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Prevalence of *papC* and *usp* Virulence Factors in Uropathogenic *Escherichia coli* Causing Asymptomatic Urinary Tract Infections in Adolescents

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Authors' contributions

This work was carried out in collaboration between all authors. Author SND under the guidance of authors KN and PT managed the literature searches, designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors KN and PT edited the write-up to enrich the intellectual content. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine the prevalence of two virulence genes associated with uropathogenic *Escherichia coli*; *pap*C gene of the P fimbriae for adherence to uro-epithelial cells and *usp* (uropathogen-specific protein) gene, a *Vibrio cholerae* toxin gene homologue.
Study Design: Cross sectional.
Place and Duration of Study: Department of Biochemistry and Biotechnology and the Clinical Analysis Laboratory, Kwame Nkrumah University of Science and Technology, Kumasi, between October 2011 and February 2012.
Methodology: *Escherichia coli* isolates (n= 149) from an adolescent population of ages 13-18 years (from a total sampled population of 85 males and 64 females) were screened for *pap*C and *usp*, using specific primers for the two genes in polymerase chain reactions.
Results: The *usp* gene was the most prevalent (72.48%), followed by *pap*C (51.00%) and *pap*C+*usp* (24.16%). Significant difference ($P = .002$) was observed between *pap*C and *usp* and also *pap*C and *pap*C+*usp* ($P < .0001$). *usp* Gene prevalence was also significantly different from that of *papC*+*usp* ($P < .0001$).

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Conclusion: This study suggests that a higher proportion of strains of uropathogenic *Escherichia coli* implicated in UTI in the studied population possess the *usp* gene whose protein product potentially serves to reduce competing microbes in the urinary tract.

Keywords: Virulence; uropathogenic; prevalence; bacteriocin; adhesion; fimbrae.

1. INTRODUCTION

Urinary tract infections are dominated by *Escherichia coli*, accounting for 70 to 90 % of all infections in both children and adults [1,2]. Uropathogenic *Escherichia coli* interacts with its host, through three major pathways, facilitated by three major groups of genes, called virulence factors [3]. These gene products are adhesion structures, exotoxins or membrane-bound toxins and iron acquisition structures. The *pap*C gene, essential for the formation of P fimbriae for adherence to uro-epithelial cells has been well studied and thought to be a primary interaction route the *Escherichia coli* adopts in order to infect its host [4,5]. A *Vibrio cholerae* toxin homologue gene, uropathogen-specific protein (*usp*) gene has also been found in uropathogenic *Escherichia coli* for the production of bacteriocin to eliminate competing uropathogens [6,7,8]. No single virulence factor has been identified in isolation but various combinations of these virulence factors have been found in isolated cultures of uropathogenic *Escherichia coli* as the cause of pathogenicity [9,10,11]. This observation therefore, suggests synergistic action of virulence factors as the mechanism of uropathogenic *Escherichia coli* invading host defence system to cause disease [3]. Variation in the distribution of these virulence factors in isolated uropathogenic *Escherichia coli* have been attributed to geographical differences, strain types and populations sampled [10,12,13,14,15,16,17]. Comparisons have been drawn between *papC* and *usp* genes from studies done in different geographical locations [13,14,15]. However, research in this field has been scarce in the origin of this study (Sub-Saharan Africa). This study used the PCR technique to determine the distribution of *pap*C and *usp* genes in uropathogenic *Escherichia coli* isolates from an adolescent population, which is known to have higher UTI prevalence than other age groups [18].

2. MATERIALS

2.1 Isolation of Bacteria

A total of 149 isolates of *Escherichia coli* from 149 patients (only one isolate from each) distributed as: 85 males and 64 females were analyzed. The participants were school-going adolescents aged between 13 and 18 years and had consented to the study. Routine urinalysis was conducted and the laboratory criterion for UTI caused by *Escherichia coli* was the presence of a positive culture response with at least 10^4 CFU of *Escherichia coli* per mL of clean-voided urine.

