.1)	
Viral load (copies/ml)			**0.203**
< 1000	71(22.6)	243(77.4)	
≥1000	14(16.3)	72(83.7)	

< 1000 copies/ml indicates viral suppression (WHO, 2013).

4.2 Distribution of uropathogens

Table 4.4 shows the profile of uropathogens isolated from the 400 participants recruited in the study. Eighty-five (85) uropathogens were identified from urine specimens that yielded significant microbial growth. The top three pathogens were, *Escherichia coli* 69.4% (59/85), *Enterococcus faecalis* 8.2% (7/85)), and *Klebsiella pneumoniae* 7.0% (6/85)).

4.3 Antibiotic Susceptibility Profile of UPEC

Figure 2 represents resistance pattern of UPEC isolates from the participants. The highest resistance among UPEC was to 100% Ampicillin and 98.3%, to sulfamethoxazole-trimethoprim, and the least resistance recorded was to Meropenem and Fosfomycin at 1.7%.

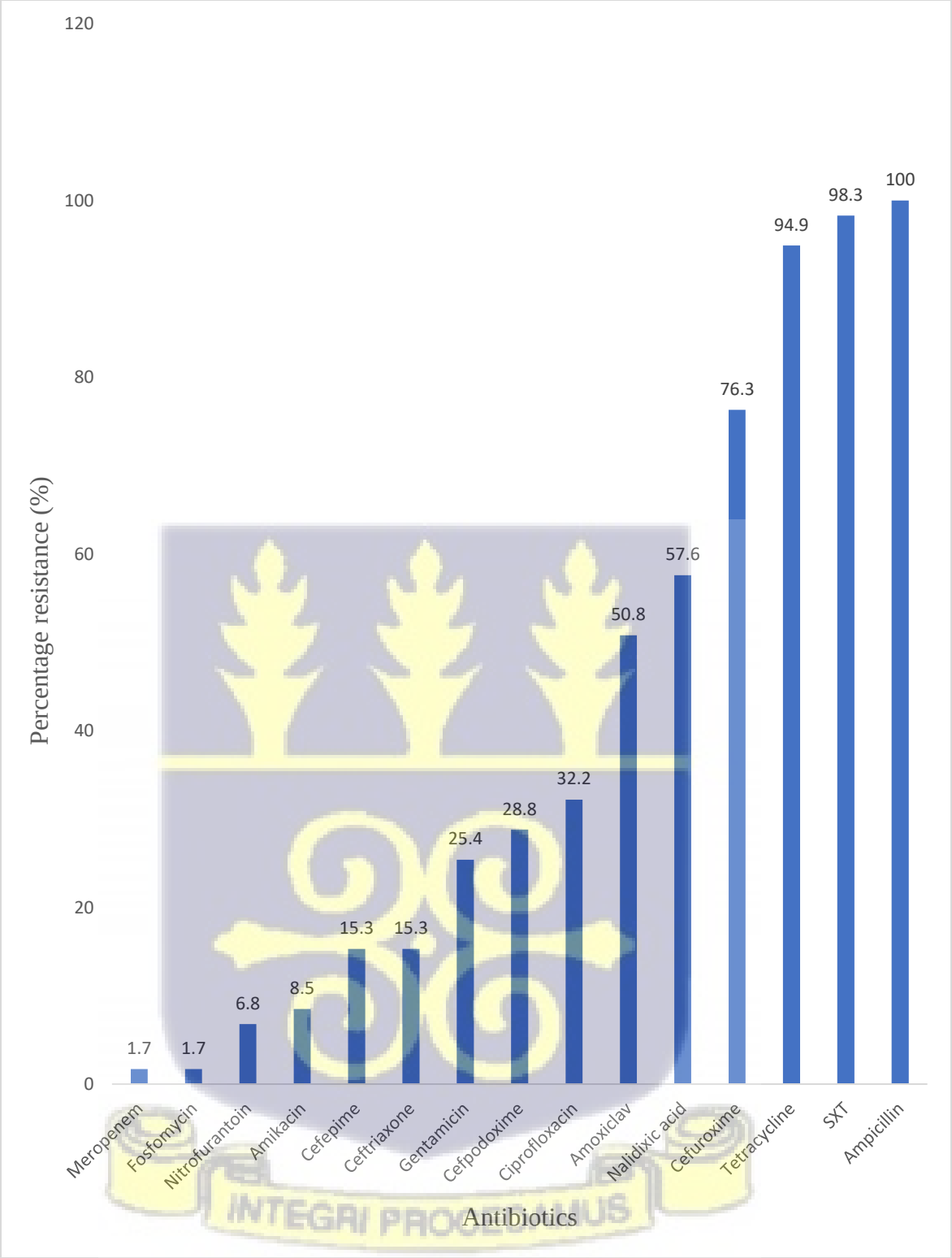


Figure 2. Proportion of resistance of UPEC isolates to antibiotics

4.4 Virulence gene distribution among UPEC isolates

The most widely distributed virulence factor gene as represented in figure 3, was *chuA* with a prevalence of 66.1% followed by *papC*, *cnf1*, *kpsMTII* and *iutA* with prevalence rates of 57.6%, 50.8%, 45.8 and 35.6% respectively.

