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ORIGINAL ARTICLE

Association of *Escherichia coli* Infection and *fimh* Virulence With Benign Prostatic Hyperplasia in Ghanaian Patients

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ABSTRACT

Background: Benign prostatic hyperplasia (BPH) is the most common urological disorder of the prostate in aged men. Oxidative stress and environmental factors have been associated with BPH. However, information on infectious agents association with BPH remains scarce. This study aims to determine *Escherichia coli* (*E. coli*) infection and virulence gene association with BPH in patients.

Methods: A case–control study was conducted with 61 BPH patients and 52 controls. Prostate volume (PV) was estimated for diagnosis of BPH using abdominal ultrasound. Serum malondialdehyde (MDA) levels were measured, and data on alcohol intake and physical exercise were obtained with questionnaire. *E. coli* DNA was extracted from urine samples, and targeted 16S rRNA and *fimH* gene primers were used for PCR amplifications.

Results: Mean difference of PV between patients (55.10 ± 27.37) and controls (26.33 ± 6.37) was statistically significant ($p < 0.01$). Serum MDA was significantly and positively correlated with PV ($p < 0.001$). Exercise correlate inversely with prostate volume. Intriguingly, alcohol intake significantly and inversely correlated with PV ($p < 0.05$). *E. coli* infection, but not virulence, was associated with an almost 12-fold increased risk of PV ($p < 0.01$). No *fimH* gene sequence variation was observed in isolates from patients and controls. However, Ghanaian isolates displayed sequence diversity when compared with isolates from other countries.

Conclusion: *Escherichia coli* infection, particularly variant carrying the *fimH* virulence gene, was more frequent among the BPH patients. These findings suggest that *E. coli* infection should be considered as a key factor in the management of BPH.

1 | Introduction

1.1 | Background

Benign prostatic hyperplasia (BPH) is a non-malignant enlargement of the prostate gland that progressively narrows the prostatic urethra and constitutes the most prevalent benign tumor in aging males [1]. This condition arises from a hormone and inflammation driven proliferation of stromal and epithelial cells in the transitional zone of the prostate, which leads to the mechanical obstruction of static (bulk) and dynamic (smooth-muscle tone) components of bladder-outlet resulting in poor urine outflow, triggering lower urinary tract symptoms (LUTS) [2, 3]. Globally, BPH affects about 94 million men aged ≥ 40 years, and histological evidence can be found in up to 90% of men by age 85 [2, 4]. Risk

factors include old age, family history, metabolic syndrome, obesity, diabetes, and sedentary lifestyle, whereas recommended preventive practices are linked to exercise and favorable cardiometabolic profiles [5]. Clinically, urinary frequency, urgency, nocturia, hesitancy, weak stream, and incomplete bladder emptying are manifested by BPH, whereas complications include recurrent urinary tract infections, retention, bladder stones, and chronic kidney injury if untreated [6].

The role of infections and associated inflammation and oxidative stress in BPH is increasingly recognized as a significant factors in its pathogenesis and progression. Chronic inflammation in the prostate is frequently observed in BPH tissues, with infiltrates mainly composed of activated T cells and macrophages [7]. Proinflammatory cytokines are produced by these immune cells, which contribute to fibromuscular growth and hyperplastic changes within prostatic tissue [8]. This inflammatory milieu facilitates the proliferation of prostatic cells via oxidative stress and immune-mediated pathways. High levels of 8-OH deoxyguanosine were reported in prostate transition zone of BPH [9]. Excessive levels of reactive oxygen species (ROS) with persistent were established to promote cell proliferation, and create antiapoptotic milieu to drive BPH. The deleterious role of ROS has been linked to the damaging effect of the free radicals on DNA and protein molecules that can alter signaling pathways that control cell proliferation and inflammation [10]. Immune response-associated local oxidative stress is considered to be triggered by factors like bacterial or viral infections as immune cells which release ROS and nitrogenous intermediates to target pathogens [10]. Therefore chronic prostatitis, whether infectious or noninfectious, creates an environment that may promote hyperproliferative cellular pathways. Studies investigating the microbial composition of BPH tissues have identified the presence of diverse bacterial populations, with *Escherichia coli* (*E. coli*) being one of the commonly detected species, particularly within the Proteobacteria phylum [11]. *E. coli* has been isolated from prostate tissues in over half of studied BPH patients, suggesting that this bacterium can colonize the prostate or urinary tract in these cases [12]. Importantly, the ability to activate the nuclear factor-κB (NF-κB) signaling pathway and induce DNA damage in prostate epithelial cells has been demonstrated by clinical isolates of *E. coli* from BPH tissues, which could contribute to local inflammation related oxidative stress and tissue remodeling [11]. Other environmental factors including lifestyle has been implicated in BPH. In a systematic review, 19 studies associated alcohol consumption with a decreased risk of BPH [13], however the mechanism is not fully understood. Nevertheless, physical activity has been found to reduce the risk of BPH [14, 15]. To date, information on the association between bacterial infection and BPH remains limited, particularly in developing countries such as Ghana. This study sought to establish an association between *E. coli* infection and virulence variant among Ghanaian BPH patients.

