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Research Article

Virulence Markers in *Escherichia coli* Isolated from the Urine of Pregnant Women and HIV Patients in Port Harcourt, Nigeria

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ABSTRACT

The possession of virulence factors that elicits pathogenicity in uropathogens is a concern in public health care. It is said that possession of many virulence gene accelerates the degree of pathogenicity and the extent at which infections occur. This study therefore, was carried out to detect and compare the prevalence of virulence genes in *Escherichia coli* isolated from 130 urine specimen of pregnant women and 130 urine specimen of HIV patients attending University of Port Harcourt Teaching Hospital. Based on previous study, A total of 25 *Escherichia coli* isolates from urine of pregnant women (13) and HIV patients (12) were selected for virulence genes detection using molecular method. Assays were carried out to detect four virulence encoding genes (*hlyA, afaC, cnf-1* and *fimH*) in *Escherichia coli*. The study revealed the highest incidence of *fimH* (68.0%) virulence gene in *Escherichia coli*. There was no detection of haemolysin A (*hylA*) and cytotoxin neutralizing factor (*cnf-1*) virulence gene. The virulence gene detection was higher in HIV patients (69.2%) when compared with that of the pregnant women (66.7%). There was a significant difference in occurrence of *fimH* in *Escherichia coli*. There is need to create awareness on uropathogens and their virulence factors, how they are acquired and measures to prevent them, effective testing/treatment are also needed to check their occurrence.

Keywords: Virulence genes, *Escherichia coli*, pregnant women and HIV patients.

Introduction

Virulence markers are those factors produced by microorganisms which evoke diseases or infections including surface coat with inhibiting effect on phagocytosis, surface receptors which binds to the host cells and toxins. The acquisition of virulence genes exhibited by microorganism has an impact in the ability of microorganisms to cause infections. An example of this is seen in *Escherichia coli*, a Gram-negative rod shaped bacterial often isolated in urine where they cause infections in the urinary tract. They are frequently isolated from the urine of people with compromised immune-system especially Human immune-deficiency virus (HIV) patients and pregnant women who harbour them as a result of anatomical and physiological changes encountered in pregnancy (Malekzadegan *et al*., 2018; Nelson and Good, 2015 and Ballesteros-Monrreal *et al.*, 2020).

They have been reported by several works to possess many bacterial virulence factors with encoding genes which enables them to attach, invade and cause disease in host (Ballesteros-Monrreal *et al.*, 2020; Abd El-Baky *et al*., 2020 and Tarchouna *et al*., 2013). In expressing the virulence factors which are the main drivers for pathogenicity, there is likelihood of therapy failure, complications and prolong hospitalization time. This is so because the possession of virulence genes affects the gravity and degree of infection especially in pregnant women and HIV patients who are on anti-retroviral drugs (Ballesteros-Monrreal *et al.*, 2020 and Tarchouna *et al*., 2013). One component that predisposes HIV patients to acquire urinary tract pathogens is mainly due the suppression of their immune system which enables uropathogens to colonize the urinary tract easily. *E. coli* possess a wide range of virulence factors that allows it successfully colonize the urinary tract leading to pathogenicity.

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The most frequent virulence factors seen in uropathogenic *Escherichia coli* (UPEC) are those involved in adherence, fimbrial and afimbrial adhesins which are encoded by *fimH* and *papG*. They are virulence genes that promote the colonization and the pathogenic potentials of *E coli* (Hojati *et al*., 2015).

They act like hemagglutinin by helping to adhere to the cells of the uroepithelial., Iron acquisition system which involves Aerobactin, toxigenic proteins which includes hemolysin A encoded by *hylA* genes, cytotoxic neutralizing factor encoded by *cnf*-1 genes, *sat*, *vat* and motility are all frequently encountered virulence factors encoded by genes in *Escherichia coli* (Ballesteros-Monrreal *et al*., 2020).

There are scarce data on detection and prevalence of virulence genes that occur in pregnant women and HIV patients in Port Harcourt, Nigeria. Since possession of virulence genes are the cause of virulence and pathogenicity associated with uropathogenic *E. coli*, there is need to identify the prevalent virulence genes associated with *E. coli* among these targeted individuals.