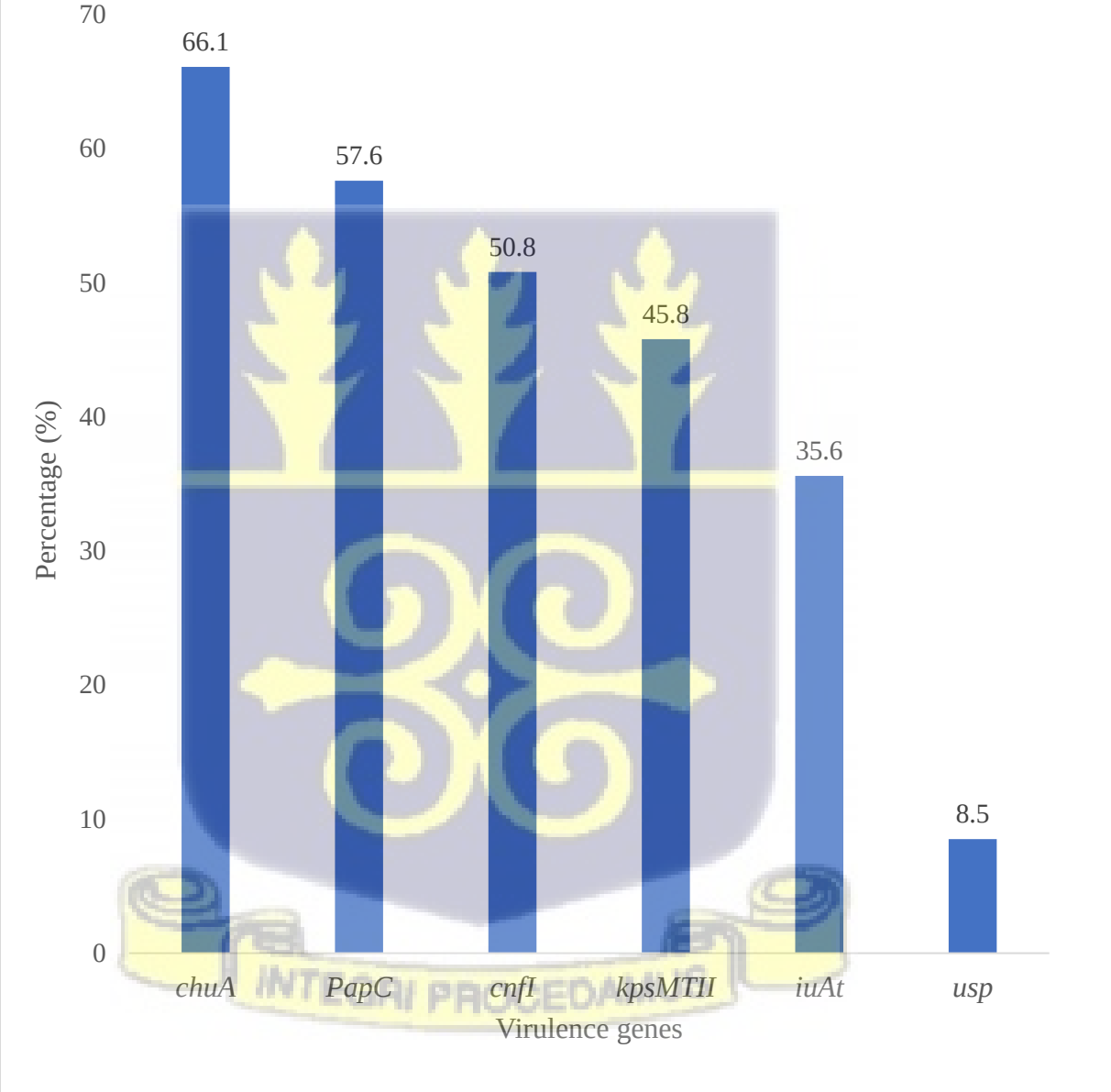


Figure 3. Distribution of virulence genes

4.5 . Distribution of Integrons in UPEC isolates

Carriage of *intI* in UPEC isolates was 42.4% (25/59) and *intII* gene 20.3% (12/59). **No isolate harbored more than one integron gene.**

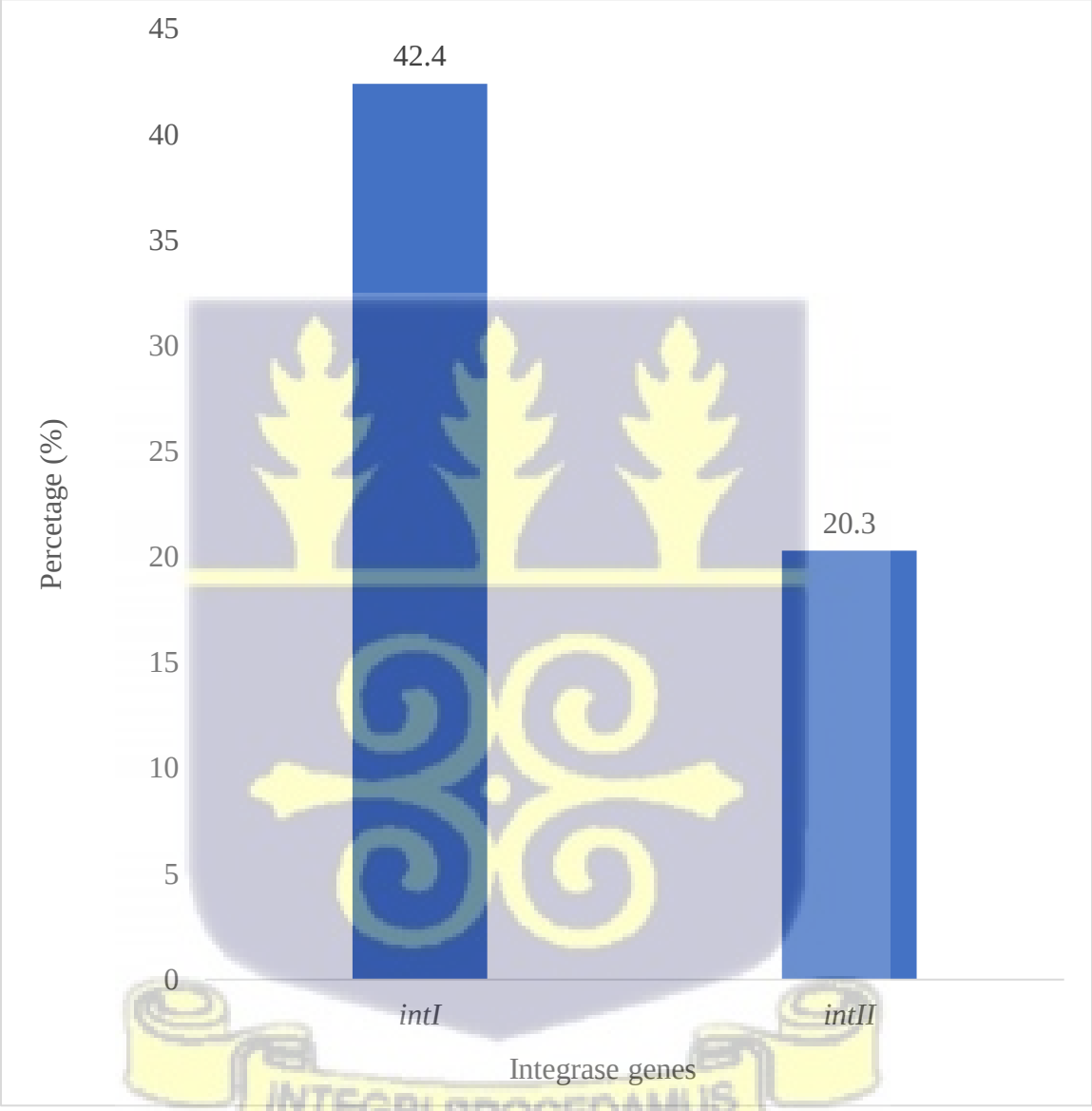


Figure 4. Distribution of integron in UPEC isolates

Table 4.3 Resistance profile and distribution of integron and virulence genes in UPEC isolates

	Integron genes		Virulence genes						Antimicrobial drug
Isolate ID	*intI*	*intII*	*chuA*	*cnf1*	*usp*	*kpsMTII*	*iutA*	*papC*	Resistance profile
E. coli 1	-	+	+	-	-	-	+	+	AMP, CXM, SXT
E. coli 2	-	+	-	+	-	+	-	-	AMP, SXT, TET
E. coli 3	-	-	+	-	-	-	+	+	AMC, AMP, SXT, CIP, CPD, CXM, GEN, TET
E. coli 4	-	-	+	+	-	+	+	+	AMP, NAL, SXT, TET
E. coli 5	-	-	+	+	+	+	-	-	AMC, AMP, CXM, SXT, TET
E. coli 6	-	-	+	-	-	-	+	+	AMP, CIP, NAL, SXT, TET
E. coli 7	-	-	+	+	+	-	-	+	AMC, AMP, CIP, CXM, GEN, NAL, SXT, TET,
E. coli 8	-	+	+	+	-	+	-	+	AMC, AMP, CXM, SXT, TET,
E. coli 9	+	-	-	-	-	-	-	-	AMC, AMP, CIP, CPD CXM, SXT, TET
E. coli 10	-	+	+	+	-	+	-	+	AMC, AMP, CPD, CXM, NAL, SXT, TET,
E. coli 11	-	+	+	-	-	+	-	-	AMP, FOS, SXT, TET
E. coli 12	-	-	+	-	-	+	-	-	AMC, AMP, CPD, CRO, CXM, GEN, FEP, SXT
E. coli 13	-	-	+	-	-	-	+	+	AMP, CIP, CPD, CRO, CXM, GEN, NIT, SXT, TET,
E. coli 14	+	-	-	-	-	-	+	-	AMC, AMP, AMK, CIP, CPD, CRO, CXM, GEN, FEP, NAL, NIT, SXT, TET.
E. coli 15	+	-	-	-	-	-	+	+	AMC, AMP, CIP, CPD, CXM, NAL, SXT, TET.
E. coli 16	-	-	+	+	-	+	-	-	AMP, CXM, SXT, TET
E. coli 17	-	-	-	-	-	-	+	+	AMP, CXM, NAL, SXT, TET

Table 4.3 Continued: Resistance profile and distribution of integron and virulence genes in UPEC isolates