2 | Materials and Methods

2.1 | Study Site

A case-control study was conducted in Greater Accra Regional Hospital (GARH): a primary referral hospital with a bed capacity of over 600 and Ledzokuku Municipal Assembly Hospital (LeKMAH) with a bed capacity of about 100.

2.2 | Study Population

Study participants were clinically diagnosed BPH patients aged between 50 and 73 years, and presenting with lower urinary tract symptoms (LUTS) and age-matched apparently healthy controls. The controls were recruited through community screening in the hospital environment. The study participants were recruited from July 2022 to July 2023. Patients with prostate cancer or other malignancies, those who had undergone prostatectomy, those who have started antibiotic treatment and catheterized patients were excluded from the study. A total of 113 participants made up of 61 BPH patients and 52 controls were recruited into the study. Socio-demographic and data on exercise and alcohol consumption were obtained using a questionnaire.

2.3 | Urine Sample Collection

Study participants were directed to collect approximately 5 mL of mid-stream urine sample by first allowing an amount of urine to flow and discard. This was followed by collection of the middle portion of the urine flow to the 5 mL mark of the sterile falcon tube. The urine sample was centrifuged at 2500 rpm for 5 min, decanted and the deposit stored at −80°C for DNA extraction. Urine crystals were dissolved with 10% of 1M Tris-EDTA (equal volumes) to concentrate possible bacteria before DNA extraction.

2.4 | Abdominal Scan

BPH was identified using prostate volume measured with Siemens SONOLINE SSI-6000 ultrasound machine (Shenzhen, China). The prostate was imaged entirely and the prostate volume determined using the inbuilt Ellipsoid formula; length x height x weight x 0.5236 (pi/6).

2.5 | DNA Extraction From Urine

DNA was extracted from urine using DNeasy Blood & Tissue Kit (Qiagen Inc. USA). The extraction was performed following the manufacturer's protocol. The concentration and purity of DNA samples were obtained with NanoDrop Ultra Spectrophotometer (ThermoFisher Scientific, USA). Extracted DNA was stored at −20°C until ready to be used.

2.6 | *Escherichia coli* Detection Using 16S rRNA Targeted PCR Method

The DNA extracted were amplified using a conventional polymerase chain reaction (PCR) approach with primers targeting 16S rRNA gene sequences of *E. coli*. Forward Primer: 5′-GGA AGAAGCTTGCTTCTTTGCTG-3′ and Reverse Primer: 5′-AGC CCGGGGATTTCACATCTGA-3′ [16]. The PCR reaction for detection of *E. coli* was carried out with thermocycler (Prime thermal cycler, Bibby Scientific Limited, UK) with the primer

set and ready-to-use OneTaq Quick-Load 2X Master Mix (BioLabs Inc., England) by following the manufacture's reaction conditions. Each 25 μL PCR reaction mixture had the following components and final concentrations: 1x reaction buffer, 0.5 μM each of the forward and reverse primers, and 0.1 μg/μL genomic DNA. Nuclease-free water was added to obtain the reaction volume. The reaction mixture was subjected to an initial denaturation at 94°C for 30 s, 35 cycles of denaturation at 94°C for 1 min, annealing at 50.5°C for 1 min, and extension at 72°C for 2 min. Final extension was performed at 72°C for 5 min.