2.1.1 Extraction of bacterial DNA

DNA to be amplified was obtained from 400 µl aliquots of urine samples initially dispensed into sterile 1500 µl Eppendorf tubes and centrifuged at 11,000 rpm for 30 seconds. Aliquots of 200 µl were pipetted from the supernatant into sterile 1500 µl Eppendorf tubes and incubated at 120ºC for 15 minutes in a water bath to lyse bacterial cells as described by Le

Bouguenec et al. [19] but without prior microbial culturing in broth. Resultant DNA solution was stored as template DNA stock and short-spun for use in PCR reaction.

2.1.2 PCR amplification of bacterial DNA

PCR was carried out in a total volume of 10.5 µl, containing 1.5 µl of the template DNA, each of the primers at 10 µM, the four deoxyribonucleoside triphosphates (each at 250 µM), 10 mM Tris hydrochloride (pH 9), 1.5 mM $MgCl_2$, 30 mM KCl and 1U of DNA polymerase (Bioneer, South Korea). The primer sequences (Wageningen University and Research Center, The Netherlands) used were as follows: *usp*-F-5'-CGGCTCTTACATCGGTGCGTTG-3',R-5'-GACATATCCAGCCAGCGAGTTC-3' and *pap*C-F-5'-GACGGCTGTACTGCAGGGTGTGGCG-3',R-5'-ATATCCTTTCTGCAGGGATGCAATA-3' [11, 17]. PCR amplifications consisted of an initial denaturation at 94ºC for 2 minutes and 30 cycles of denaturation at 94ºC for 30 seconds, annealing at 65ºC for 1 min, and extension at 72ºC for 2 minutes and finally held at 4ºC for 7 minutes in a Thermal Cycler. Five microliters of the reaction mixture was then analyzed by gel electrophoresis on 2% agarose gel, and the reaction products visualized by staining with ethidium bromide, compared to a 1kb DNA size ladder (Wageningen University and Research Center, The Netherlands) corresponding to the products of the amplification.

2.2 Statistical Analysis

The data were entered and analyzed using the Fischer's exact or Chi-square. The statistical software used was GraphPad Prism version 5 from GraphPad Software Inc USA. The statistical significance was set at $P = .05$.

3. RESULTS AND DISCUSSION

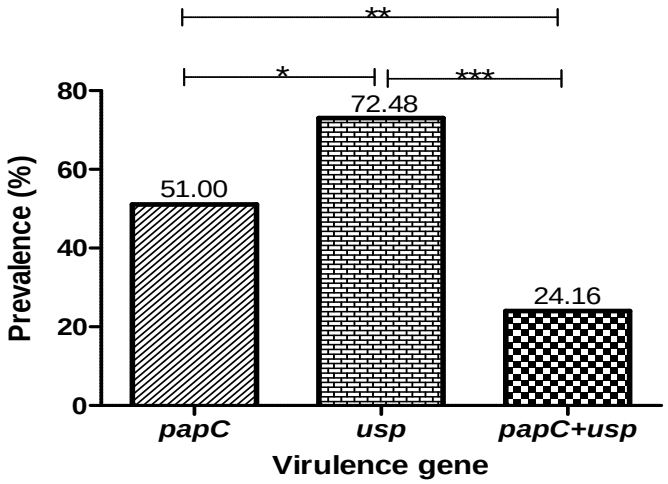

Fig. 1. Distribution of virulence genes in *E. coli* isolates
=significant difference p= .0021, **=significant difference p= .0001, *= significant difference p< .0001 at 95% confidence interval*

The *usp* gene was the most prevalent (72.48%), followed by *papC* (51.00%) and *papC+usp* (24.16%) (Fig.1). There was significant difference (*P*= .0021) between *papC* and *usp* and

also *papC* and *papC+usp* (*P*= .0001). The prevalence of *usp* gene was also significantly different from that of *papC+usp* (*P*< .0001).

The *papC* gene was more predominant in males (56.47%) than females (43.75%). Prevalence from isolates obtained from males increased through the pubertal stage (13 to 16years) before finally declining to 40.00% at age 18. However, that for females increased from age 14 to 16 and finally peaked at 18years with 100% (Table 1). At age 14years, there was significant difference (*P*= .03) in the presence of *papC*, with males having a higher prevalence compared to females. It was found, usp gene was predominant in both sexes; the females had a higher percentage (76.56%) than males (69.41%).