E. coli 18	+	-	-	-	-	-	-	-	AMP, CXM, SXT, TET
E. coli 19	-	-	+	+	-	-	-	+	AMC, AMK, AMP, CXM, GEN, NAL, SXT, TET,
E. coli 20	+	-	+	+	-	+	-	+	AMK, AMP, CIP, CPD, CRO, CXM, GEN, FEP, NAL, SXT, TET.
E. coli 21	-	+	+	+	-	+	-	+	AMC, AMP, CXM, SXT, TET
E. coli 22	+	-	+	+	-	+	-	+	AMP, CXM, SXT, TET
E. coli 23	-	-	+	+	-	+	-	+	AMP, CXM, SXT, TET
E. coli 24	+	-	+	+	-	-	+	+	AMP, CXM, SXT, TET
E. coli 25	-	-	-	-	-	-	-	-	AMP, CIP, CXM, GEN, NAL, SXT, TET
E. coli 26	+	-	-	-	-	-	-	-	AMC, AMP, CXM, NAL, SXT,
E. coli 27	+	-	+	+	-	-	+	+	AMC, AMP, CXM, SXT, TET, NA
E. coli 28	+	-	-	-	-	-	+	-	AMP, CXM, GEN, NAL, SXT, TET,
E. coli 29	-	+	-	-	-	-	-	-	AMC, AMP, CXM, NAL, SXT, TET,
E. coli 30	+	-	+	-	+	+	-	-	AMP, CXM, SXT, TET
E. coli 31	-	-	-	-	-	-	-	+	AMP, CXM, SXT, TET
E. coli 32	+	-	+	+	-	+	-	-	AMP, CXM, GEN, SXT, TET
E. coli 33	+	-	+	+	-	+	-	-	AMP, CRO, CXM, GEN, NAL, SXT, TET
E. coli 34	+	-	-	-	-	-	-	-	AMC, AMP, CRO, CXM, GEN, NAL, SXT, TET
E. coli 35	-	+	+	+	-	+	-	-	AMC, AMP, CIP, CPD, CXM, FEP, NAL, SXT, TET
E. coli 36	+	-	+	-	-	+	-	+	AMC, AMP, SXT, TET
E. coli 37	-	+	+	+	-	+	-	+	AMP, SXT, TET

Table 4.3 Continued: Resistance profile and distribution of integron and virulence genes in UPEC isolates

E. coli 38	-	-	-	-	-	-	-	-	AMC, AMP, NAL, SXT, TET
E. coli 39	-	-	-	-	-	-	+	-	AMC, AMP, NAL, SXT, TET
E. coli 40	+	-	+	+	-	-	+	+	AMC, AMP, NAL, TET
E. coli 41	+	-	+	+	-	+	-	+	AMP, CXM, SXT, TET
E. coli 42	+	-	+	+	-	-	-	+	AMP, SXT, TET,
E. coli 43	+	-	-	-	-	-	+	-	AMP, CIP, CXM, GEN, NAL, SXT, TET
E. coli 44	-	+	+	+	-	+	-	+	AMP, CXM, SXT, TET,
E. coli 45	-	-	+	+	-	+	-	+	AMK, AMC, AMP, CIP, CXM, GEN, SXT, TET
E. coli 46	-	-	-	-	-	-	-	+	AMK, AMP, CIP, NAL, SXT, TET
E. coli 47	-	-	+	-	-	-	-	+	AMC, AMP, MEM, CRO, CPD, CXM, GEN, FEP, NAL, SXT, TET
E. coli 48	+	-	+	-	-	+	+	+	AMP, NAL, SXT, TET
E. coli 49	-	+	-	-	-	-	+	-	AMP, NAL, SXT, TET
E. coli 50	+	-	+	+	-	+	-	-	AMC, AMP, NAL, SXT, TET
E. coli 51	+	-	-	-	-	-	-	-	AMC, AMP, CPD, CXM, SXT, TET
E. coli 52	-	+	+	+	-	+	-	+	AMC, AMP, CPD, CXM, SXT, TET
E. coli 53	-	-	-	+	-	-	+	+	AMC, AMP, CIP, CPD, CRO, CXM, FEP, NAL, NIT, SXT, TET,
E. coli 54	+	-	-	-	+	-	+	+	AMC, AMP, CIP, NAL, SXT, CXM, TET
E. coli 55	+	-	+	+	-	+	+	+	AMC, AMP, NAL, SXT, CXM, TET
E. coli 56	+	-	+	+	-	+	-	+	AMC, AMP, NAL, SXT, CXM, TET
E. coli 57	-	-	+	-	+	-	+	+	AMC, AMK, AMP, CRO, CXM, GEN, FEP, NIT, NAL, SXT, TET

Table 4.3 Continued: Resistance profile and distribution of integron and virulence genes in UPEC isolates

E. coli 58	-	-	+	+	-	-	+	-	AMC, AMP, CIP, CPD, CRO, CXM, GEN, FEP, NAL, SXT, TET
E. coli 59	-	-	+	+	-	+	-	-	AMC, AMP, CPD, CRO, CXM, FEP, SXT, TET

4.6 HAART duration as a predictor of Antibiotic resistance

Table 4.4 Binary logistic regression analysis of HAART duration as a predictor of resistance

	Antibiotics												
	MEM	SXT	CRX	FOS	FOT	TET	CIP	AMK	CTR	FEP	CPD	AMC	NAL
HAART duration													
OR	1.049	0.877	1.009	0.917	1.011	1.031	1.048	0.860	0.996	1.037	1.083	**1.329**	0.697
p	0.861	0.408	0.917	0.917	0.942	0.854	0.541	0,230	0.971	0.715	0.316	**0.002**	1.029

OR=odds ratio, *p*=p-value; **Bold** *p* value means Significant association ($p \leq 0.05$). If $p \leq 0.05$ and OR > 1 it means risk factor is a predictor of resistance. (MEM=Meropenem, SXT = Trimethoprim Sulfamethoxazole, CXM= Cefuroxime, FOS = Fosfomycin, TET= Tetracycline, CIP=Ciprofloxacin, AMK = Amikacin, CTR = Ceftriaxone, FEP = Cefepime, AMP = Ampicillin, CPD = Cefpodoxime AMC=Amoxiclav, NAL=Nalidixic

CHAPTER 5

DISCUSSION

Asymptomatic bacteriuria (ASB) is reportedly high among PLHIV (Skrzat-Klapaczyńska *et al*., 2018) and uropthagenic *E. coli* is the most prevalent organism. Most individuals may develop asymptomatic bacteriuria because, bacterial colonization of the bladder even in high numbers seldomly results in symptoms (Foxman, 2010).

The prevalence of ASB among WLHIV in this study was 21.25%. This indicates that WLHIV are at a high risk of developing UTI as it is known that 30% of people with ASB may develop symptomatic UTI if not treated (Smaill, 2007). This result is at variance with the 57.8% and 51.8% reported in Nigeria and Poland respectively (Akinbami *et al*., 2013; Skrzat-Klapaczyńska *et al*., 2018), but agrees with 21.8% reported by Ojoo *et al*., (1996) among HIV positive commercial sex workers in Nairobi. In further comparison, the prevalence in this study is also higher than the 12.3%, 7.0% and 18% reported in Tanzania, Ethiopia and South Africa respectively (Fenta *et al*., 2016; Laker *et al*; 2004; Ngowi *et al*., 2021) but slightly lower than 36.7% and 31.3% reported in India and Nigeria respectively (Akadri & Odelola, 2020; Yadhav & Samreen, 2017). The comparatively lower prevalence in this study could be due to the inclusion criteria which accommodated participants on prophylactic cotrimoxazole. The intake of antibiotics can result in negative or insignificant culture results in participants with susceptible isolates. The difference in the location may also play a role in the differences in the prevalence rates as the study sites are geographically distinct.

In Ghana, higher ASB rates of 56.1%, 52.6%, and 33.3% were reported among pregnant HIV negative women (Afoakwa *et al*., 2018; Boye *et al.*, 2012; Obirikorang *et al*., 2012). The comparatively lower prevalence reported in this study could be attributed to the difference in the study population (pregnant women) as it is known that anatomical and physiological changes in pregancy increase uropathogen colonization in pregnant women (Labi *et al*., 2015).