2.7 | Type 1 Fimbriae (fimH) Detection

Using forward Primer: 5′-AACAGCGATGATTTCCAGTTTGT GTG-3′and reverse Primer: 5′-ATTGCGTACCAGCATTAGCA ATGTCC-3′ [17]. The following PCR conditions for *E. coli* virulence (*fimH*) identification were used: initial denaturation at 94°C for 10 min followed by 30 cycles of 2 min at 94°C, 30 s at 60°C for annealing followed by extension for 1 min at 72°C. A final extension for 10 min at 72°C terminates the process.

Following the PCR, a 10 µL aliquot of the amplicon was subjected to electrophoresis at 120 volts using a Fisher Biotech electrophoresis system FB-SB-B16 (Fisher Biotech, USA). Electrophoresis was performed in a 2% agarose gel (Biopioneer Co, USA) containing 0.5 µg/ml ethidium bromide (Life Technologies Co, USA) in 1X Tris-acetate-EDTA (TAE) running buffer (Biopioneer Co, USA). Samples were prepared with 2 µl of blue/orange DNA loading dye (6X) (Promega Co, USA). A 100 base pair nucleotide sequence molecular size marker (Sigma Mo, USA) was included alongside the PCR products. Gel documentation was carried out using UV Bio Dock Its imaging system (Analytic Jena US/Canada) (California, USA).

2.8 | Evolutionary Analysis by the Maximum Likelihood Method for *Escherichia coli*

Phylogenetic and molecular evolutionary analyzes were conducted using MEGA version 11. Ancestral states were inferred using the Maximum Likelihood method and Tamura-Nei model [18]. Initial tree topologies for the heuristic search were automatically generated using the Neighbor-Joining and BioNJ algorithms, applied to a matrix of pairwise distances estimated with the Tamura-Nei model. The topology with the highest log-likelihood value was then selected. Rates among sites were treated as uniform (Uniform rates option). This analysis incorporated 22 nucleotide sequences, encompassing the 1st, 2nd, and 3rd codon positions, along with non-coding regions. The final dataset comprised a total of 661 positions.

2.9 | Evolutionary Analysis by the Maximum Likelihood Method for fimH

The phylogeny was inferred using the Maximum Likelihood method and Tamura-Nei model of nucleotide substitutions [18]. The initial phylogenetic tree for heuristic searches was selected based on a comparative log-likelihood analysis between a Neighbor-Joining (NJ) tree and a Maximum Parsimony (MP) tree. The NJ tree was constructed from a matrix of pairwise genetic distances calculated using the p-distance method. The MP tree was determined by selecting the shortest tree from 10 independent MP tree searches, each initiated with a randomly generated starting tree [19]. The analytical procedure encompassed 13 coding nucleotide sequences, incorporating first, second, and third codon positions, as well as non-coding regions, resulting in a final dataset comprising 504 nucleotide positions [20]. Evolutionary analyzes were performed using MEGA11, employing up to four parallel computing threads.

2.10 | Statistical Analysis

Data obtained were recorded and cleaned with Microsoft excel version 10 and analyzed using the Statistical Package for Social Sciences (SPSS), version 26.0 and GraphPad version 8.01. Qualitative data were expressed as frequency and percentage whiles quantitative data were presented as mean ± SD. Categorical parameters were analyzed using chi-square (χ^2) test to determine the significant difference between two groups. Binary logistic regression was used to predict the outcome of categorical data. *p-value* < 0.05 was considered statistically significant.

3 | Results

3.1 | Baseline Characteristics of Participants

The mean difference of MDA levels between the patients and the controls was statistically significant ($p < 0.05$). Prostate volume was significantly greater in the patients than the control group ($p < 0.001$) (Table 1).