Prevalence of *usp* gene was higher in females from 13-15 and 17years with no significant difference between corresponding males of the same age. A general declining trend was observed for *usp* gene for isolates obtained from females in contrast to the gradual rise in that from males through the age range. Both males and females recorded the lowest prevalence for the presence of *papC+usp* genes; 25.88% was observed for males and 21.88% for females. A rise in prevalence was observed for isolates obtained from males (from age 14 to 18) whiles that from females showed fluctuation and finally declining at age 18 (0.00%). There was no significant difference between males and females for *papC+usp*.

Table 1. Prevalence of virulence genes of uropathogenic *E. coli* strains in both males and females stratified by age

Virulence gene	Age	Male (n=85)	Female (n=64)	*P value*
papC	13	6 (42.86)	7 (46.67)	1.00
	14	10 (55.56)	2 (13.33)	.03*
	15	13 (61.90)	10 (52.63)	.75
	16	12 (66.67)	6 (66.67)	1.00
	17	5 (55.56)	2 (40.00)	1.00
	18	2 (40.00)	1 (100.00)	1.00
	Total	**48 (56.47)**	**28 (43.75)**	**.14**
usp	13	11 (78.57)	13 (86.67)	.65
	14	10 (55.56)	13 (86.67)	.07
	15	14 (66.67)	15 (78.95)	.49
	16	12 (66.67)	4 (44.44)	.41
	17	7 (77.78)	4 (80.00)	1.00
	18	5 (100.00)	0 (0.00)	.17
	Total	**59 (69.41)**	**49 (76.56)**	**.36**
papC+usp	13	3 (21.43)	5 (33.33)	.68
	14	2 (11.11)	2 (13.33)	1.00
	15	6 (28.57)	5 (26.32)	1.00
	16	6 (33.33)	1 (11.11)	.36
	17	3 (33.33)	1 (20.00)	1.00
	18	2 (40.00)	0 (0.00)	1.00
	Total	**22 (25.88)**	**14 (21.88)**	**.70**

**= Significant difference.*

The study population showed a higher prevalence of *usp* gene over *pap*C gene (Fig. 1), (*P* = .002). The overall prevalence of 72.48% (Fig. 1) for the *usp* gene was close to the 63.7% determined for women by Bauer et al. [18] and much closer to the 79-93% range in mixed

populations in Japanese studies [7]. This could be an indication of genetic relatedness of *Escherichia coli* strains causing UTI in this study and that in Japan. It may also suggest the importance of the *usp* gene for *E. coli* survival in the microbial community involved in UTI. Moreover, the higher percentage of *usp* gene compared to *pap*C gene observed in isolates from females could be due to the fact that the shorter urinary tract of the female, making it more prone to infection, has resulted in the adaptive response of the *Escherichia coli* in resorting to the acquisition of the *usp* gene as a survival tool, in the midst of potential competing pathogens. There could be increased diversity of microbial community within the urinary tract, leading to the acquisition of the *usp* gene by *Escherichia coli* to produce bacteriocin to eliminate competing uropathogens. It can also be argued that some of the UTI recorded might not have been first episode infection, but re-infections made possible by reservoir uropathogens in the urinary tract. Thus, these *Escherichia coli* forming part of the reservoir microbial community must have adapted by acquiring *usp* gene, whose protein product bacteriocin, acts to eliminate other competing microbes.

The *pap*C gene recorded for *Escherichia coli* strains from both males and females were 56.47% and 43.75% respectively (Table 1) and an overall prevalence of 51.00% (Fig. 1). This means that about half the numbers of *Escherichia coli* isolates from adolescents possess the gene for fimbriae formation for adherence to uro-epithelial cells to cause UTI. This prevalence (51.00%) may also be an indication of the importance of the *papC* gene product for initiating UTI.