From this study, the highest prevalence of bacteriuria was found among women within the age category of 15 to 25 years. This is in agreement with reports by Karikari *et al*., (2020) who recorded high bacteriuria among the age group, 20 to 29 years. A possible explanation could be because they are more sexually active and sex is risk factor for ASB and UTI (Shefield & Cunnigham, 2005; Vincent *et al*., 2013; Labi *et al*., 2015). ASB was also very high among women above 56 years. This could be due to genital prolapse which is reportedly prevalent in elderly women (Skrzat-Klapaczyńska *et al*., 2018; Storme *et al*., 2019). This condition leads to voiding dysfunction which has been reported as a very common condition among the elderly, leading to an increased risk of UTI (Aytan *et al*., 2014). Our finding are contrary to those of Olowe *et al*., (2015) in Nigeria, who recorded the highest bacteriuria among the age group, 30 to 39 years and also found age to be a predictor of ASB (p=0.02) among this population.

Participants assigned to WHO disease stage 1 had the highest bacteriuria. This is because women in this category are usually asymptomatic and may appear healthy to actively engage in sexual activities. The study however found no association between WHO disease stage and ASB (p>0.05). This is similar to the findings of Duoriyekemwen *et al*., (2012) who also found no association between bacteriuria and WHO disease stage in

children with HIV/AIDS but does not agree with that of Okechukwu & Thairu., (2019) and Adekunle & Adetokunbo, (2014) who established an association between bacteriruia and WHO disease stages. This could be because of differences in treatment adherence and immnue status of the participants.

In this study, the predonimant uropathogen isolated was *Escherichia coli* (UPEC) and this accounted for 69.4% of the total uropathogens. This concurs with the 62.5% reported by Acheampong *et al.*, (2018) in Ghana, 63% by Widmer *et al.*, (2010) in South Africa, 54.3 % by Debalke *et al.*, (2014) in Ethiopia but slighlty higher, though predominant, than the 44.3% by Adekunle & Adetokunbo, (2014) in Nigeria, 48% by Awolude *et al.*, (2010), 47.0% by Afoakwa *et al.*, (2018), 48%by Boye *et al.*, (2012), 46.4% by Afriyie *et al.*, (2015), 36.0% by Gambrah *et al.*, (2021), 36.8% by Obirikorang *et al.*, (2012), 37% by Turpin *et al.*, (2007) and 46.6% reported by Yenli *et al.*, (2019) in Ghana. This finding however does not align with reports from other studies where *Staphyloccus, Enterococcus* and *Klebsiella species* were the predominant uropathogens (Ambrose *et al.*, 2009; Donkor *et al.*, 2017; Donkor *et al.*, 2019; Labi *et al.*, 2015; Omoregie & Eghafona, 2016; Samje *et al.*, 2020). This could be due to differences in the study population and some social factors such as personal hygiene and frequency of sexual activiy, hypoestrogenism (Raz & Stamm, 1993), presence of hydrogen peroxide producing *Lactobacillus* strains (Gupta *et al.*, 1998) and ABO secretor status. Non-secretors of the ABO antigen are four times more prone to UPEC colonization, chiefly because UPEC has a better adherence to uro-epithelial cells.

Multidrug resistance was almost non-existent three decades ago with causes limited to mutations (Hasan *et al.*, 2007). In this study, all the UPEC isolates identified displayed

resistance to three or more antibiotics from different classes qualifying them as multidrug resistant (MDR) (Magiorakos *et al*., 2011). This is of concern as it suggests that the likelihood of treatment failure among this population is high and increased cost of care can be considered. This report is higher than the 78% reported by Fenta *et al*., (2016) in Ethiopia. UPEC isolates were resistant to ampicillin, trimethoprim-sulfamethoxazole and tetracyclines with resistance rates of 100%, 98.3% and 94.9% respectively. This suggests that these drugs may not be suitable for empirical treatment among this population. The high resistance to these drugs could be attributed to affordability and accesibility making it possible for self medication, overuse and misuse (Donkor *et al*., 2012; Klee *et al*., 2018). **Reports from this study corroborates earlier findings from Ghana (Acheampong *et al*., 2011; O. Adjei & Opoku, 2004; Labi *et al*., 2015; Lutterodt *et al*., 2014; Newman *et al*., 2011; Yenli *et al*., 2019) as well as reports from Tanzania and Nigeria where 71% to 90% of uropathogens from the study population were resistant to trimethoprim-sulfamethoxazole (Ambrose *et al*., 2009; Chaula *et al*., 2017; Marwa *et al*., 2015)**. It further aligns with the findings of Okechukwu & Thairus., (2019) who have reported a 100% and 95% resistance to trimethoprim-sulfamethoxazole and ampicillin respectively among PLHIV in Nigeria. These findings propose that UPEC isolates may have acquired resistance to trimethoprim-sulfamethoxazole from selective pressure as this drug has been used for prophylactic purposes for decades in this population. **The least resistance was observed to Fosfomycin, Amikacin, Nitrofurantoin and Meropenem. This is in keeping with reports in Ghana, Nigeria and Ethiopia (Afoakwa *et al*., 2018; Dzifa *et al*., 2021; Fenta *et al*., 2016; Karikari *et al*., 2020; Kemajou & Ajugwo, 2016; Lutterodt *et al*., 2014; Yenli *et al*., 2019).** The

low resistance reported to these drugs could be the result of their infrequent use in the health care settings and possibly their route of administration, making self medication and abuse difficult. These drugs will be ideal for the management of UTI among this population. However, the challenge of administering aminoglycosides is that it has nephrotoxicity potentials (Martínez-Salgado *et al*., 2007). Given the high renal problems recorded among this population in Ghana (Obiri-Yeboah *et al*., 2018), it is advisable that amikacin should only be used when the renal function status of this population has been ascertained or treatment options are exhausted. On the other hand, Nitrofurantoin and Fosfomycin are well tolerated with rare adverse events (Gardiner, 2019) and can be a better alternative for the treatment of UTI among this population.

Integrons play an important role in antibiotic resistance by capturing, integrating and expressing antibiotic resistant genes (Hall & Collis, 1995). From the study, carriage of *intI* and *intII* genes were 42.4% and 20.3% respectively. The carriage of these two genes is higher than figures reported in an earlier study conducted in Ghana where carriage of *intI* and *intII* genes isolated from pregnant women was 12.2% and 2.4% respectively (Forson *et al*., 2019). This study also investigated the association between integrase genes and resistance to trimethoprim-sulfamethoxazole, even though no significant association was found ($p>0.05$), it was observed that 41.4% of the trimethoprim-sulfamethoxazole resistant isolates, carried the *intI* gene. This is because the TMP-SMX resistance genes (*sul* and *dhfr*) are predominantly carried on *intI* cassettes (Elias *et al*., 2021). The findings here are similar to reports from Syria and Europe where 54.66% and 57.6% of trimethoprim-sulfamethoxazole resistant UPEC isolates respectively, carried *intI* genes (Al-assil *et al*., 2013) but lower than reports from Korea (69.0%) and California (71.6%)

(Solberg *et al*., 2006; Yu *et al*., 2004). Over 40% of UPEC isolates having resistance to nalidixic acid, amoxiclav, trimethoprim sulfamethoxazole, cefuroxime and tetracyclines also haboured *intI* gene. This agrees with reports by Al-assil *et al*., (2013) and Khan *et al*., (2018) who established a strong association between *intI* and multidrug resistance including ESBL production. This could explain the high resistance recorded to these antibiotics. HAART duration has been observed to be a predictor of resistance to amoxiclav [(OR=1.329, 95% CI, 1.113-1.588) (p= 0.002)]. It was further observed that there was higher resistance to commonly prescribed antibiotics; amoxiclav, nalidixic acid, ceftriaxone, ciprofloxacin and cefuroxime among participants with HAART duration above five (5) years. This could be due to prior antibiotic exposure from previous hospital visits or admissions (Olaru *et al*., 2021).