This study was therefore aimed at detecting and comparing the prevalence of the virulence genes *hlyA, afaC, cnf-1* and *fimH in Escherichia coli* isolated from urine samples of pregnant women and HIV patients attending anti-retroviral clinic at the University of Port Harcourt Teaching Hospital.

Materials and Methods

Sample Collection.

Twenty-five (25) uropathogens identified to be *Escherichia coli* was selected from isolates in urine of HIV patients (13) and pregnant women (12) from previous study.

Identification of virulence gene in *Escherichia coli* using molecular methods

Three Sequential methods, DNA extraction, DNA quantification and polymerase chain reaction was used to detect the virulence genes associated with *Escherichia coli* isolated from urine of pregnant women and HIV patients.

Extraction of DNA (Boiling method) and DNA quantification

Bacterial isolate which was 48 hours old in normal saline (1 micro-liter) was transferred to an Eppendorf tube and spun in a centrifuge (Biologix-USA) for 5min at 15000rpm at a temperature of 29^0C. The pellet (cells) left after discarding the supernatant was re-dissolved in 1ml of nuclease free water. The mixture was heated for 10 minutes at a temperature of 100^0C with the use of a heat block machine (Biologix-USA). The bacterial suspension at room temperature was spun using a centrifuge machine at 10000rpm for 5 minutes. The supernatant containing the DNA was then transferred into a fresh Eppendorf tube and stored. The extracted genomic DNA was quantified with the use of 1000 spectrophotometer Nanodrop.

Polymerase chain reaction of virulence genes

Specific primers were used to amplify sequences of the *hlyA, afaC, cnf-1* and *fimH* operons for the targeted genes of virulence factors, Hemolysin, Afa adhesions, Cytotoxic necrotizing factor and Type 1 fimbriae respectively. This was achieved by employing the primer sequences, predicted sizes of the amplified products, and specific annealing temperatures of Tarchouna *et al*. (2013), Soto, *et al*. (2011), Licznar *et al*. (2003) and Tarchouna *et al*. (2013), for the targeted genes respectively.

Cocktail preparation for PCR reactions

The cocktail was prepared for each of the 25 isolates. Each cocktail consists of the reverse primer (0.3µL) and forward primers (0.3µL), nuclease free water (5.4 µL) and master mix (2 µL) forming a total of 8µL for each isolate with two (2) DNA templates. The amplification was carried out in a PCR machine (Edvotek-USA). A 10-ml aliquot of the PCR product underwent gel electrophoresis on 2% agarose, followed by staining with ethidium bromide solution. Amplified DNA fragments of specific sizes were detected by UV-induced fluorescence and the size of the amplicons was estimated by comparing them with the 1 kb DNA ladder (Promega) included on same gel.

Statistical Analysis

Data gotten by Statistical analysis from two independent determinations was analyzed by the use of Chi-square test and the significance of the mean differences was determined at $p< 0.05$.

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Results

The result of *Escherichia coli* total virulence gene in urine of pregnant women and HIV patients is presented in Figure 1. The result disclosed that, *fimH* (n=17, 68%) was the highest occurring virulence genes in *Escherichia coli* in urine of pregnant women and HIV patients. It also revealed absent of two genes *cnf-1* and *hylA*.

Figure 2 shows the total virulence gene occurrence in pregnant women and in the HIV patients. The result revealed a higher prevalence of *fimH* (n=9, 69.2%) and *afaC* (n=7, 53.8%) in *Escherichia coli* in urine of the HIV patients. There was significant difference in the occurrence (x^2 = 23.833, *P* = 0.000).

