ISOLATION AND CHARACTERIZATION OF UROPATHOGENIC ESCHERICHIA COLI IN WATER CLOSET USED BY MALE STUDENTS IN A TERTIARY INSTITUTION IN NIGERIA.

BY

Adedoyin EXCEL ADEOLU

18010104004

A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY.

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DECLARATION

I hereby declare that this project has been written by me and is a record of my research work. It has not been presented in any previous application for a higher degree at this or any other University. All citations and sources of information are acknowledged by means of reference.

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ADEDOYIN, EXCEL ADEOLU

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Date

CERTIFICATION

This is to certify that the content of this project entitled **'Isolation and Characterization of Uropathogenic Escherichia coli in Water Closet used by Male Students in a Tertiary Institution in Nigeria'** was prepared and submitted by **ADEDOYIN, EXCEL ADEOLU** in partial fulfilment of the requirements for the degree of **BACHELOR OF SCIENCE IN BIOTECHNOLOGY.** This original research work was carried out under my supervision and is hereby accepted.

_____ (Signature and Date)

Mr. Tosin. Ogunbiyi

Supervisor

_____ (Signature and Date)

Dr. Moses Abiala

Head of Department

DEDICATION

This project work is dedicated to the Almighty God, by whose grace and mercy I was able to complete this project.

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ABSTRACT

Background of study: Urinary Tract Infection (UTIs) are the most prevalent urinary tract diseases in the world. The most typical origin of UTIs is Uropathogenic *Escherichia coli* (UPEC) strains, which have a variety of virulence factors for their pathogenicity in the urinary system. This study aims to investigate the prevalence of Uropathogenic *E. coli* in male hostels of a tertiary institution in Ogun state.

Aim: To identify the Uropathogenic *E. coli* causing urinary tract infection and its impact on the health of male students.

Methodology: Using a sampling technique known as the swab-rinse technique, fifty samples were randomly collected from male toilet hostels in a tertiary institution in Ogun state, Nigeria. The samples were cultured on MacConkey agar after which the DNA of distinct isolates were extracted for multiplex polymerase chain reaction using virulence gene primers *papC, papEF, papGclassicI, Cdbt,* and *hlyA*. The amplicon generated were viewed using 1.8% agarose gel on UV transilluminator.

Result: Of the 50 samples screened, this study showed prevalence of Uropathogenic *E. coli* to be (44.6%). *Pap* gene was the most prevalent (42.2%) followed by *hlyA* (4.4%). The PCR reaction results identified 11*PaPC* (24.4%), 4 *PaPGclassic*(8.8%), 3 *PaPE/F*(6.7%), 2*HlyA*(4.4%), 1 *Cdbt*(2.2%). All these strains were negative for *Usp* and *CNF*. *PaPC* was identified to be having occurred, the most pervasive virulence factor found in 24.4% of strains.

Conclusion: This study findings showed the presence of Uropathogenic *E. coli* in the study population, and this suggests that Uropathogenic *E. coli* is a prevalent and widespread cause of Urinary Tract Infection within Ogun state. This study provides valuable insight into the prevalence of UPEC in male hostels of a tertiary institution. It also highlights the importance of regular monitoring and screening of individuals with UTI symptoms to prevent the spread of UPEC. The high prevalence of uropathogenic E. coli suggests that there may be a need for intervention to control and prevent the spread of this pathogen.

Keywords: Urinary Tract Infection, Prevalence, Virulence factor, Uropathogenic *E. coli*, *Escherichia coli*

CHAPTER ONE

INTRODUCTION

1.1.   Background of the study

Urinary tract infections (UTIs) are primarily caused by the presence and multiplication of microorganisms in the urinary tract (McDaniel and Kaper, 1997). *Escherichia coli* is the causative pathogen in 70–95% of acute, uncomplicated UTIs in adults, followed by other Enterobacteriaceae, like *Proteus mirabilis* and *Klebsiella spp*., and by *Staphylococcus saprophyticus* in 5–10% of cases (Garbe *et al*., 2015). The onset of UTIs is primarily caused by the ascent of microorganisms from the urethra. When the pathogen can penetrate the urinary tract system and accumulates to more than 10^5 colonies/ml in urine, a urinary tract infection (UTI) result (Smelov *et al*., 2010). One to three percent of consultations is for urinary tract infections (UTI), the most frequent bacterial illness seen in general practice (Goddard *et al*., 2010). About 150 million individuals worldwide are diagnosed with (UTI) each year, and it affects all age groups, from neonates to the elderly. Financially, community-acquired UTI's expensive, costing the world economy more than six billion US dollars. Lower UTI is defined as the involvement of the urethra, bladder, prostate, epididymis, and testis (Das and Banerjee, 2015). Upper UTI is the term for the condition that affects the kidneys and ureters. Bacteria most frequently enter the urinary system by the ascending transurethral pathway, but they can also enter through the bloodstream or lymphatics (Yagoob, 2009). Every year, 150 million humans around the world are affected with UTIs, one of the most frequent bacterial diseases (Harding and Ronald, 2016). Although both men and women can get infected, UTIs are typically thought of as a condition that only affects women, who have a 50% lifetime prevalence (Foxman, 2014). Because multidrug-resistant Uropathogens are more common and antibiotic treatment for acute infections does not prevent recurrences, current treatments are not optimum (Gupta K *et al*., 2001; Al-Badr and Al-Shaikh, 2013). The majority of UTIs are caused by Uropathogenic *Escherichia coli* (UPEC) strains, which have a variety of virulence characteristics for their pathogenicity in the urinary system, including fimbriae, capsules, iron scavenger receptors, flagella, toxins, and lipopolysaccharide. Due to differences in anatomical structure and the greater risk of infection following bladder catheterization, which weakens natural defence mechanisms, women have UTIs more frequently than males. Only a small percentage of UTIs are hematogenous in origin, and these infections are typically brought on by a few relatively rare bacteria (such as *Staphylococcus aureus, Candida*

spp., and *Mycobacterium tuberculosis*), which cause primary infections elsewhere in the body and then spread to the urinary tract (Wittenberg *et al*., 2014). Both sexes are susceptible to urinary tract infections. However, women are more likely to experience it, particularly when they are sexually active for reproduction. This is brought on by their narrow urethra, injuries sustained during sexual activity, and lack of bactericidal secretions (Ronald, 2003). If the lower urinary tract is affected, urinary tract infection (UTI) symptoms such as dysuria, frequency, urgency, strangulation, and suprapubic pain may also be present (Biswas *et al*, 2010). Fever, sensitive and painful loins are the symptoms of an upper (UTI). Asymptomatic urinary tract infections may be found during routine examinations (Goddard *et al*., 2010). When looking at the urine under a microscope for a UTI, pus cells (white blood cells, WBCs, or red blood cells) may be visible (RBCs). Pyuria is defined as having six to ten or more pus cells per High Power Field (HPF) of freshly voided midstream urine that are unspun (Mangula *et al*., 2013). When there are two or more leucocytes in the urine, it is considered to be significant pyuria (Anígilájé and Bitto). One of the most frequent locations for bacterial infections is the urinary system, where *E. coli* is by far the most prevalent pathogen. The strains of commensal *E. coli* that make up the majority of the *E. coli* infecting the lower colon of people are different from the subgroup of *E. coli* that causes simple cystitis and acute pyelonephritis. Six O serogroups account for 75% of UTIs caused by *E. coli*, and these six groups feature characteristics that are epidemiologically linked to acute pyelonephritis and cystitis in the normal urinary tract, including expression of P fimbriae, haemolysin, aerobactin, serum resistance, and encapsulation (Turner *et al*., 2006).

1.2 STATEMENT OF PROBLEM

Studies on Urinary Tract Infections (UTIs) associated with Pathogenic bacterium have revealed that Uropathogenic *E. coli* continues to be the primary cause of UTIs and is also the cause of multifactorial nosocomial infections (Bigwan and David, 2013). According to estimates, *E. coli* causes 80% of all uncomplicated UTIs and 18–35% of UTIs linked with long-term indwelling catheters. From numerous studies on the Uropathogens obtained from inpatients and outpatients revealed that UPEC isolates were the most prevalent isolate in UTI patients (Salvador *et al*., 2012). This study's objectives include determining the prevalence of *E. coli* species that have not yet been examined, evaluating and validating the claims made about the species, and determining the

percentage of a particular population that is impacted by Uropathogenic *E. coli* species (Muller *et al*., 2009).

1.3   AIM AND OBJECTIVES OF STUDY

The purpose of the study is to identify the Uropathogenic *E. coli* causing urinary tract infection and its impact on the health of male students.

The specific objectives of this study are to:

1. Detect Uropathogenic bacteria in swabs from male students' toilets.
2. Isolate and identify Uropathogenic *E. coli* from toilet swab samples.
3. Determine the prevalence of Uropathogenic *E. coli* among male students on and off the campus premises.