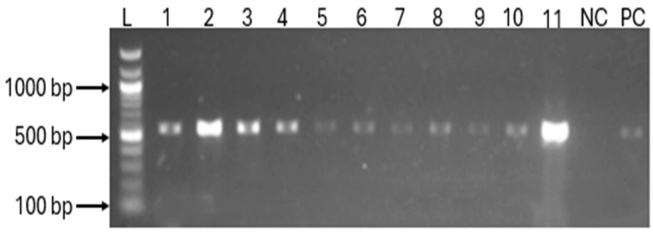

FIGURE 1 | Gel image for *Escherichia coli* gene amplification using 16 S RNA primer for conventional PCR. PC: Positive control (well 13) and NC: Negative controls [12]. Well 1–11 had the gene of interest (*E. coli* gene, 544 bp), L: 100 bp ladder.

TABLE 1 | Association of baseline characteristics with benign prostate hyperplasia.

Parameter	BPH patients ($N = 86$)	Controls ($N = 82$)	95% CI of mean difference	*p*-value
Age (years)	64.22 ± 5.38	64.30 ± 4.31	63.35–65.25	0.9116
MDA level (µmol/l)	1.22 ± 0.68	1.01 ± 0.49	−0.38–(−0.02)	0.0295*
Prostate volume (cm^3)	57.12 ± 27.15	25.86 ± 6.21	−37.32–(−25.18)	< 0.001*

Abbreviation: *N*, population.
*$p < 0.05$ was considered statistically significant.

3.2 | *Escherichia coli* Identification Among Study Participants

Figure 1 shows the *Escherichia coli* gene (specific for 16 S rRNA) identified from the urine specimen of participants on 2% agarose gel with a band size of 544bp after a conventional PCR amplification.

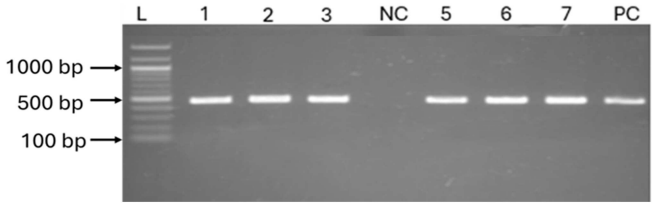

FIGURE 2 | A gel image for *Escherichia coli* virulence gene (*fimH*) amplification using conventional PCR. PC: Positive control (well 8), NC: Negative controls [4]. and wells 1–3 and 5–7 had genes of interest (*E. coli* virulence gene, 465 bp) L: 100 bp ladder.

3.3 | *Escherichia coli* Virulence Identification in Benign Prostatic Hyperplasia

Figure 2 shows the *Escherichia coli* virulence type 1 *fimbriae* (*fimH*) gene with a band size of 465 bp identified in the urine specimen of participants after a conventional PCR amplification.

3.4 | Genetic Diversity and Clustering Patterns of *E. coli*

The tree shown in Figure 4 reveals a phylogenetic distribution among the studied *E. coli* isolates. The isolates from the patients