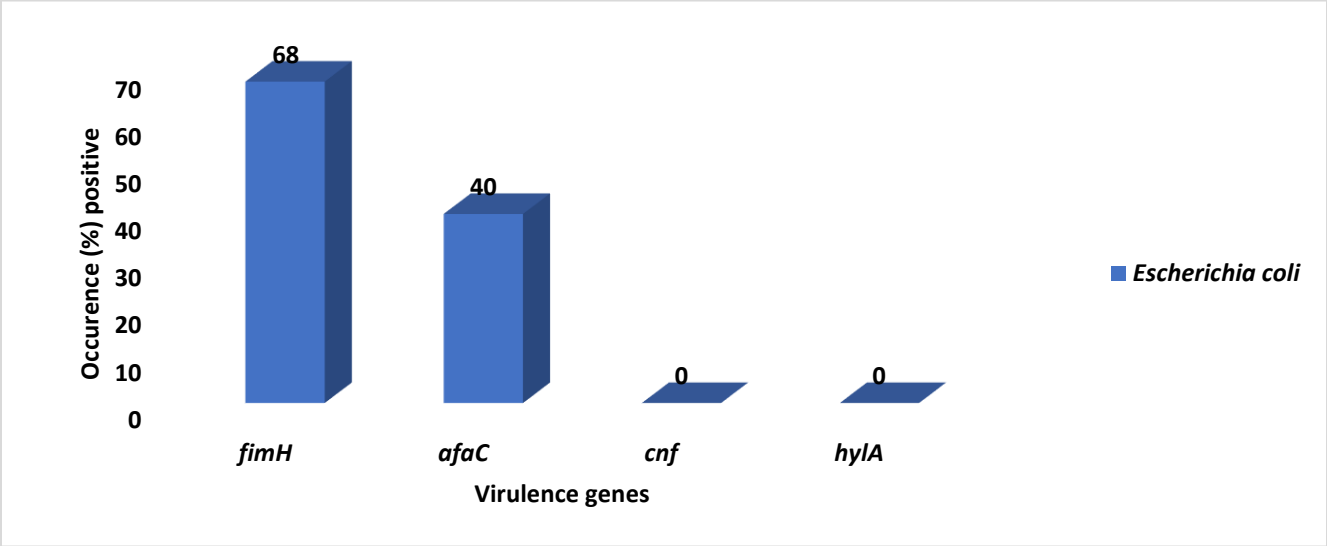

Figure 1: Total detected virulence gene for all the *Escherichia coli* isolates

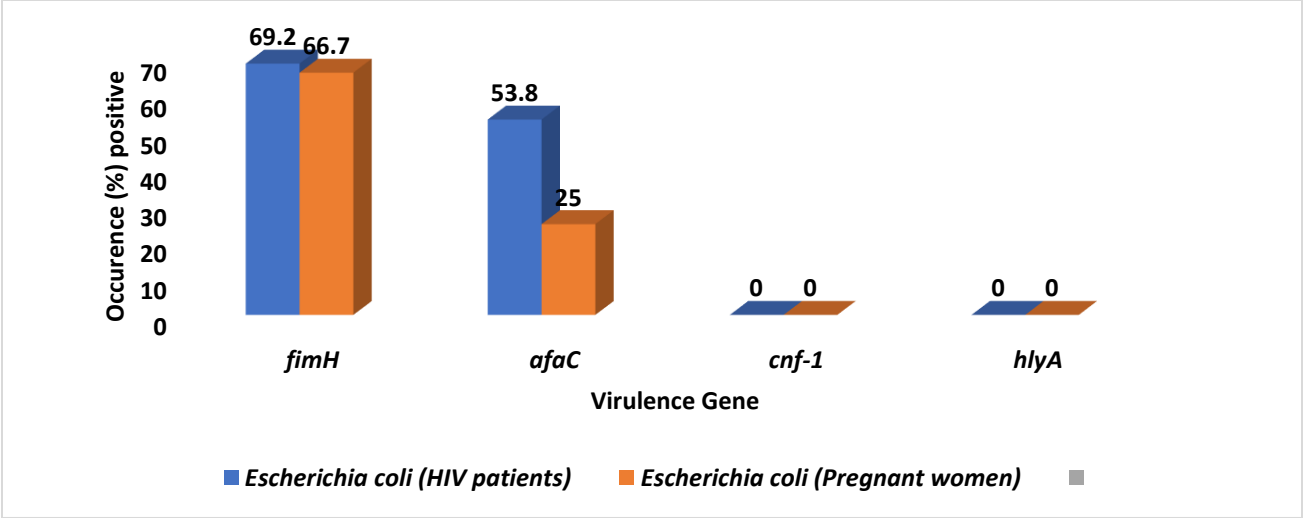

Figure 2: Total virulence gene occurrence in pregnant women and in HIV patients

The Co-occurrence prevalence of virulence genes in *Escherichia coli* in urine of pregnant women and HIV patients is shown in Figure 3. The results disclosed *Escherichia coli* in HIV patients harboring the highest prevalence of co-occurrence (n=7, 53.8%) of virulence genes. While the virulence gene profile of *Escherichia coli* determined by PCR based on pregnant