The *pap*C prevalence of 51.00% is greater than the 21.1% determined in a Brazilian study 15 and the 48% for immuno-compromised populations in Slovenia [16].These differences might have arisen from both the sample populations and the relative proportions of males and females in these populations. It is also possible, strain differences due to geographic locations can be a contributory factor to the difference from results from the other studies. However, the difference in distribution with respect to gender, although not statistically significant, could be justified by the fact that isolates from cystitis, a condition that predominates in females, have been found to have low prevalence of *pap*C, hence the lower percentage of 43.75% (Table 1) determined in females [14]. In Table 1, both males and females recorded *usp* gene as the predominant virulence gene, with 69.41% and 76.56% respectively, although there was no significant difference between genders. This could be explained by the relatively even distribution of *usp* gene in *Escherichia coli* strains, an adaptation employed by the bacteria to persist in a competitive microbial community through series of re-infections.

Relatively lower prevalence of the combined presence of both *pap*C and *usp* (*papC*+*usp*) genes could mean that the *Escherichia coli* strains survived in the urinary tract through the genetically modulated inclination to produce either the adherence gene or by producing bacteria toxins against other uropathogens but not both. Thus, most of the bacteria strains tend to be inclined towards bacteriocin production as shown in Fig. 1. It is also possible persistent untreated UTI conditions may have led to more competition, hence the uropathogenic *Escherichia coli* acquiring the *usp* gene.

4. CONCLUSION

Our adolescent population studied for UTI, has shown a significantly higher prevalence of the *usp* gene compared to *papC* and the combined presence of both genes (*papC*+*usp*). This may have been due to re-infections which may have caused these microbes to acquire the *usp* gene.

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CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

The authors declared that they have no competing interests exist.

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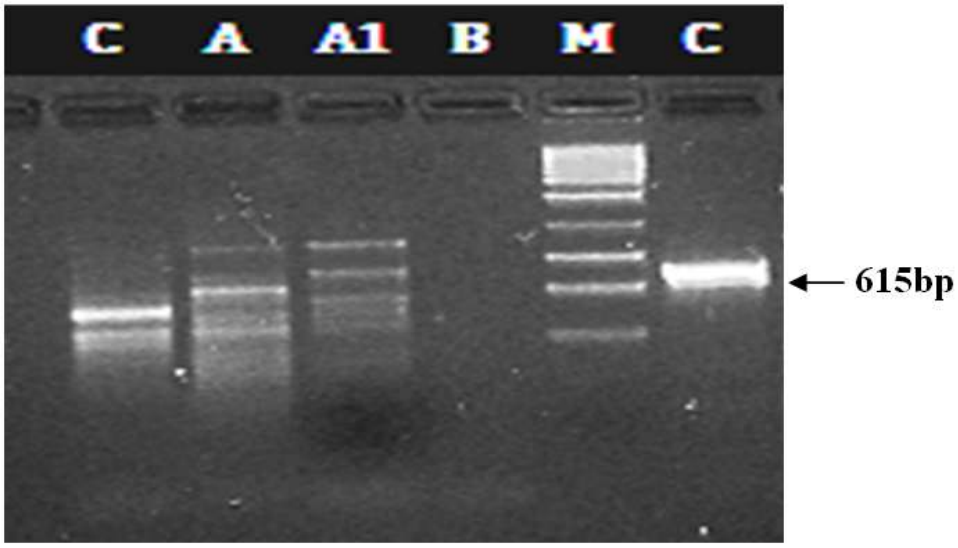
APPENDIX



An ethidium bromide-stained agarose gel electrophoregram of amplified *usp* genes of uropathogenic *E. coli* DNA of different template preparation. [C= Control, A and A1= Denatured samples at 120ºC, B= Sample without denaturation and M= DNA Marker].

Virulence genes and primer sequences used for DNA amplification

Virulence gene	Primer sequence	Product size	Reference
usp	F-5'-CGGCTCTTACATCGGTGCGTTG-3' R-5'-GACATATCCAGCCAGCGAGTTC-3'	615bp	17
papC	F-5'-GACGGCTGTACTGCAGGGTGTGGCG-3' R-5'-ATATCCTTTCTGCAGGGATGCAATA-3'	328bp	11

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