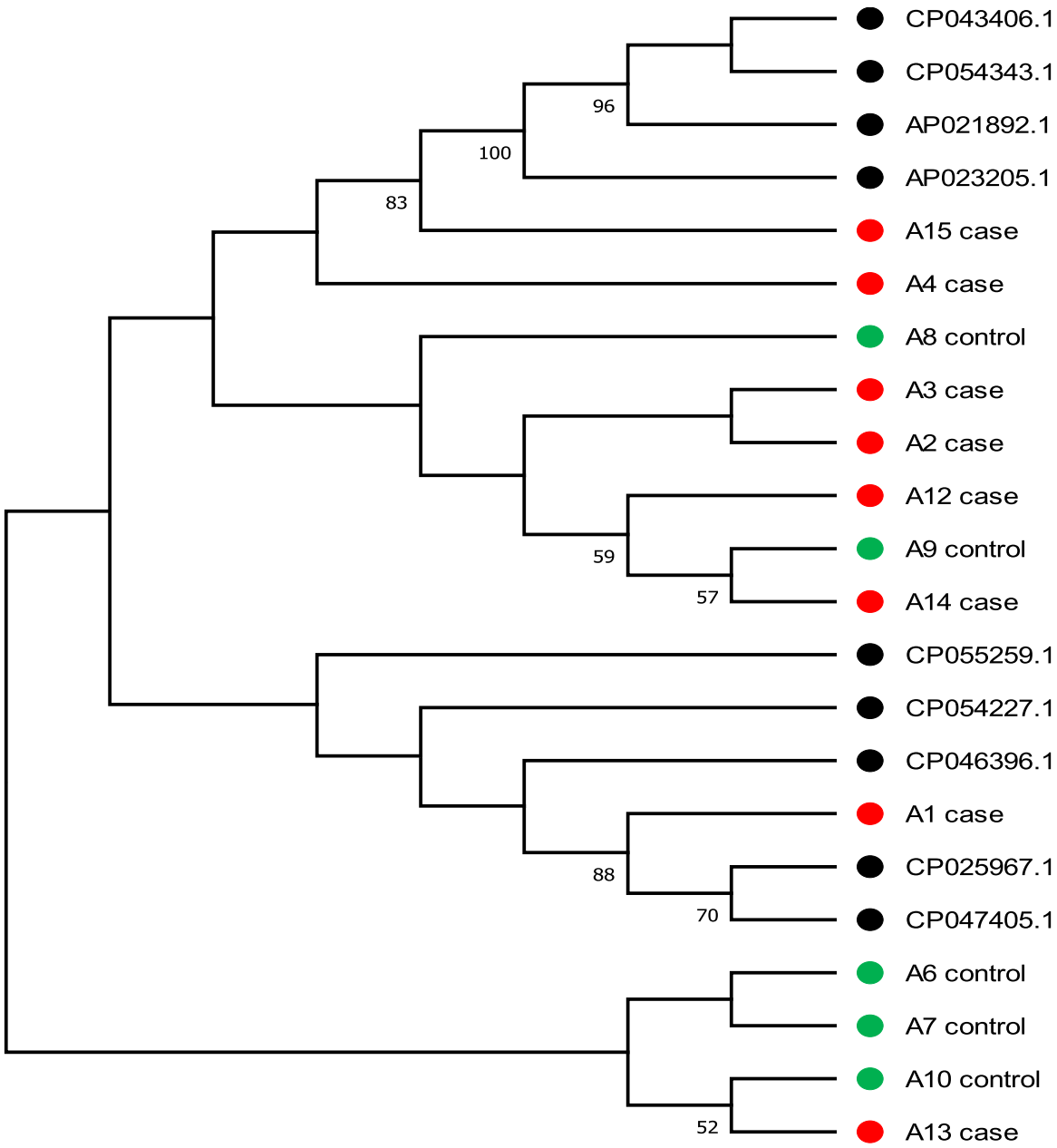

FIGURE 3 | Genetic diversity and the clustering patterns of *Escherichia coli* isolates. [Color figure can be viewed at wileyonlinelibrary.com]

and controls are dispersed across multiple clades, suggesting that pathogenicity does not strictly correlate with a single evolutionary lineage but rather may arise from genetically deferent backgrounds. The close clustering of some patients and controls isolates indicates potential genetic similarities, possibly due to horizontal gene transfer or the presence of shared evolutionary origins.

CP- and the AP- are reference isolate sequences and the sequences from isolates of patients and controls are designated Ax Case and Ax control, respectively when x is the sample number.

3.5 | Genetic Diversity and Clustering Patterns of *E. coli* Virulence

The phylogeny tree shown in Figure 4 reveals a strongly supported monophyletic clade (bootstrap 100, 99, 61), indicating evolutional conservation in isolates. However, the isolates showed a diverse sequences when compared to the isolated reported from other countries Figure 3.