women and HIV patients is shown in Table 1. The result disclosed *fimH-afaC* profile as the most occurred virulence gene profile (n=7, 53.8%) in HIV patients.

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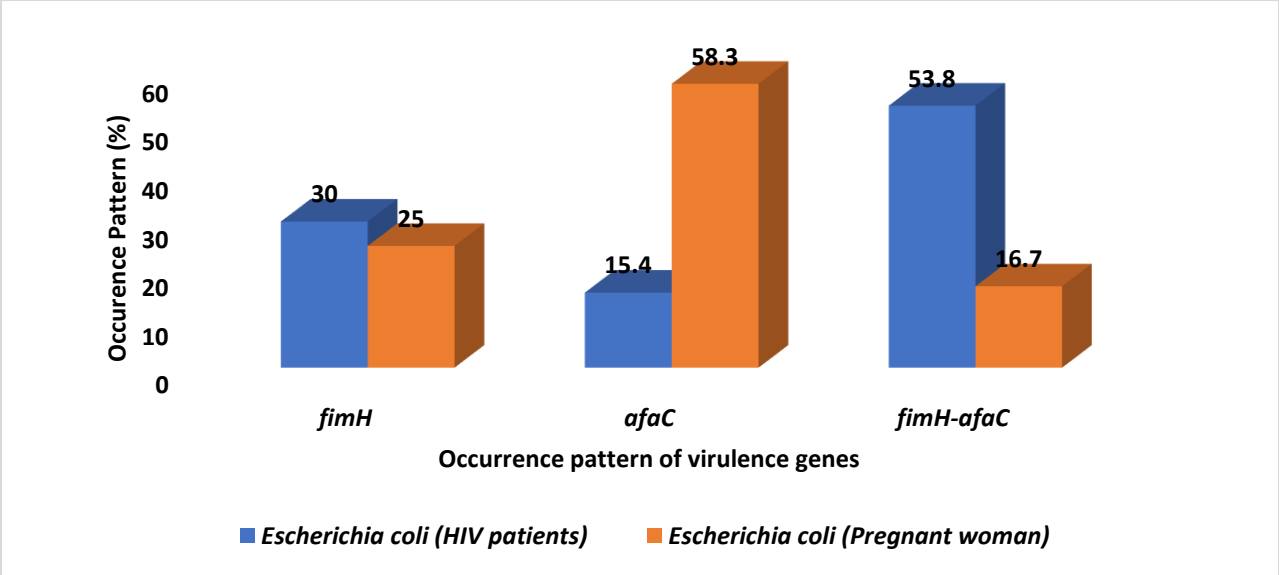

Figure 3: Occurrence pattern of virulence genes from *E. coli* between pregnant women and HIV patients

Table 1: Virulence gene profile of *E. coli* determined by PCR based on pregnant women and HIV patients

Virulence gene pattern *Escherichia coli*	HIV patients (n-13) (%)	Pregnant women (12) (%)
fimH	1(7.7)	6(50)
afaC	0(0)	1(8.3)
fimH- afaC	7(53.8)	2(16.7)