1.4   SCOPE OF STUDY

- Sample collection and processing: Develop a standardized procedure for collecting toilet swab samples from the identified students, process the samples using appropriate laboratory techniques to isolate and identify uropathogenic E. coli strains.

- Characterization of Uropathogenic E. coli strains: Determine the virulence factors and antibiotic resistance profiles of the isolated uropathogenic E. coli strains to help understand their pathogenic potential.

- Impact on health: Evaluate the impact of uropathogenic E. coli on the health of male students by assessing the severity of the infection, its recurrence rate, and the need for hospitalization or antibiotic treatment.

- Data analysis: Analyze the data collected from the study and draw conclusions based on the findings.

- Recommendations: Provide recommendations based on the study findings to guide interventions aimed at reducing the spread of uropathogenic E. coli and urinary tract infections among male students in the tertiary institution.

Overall, this scope of study focuses on identifying the uropathogenic E. coli causing urinary tract infections among male students in a tertiary institution and investigating its potential source in the water closets used by these students. The study also aims to assess the impact of this infection on the health of male students and provide recommendations for interventions to control and prevent its spread.

1.5 SIGNIFICANCE OF STUDY

Based on a report and research study on Uropathogenic *E. coli* in various samples taken from the university. This study will provide useful information on Uropathogenic *E. coli* identification.

1.6 DEFINITION OF TERMS

Prevalence: This refers to the percentage of people in a population who, at a certain time or over a given length of time, have a particular disease or condition. Prevalence in the context of urinary tract infections refers to the proportion of UTI cases within a population.

Uropathogens: A uropathogen is a kind of pathogenic microbe, such as a virus or bacteria, that causes infections in the urinary tract. *Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis* are a few prevalent uropathogens that result in UTIs.

Nosocomial Infections: These diseases, also referred to as hospital-acquired infections, are developed by patients while they are staying in a hospital or other healthcare institution. UTIs caused by nosocomial infections, such as catheter-associated UTIs and surgical site infections, are just two examples.

Urinary Tract Infection (UTIs): A urinary tract infection (UTI) is an infection that can affect the kidneys, bladder, ureters, and urethra as well as other parts of the urinary system. UTIs are frequently brought on by bacterial infections, and they can produce symptoms like lower abdomen pain, frequent urination, and painful urination.

Upper UTIs: This is a term used to describe a urinary tract infection that affects the ureters, the tubes that join the kidneys and bladder, or the kidneys. Fever, chills, pain in the flank, and nausea are all indications of an upper UTI.

Asymptomatic UTI: This is a urinary tract infection where unnoticeable signs, such as pain or discomfort when urinating, are absent. However, the bacteria may still be present in the urine and cause problems if untreated, particularly in vulnerable groups like pregnant women and the elderly.

Catheterization: This is a medical technique is inserting a flexible tube called a catheter into the bladder through the urethra to drain urine. When a person is unable to pee normally, such as in cases of urinary retention, or during specific medical procedures like surgery, catheterization is utilized.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION TO E. COLI

Escherichia coli (E. coli) is a facultatively anaerobic, rod-shaped, Gram-negative bacterium. Dr Theodor Escherich (1857–1911), a German–Austrian pediatrician, made the discovery of the bacterium *E. coli* in 1885 (Nataro and Mobley, 2004). The majority of *E. coli* strains are a typical component of the flora that inhabits the gastrointestinal tracts of both humans and animals. Many mammals, including humans, have it in their guts (Elsas *et al*., 2011). The growth rate of this bacterium is rapid. It is very simple to grow and doesn't require any additional dietary supplements. It can develop at room temperature as well as at human body temperature, which is around 37°C. It is a multipurpose bacterium that can thrive on a variety of surfaces. The bacteria grow well in liquid media with or without aeration (Fotadar *et al*., 2004). Additionally, they thrive on solid surfaces, such as Petri dish-made lab agar medium. There are about 4,000 protein-encoding genes in the *E. coli* lab strain. The genetic flexibility of the bacteria allows for the exchange of DNA through conjugation (Tortara, 2010). *E. coli* grows best at a temperature of 37 °C (99 °F), while some lab strains can grow as high as 49 °C (120 °F) (Fotadar *et al*., 2004). When treated with divalent cations like magnesium and calcium in the lab, they can absorb DNA from the surrounding environment (transformation). One of the first bacteria to colonize the gut after birth is the well-known commensal bacterium *E. coli*. The beneficial strains, which are a normal component of the gut's microbiota and have a mutualistic relationship with their hosts, produce vitamin K2 and guard against the colonization of the intestine with pathogenic bacteria (Bentley and Meganathan, 2012). The sequence of the *E. coli* strain K-12 MG1655, which was published in 1997, contained the first complete *E. coli* genome (Blattener *et al*., 1997). With just the temperate bacteriophage lambda and F plasmid removed, the sequenced strain has been kept as a laboratory strain and has undergone minimal genetic modification. 4,639,221 base pairs make up the genome's compiled version. The genome is made up of non-coding repeats (0.7%), stable RNAs (0.8%), and protein-coding genes (81.8%). Gene expression regulation and other tasks are carried out by 11% of the genome. Genomes of pathogenic E. coli strains are often larger because these strains need several unique traits, or "virulence factors," to be pathogenic. These are contained in virulence-associated genes (VAGs), which are typically grouped in DNA areas known as pathogenicity islands (PAIs)

(Schmidt and Hensel, 2004). Despite being a well-known commensal bacterium, there are numerous harmful strains of *E. coli*. Certain virulence factors have been acquired by several highly adapted *E. coli* clones, increasing their capacity to adapt to novel environments and enabling them to cause a variety of diseases, including intestinal and extraintestinal infections (Houghteling and Walker, 2015).

As one of the first bacteria to colonize the gut, the facultative anaerobe *E. coli* contributes to creating the anaerobic conditions necessary for subsequent anaerobic bacteria to invade the gut. After *E. coli* colonization, the host and *E. coli* typically survive in a mutually beneficial relationship for decades. Due to the production of vitamin K by *E. coli* and the so-called colonization resistance, *E. coli* receives "food and shelter," and the host benefits. The phenomenon of protection against colonization by pathogenic bacteria, especially pathogenic *E. coli*, is known as colonization resistance (Stecher and Hardt, 2011). Due to its involvement in numerous forms of infections, the *E. coli* species is also significant from a medical standpoint. Extraintestinal pathogenic *E. coli* (ExPEC), which is linked to infections of extraintestinal anatomic locations, and intestinal pathogenic *E. coli* (IPEC), which is linked to infections of the gastrointestinal system, are the two main categories of pathogenic *E. coli*.

2.2   UROPATHOGENIC E. COLI

Uropathogenic *Escherichia coli* (UPEC) are more of a general category of extraintestinal pathogenic *E. coli*, from a varied group of strains (Muller *et al*., 2009). There is no universally shared core set of virulence traits across UPEC. The majority of UPEC belong to the B2 and D phylogroups, and according to the O (lipopolysaccharide) serotype, they can be split into uropathogenic clones, with 58% of UPEC isolates belonging to one of eight serogroups (O1, O2, O4, O6, O8, O9, O18, and O83) (Blanco *et al*.,1996). The type or severity of infection from which the bacteria are obtained can be used to categorize UPEC. The most serious UTIs are caused by isolates of pyelonephritis or urosepsis, which infect the kidneys and enter the bloodstream. Cystitis isolates result in bladder infections, while asymptomatic bacteriuria (ABU) strains persistently colonize the urinary system in a nearly commensal manner without presenting clinical symptoms (Simms and Mobley, 2008). Ten pathogen-specific genomic islands making up 13% of the genome of the pyelonephritis isolate *E. coli* CFT073 were discovered by comparative genomic

hybridization, which compared the genomic content of ten UPEC strains and four fecal/commensal strains (Lloyd *et al*., 2007). There were three islands with a recognized pathogenic potential, P fimbriae, and other characterized virulence components. Additionally, commensal isolates and UPEC isolates share 52% of the genome, and just 131 of the 5379 genes in *E. coli* CFT073 were distinct to UPEC (Lloyd *et al*., 2007). When compared to fecal commensal *E. coli*, ABU isolates, like UPEC isolates that cause symptomatic disease, have enlarged genomes. However, a study of 112 ABU isolates that had at least one urine culture with 105 CFU/ml and bacteriuria lasting 1-74 days indicated that reductive evolution of ABU isolates inside human hosts. Over time, ABU isolates with point mutations and deletions in genes encoding virulence components become less virulent (Salvador *et al*., 2012). As a result, horizontal gene transfer events like the acquisition of PAIs allowed UPEC to gain virulence, but reductive evolution reduces the virulence of ABU strains to a more commensal-like condition.