The severity of UTI is largely dependent on the array of virulence genes present and the level at which they are expressed. This study determined the prevalence of virulence genes in UPEC isolates. From the study, about 76.3% of the isolates haboured one or more virulence genes. *ChuA* was the most prevalent gene and was haboured by 66.1% UPEC. The *chuA* gene codes for heme receptor protein and is ubiquitous in UPEC assisting in the importation of iron (Torres & Payne, 1997; Wyckoff *et al*., 1998; Nagy *et al*., 2001). It aids in the formation of intracellular bacterial communities and displays biofilm-like properties which enable UPEC to resist immune response by the development of a torpid pool of bacterial cells inside uroepithelial cells (Anderson *et al*., 2004; Reigstad *et al*., 2007).

PapC, *cnf1*, *kpsMTII* and *iutA* was prevalent in 57.6%, 50.8%, 45.8% and 35.6% UPEC isolates respectively. Adherence is the first step to colonization and the most important

determinant of pathogenicity (Mulvey, 2002), a possible explanation for the high prevalence of *papC*. The *papC* gene encodes a protein that is necessary for the fimbriae P bio- genesis regulation and had been reported as a precursor for pyelonephritis (Antao *et al*., 2009; Lane & Mobley., 2007). *Cnf1* on the other hand is a toxin shown to induce apoptosis in bladder cell lines of human (Mills *et al*., 2000) and has also been shown to also decrease the phagocytic function of polymorphonuclear neutrophils (Hofman *et al*., 2000). Horcajada *et al*., (2005) revealed that *cnf1* is a good promoter of bacteremia emanating from urinary tract infections. Therefore, the combined prevalence (>50%) of these genes suggest that this population are at increased risk of renal complication and bacteremia of urologic origin.

Usp gene was the least prevalent among the UPEC isolates and this is consistent with reports from Ghana where *usp* and some adhesin genes were less prevalent in UPEC isolate from pregnant women (Forson *et al*., 2019).

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Findings from this study demonstrate that asymptomatic bacteriuria is common among women living with HIV (WLHIV) with no association with age, viral load, WHO disease stage and HAART duration. High prevalence of multidrug resistant UPEC coupled with high carriage of virulence genes indicates that WLHIV are at a **high** risk of developing invasive infection of urologic origin. There is a need for regular monitoring of bacteriuria and antibiotic susceptibility testing among this population.

6.2 LIMITATION

- There was no HIV negative control group in this study to help appreciate if the level of asymptomatic bacteriuria and resistance is as a result of HIV infection.
- The study did not evaluate the immune status, $CD4^+$ count, of the participants since ASB is less prevalent in people with $CD4^+$ counts ≥200 copies/ml
- Only a limited number of virulence genes were screened and the absence of other genes cannot be verified.

6.3 RECOMMENDATION

- According to IDSA, trimethoprim-sulfamethoxazole should not be used in a community in which resistance level to this drug is ≥10 to 20%. The 98.3% reported suggests that SXT should not be used in empirical therapy among this population.

- Further studies should be conducted on a wider range of virulence genes to determine their role in antibiotic resistance.
- High level of resistance to cotrimoxazole raises questions about its prophylactic efficacy against fungal and protozoan microbes. Therefore, further studies should be carried out to determine if this drug still offers protection against malaria, pneumonia and other opportunistic infection among PLHIV.
- Findings from this study echoes the need for the establishment and equipping of microbiological laboratories in the country to undertake nationwide surveillance on antimicrobial resistance, to generate data which will inform clinical decisions and health policies.

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APPENDICES

Appendix 1
Informed Consent Form

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA MEDICAL SCHOOL

INFORMED RESEARCH CONSENT FORM

Title of Research Project: Characterization of Uro-Pathogenic *E. coli* (UPEC) in HIV Seropositive Women with Asymptomatic Bacteriuria.

Principal Investigator: Harold Kwadzo Amegbletor, (University of Ghana Medical School, Department of Medical Microbiology, P. O. Box 4236 Accra Ghana *hkamegbletor001@st.ug.edu.gh* 0505557062)

General information about the Research

Asymptomatic bacteriuria (ASB) is a precursor for Urinary tract infection (UTI) and has been reportedly high among People living with HIV (PLHIV) especially women in this category. Amongst the implicating organisms, uro-pathogenic *Escherichia coli* (UPEC) is the most frequently reported and the pathogenicity is conferred by the acquisition and expression of virulent factor genes. Considering the high level of UTI among PLHIV, it is imperative to determine the prevalence of ASB among this category and the associated risk factors. Additionally, limited treatment options resulting from the high level of resistance by uropathogens has called for the need to gain further knowledge on the role of virulence factors genes in the development of resistance to commonly prescribed

antibiotics. This study seeks to determine the prevalence of Uro-pathogen (UPEC) amongst WLHIV, virulence genes and resistance to commonly prescribed antibiotics. It also seeks to determine the correlation between high viral loads and bacteriuria.

Items Required from participants		Clinical/personal information through questionnaires	5mls of venous Blood	Mid-stream clean catch urine
Risk		A little pain at the site of venipuncture		
Benefits	Short term	Study participants with positive urine cultures will be referred to the ART clinic		
	Long term	The pattern of antimicrobial resistance to uro-pathogens will be communicated to the appropriate authorities to aid in policy making		

Confidentiality

Information provided in this research will be treated with all confidentiality and protected to the best of our ability. Unique Identification numbers will be used in this study in place of names.

Contacts for Additional Information

In-case of any questions appertained to this research or any research related injury, please contact Dr. Japheth Opintan on +233 (0) 244-789-209, Harold Kwadzo Amegbletor on +233 (0) 505- 557-062 or Mr. Nana Yaw Abankwah (EPRC administrator) on +233 (030) 294 0528, +233 (030) 266 5103.

Your right as a participant

Your decision to participate in this study is voluntary. You have the right to ask questions before giving your full consent, you have the right to information on the outcome of this study, right to discontinue at any stage and the right to refuse to participate in this study. This research has been reviewed by the Ethical and Protocol Review Committee (EPRC) of the College of Health Sciences, University of Ghana. Any question about your rights as a research participant can be directed to the EPRC Office between the hours of 8am-5pm on +233 (030) 294 0528, +233 (030) 266 5103 or email address: eprc@chs.edu.gh.

Voluntary Participation and Right to Leave the Research

This study is voluntary and you have the right to leave at any stage without any penalty.

STATEMENT OF CONSENT:

The purpose, benefits, risks and procedures described in the above document for the research, **Characterization of Uro-pathogenic *E. coli* in HIV seropositive women with asymptomatic bacteriuria,** has been read and explained to me in language I understand and speak. I had the opportunity to ask questions which have been answered to my satisfaction. I have been told I can contact Mr. Nana Yaw Abankwah (administrator) on +233 (030) 294 0528, +233 (030) 266 5103 or email address: eprc@chs.edu.gh and Harold Kwadzo Amegbletor on 0505557062/haroldam@yahoo.com if I have questions

about my rights as a study participant, to discuss problems, concerns or suggestions related to the research

I understand a copy of the signed consent form will be given to me to take home. My signature or thumbprint below confirms my agreement to participate.

Participant's Signature/ Thumbprint: ……..…… Date: ……

Investigator's signature/Thumbprint: ……..…… Date: ……

STATEMENT OF WITNESS

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research voluntarily.

Witness' Signature/ Thumbprint :……..…… Date: ……

Investigator's signature/Thumbprint: ……..…… Date: ……

Appendix II

Questionnaires

UNIVERSITY OF GHANA MEDICAL SCHOOL

RESEARCH TOPIC: Characterization of Uro-Pathogenic *E. coli* (UPEC) in HIV Seropositive Women with Asymptomatic Bacteriuria**.**

SM #:.		Study ID:	
AGE:		DATE:	

1. For the past two weeks, have you had any discomfort whiles urinating? **YES** **NO**

   If yes, please describe the type of discomfort

   Pain ☐ Burning sensation ☐ unusual smell ☐ Difficulty urinating ☐

   How long has it been? ……..