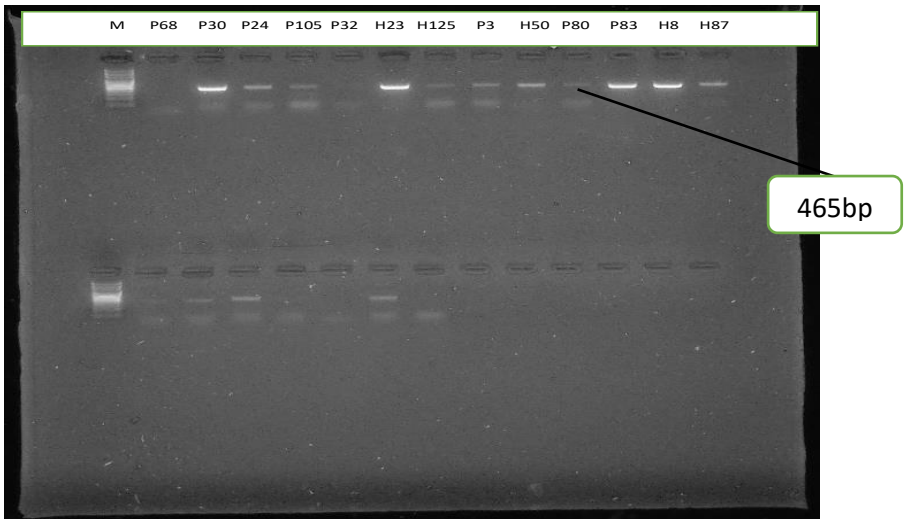

Figure 4: PCR product of *fimH* virulence gene in *Escherichia coli* (465bp)

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Discussion

The ability to possess many bacterial virulence genes was reported to add to the gravity, degree of infection and the capability of uropathogenic *Escherichia coli* to cause disease (Dale and Woodford, 2015 and Jaureguy *et al.*, 2007). *Escherichia coli* being a commensal frequently isolated from human faces in acquisition of different virulence factors poses a great threat to public health. This is because having numerous virulence determinants can give rise to great possibility of therapy failure which is of great concern. The present study investigated the prevalence of virulence genes from 12 *Escherichia coli* isolates from urine of pregnant women and 12 *Escherichia coli* from urine of HIV patients, in addition, it compared the prevalence of the virulence genes *hlyA, afaC, cnf-1* and *fimH in Escherichia coli* isolated from urine samples of pregnant women and HIV patients on ante-natal care and on anti-retroviral drugs for not less than 1 year respectively.

The total virulence gene detection revealed *fimH* (68.0%) in *Escherichia coli* as the highest occurred virulence gene in pregnant women and HIV patients (Figure 1). Detecting *fimH* as the highest occurring virulence gene in *Escherichia coli* is in line with the discoveries of other researchers. In illustrating this, some studies disclosed *fimH* as the highest occurred virulence genes at 80%, 78.4% and 78.0% respectively among uropathogenic *Escherichia coli* isolated from urine in their studies (Mahdis *et al*., 2020 and Malekzadegan *et al*., 2018).

Furthermore, some researchers, Ballesteros-Monrreal and his group also disclosed that *fimH* occurred at 100% which was the most occurring virulence genes among *Escherichia coli* isolates in their study on detection of virulence genes in urine of pregnant and non-pregnant women (Ballesteros-Monrreal *et al*, 2020). A publication in 2017 by some researchers also disclosed *fimH* virulence genes occurring most in their study (Parniaga-Contreras *et al*., 2017). These results proved a point that *fimH* virulence gene is importance in establishment of infection by *Escherichia coli* in the urinary tract. The *fimH* virulence codes for type 1 fimbriae which is very important for attachment purposes in the epithelial cells of a host. Studies carried out by Mulvey (2002) indicated that virulence factors in *Escherichia coli* are usually genes that encodes for adhesion such as the fimbriae used to invade and colonize host cells (Mulvey, 2002). Perhaps, this brought about the high occurrence displayed. Furthermore, the virulence genes revealed in HIV patients (Figure 2) showed a higher prevalence (*fimH*-69.2% and *afaC*-53.8%) in both *fimH* and *afaC* genes when compared with virulence genes (*fimH*-66.7 and *afaC*-25.0%) seen in pregnant women. This can be explained with reasons that there is always the existence of opportunistic infections in HIV patients which takes place due to fluctuating and waning immunity experienced by these group due to their inability to keep to recommended diet and required therapy. Therefore, *Escherichia coli* which is an opportunistic pathogen will access an enabling environment to attack and cause infections. This is unlike in the case of pregnancy where there is a temporary suppressed immunity and not suppression as a result of virus invasion. Perhaps, opportunistic infections may not gain more grounds as they would in HIV cases. There was a statistical difference (X^2 = 23.833, P = 0.000) the occurrence of *fimH* virulence genes in HIV patients.