2.3   **PATHOGENESIS OF UROPATHOGENIC E. COLI**

E. coli-related UTIs are ascending infections in which periurethral bacterial contamination gives UPEC access to the otherwise sterile urinary system (Lane *et al*., 2007). The action of flagella, which propel bacteria up the urethra to the bladder, is what causes UPEC to ascend the urinary tract (Walters *et al*., 2012). The bacteria then use a variety of fimbriae and non-fimbrial adhesins to colonize, iron acquisition systems to facilitate growth, and toxins to evade the innate immune response. Flagella allow a subset of E. coli to separate from the bladder and go through the ureters to the kidneys (Lane *et al*., 2007). Since non-motile mutants of UPEC that are unable to express *FliC*, the primary component of the flagellum, are attenuated in the mouse model of ascending UTI, flagellar motility is crucial for Uropathogenesis (Wright *et al*., 2005). *E. coli* controls the production of fimbriae and flagella in a reciprocal manner, switching between motile and sessile lifestyles when fimbriae are produced and flagella are down-regulated (Lane *et al*., 2007). The transition between motility and sessility can be influenced by a number of transcription factors, including H-NS (Korea *et al*., 2010), *Lrp* (Simms and Mobley, 2008), cAMP-CRP (Yokota and Gots, 1970; Muller *et al*., 2009), and LeuX (Ritter *et al*., 1995), as well as non-specific regulators, like DNA topology. The majority of these transcription factors control the activity of the recombinases *FimB* and *FimE*, which have an impact on how the type 1 fimbrial operon's promoter is oriented as explained below (Ritter *et al*., 1995; Dorman and Corcoran, 2009). The transcription

of *flhDC*, which encodes the master regulator of flagellar biosynthesis, is simultaneously affected by the same regulators in a different way so that when flagella are expressed, fimbrial production is suppressed. Fimbrial operons can encode regulatory components that control the expression of the flagella and motility. For instance, when *sfaXII*, a regulatory gene linked to the *SfaII* fimbrial operon, is overexpressed, *FliC* is reduced, which results in decreased motility. Type 1 fimbrial expression is also negatively impacted by this protein (Sjostrom *et al*., 2009). Inhibiting flagellar gene expression in UPEC is also *PapX*, a protein encoded by the P fimbrial operon that shares 96% of its amino acid sequence with *SfaX* (Simms and Mobley, 2008). The expression of type 1 and Yad fimbriae inhibits flagellar expression as well, however, the mechanisms are unknown (Lane *et al*., 2007; Simms and Mobley, 2008; Spurbeck *et al*., 2011), showing that the creation of fimbriae might have indirect impacts on motility.

2.4   VIRULENCE FACTORS OF UROPATHOGENIC E. COLI

A crucial stage in the infection process is the colonization of the urinary system. The power of urination would propel germs out of the urinary system if they did not bond to the host epithelium (Kaper *et al*., 2014). Fimbriae, rod-like appendages that protrude from the bacterial cell surface and end in a tip adhesin, fibrillae, flexible, extended conformations with adhesins present throughout the structure (i.e., not just at the tip), and afimbrial adhesins like autotransporters are just a few of the different mechanisms used by UPEC to adhere to the uroepithelium (Boisen *et al*., 2012). These various adhesins are coordinated by complex regulatory mechanisms (Snyder *et al*., 2005). Research on the regulatory networks controlling the expression of fimbrial adhesins is ongoing and may eventually result in the creation of new therapeutic targets.
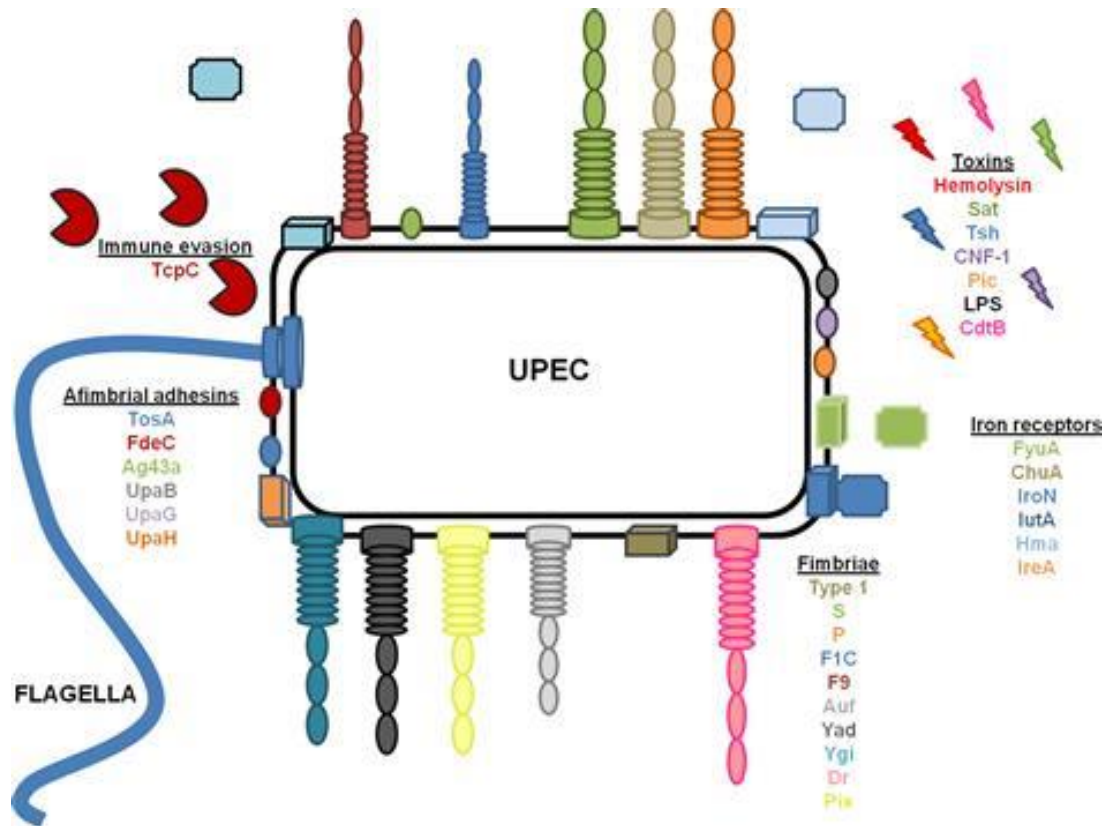
Fig 2.1: A diagram of a UPEC strain showing its virulence factors

Fimbriae

The genome of the prototype pyelonephritis strain of *E. coli* CFT073 has 12 fimbrial operons, including 10 chaperone-usher fimbriae and two putative type IV pili. *Type 1*, *F1C*, *Auf*, Ygi, Yad, F9, and two P fimbriae are the eight chaperone-usher fimbriae whose impacts on Uropathogenesis have been investigated. Pix fimbriae have been investigated in the pyelonephritis isolate *E. coli* 536 and have been related to Uropathogenic E. coli; nonetheless, molecular epidemiologic data show that this fimbria is uncommon (Spurbeck *et al*., 2011). Dr fimbriae are fibrillar structures that have been connected to both diffuse adherent E. coli and uropathogenic strains of *E. coli* (DAEC) (Wright *et al*., 2005)

Type 1 Fimbriae

The majority (99%) of *E. coli* strains genomes contain type 1 fimbriae, which are required for colonization of the oropharynx as a step before colonization of the intestine (Vigil *et al*., 2011) (Orndorff and Bloch, 1990). The adhesin, *FimH*, which is present at the tip of the fimbriae as well as sporadically along the shaft, is the primary structural subunit, *FimA*, along with a few other smaller subunits (Klemm *et al*., 1990; Krogfelt *et al*., 1990). These fimbriae attach to muscle cells and vascular walls, as well as mannose moieties (Brinton, 1959) and uroplakin 1a with the tip adhesin FimH to facilitate adhesion to and invasion of bladder epithelial cells (Virkola *et al*.,1988). The urinary system is harmed by Type 1 fimbriae because they exacerbate infection-related inflammation (Connell *et al*., 1996). Because type 1 fimbriae's promoter is located within an invertible element (known as the *fimswitch* or *fimS*), type 1 fimbriae are prone to phase fluctuation during transcription. The *FimB* and *FimE* recombinases' activity on the *fim* switch controls this phase variation.