   Did you report to any hospital or pharmacy? **YES** ☐ **NO** ☐

   Were you given any drugs? **YES** ☐ **NO** ☐

   Were you given any antibiotic? **YES** ☐ **NO** ☐

   Are you still taking the drug given you on account of your complaints? **YES** ☐ **NO** ☐

   Do you remember the name of the drug? **YES** ☐ …… **NO** ☐

2. How long have you known your status? ……..……

3. Year of treatment (ART) initiation …….

4. Have you ever defaulted on taking your ART drugs? **YES** ☐ **NO** ☐

   If yes, how long? ……..

5. Have you ever run out of drug since you started this therapy? **YES** ☐ **NO** ☐

6. Have you been taking any herbal drugs aside the ARTs? **YES** ☐ **NO** ☐

   If yes, for how long? …….

   Are you still taking it? …….

7. Have you been on admission in the past three months? **YES** ☐ **NO** ☐

   If yes, on what account were you admitted?

   ……

   ……

8. Have you ever taken cotrimoxazole prophylaxis? **YES** ☐ **NO** ☐

9. Have you ever been taken off the cotrimoxazole? **YES** ☐ **NO** ☐

   If yes,

   What accounted for that? **Viral suppression** ☐ **Improved CD4** ☐ **Allergies with Complications** ☐

   How long were you taken off the prophylaxis?.....

THANKS FOR YOUR PARTICIPATION

Appendix III

Laboratory Protocols

1. Preparation of Agarose Gel (Lee *et al.*, 2012)

i. Weigh out the appropriate mass of agarose into an Erlenmeyer flask. Agarose gels are prepared using a w/v percentage solution. The concentration of agarose in a gel will depend on the sizes of the DNA fragments to be separated, with most gels ranging between 0.5%-2%. The volume of the buffer should not be greater than 1/3 of the capacity of the flask.

ii. Add running buffer to the agarose-containing flask. Swirl to mix. The most common gel running buffers are TAE (40 mM Tris-acetate, 1 mM EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA).

iii. Melt the agarose/buffer mixture. This is most commonly done by heating in a microwave, but can also be done over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.

iv. Add ethidium bromide (EtBr) to a concentration of 0.5 µg/ml. Alternatively, the gel may also be stained after electrophoresis in running buffer containing 0.5 µg/ml EtBr for 15-30 min, followed by destaining in running buffer for an equal length of time.

v. Allow the agarose to cool either on the benchtop or by incubation in a 65 °C water bath. Failure to do so will warp the gel tray.

vi. Place the gel tray into the casting apparatus. Alternatively, one may also tape the open edges of a gel tray to create a mold. Place an appropriate comb into the gel mold to create the wells.

vii. Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature. Remove the comb and place the gel in the gel box. Alternatively, the gel can also be wrapped in plastic wrap and stored at 4 °C until use.

2. Setting up of Gel Apparatus and Separation of DNA Fragments

i. Add loading dye to the DNA samples to be separated. Gel loading dye is typically made at 6X concentration (0.25% bromphenol blue, 0.25% xylene cyanol, 30% glycerol). Loading dye helps to track how far your DNA sample has traveled, and also allows the sample to sink into the gel.

ii. Program the power supply to desired voltage (1-5V/cm between electrodes).

iii. Add enough running buffer to cover the surface of the gel. It is important to use the same running buffer as the one used to prepare the gel.

iv. Attach the leads of the gel box to the power supply. Turn on the power supply and verify that both gel box and power supply are working.

v. Remove the lid. Slowly and carefully load the DNA sample(s) into the gel. An appropriate DNA size marker should always be loaded along with experimental samples.

vi. Replace the lid to the gel box. The cathode (black leads) should be closer the wells than the anode (red leads). Double check that the electrodes are plugged into the correct slots in the power supply.

vii. Turn on the power. Run the gel until the dye has migrated to an appropriate distance.

3. Observing Separated DNA fragments

i. When electrophoresis has completed, turn off the power supply and remove the lid of the gel box.

ii. Remove gel from the gel box. Drain off excess buffer from the surface of the gel. Place the gel tray on paper towels to absorb any extra running buffer.

iii. Remove the gel from the gel tray and expose the gel to uv light. This is most commonly done using a gel documentation system. DNA bands should show up as orange fluorescent bands. Take a picture of the gel.

iv. Properly dispose of the gel and running buffer per institution regulations.

Appendix IV

DNA extraction protocol

Extraction Protocol: Modified from Ribeiro *et al*., (2016) Protocol

- Pipette 200µl of double distilled water into Eppendorf tubes
- Transfer three (3) well isolated colonies in the tube and heat at 98^{o}C for 10 minutes.
- Centrifuge at 1,350rpm for 5minutes and discard supernatant
- Aliquot 150µl of the supernatant (DNA template) into 2ml Eppendorf tubes and store in -20^{o}C until further works.

Appendix V

Pre, During and Post PCR Activities

Table A1: Proportions of ingredients for the preparation of Master mix

Components	15µl Reaction Volume(15µl)	Volume (µl) for 33 isolates
One Tag Quick-Load 2× Master mix	6.25	206.25
10µM Forward primer	1	33
10 µM Reverse primer	1	33
Template DNA	1.5	X
Nuclease-free water	3.25	17.25

Table A2: Thermocycling conditions for the Virulence genes (30cycles)

Components	Temperature (^{0}C)	Time
Initial Denaturation	95	7minutes
Final Denaturation	94	30 seconds
Annealing	63	45 seconds
Extension	72	5minutes
Final Extension	72	4minutes
Hold	4	

Table A3: Thermocycling conditions for integrase genes (30cycles)

Components	**Temperature (^{0}C)**	**Time**
Initial Denaturation	94	5minutes
Final Denaturation	94	30 seconds
Annealing	64	30 seconds
Extension	68	3minutes
Final Extension	72	10minutes
Hold	4	

Appendix VI
Gel images

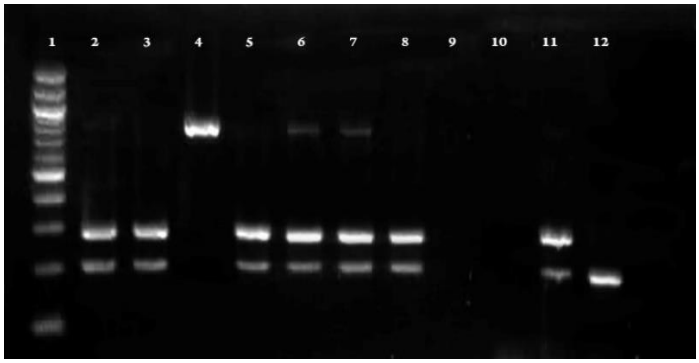


Figure 5.0

Gel 1
Lane 1 – ladder
Lane 2,3,5,6,7,8 and 11 – samples with both PAPC and CHUA genes
Lane 12 – sample with PAPC
Lane 4,6, and 7 – Int II

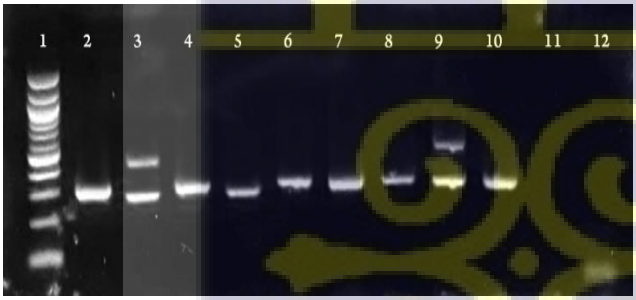


Figure 6.0

Gel 2
Lane 1 – ladder
Lane 2,4,6,7,8,9,10– samples with IUTA (300bp)
Lane 3 & 5 – sample with CHUA (279bp)
Lane 3&9 – USP (440bp)
Lane 12 – Negative control