This result is not consistent with reports from Raeispour and Ranjbar, (2018). Their discovery had it that aerobactin (aer) (90%) virulence gene was the highest detected virulence gene in their research. This is possible because variations may arise due to the source of isolates and the predominant organism in that particular source. Other factors may include, number of isolates studied and the type of virulence genes selected. The prevalence of *fimH* virulence genes at 66.7% and 69.2% in *Escherichia coli* from HIV patients and pregnant women respectively presented in this study is similar with report of 74.4% and 78.4% seen in other studies (Haghighatpanah and Mojtahedi, 2019 and Mahdis *et al*, 2020). Some investigators revealed a low prevalence of 30% and 28% (Bahalo *et al*., 2013 and Hassan *et al*., 2011). The occurrence of higher and lower variations in the prevalence of *fimH* in *Escherichia coli* can be attributed to sampling techniques, health status of the participants, climate and environmental conditions customs associated with these studies. Furthermore, there is no data on virulence gene of *Escherichia coli* in urine of pregnant women and HIV patients previously published in Port Harcourt, Nigeria to the author's knowledge which can be used for comparison except for studies outside Nigeria where similar study was carried out on clinical samples. The *hylA (*Hemolysin A) and *cnf-1* (Cytotoxin neutralizing factor) virulence genes were not detected in *Escherichia coli* in urine of two groups studied.

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Report had it that these virulence gene are usually seen in patients with infections in the urinary tract that led to and hemolytic activity or bacteremia which is vital in tissue damage (Daga *et al*., 2019 and Sonnen and Henneke, 2013). Their absence points to the fact that participants involved do not have bacteremia but only visited the clinic because of their weekly or monthly routine check-up and showed no sign of being ill.

The *fimH* and *afaC* co-occurrence (Figure 3) were more common among *Escherichia coli* in urine of HIV patients when compared with those in urine of pregnant women. This buttresses the fact that *Escherichia coli* in HIV patients had more virulence gene than in pregnant women.

The virulence gene profile revealed that *Escherichia coli* exhibited 3 virulence gene profile (Table 1). Other researchers have discovered more than this number of virulence gene profile. The uropathogenic *Escherichia coli* isolates from HIV patients revealed a virulence gene pattern referred to as *fimH-afaC* (53.8%) which was the most predominant virulence gene pattern seen among the isolates characterized by the presence of only *fimH and afaC* virulence genes. This pattern was found in 7 isolates out of the 13 *Escherichia coli* studied from HIV patients. At the other hand, the pregnant women had a lower *fimH-afaC* (16.7%) pattern compared to HIV patients. This is due to the higher virulence gene seen in *Escherichia coli* in HIV patients than it occurred in pregnant women.

There was no occurrence of multiple virulence genes in isolates unlike in other studies (Tabasi *et al*., 2016). This has to do with the limited number of isolates and virulence gene studied and the absence *hylA* and *cnf-1* in *Escherichia coli*. Generally, it was observed that uropathogens isolated from HIV patients expressed higher level of virulence genes than those isolated from pregnant women.

In conclusion, *fimH* was found to be the most prevalent virulence gene among *Escherichia coli*, isolates respectively. In-spite of this information, further epidemiological studies are needed to determine the prevalence of these genes and other virulence genes associated with *Escherichia coli* to further verify the association between virulence factors associated with different clinical types of urinary tract infection. This study could pave the new way to understand the roles of these virulence factors and in turn development of anti-virulence antibiotics.

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