P fimbriae

The first UPEC virulence factor to be identified was P fimbria (Eden *et al*., 1976). There are 11 genes in the pap (pyelonephritis associated pili) operon that code for P fimbriae, with *papA* encoding the major fimbrial subunit and *papG* the tip adhesin (Hull *et al*., 1981). Contrary to type 1 fimbriae, P fimbriae promote adhesion that is mannose-resistant and specific to the P blood group antigen, a glycosphingolipid with the major epitope being the digalactoside -D-Gal-(1-4)—D-Gal (Källenius and Möllby, 1979). Each of the three *papG* alleles has a different area of the Gal-Gal disaccharide-containing glycosphingolipids as its preferred substrate (Stromberg *et al*., 1991). P fimbriae are encoded in the genomes of 54–70% of UPEC isolates compared to 20–25% of fecal

E. coli strains; the distribution of papG allelic types is as follows: papG1 (0–1% UPEC, 0-1% fecal), papG2 (36-46% UPEC, 16–18% fecal), and papG3 (17–23% UPEC, 5-9% fecal) P fimbriae increase the severity of UTI by encouraging mucosal inflammation, robust adhesion to vascular endothelium, the muscular layer, and weak adherence to bladder epithelial surfaces (Virkola *et al*., 1988). There is no defect in the colonization of the mouse model of ascending UTI in the P fimbria isogenic mutant, despite the fact that there is a positive correlation between infection severity and the presence of P fimbriae (Spurbeck *et al*., 2011; Vigil *et al*., 2011), and antibodies to P fimbriae are present in the serum of infected individuals (de Ree and van den Bosch, 1987). (Mobley *et al*., 1993). However, E. coli DS17-8 (papG) was cleared quicker and did not result in as much kidney damage in a pyelonephritis model in cynomolgus monkeys. Wild-type E. coliDS17 colonized the urinary tract for a longer period and caused a loss of kidney function (Roberts *et al*., 1994). The presence of the *pap* operon is unmistakably a diagnostic of highly virulent strains, despite the fact that P fimbriae are supposed to only have a minor role in Uropathogenesis.

F1C fimbriae

In a recent study, there was no significant difference in the prevalence of *F1C* fimbriae between UPEC (16%) and fecal *E. coli* strains (10%); nevertheless, other investigations have demonstrated a correlation between the presence of *F1C* fimbriae and isolates with pyelonephritis (Johnson *et al*., 2005). The *F1C* fimbria are encoded by an operon of seven genes called focAICDFGH (Riegman *et al*., 1990), with *FocA* standing in for the main subunit of the pilus and *FocH* for the tip adhesin. F1C fimbriae bind to receptors found in the kidneys and bladder (Virkola *et al*., 1988). In contrast to the epithelium, *F1C* fimbriae mediate attachment to the bladder's endothelium and muscle layer (Virkola *et al*., 1988). F1C primarily mediates adhesion to the distal tubules and

collecting ducts, despite the fact that these fimbriae also bind in the glomeruli and to the vascular endothelium in the kidneys (Virkola *et al.*, 1988). GalNAc1-4Gal sequence of glycolipids was shown to be the receptor structure of *F1C* fimbriae (Khan *et al.*, 2000). As a result, *F1C* fimbriae may have a role in the colonization of the urinary system as a virulence factor. However, no experimental infection has been performed to show the significance of *F1C* fimbriae during a urethral tract infection.

S fimbriae

S fimbriae are highly related to cystitis and ABU isolates and are encoded by 15% of UPEC isolates compared to 5% of fecal *E. coli* strains (Spurbeck *et al.*, 2011). S fimbriae are frequently included with *F1C* fimbriae in epidemiological studies because they are genetically identical to *F1C* fimbriae except the tip adhesin (Ott *et al.*, 1988). However, the *F1C* and *S* fimbriae's various tip adhesins confer different binding specificities, hence these related fimbriae should be regarded as distinct virulence factors for UPEC (Marre *et al.*, 1990). Additionally, *S* fimbriae bind to the glomerular epithelium, collecting duct epithelium, and epithelium in the lumen of the proximal and distal tubules (Korhonen *et al.*, 1986). *S* fimbriae bind to connective tissue, muscle, and epithelial layers in the bladder (Virkola *et al.*, 1988). *S* fimbriae thus meet the requirements for a virulence determinant in UPEC (Marre *et al.*, 1986).

F9 fimbriae

Compared to 56% of fecal E. coli strains, 78% of UPEC isolates encode the F9 operon's fimbriae (Spurbeck et al., 2011). When expressed in a non-fimbriated strain of E. coli, F9 fimbriae promote adherence to HeLa epithelial cells but do not agglutinate red blood cells from people, dogs, horses, or sheep. *coli*, this fimbria promotes biofilm formation in M9 minimal salts medium (Ulett *et al.*,

2007). As a result, *F9* fimbriae only contribute minimally to the production of UPEC biofilms on abiotic surfaces. When *E. coli* CFT073 is grown in human urine, *F9* fimbriae are expressed, which may suggest that they play some sort of virulence-related role (Spurbeck *et al.*, 2011). *F9* fimbriae's impact on uroepithelial cell adhesion in vivo or elsewhere has not been studied.

Ygi fimbriae

In contrast to 24% of fecal *E. coli* strains, 61% of UPEC isolates encode Ygi fimbriae, suggesting that these fimbriae may represent Urovirulence factors (Spurbeck *et al.*, 2011). According to Spurbeck *et al.* (2011), deletion of the ygi operon from the pyelonephritis strain *E. coli* CFT073 was sufficient to lessen adhesion to the human kidney epithelial cell line HEK 293 and lessen the production of biofilm on abiotic surfaces. The addition of the *ygi* operon on a plasmid completely compensated for these inadequacies. Both human urine and LB medium express *Ygi* fimbriae. The ygi deletion mutant was outcompeted by wild-type *E. coli* CFT073 in the kidneys of mice in the mouse model of ascending UTI, and this phenotype was fully complementable, proving that these fimbriae are involved in the pathogenesis of UTI and meeting molecular Koch's postulates (Hicks *et al.*, 1996).

Yad fimbriae

Compared to 21% of fecal E. coli strains, 46% of UPEC strains had Yad fimbriae encoded (Spurbeck et al., 2011). Itoh et al. (1997) found that deletion of the yad operon from E. coli CFT073 decreased the bacteria's ability to move, form biofilms on inanimate surfaces, and adhere to the human bladder epithelial cell line UM-UC-3. In the bladder and kidneys of the mouse model of ascending UTI, a double mutant, in which both the yad and ygi fimbrial operons are deleted from the chromosome, was defeated by wild-type E. coli CFT073. Yad fimbriae are also expressed

when the bacteria are cultivated in human urine. This shows that *Yad* fimbriae, along with Ygi fimbriae, are important in the colonization of the bladder because the ygi deletion mutant is only outcompeted by wild-type *E. coli* CFT073 in the kidneys (Fang *et al.*, 1995).

Auf fimbriae

In comparison to 27% of fecal *E. coli* strains, 67% of UPEC encodes the *Auf* fimbriae (another upec fimbria, encoded by aufABCDEFG) (Spurbeck *et al.*, 2011). *Auf* fimbriae did not mediate adhesion to T24 bladder epithelial cells or HEp-2 laryngeal carcinoma cells when produced in *E. coli* BL21-AI cells, nor did it agglutinate humans, guinea pig, or sheep erythrocytes (Buckles *et al.*, 2004). No protein was found by immunoblot in vitro despite *E. coli* CFT073 and 10 additional UPEC strain's transcription of the auf operon. In a study, mice were infected with either wild-type *E. coli* CFT073 or an *aufC* mutant, and after infection, the animals infected with the wild-type strain generated an immunological response to the *AufA* antigen as measured by ELISA, while just one out of ten mice infected with the mutant strain did. According to this, *Auf* fimbriae are created in the human body (Frenzen *et al.*, 2005).

Pix fimbriae

Compared to 4% of fecal *E. coli* strains, *Pix* fimbriae are a comparatively uncommon fimbrial form present in 8% of UPEC (Spurbeck *et al.*, 2011). Pix fimbriae were first identified in the Uropathogenic *E. coli* strain X2194 on a pathogenicity island (Lugering *et al.*, 2003). Due to a truncation in the regulatory region linked to P fimbriae, *Pix* fimbriae, which are related to *P* fimbriae, are differentially regulated. Temperature was observed to affect *Pix* expression during the stationary phase. Pix fimbriae, despite being uncommon, can be a significant virulence factor

for some UPEC, as shown by the fact that they increase adhesion to HeLa cells (Lugering *et al*., 2003).

Dr/Afa fimbriae

The receptor identified by the Dr and Afa adhesin families of E. coli adhesins is the Dr blood group antigen, a cell membrane protein in erythrocytes also known as the decay accelerating factor (Nowicki et al., 1990). AFA-I and AFA-III are examples of afimbrial adhesins from the Dr family, whereas F1845 and Dr fimbriae are examples of fimbrial adhesins (Nowicki et al., 1990). In comparison to 2% of faecal E. coli strains, 7% of UPEC have the Dr fimbriae (also known as O75X), the most researched member of the Dr family of adhesins, which bind type IV collagen in vitro (Westerlund et al., 1989). In vivo, Dr fimbriae bind to human neutrophils, the connective tissue between muscle cell layers in the bladder, the peritubular connective tissue, and only weakly to epithelial cells (Nowicki et al., 1986). (1988, Virkola et al.). Dr fimbriae also assist in cell invasion (Goluszko et al., 1997; Fang et al., 2004; Das et al., 2005), allowing bacteria to conceal themselves inside of cells and avoid the humoral immune response. A vaccination against Dr fimbriae would only stop a small subset of UPEC infections, though, as the bulk of UPEC do not encode these fimbriae.