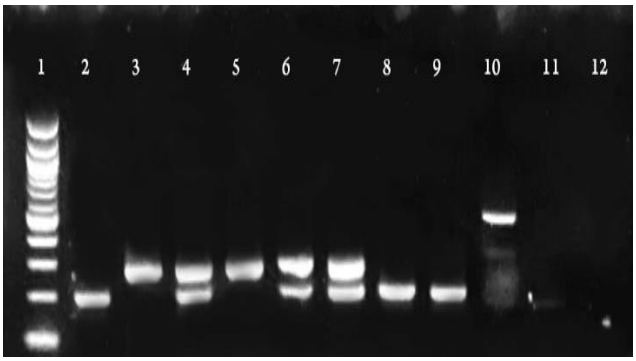


Figure 7.0

Gel 3
Lane 1 – ladder
Lane 2,4,6,7,8 and 9 – samples with both PAPC
Lane 3,4,5,6,7, – sample with KPSMTII
Lane 10 – CNFI
Lane 12 – Negative control

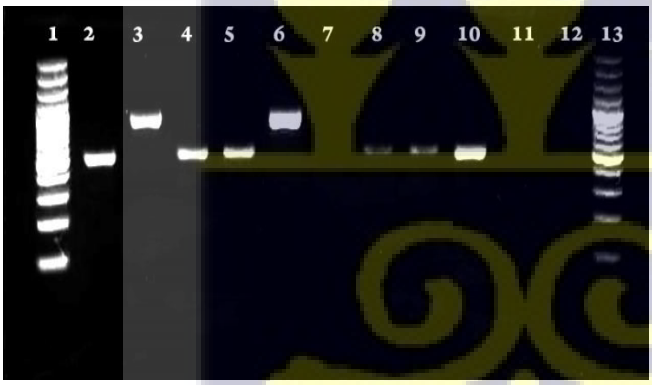


Figure 8.0

Gel 4
Lane 1 – ladder
Lane 2 & 3 – Positive controls INT I (483bp)/INT II (788bp)
Lane 4,5,8,9 & 10 – sample INTI (483bp)
Lane 6 – Sample with INT II (788bp)
Lane 12 – Negative control

Appendix VII

ADDITIONAL RESULTS

Table A4 Distribution of virulence genes in antibiotic resistant and susceptible isolates

Antibiotics	R or S	Virulence genes n (%)					
	(n)	*iutA*	*cnf1*	*papC*	*usp*	*chuA*	*KpsMTII*
Meropenem	**R**(1)	0	0	0	0	1(100)	0
	S(58)	21(36.2)	30(51.7)	33(56.9)	5(8.6)	38(65.5)	27(46.6)
Gentamicin	**R**(15)	6(40.0)	7(46.7)	7(46.7)	3(13.3)	11(73.3)	5(33.3)
	S(44)	15(34.1)	23(52.3)	27(61.4)	3(6.8)	28(68.3)	22(50.0)
SXT	**R**(58)	20(34.5)	29(50.0)	33(56.9)	5(8.6)	38(65.5)	27(46.6)
	S(1)	1(100)	1(100)	1(100)	0	1(100)	0
Cefuroxime	**R**(45)	15(33.3)	24(53.3)	25(55.6)	5(11.1)	30(66.7)	20(44.4)
	S(14)	6(42.9)	6(42.9)	9(64.3)	0	9(64.3)	7(50.0)
Fosfomycin	**R**(1)	0	0	0	0	1(100)	1(100)
	S(58)	21(36.2)	30(51.7)	34(58.6)	5(8.6)	38(65.5)	26(44.8)
Nitrofurantoin	**R**(4)	4(100)	1(25.0)	3(75.0)	3(25.0)	2(50.0)	0
	S(55)	17(30.9)	29(52.7)	31(56.4)	4(7.3)	37(67.3)	27(49.1)
Tetracycline	**R**(56)	20(35.7)	30(53.6)	33(59.8)	5(8.9)	37(66.1)	26(46.4)
	S(3)	1(33.3)	0	1(33.3)	0	2(66.7)	1(33.3)
Ciprofloxacin	**R**(19)	9(47.4)	8(42.1)	11(57.9)	2(10.5)	10(52.6)	4(21.1)
	S(40)	12(30.0)	22(55.0)	23(57.5)	3(7.5)	29(72.5)	23(57.5)
Cefepime	**R**(9)	4(44.4)	5(55.6)	4(44.4)	1(11.1)	7(77.8)	4(44.4)
	S(50)	17(34.0)	25(50.0)	30(60.0)	4(8.0)	32(64.0)	23(46.0)

Table A4 continued

Distribution of virulence genes in antibiotic resistant and susceptible isolates

Amikacin	**R**(5)	2(40.0)	3(60.0)	4(80.0)	1(20.0)	4(80.0)	2(40.0)
	S(54)	19(35.2)	27(50.0)	30(55.6)	4(7.4)	35(64.8)	25(46.0)
Ceftriaxone	**R**(9)	5(55.6)	4(44.4)	5(55.6)	1(11.1)	2(77.8)	3(33.3)
	S(50)	16(32.0)	26(52.0)	29(58.0)	4(8.0)	32(64.0)	24(48.0)
Cefpodoxime	**R**(17)	7(41.2)	7(41.2)	10(58.8)	1(5.9)	12(70.6)	7(41.2)
	S(42)	14(33.3)	23(54.8)	24(57.1)	4(9.5)	27(64.3)	20(47.6)
Ampicillin	**R**(59)	21(35.6)	29(49.2)	34(57.6)	5(8.5)	39(66.1)	27(45.8)
	S(0)	0	0	0	0	0	0
Amoxiclav	**R**(29)	10(33.3)	15(50.0)	16(53.3)	4(13.3)	18(60.0)	12(40.0)
	S(30)	11(37.9)	15(51.7)	18(62.1)	1(3.4)	21(72.4)	15(51.7)
Nalidixic acid	**R**(34)	19(55.9)	15(44.1)	20(58.8)	3(8.8)	19(55.5)	10(29.4)
	S(25)	2(8.0)	15(50.0)	14(56.0)	2(8.0)	20(80.0)	17(68.0)

R= Resistant, S= Sensitive

Table A5 Distribution of integrase genes in antibiotic resistant and susceptible isolates

Antibiotics	R or S (n)	Integrase genes n (%)	
		intI	*intII*
Meropenem	R	0	0
	S	25(43.1)	12(20.7)
Gentamicin	R	6(40.0)	0
	S	19(43.2)	12(27.3)
SXT	R	24(41.4)	12(20.7)
	S	1(100)	0
Cefuroxime	R	20(44.4)	8(17.8)
	S	5(35.7)	4(28.6)
Fosfomycin	R	0	1(100)
	S	25(43.1)	0
Nitrofurantoin	R	1(25.0)	0
	S	24(43.6)	12(21.8)
Tetracycline	R	24(42.9)	11(19.6)
	S	1(33.3)	1(33.3)
Ciprofloxacin	R	9(47.4)	1(5.3)
	S	16(40.0)	11(27.5)
Cefepime	R	2(22.2)	1(11.1)
	S	23(46.0)	11(22.0)
Amikacin	R	2(40.0)	0
	S	23(42.6)	11(22.0)

Table A5 continued

Distribution of integrase genes in antibiotic resistant and susceptible isolates

Ceftriaxone	R	3(33.3)	12(22.)
	S	22(44.0)	3(17.6)
Cefpodoxime	R	6(35.3)	9(21.4)
	S	19(45.2)	3(17.6)
Ampicillin	R	25(42.2)	12(20.0)
	S	0	0
Amoxiclav	R	12(40.0)	6(20.0)
	S	13(44.8)	6(20.7)
Nalidixic acid	R	15(44.1)	4(11.8)
	S	10(40.0)	8(32.0)

R = Resistant, S= Sensitive

Risk factors as predictors of growth

Table A6 Binary logistic regression analysis of risk factors as predictors of growth

	Risk Factors							
	Age		HAART duration		Viral load		WHO disease stage	
	p	OR	*p*	OR	*p*	OR	*p*	OR
Bacterial Growth	0.06	1.020	0.287	1.036	0.836	1.115	0.885	1.286

OR=odds ratio, *p*=p-value; Significant association ($p \leq 0.05$). If $p \leq 0.05$ and OR > 1 it means risk factor is a predictor of bacterial growth.