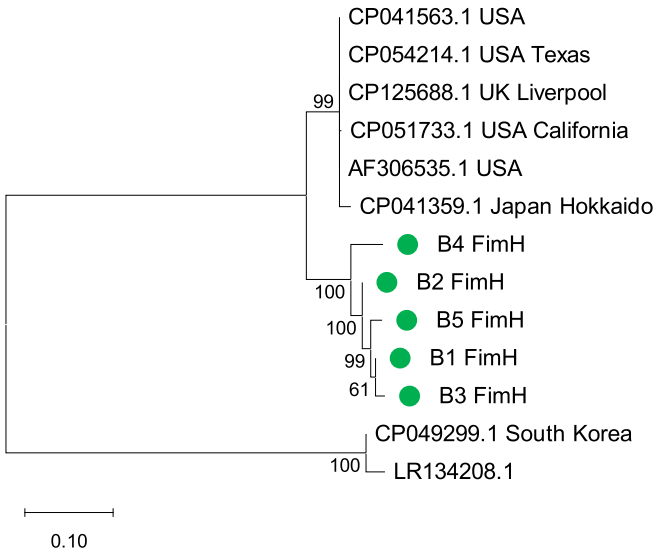

FIGURE 4 | Genetic diversity and the clustering pattern of *Escherichia coli* virulence *FimH*. [Color figure can be viewed at wileyonlinelibrary.com]

3.6 | Association of Risk Factors With Benign Prostate Hyperplasia

Proportion of patients involve in exercise, and consume alcohol compared to the control group showed no significant difference ($p > 0.05$) (Table 2). However, *E. coli* infection was significantly higher among the patients than the controls ($p < 0.01$). Although the difference in proportion of virulent bacterial infection among the study populations was not statistically significant, *E. coli* carrying the virulent type 1 *fimbriae* (*fimH*) gene was overrepresented among the patients.

Relationship of risk factors with prostate volume (BPH) is presented in Table 3. A unit increase of MDA level significantly increases prostate volume by almost 13 units when unadjusted and 11 units when adjusted for exercise, alcohol intake and *E. coli* infection ($p < 0.001$). Exercise although not statistically significant, decreases prostate volume by 5 units per a unit exercise in both unadjusted and the adjusted models. Intriguingly, alcohol intake has positive effect on prostate volume. A unit alcohol intake significantly decreases prostate volume by almost 9 units in the unadjusted model ($p < 0.05$), and almost 7 units when model was adjusted. *E. coli* infection increases prostate volume by 12 units compared those not infected ($p < 0.01$).

4 | Discussion

E. coli infection was high among patients than controls, and the virulence type, type 1 fimbriae (fimH) gene was overrepresented. *E. coli*, a uropathogenic bacterium is the commonest causal agent of UTIs in humans, and a prevalence between 21% and 54% has been reported among UTIs patients [21]. In an Afghanistan study to determine the prevalence of UTIs among BPH patients, *E. coli* infection accounted for about 25% of UTIs cases in the patients [22]. Two independent study conducted among Nigerian population also reported 62.2% and 16.1% [22, 23]. Furthermore, a study in Iraq to investigate infection associated prostate

TABLE 2 | Factors association with benign prostate hyperplasia.

Parameter	BPH patients ($N = 86$)	Controls ($N = 82$)	Chi square (χ^2)	*p*-value
Exercise				
No	35 (40.7)	31 (37.8)	0.19	0.6644
Yes	51 (59.3)	51 (62.2)		
Alcohol intake *n* (%)				
No	34 (39.5)	31 (37.8)	0.08	0.7718
Yes	52 (60.5)	51 (62.2)		
E. coli infection: *n* (%)				
No	20 (32.8)	35 (67.3)	23.12	< 0.001*
Yes	41 (67.2)	17 (32.7)		
E. coli virulence: n_1 (%)				
No	32 (78.0)	16 (94.1)	0.25	> 0.05
Yes	9 (22.0)	1 (5.9)		

Note: N = population, n = subpopulation, n_1 = proportion with patients with or without virulence bacteria. Fisher's Exact test was perform for proportions less than 5.
*$p < 0.05$ was considered statistically significant.

TABLE 3 | Risk factors of benign prostate hyperplasia.