Fimbrial Adhesins

Mannose-sensitive and mannose-resistant pili, two types of pili, have been discovered in UPEC strains (Schembri *et al*., 2001). The most significant mannose-sensitive pili in these strains appear to be type 1 pili, which are encoded by a gene cluster in the bacterial chromosome. FimA, a heterodimer, and the minor components FimC, FimD, FimF, FimH, and FimG were used to create the pili's big component (Schembri *et al*., 2001; Thankavel *et al*., 1997). FimH adhesin at the tip of the pili most likely attaches UPEC to the mannosylated uroplakins on the surface of the bladder epithelial cells. These traits imply that type 1 pili play a part in IBC formation, cell invasion, and the development of resistant biofilm-like formations. According to Dhakal et al. (2008) and Eto et al. (2007), the binding of bacteria through the FimH adhesin promotes both bacterial penetration into bladder epithelial cells and epithelial cell exfoliation or death.

In UPEC strains, mannose-resistant pili are extremely varied and classified into many kinds according to the qualities of the receptors and other factors. One of the most well-known fimbriae is the P pili, which are composed of repeating units of PapA. At the tip of this structure is an adhesin called PapG, which is identical to FimH in type 1 pili and communicates with three additional subunits termed PapE, PapF, and PapK. (Busch and Waksman, 2012; Kline *et al*., 2009; Mulvey, 2002). Our research team's findings have demonstrated that P pili play a significant role in the colonization of UPEC in the kidneys (Roberts *et al*., 1994). (Tabasi *et al*., 2015, 2016). As with other mannose-resistant pili, S pili are composed of a main component called SfaA and three smaller subunits called SfaG, SfaH, and SfaS. SfaS functions as the pili's adhesin and attracts bacteria to sialic acid sites on kidney epithelial cells. *E. coli* strains that cause more serious illnesses such as pyelonephritis, meningitis, and bacteremia frequently include pili (Parkkinen *et al*., 1988). The bladder and kidney epithelial cells' glycolipid receptors, which harbour -galactosidase, are the F1C pili's unique receptors, making them analogues of S pili (Khan *et al*., 2000). Another family of mannose-resistant pili is the Dr family, which can bind human Decay-accelerating factor (DAF) molecules on red blood cells (RBCs) and epithelial cells. By binding to kidneys, this family of pili makes it easier for bacteria to colonize and extends their time of survival in the urinary tract. (Mulvey, 2002; Nowicki *et al*., 2001).

Nonfimbrial(afimbrial) adhesins

TosA, a potential member of the repeat-in-toxin (RTX) family and a recognized nonfimbrial adhesin, contributed to the colonization of UPEC in an animal model's urinary tract (Vigil *et al*., 2011). Other significant nonfimbrial adhesins among the UPEC strains were identified as members of the autotransporter family, including FdeC (for factor adherence *E. coli*), Antigen-43 (Ag43), UpaH, UpaC, and UpaG (Allsopp *et al*., 2012b; Nesta *et al*., 2012; Wells *et al*., 2010). Ag43 played a variety of roles in the pathogenicity of UPEC strains as a surface factor, including binding, colonization, the development of IBCs and biofilm, and long-term stability of bacteria in the bladder (Ulett *et al*., 2007). Additionally, the UpaG trimeric autotransporter is involved in cell aggregation, the generation of biofilms, and the binding of bacteria to bladder epithelial cells and extracellular matrix proteins like fibronectin (Valle *et al*., 2008). Numerous studies have demonstrated the role of UpaH in the development of biofilms and the colonization of the juvenile bladder in both in vitro and in vivo settings (Allsopp *et al*.,2012). It is noted that a research team was still doing additional tests on the immunogenicity and protective effectiveness of UpaH as a vaccine candidate against clinical UPEC isolates. In addition to other nonfimbrial adhesins, it has been demonstrated that Iha (IrgA homologue adhesin), an iron-regulating adhesin, plays a part in how bacteria adhere to bladder epithelial cells (Johnson *et al*., 2005).

Iron scavenger receptors

UPEC strains use a variety of techniques to take iron from the host's urinary system. One of these approaches involves using siderophores, which are iron chelators, to bind iron and transport it to the cytoplasm of bacteria (Reigstad *et al*., 2007). These siderophores have been identified in investigations conducted by various researchers (Caza and Kronstad, 2013; Garcia *et al*., 2011; Habibi *et al*., 2017) and include salmochelin, aerobactin, enterobactin, and yersiniabactin. Additionally, heme is taken up and transferred into the periplasm of UPEC via the iron receptors Hma and ChuA generated by UPEC strains (O'Brien *et al*., 2016; Reigstad *et al*., 2007). According to studies, UPEC's outer membrane iron receptors also play vital roles in colonization, the creation of biofilms, and the development of IBC reservoirs (Flores-Mireles *et al*., 2015).

Toxins

UPEC strains release toxins using the type I and V secretion systems instead of the type III secretion system, which is present in diarrheagenic *E. coli* (Henderson *et al*., 2004). These toxins include vacuolating autotransporter cytotoxin (Vat), secreted autotransporter toxin (Sat), and alpha-hemolysin (hlyA), as well as cytotoxic necrotizing factor 1 (CNF1) (Wiles *et al*., 2008). These toxins disrupt the host cells' structure or function, interrupt the cell cycle, or cause cell lysis (Wiles *et al*., 2008). Half of the UPEC strains generate alpha-hemolysin, a calcium-dependent secretory protein that can invade, lyse bladder and kidney cells, stimulate the production of cytokines, and cause inflammatory reactions (Marrs *et al*., 2005; Nielubowicz and Mobley, 2010). A cytoskeleton disturbance in the host cells is the result of the CNF1 toxin, which has been found in about 30% of UPEC strains. It causes the constitutive activation of the Rho family from GTP-binding proteins, including Cdc42. Additionally, this toxin aids in the attachment of UPEC and its penetration into the host cells (Lemonnier *et al*., 2007; Tabasi *et al*., 2016). In a mouse model, Sat and Vat autotransporter toxin, a subclass of serine proteases, caused tissue damage, particularly glomerular damage and vacuolization of the kidneys (Guyer *et al*., 2002; Maroncle *et al*., 2006; Wiles *et al*., 2008). They also showed lethal effects on the bladder and kidney cells in in vitro experiments.

Flagella

A filament, hook, motor, and basal body make up bacterial flagella (Honko and Mizel, 2005). Flagellin (FliC), a protein monomer that is utilized to make the flagella, has been employed in vaccines (Honko and Mizel, 2005; Salazar-Gonzalez and McSorley, 2005) via activating the Toll-Like Receptor-5 (TLR5). The flagellum is employed by UPEC strains to get new resources and evade harsh environmental conditions and the host immune system (Lane *et al*., 2007). Flagella play a crucial role in the transfer of bacteria from the bladder to the kidneys, as demonstrated by the correlation between the expression of flagella in UPEC strains and the ascent of the bacteria into the upper urinary tract (Lane *et al*., 2007; Schwan, 2008; Wright *et al*., 2005). Additionally, research has demonstrated the significance of flagella in various stages of the production of biofilms in the urinary tract (Pratt and Kolter, 1998).

LPS and capsule

Different polysaccharides can be found on the surface of UPEC strains. In the majority of cases, a capsular layer (K antigen) covers the lipopolysaccharide (LPS) that is recognized as O antigen on the exterior side of the UPEC outer membrane (Russo *et al*., 2009; Whitfield, 2006). The O and K antigens of UPEC strains have a significant degree of antigenic variability (Whitfield and Roberts, 1999). For instance, it has been found that the UPEC isolates have a high frequency of the antigens O1, O2, O4, O6, O7, O8, O16, O18, O25, and O75 (Bidet *et al*., 2007; Lloyd *et al*., 2007). Studies have shown that UPEC strains may be able to avoid host immune response mechanisms such as opsonophagocytosis, complement-mediated bactericidal, and death by antimicrobial peptides by using LPS and capsular polysaccharides as virulence factors (Buckles *et al*., 2009; Whitfield, 2006). Some capsular types, such as K1 and K5 block the humoral response to urine infections by mimicking tissue components at the molecular level (Johnson, 1991). Additionally, LPS appears to contribute to the colonization of UPEC in the bladder, the development of IBC reservoirs, and antibiotic resistance to hydrophobic substances (Aguiniga *et al*., 2016; Zhang *et al*., 2015)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area and Sample Site

A tertiary institution in Ogun state was selected as the sampling site for this study. Prior to sample collection, the consent of the occupants of each room was obtained. An average of four students occupies a room in each of the male dormitories. The samples were obtained from the toilet bowl of the occupant's water closet.

A total of 50 toilet swab samples were obtained from the male toilet. Samples were collected from the toilet using a swab stick preserved in normal saline to keep the pathogens alive. 2 ul of normal saline was dispensed into each swab bottle. The swab stick was used to swab the water closet and then inserted back into the bottle containing normal saline and later transported to the laboratory.

3.2 REAGENTS AND TYPES OF EQUIPMENT USED

REAGENTS: MacConkey agar, Brain Heart Infusion (BHI), glycerol, chloramphenicol, serum, distilled water, normal saline.