Table A7 Binary logistic regression analysis of virulence genes as predictors of resistance

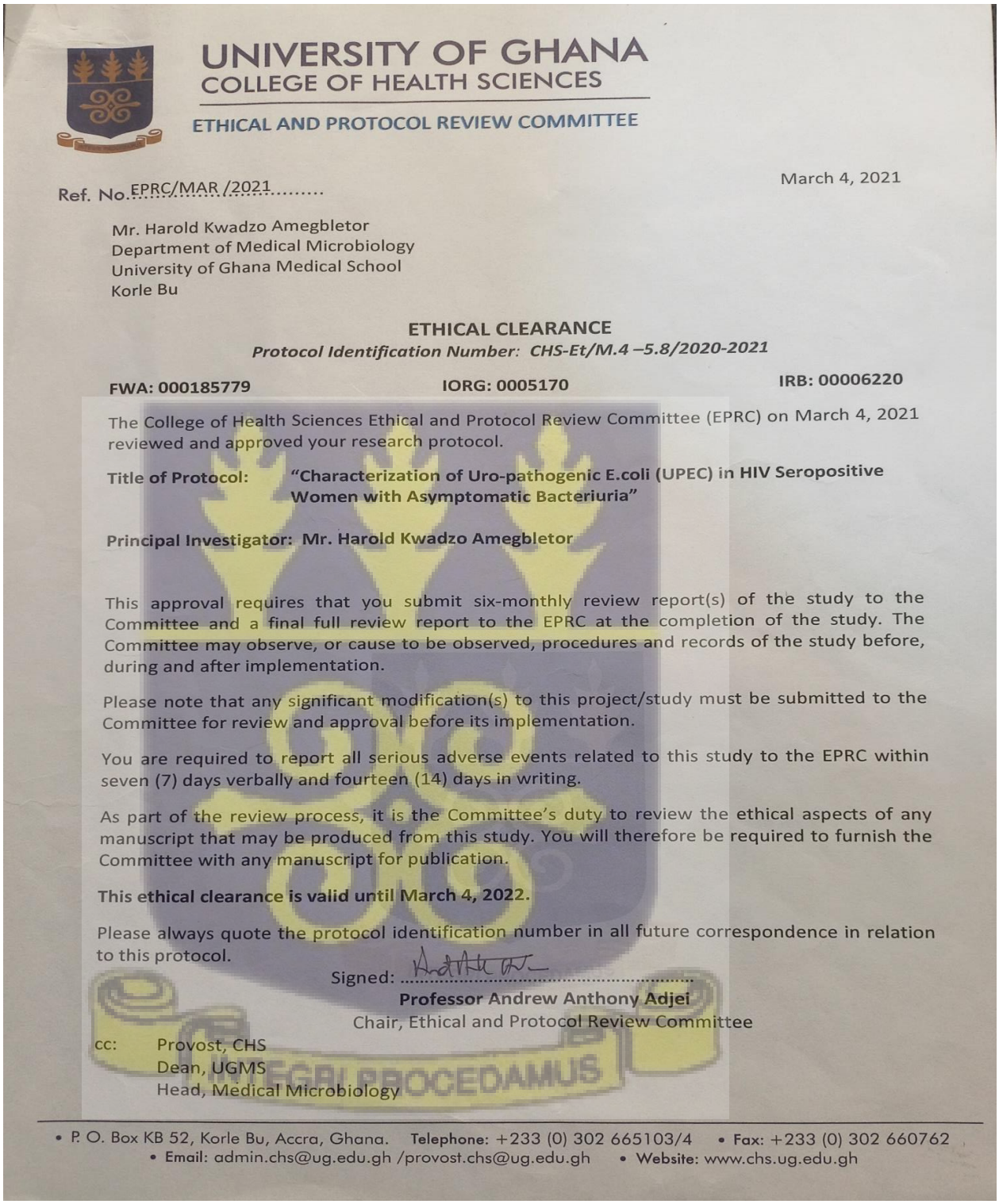
	Virulence genes					
	iutA	*cnf1*	*papC*	*usp*	*chuA*	*KpsMTII*
Meropenem						
OR	4.111	0.000	0.271	8.183	0.000	0.000
p	0.997	0.996	1.000	1.000	0.997	0.998
Gentamicin						
OR	1.061	1.339	5.343	1.113	0.099	4.820
p	0.942	0.747	0.068	0.922	0.064	0.138
SXT						
OR	0.000	20.00	4.344	0.721	40.178	0.000
p	0.997	1.000	1.000	1.000	1.000	0.998
Cefuroxime						
OR	2.071	0.332	1.872	0.000	0.596	3.787
p	0.360	0.215	0 .432	0.999	0.614	0.237
Fosfomycin						
OR	4.293	0.000	94.081	267.326	0.003	0.000
p	1.000	0.996	1.000	1.000	1.000	0.999
Nitrofurantoin						
OR	0.000	0.786	0.960	0.208	1.756	0.000
p	0.997	0.873	0.978	0.389	0.682	0.998
Tetracycline						
OR	0.492	0.000	0.000	0.000	0.000	0.000
p	0.700	0.995	0.995	0.998	0.995	0.995
Ciprofloxacin						
OR	0.918	0.526	1.000	0.813	1.544	4.350
p	0.902	0.456	1.000	0.844	0.650	0.117

Table A7. continued: Binary logistic regression analysis of virulence genes as predictors of resistance

Amikacin						
	OR	1.230	0.702	0.340	0.548	0.700	1.104
	p	0.852	0.767	0.400	0.451	0.812	0.939
Ceftriaxone						
	OR	0.409	0.626	2.847	1.462	0.160	1.803
	p	0.342	1.605	0 .288	0.768	0.167	0.606
Cefepime						
	OR	0.445	0.712	4.528	1.221	0.271	1.458
	p	0.415	0.732	0.121	0.879	0.306	0.760
Cefpodoxime						
	OR	0.867	2.607	1.222	2.641	0.324	1.222
	p	0.844	0.216	0.776	0.429	0.247	0.827
Amoxiclav						
	OR	1.535	0.340	0 .714	0.178	0.467	0.395
	p	0.531	0.481	1.266	0 .155	1.893	2.126
Nalidixic acid						
	OR	**14.567**	0.202	1.109	1.006	5.539	0.747
	p	**0.001**	0.276	0.898	0.996	0.160	1.391

OR=odds ratio, *p*=p-value; **Bold** *p* value means Significant association ($p \leq 0.05$). If $p \leq 0.05$ and OR > 1 it means risk factor is a predictor of resistance.

Appendix VIII
Ethical Clearance



UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No. EPRC/MAR /2021

March 4, 2021

Mr. Harold Kwadzo Amegbletor
Department of Medical Microbiology
University of Ghana Medical School
Korle Bu

ETHICAL CLEARANCE
Protocol Identification Number: CHS-Et/M.4 –5.8/2020-2021

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) on March 4, 2021 reviewed and approved your research protocol.

Title of Protocol: **"Characterization of Uro-pathogenic E.coli (UPEC) in HIV Seropositive Women with Asymptomatic Bacteriuria"**

Principal Investigator: Mr. Harold Kwadzo Amegbletor

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid until March 4, 2022.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, UGMS
Head, Medical Microbiology

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