Parameter	Linear regression model			
	Crude coeff. (95% CI)	*p*-value	Adjusted coeff. (95% CI)	*p*-value
MDA level	12.63 (6.46–18.79)	< 0.001*	11.41 (5.44–17.37)	< 0.001*
Exercise				
No	0 (base)		0 (base)	
Yes	−5.24 (−13.11 to 2.63)	0.191	−5.45 (−12.92 to 2.02)	0.151
Alcohol intake				
No	0 (base)		0 (base)	
Yes	−8.95 (−16.72 to −1.18)	0.024	−6.92 (−14.28 to 0.44)	0.065
E. coli infection				
No	0 (base)		0 (base)	
Yes	12.32 (4.42–20.23)	0.002	12.30 (4.23–20.37)	0.003*

Note: N = population size of each group, n = subgroup screened for *E. coli* infection (patients = 61 and controls = 52), n_1 = proportions of participants with *E. coli* infection screened for virulent sequence (Patients = 41 and Controls = 17). Fisher Exact Test was used for counts less than 5.
*$p < 0.05$ was considered statistically significant.

hypertrophy, observed *E. coli* as the most common uropathogenic bacterium (37.5%) in pre-operative BPH patients [24]. An observational study reported 82% *fimH* virulent factor in *E. coli* isolated from Ethiopian population with BPH [17], and another study associated cytotoxic necrotizing factor 1 (cnf 1) virulence gene with the condition [25].

In BPH, evidence implicated *Escherichia coli* in the pathogenesis mainly through inflammation-mediated pathways. *E. coli* has been cited to activate NF-kB pathway and induce DNA damage in prostate epithelial cells [11], suggesting a link to prostate inflammation and cellular injury. FimH enhance bacterial attachment to the host tissue which is a major step in colonization. The infection is reported to evade immune system and produce toxin like Shia toxin that hijack host signaling pathways to induce diseases [26]. The bacteria use the fimbriae adhesion structure to induce activation of Toll-like receptor-4 resulting in immune cells activation including proliferation of T cells, production of IFN-γ and TNF-α and activation of the immune system which is being explore for therapeutic purposes [27]. Sustained activation of the immune system or inflammatory response is strongly linked with production of reactive oxygen species (ROS) with damaging effect on DNA molecules, and dysregulation of DNA repair-related gene resulting in cell proliferation [28]. The current study reports elevated serum MDA levels in patients compared with controls. The finding supports earlier studies that have implicated oxidative stress in BPH among patients [10, 29]. Lipid peroxidation as indicated by high MDA levels may be attributed to the infection among the patients. Chronic infection of *E. coli* is dependent on the variants characterized by gene sequence diversity. A close clustering pattern of E coli isolates gene sequences in the patients and controls indicate a potential genetic similarity, however, *fimH* gene sequences compared with sequences from other countries revealed sequences diversity. The observed phylogenetic similarity between patients and virulence (fimH) sequences and the international reference strains may indicate the co-selection of virulence and antibiotic resistance traits. Virulence genes like *fimH*, inhabit mobile genetic elements such as plasmids and transposons which carry antibiotic resistance genes simultaneously [20, 26]. This link enables horizontal gene transfer which allows strains to acquire adhesive factors and resistance concurrently, mainly under antibiotic pressure. The reported high *E. coli* infection may be due to urinary obstruction which allows enough time for bacteria adherence and growth leading to UTIs.

The current study support findings from previous studies on lifestyle and BPH. Alcohol intake was established to have a positive effect on prostate health and supports earlier report [13], however, the molecular mechanism is not immediately known. Physical activity was found to show reverse relationship with prostate volume, and the finding was in agreement with previous reports [14, 15]. Moderate physical activity has been reported to be beneficial, as it improves immunological responses and antioxidant defenses [30].

5 | Conclusion

In conclusion, this study reports for the first time *E coli* infection and bacteria virulent factor association with BPH among Ghanaian patients. This study suggests further investigation to identity risk factors associated with *E. coli* virulence and promotes holistic treatment plans for BPH patients.

Author Contributions

E.A.T., R.A.N., S.N.D., and G.A.A. contributed to the study concept and design. Data acquisition was performed by Y.A., G.A.A., O.Q., and S.S., while data analysis was carried out by Y.A., O.Q., R.A.N., and E.A.T. Y.A., S.N.D., S.S., and G.A.A. were involved in drafting the article. All authors read and approved the final article.

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Ethics Statement

The study was approved by the Ghana Health Service Ethics Review Committee (GHS-ERC 005/04/22).

Consent

All the study participants consented to be part of the study by signing an informed consent.

Conflicts of Interest

The authors have no competing interests to declare.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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