TYPES OF EQUIPMENTS USED: Test tube, thin foil, weighing balance, Bunsen burner, incubator, centrifuge, microscope, glass slides, cover slide inoculating loop, Heating block, Thermal cycler, Gel electrophoresis tanks, Gel documentation system, Incubator (37℃), Vortex mixer.

APPARATUS USED: Stomacher bags, wash bottles, Petri-dishes, measuring cylinder, glass pipettes, beakers, conical flasks, glass spreader, inoculating loop, wash bottles, Eppendorf tubes, micropipette (with their tips), test tubes and foil corks (with their racks), PCR tubes, glass slides, oxidase test disc, thin foil.

3.3   PREPARATION OF CULTURAL MEDIA

MacConkey Agar

MacConkey Agar was used for the identification and enumeration of Coliforms in the isolate and it was prepared based on the manufacturer's instruction Sorbitol MacConkey Agar was used for the presumptive identification, enumeration and isolation of Escherichia coli strains in the isolate. It was prepared according to the manufacturer's instruction (Lilfilchem). 65.0 g of powdered media was dissolved in 1000 ml of distilled water, it was boiled for a few minutes. Items were sterilized in an autoclave for 15 minutes at 15 pounds of pressure (121°C to 45-50°C). This was well combined before being put into sterile Petri dishes.

Brain Heart Infusion (BHI)

Microorganisms can be grown using brain heart infusion (BHI), a growth media. It is a nutrient-rich medium that can be used to cultivate a wide range of discerning organisms. BHI is widely utilized in both clinical and research settings to cultivate a wide range of microorganisms. 37 g of the dehydrated medium was dissolved in 1000 ml of distilled water based on the manufacturer's instructions in a conical flask and mixed thoroughly. The conical flask is then closed using a foil cork (made up of cotton wool wrapped in aluminum foil). The mixture was stirred for a while using the magnetic stirrer to completely dissolve the powder. 5ml of the media was then dispensed into various Eppendorf tubes and then sterilized by autoclaving at 121^{0}C for 15 minutes.

Subculturing

Sub-culturing is the process of moving microorganisms from their stock culture into a new nutritive medium. It involves moving cultures from solid medium to broth and from solid media to slant, plate to plate. Sub-culturing is done to keep cultures in their active state (extend life and/or enhance cell count) for a variety of purposes. The plates were checked after the required duration for the growth a sub-culturing needs to done. Subculturing was done to purify the isolated bacterial colonies from a mixed culture to a new and single culture, the bacterial isolates transferred or sub-

cultured were those were differentiated on the basis of their colony morphology, shape, color, elevation and other physical characteristics. Colonies differentiated by morphological characteristics are transferred onto fresh petri dishes containing MacConkey agar. A loopful of preferred isolate was taken using the inoculating loop (the inoculating loop is heated using the Bunsen burner and allowed to cool for like 5 seconds before taking the loop from the original mixed culture and streaked onto the new petri-dish). For sub-culturing, the isolate containing loop is transferred to the new petri-dish using the streaking method procedure.

3.4   ISOLATION OF UROPATHOGENIC E. COLI

The swab bottle containing the swab stick and normal saline was vortexed for 1 min. Uropathogenic *E. coli* was isolated from toilet swabs as follows; the toilet swabs sample was vortexed at a speed of 3000 rpm for 1 min after which the supernatant was decanted and the sediment was reconstituted with 1 ml of normal saline. Serial dilution was done from 10-1 to 10-4 and inoculated into a plate of MacConkey agar plates and incubated at 37ºC for 72 hr. During the subculturing procedure, the inoculation loop was sterilized in an upright position above flame from the Bunsen burner until it got hot, then it was cooled in the air; the upper lid of the petri dish containing stock culture was opened and the pure culture of Uropathogenic *E. coli* was picked with the help of the inoculation needle; then the media plates were steaked with Uropathogenic *E. coli*. Later, two isolates were picked and inoculated on Brain Heart Infusion Broth (BHI). Then, isolates were incubated for two days. Glycerol was added to the isolate and put in the freezer for storage.

3.5   ACTIVATION OF ISOLATES

1. Isolates were taken out of the freezer and allowed to thaw at room temperature.
2. 5ml of BHI was added to Eppendorf tubes and autoclaved at 15 pounds of pressure (121°C) to 45–50°C for 15 minutes.
3. 100µl of the isolates were added to the Eppendorf tubes containing the BHI and incubated at 37◦C for 24 hrs bringing about the activation of the isolates.

3.6   DNA EXTRACTION

1. The isolates were centrifuged for 3 mins at 5,000 x g, and then the supernatant was.

2. The Eppendorf tube was filled with 600µl of sterile distilled water, vortexed, and centrifuged at 5,000 x g for 3 mins, the supernatant was discarded, and the process was repeated.

3. After which, 300 µl of double distilled water was pipetted into the Eppendorf tube containing the pellets, vortexed, and then placed in the heating block to boil at 100∘C for 15 mins, the solution was then placed in ice to cool for 5-10 mins, and the contents of the Eppendorf tube were centrifuged for 6 mins at 7,000 x g.

4. A new set of Eppendorf tubes were labelled with the corresponding codes of the isolates and 150µl supernatant containing the extracted DNA was then transferred into the new Eppendorf tubes and stored in the freezer at -20ºC for further analysis.

POLYMERASE CHAIN REACTION

The components of the PCR used to identify Uropathogenic *E. coli* are listed in the table below.

1. The PCR cocktail was made and then transferred to a PCR tube before being placed in the thermocycler.

2. Initial denaturation was performed at 95°C for 5 mins, followed by 35 cycles of 95°C for 2 mins, 42°C for 30 s, and 72°C for 4 mins, followed by a final elongation step at 72°C for 10 mins.

3. There were also negative control reactions provided. The template DNA was replaced with sterile water for the negative controls.

4. Electrophoresis was used to confirm the PCR products, and a Gel Documentation system was used to visualize them under UV light.

Polymerase Chain Reaction Process

Table 3.1: Uropathogenic Escherichia coli 1ˢᵗ Round PCR Reaction Table for samples 1 to 90

Reagents	Initial Concentration (μm)	Final Concentration (μm)	Volume/Reaction (V/R) (μl)	Number of samples (n=92) (μl)
Master Mix	5x	1x	2.4	216
PaPC$_F$	20	0.6	0.36	32.4
PaPC$_R$	20	0.6	0.36	32.4
PaP$_{G\ class\ F}$	20	0.6	0.36	32.4
PaP$_{G\ class\ R}$	20	0.6	0.36	32.4
CdtB$_F$	20	0.6	0.36	32.4
CdtB$_R$	20	0.6	0.36	32.4
HLYA$_F$	20	0.6	0.36	32.4
HLYA$_R$	20	0.6	0.36	32.4
MgCl$_2$	25μm	0.5	0.24	21.6
PCR Grade Water (dH20)			4.48	403.2
DNA Template			2	
Total			12	

Preparation of the stock solution of the primers $PaPC_F$, $PaPC_R$, $PaP_{G\ class\ F}$, $PaP_{G\ class\ R}$, $CdtB_F$, $CdtB_R$, $HLYA_F$ **and** $HLYA_R$

Add 403.2 µl of PCR grade water to the lyophilized primers to get 12 µm concentration of $PaPCF$, $PaPC_R$, $PaP_{G\ class\ F}$, $PaP_{G\ class\ R}$, $CdtB_F$, $CdtB_R$, $HLYA_F$ and $HLYA_R$ primer. Pulse the centrifuge for 15 secs.

Pipette 20 µl of the prepared stock solution and 403.2 µl of PCR grade water (dH20) into a new Eppendorf tube labelling it $PaPC_F$, $PaPC_R$, $PaP_{G\ class\ F}$, $PaP_{G\ class\ R}$, $CdtB_F$, $CdtB_R$, $HLYA_F$ and $HLYA_R$, pulse vortex and pulse centrifuge.

Master Mix Cocktail Preparation

403.2 µl of PCR grade water was added into a new Eppendorf tube labelled mm (master mix), 216 µl of master mix, 32.4 µl of $PaPC_F$, $PaPC_R$, $PaP_{G\ class\ F}$, $PaP_{G\ class\ R}$, $CdtB_F$, $CdtB_R$, $HLYA_F$ and $HLYA_R$ primers were added, and 2µl of the DNA template was added and pulsed centrifuged to prepare the cocktail. 12 µl of the cocktail was dispensed into each PCR strip tube along with 2 µl of DNA template. The cycling condition of this reaction was initial denaturation 94ºc for 2 mins then coupled with final denaturation at 94ºc for 30 secs, the annealing temperature was 72 ºc for 5mins and extension was at 10ºc for ∞ that made a total of 40 cycles.

Table 3.2: 2ⁿᵈ Round Reaction Table for *PaPC$_F$, PaPC$_R$, USP$_f$, USP$_R$, CNF$_F$,* **and** *CNF$_R$* **Primers for samples 1v to 30v (MULTIPLEX PCR)**

Reagents	Initial Concentration (µm)	Final Concentration (µm)	Volume/Reaction (V/R) (µl)	Number of samples (n=92) (µl)
Master Mix	5x	1x	2.4	216
PaPC$_F$	20	0.6	0.36	32.4
PaPC$_R$	20	0.6	0.36	32.4
USP$_f$	20	0.6	0.36	32.4
USP$_R$	20	0.6	0.36	32.4
CNF$_F$	20	0.6	0.36	32.4
CNF$_R$	20	0.6	0.36	32.4
MgCl$_2$	25	0.5	0.24	21.6
PCR Grade Water (dH20)			5.2	468
DNA Template			2	
Total			12	

Master Mix Cocktail Preparation

468 µl of PCR grade water was added into a new Eppendorf tube labelled mm (master mix), 216 µl of master mix, 20 µl of PaPC$_F$, PaPC$_R$, USP$_f$, USP$_R$, CNF$_F$, and CNF$_R$ primers were added, and 2 µl of the DNA template was added and pulsed centrifuged to prepare the cocktail. 12 µl of the

cocktail was dispensed into each PCR strip tube. The cycling condition of this reaction was initial denaturation 94ºc for 2 mins then coupled with final denaturation at 94ºc for 30 secs, the annealing temperature was 72ºc for 5mins and extension was at 10ºc for ∞ that made a total of 40 cycles.

Agarose Gel Electrophoresis

Dry Agarose powder was used to make the agarose, and 1.8g of it was dissolved in 100ml of 1x TAE buffer. An amount of 45mls of TBE was measured and dispensed in a conical flask and 0.81g of agarose powder was added to it. It was microwaved at consecutive times to prevent solidification and dispensed into a falcon tube in which 4 µl of ethidium bromide was added and the gel solution was swirled to mix and the solution was dispensed into the tank and was left to solidify. One end of the box was connected to a positive electrode while the other end was connected to a negative electrode. The inner part of the gel tank contained a Tris Borate EDTA (TBE) buffer solution The end of the gel with the well was positioned towards the negative electrode while the other end was positioned towards the positive electrode. Each PCR reaction was transferred into each of the wells, one well was reserved for a DNA ladder (a standard reference that contains DNA fragments of known lengths). The power to the gel box was switched on, and current began to flow through the gel. The gel ran for 30 minutes before being viewed using the gel documentation system which is the UV transilluminator.

Table 3.3: 1st Round Reaction Table for $PaPC_F$, $PaPC_R$, $PaPC_{classic}L_F$, $PaPC_{classic}L_R$, $HLYA_F$ and $HLYA_R$ for samples 31v to 100v

Reagents	Initial Concentration (µm)	Final Concentration (µm)	Volume/Reaction (V/R) (µl)	Number of samples (n=92) (µl)
Master Mix	5x	1x	3	276
$PaPC_F$,	20	0.6	0.45	41.4
$PaPC_R$	20	0.6	0.45	41.4
$PaPC_{classic}L_F$	20	0.6	0.45	41.4
$PaPC_{classic}L_R$	20	0.6	0.45	41.4

HLYA$_F$	20	0.6	0.45	41.4
HLYA$_R$	20	0.6	0.45	41.4
MgCl$_2$	25	0.5	0.3	27.6
PCR Grade Water (dH20)			7	644
DNA Template			20	
Total			15	

Master Mix Cocktail Preparation

644 µl of PCR grade water was added into a new Eppendorf tube labelled mm (master mix), 276 µl of master mix, 41.4 µl of PaPC$_F$, PaPC$_R$, PaPC$_{classic}$L$_F$, PaPC$_{classic}$L$_R$, HLYA$_F$ and HLYA$_R$ primers were added, 15 µl of the cocktail was dispensed into each PCR strip tube along with 2 µl of DNA template. The cycling condition of this reaction was initial denaturation 94ºc for 2 mins then coupled with final denaturation at 94ºc for 30 secs, the annealing temperature was 72ºc for 5mins and extension was at 10ºc for ∞ that made a total of 40 cycles.

Table 3.4: 2nd Round Reaction Table for *CdtB$_F$, CdtB$_R$, UsP$_F$, UsP$_R$* and *UsP$_R$* Primers for samples 1 to 79 (MULTIPLEX PCR)

Reagents	Initial Concentration (µm)	Final Concentration (µm)	Volume/Reaction (V/R) (µl)	Number of samples (n=92) (µl)
Master Mix	5x	1x	3	276
CdtB$_F$	20	0.6	0.45	41.4
CdtB$_R$	20	0.6	0.41	41.4
UsP$_F$	20	0.6	0.45	41.4

UsP$_R$	20	0.6	0.45	41.4
MgCl$_2$	25	0.5	0.3	27.6
PCR Grade Water (dH20)			7.9	726.8
DNA Template			2	
Total			15	

Master Mix Cocktail Preparation

726.8 µl of PCR grade water was added into a new Eppendorf tube labelled mm (master mix), 276 µl of master mix, 41.4 µl of *CdtB$_F$, CdtB$_R$, UsP$_F$, UsP$_R$* and *UsP$_R$* primers were added, 15 µl of the cocktail was dispensed into each PCR strip tube along with 2 µl of DNA template. The cycling condition of this reaction was initial denaturation 94ºc for 2mins then coupled with final denaturation at 94ºc for 30 secs, the annealing temperature was 72ºc for 5mins and extension was at 10ºc for ∞ that made a total of 40 cycles.

Agarose Gel Electrophoresis

Dry Agarose powder was used to make the agarose, and 1.8g of it was dissolved in 100ml of 1x TAE buffer. An amount of 45ml of TBE was measured and dispensed in a conical flask and 0.81g of agarose powder was added to it. It was microwaved at consecutive times to prevent solidification and dispensed into a falcon tube in which 4 µl of ethidium bromide was added and the gel solution was swirled to mix and the solution was dispensed into the tank and was left to solidify. One end of the box was connected to a positive electrode while the other end was connected to a negative electrode. The inner part of the gel tank contained a Tris Borate EDTA (TBE) buffer solution The

end of the gel with the well was positioned towards the negative electrode while the other end was positioned towards the positive electrode. Each PCR reaction was dispensed into each of the wells, one well was reserved for a DNA ladder (a standard reference that contains DNA fragments of known lengths). The power to the gel box was switched on, and current began to flow through the gel. The gel ran for 30 mins at 100 volts before being viewed using the gel documentation system which is the UV transilluminator.

Table 3.5: 3rd Round Reaction Table for *PaPE/F$_F$, PaPE/F$_R$, CNF$_F$, and CNF$_R$* for samples 1 to 79(MULTIPLEX PCR).

Reagents	Initial Concentration (μm)	Final Concentration (μm)	Volume/Reaction (V/R) (μl)	Number of samples(n=92) (μl)
Master Mix	5x	1x	3	276
PaPE/F$_F$	20	0.6	0.45	41.4
PaPE/F$_R$	20	0.6	0.45	41.4
CNF$_F$	20	0.6	0.45	41.4
CNF$_R$	20	0.6	0.45	41.4
MgCl$_2$	25	0.5	0.3	27.6
PCR Grade Water (dH20)			7.9	726.8
DNA Template			2	
Total			15	

Master Mix Cocktail Preparation

726.8 µl of PCR grade water was added into a new Eppendorf tube labelled mm (master mix), 276 µl of master mix, 41.4 µl of PaPE/F$_F$, PaPE/F$_R$, CNF$_F$ and CNF$_R$ primers were added, 15 µl of the cocktail was dispensed into each PCR strip tube along with 2 µl of DNA template. The cycling condition of this reaction was initial denaturation 94ºc for 2mins then coupled with final denaturation at 94ºc for 30 secs, the annealing temperature was 72ºc for 5mins and extension was at 10ºc for ∞ that made a total of 40 cycles.

Agarose Gel Electrophoresis

Dry Agarose powder was used to make the agarose, and 1.8g of it was dissolved in 100ml of 1x TAE buffer. An amount of 45ml of TBE was measured and dispensed in a conical flask and 0.81g of agarose powder was added to it. It was microwaved at consecutive times to prevent solidification and dispensed into a falcon tube in which 4 µl of ethidium bromide was added and the gel solution was swirled to mix and the solution was dispensed into the tank and was left to solidify. One end of the box was connected to a positive electrode while the other end was connected to a negative electrode. The inner part of the gel tank contained a Tris Borate EDTA (TBE) buffer solution The end of the gel with the well was positioned towards the negative electrode while the other end was positioned towards the positive electrode. Each PCR reaction was dispensed into each of the wells, one well was reserved for a DNA ladder (a standard reference that contains DNA fragments of known lengths). The power to the gel box was switched on, and current began to flow through the gel. The gel ran for 30 mins at 100 volts before being viewed using the gel documentation system which is the UV transilluminator.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

A total of 50 samples were cultured, out of which 45 (90%) samples were shown to be positive for E. *coli* isolates using MacConkey agar. The number of isolates identified to be positive for Uropathogenic *E. coli* (UPEC) isolates are 21(46.6%). Growth on MacConkey agar indicates the presence of *E. coli.* The isolates were genotypically analyzed with the use of Multiplex PCR which revealed the presence of potential Uropathogenic *E. coli* strains in all the toilet swab samples analyzed. The isolates were observed so as to predict the possible presence of suspected pathogenic microorganisms of interest which is Uropathogenic *E. coli.*

The PCR reaction results identified 11$PaPC$ (24.4%), 4 $PaPG_{classic}$(8.8%), 3 $PaPE/F$(6.7%), 2$HlyA$(4.4%), 1 $Cdbt$(2.2%). All these strains were negative for Usp and CNF. $PaPC$ was identified to be the most prevalent virulence factor detected, having occurred in 24.4% of strains. The total PaP gene is shown to be 42.2%.

Table 4.1: Test results of isolates from male dormitory

S/N	SAMPLE CODE	E. COLI	UROPATHOGENIC *E. COLI*	NEGATIVE FOR *E. COLI*
1.	MNDaG1			-
2.	MNDaG2	+	+	
3.	MNDaG3			-
4.	MNDaG4			-
5.	MNDaG5			-
6.	MNDaS1	+	+	
7.	MNDaS2			-
8.	MNDaT1	+	+	
9.	MNDaT2			-
10.	MNDaL1	+	+	
11.	MNDaL2			-

12.	MNDaL3	+	+	
13.	MNDbG1			-
14.	MNDbG2	+	+	
15.	MNDbG3	+	+	
16.	MNDbS1			-
17.	MNDbS2	+	+	
18.	MNDbT1			-
19.	MNDbT2			-
20.	MNDbL1			-
21.	MNDbL2			-
22.	MNDbL3	+	+	
23.	MD1G1	+	+	
24.	MD1G2	+	+	
25.	MD1G3			-
26.	MD1G4			-
27.	MD1M1	+	+	
28.	MD1M2	+	+	
29.	MD1M3	+	+	
30.	MD1M4	+	+	
31.	MD1L1	+	+	
32.	MD1L2			-
33.	MD1L3	+	+	
34	MD1L4	+	+	
35.	MHODG1	+	+	
36.	MHODG2			-
37.	MHODG3			-
38.	MHODG4	+	+	
39.	MHODG5			-
40.	MHODM1			-
41.	MHODM2			-
42.	MHODM3			-
43.	MHODM4			-
45.	MHCL1			-
46.	MHCL2			-
47.	MCBAS1			-
48.	MCBAS2			-
49.	MCHMS1			-
50.	MCHMS2	+	+	

The suspected presence of Uropathogenic *E. coli* strains in the samples is shown in the DNA amplification image in figure 4.1.

DNA ladder
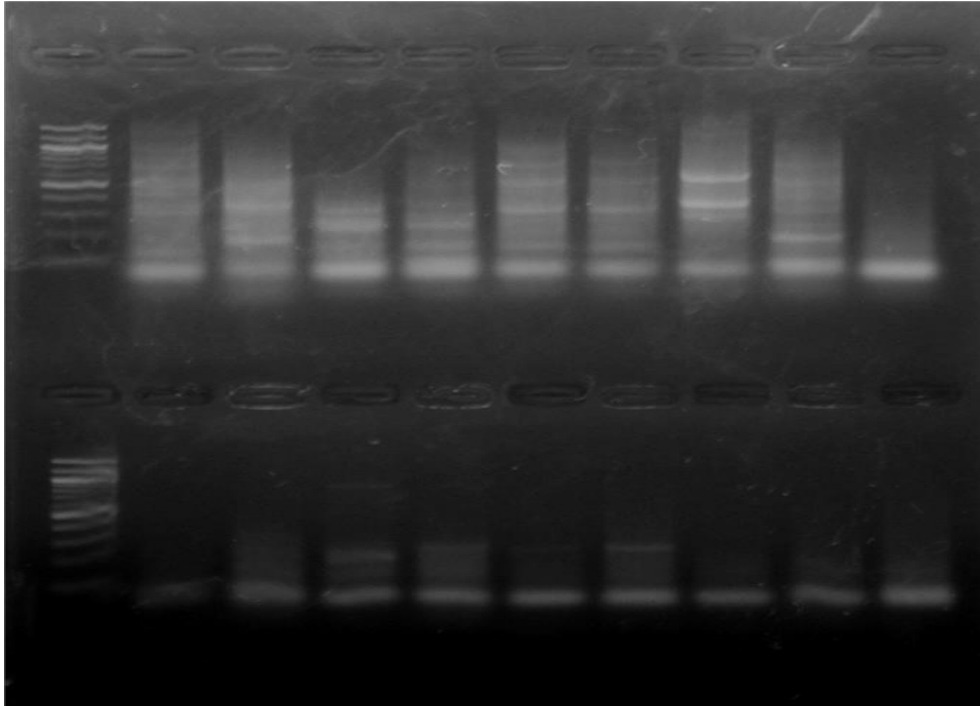

Fig 4.1: DNA amplification of toilet swab samples taken from the male dormitory

4.2   DISCUSSION

Escherichia coli is the primary cause of one of the most prevalent bacterial infections in humans UTIs. This study was carried out to identify the prevalence of UTI pathogens (Uropathogenic *E. coli*) in toilet swab samples gotten from the male dormitory. P fimbriae and G adhesins associated with fimbriae are encoded by the pap gene clusters. Three G adhesin variants that differ in receptor specificity and consequently in their ability to attach to epithelial cells have been identified using DNA sequence analysis (Arisoy *et al*., 2006). The Uropathogenic *E. coli* cells selectively adhere to the mucosa Uro-epithelium, encouraging colonization and persistence in the urinary tract, which causes a local inflammatory response and occasionally leads to the development of tissue lesions (Yamamoto, 2007). Multiplex PCR reaction was used to detect and identify the presence of Uropathogenic *E. coli* and their virulence factors.

The adhesin-encoding operons and other virulence factors that can also contribute to virulence in UTI were easily detected using this technique, which also showed to be highly specific and informative. In this study, the prevalence of *PaPC* among UPEC strains was confirmed (Johnson *et al*.,2000). This result showed that a crucial and pertinent virulence factor (VF) can also increase the pathogenicity of *E. coli*. The main mannose-resistant adhesion organelles of extraintestinal pathogenic *Escherichia coli*, the *P fimbriae*, are known to contribute to pathogenesis by encouraging bacterial colonization of host tissues and by inducing a harmful host inflammatory response (Kuehn *et al*., 1994). The *HLY* gene was discovered together with *P fimbriae*. Other two urovirulent factors, *CNF-1* and *P fimbriae*, are typically produced by *E. coli* strains that also express *HLY*. First isolated from children with enteritis were *Cdt*-producing E. coli strains (Johnson *et al*., 1988). A permanent stop in the cell cycle at the G2/M stage, which results in cellular death, is what causes CDTs to create enormous mononucleated cells.

CHAPTER FIVE

RECOMMENDATION AND CONCLUSION

5.1   CONCLUSION

The suspected presence of Uropathoegenic *E. coli* strains in the toilet swab samples reveals that they are present in the water closets used by male students in the tertiary institution. The toilet in the male dormitories of the tertiary institution under investigation contain Uropathogenic *E. coli* which can create a health hazard both to the school and to the public.

5.2   RECOMMENDATION

Male students should ensure adequate sanitation and hygiene of their toilets to assure that their toilets are free of any pathogenic microorganisms.

Students should have access to sufficient hand sanitizers and hand washing stations, and the institution should promote frequent use of these amenities. This will aid in limiting the spread of illnesses like Uropathogenic *E. coli*. More investigation is required to find out the prevalence of Uropathogenic E. coli in the institution's other sanitation facilities and to pinpoint additional probable sources of the disease on campus. This will provide information that will be used to create more thorough and successful plans for stopping the spread of UTIs among students.

In addition, the school authority and its management should implement programs to teach students about the occurrence and prevalence of Urinary Tract Infections (UTI'S) brought about by poor hygiene, indiscriminate use of antibiotics and so on.

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Appendix 1

Materials and Apparatus

TYPES OF EQUIPMENTS USED: Test tube, thin foil, weighing balance, Bunsen burner, incubator, centrifuge, microscope, glass slides, cover slide inoculating loop, Heating block, Thermal cycler, Gel electrophoresis tanks, Gel documentation system, Incubator (37℃), Vortex mixer.

APPARATUS USED: Stomacher bags, wash bottles, Petri-dishes, measuring cylinder, glass pipettes, beakers, conical flasks, glass spreader, inoculating loop, wash bottles, Eppendorf tubes, micropipette (with their tips), test tubes and foil corks (with their racks), PCR tubes, glass slides, oxidase test disc, thin foil.

Appendix 2
Reagents

Reagents used: MacConkey agar, Brain Heart Infusion (BHI), glycerol, chloramphenicol, serum, distilled water, normal saline.