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GENOTYPING OF VIRULENCE FACTORS OF ESCHERICHIA COLI

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Abstract
The most common bacteria that can cause UTIs is Escherichia coli. One of the most common types of infectious disorders in humans is urinary tract infections (UTIs). The bacteria Escherichia coli typically causes pyelonephritis and cystitis and is considered a dominant bacterium. The purpose of this study was to identify the presence of certain virulence genes, such as those encoding fimbriae, hemolysin synthesis, and aerobactin, in one hundred Escherichia coli isolates collected from patients presenting with UTI symptoms in both the lab and the clinic at Al zahraa hospital in Wasit province. Among the virulence factors examined, fimH was the most prevalent. There was a 73% prevalence of the fimbriae type 1 (fimH) gene, a 46% prevalence of pyelonephritis associated pili (pap) gene, a 32% prevalence of S-family adhesions (sfa) gene, and a 47% prevalence of hemolysin (hly) gene.

Keywords: Escherichia coli, UTI, Genotyping.

Introduction

In humans, Escherichia coli causes UTIs infection. Acute symptomatic UTIs affect at least 20% of females at some point in their life. Both the virulence of the invading bacteria and the sensitivity of their hosts determine the severity of the illness (1). Urinary tract infections, which entail the natural climb of bacteria from the urethra to the bladder and, in rare cases, the kidneys, can happen even in people with structurally and functionally normal urinary tracts. It is possible that Escherichia coli can increase their reproductive capacity and ability to penetrate renal tissue by adhering to uroepithelium, which protects the bacteria from urine lavage (2). Many different virulence factors are present *in E. coli*, which increases the severity of UTIs caused by these bacteria. The most common virulence factors are sticky agents, which aid bacterial colonization in vulnerable areas like the urethra, and toxins, which harm the host (3). To successfully initiate infections, the UPEC have adhesion elements termed pili or fimbriae. Bacterial proteins called adhesions mediate certain adhesions; fimbriae are not always involved (4). Most often identified operons encoding S fimbrial adhesin and pyelonephritis related pili

are (5, 6). The pathogenicity of UPEC may be due to bacterial adhesion as well as other virulence factors, such as hemolysin production (7).

In order to understand the pathogenic mechanisms of *Escherichia coli* (E. coli) and to develop targeted intervention techniques, it has been crucial to understand the genetic landscape dictating the virulence of the bacteria (8). The pathogenic potential and illness symptoms of this Gram-negative bacterium are dictated by its complex molecular components, which include a varied repertoire of virulence factors. The study of *E. coli's* virulence factors has been accelerated by genomic developments, which have allowed for the use of advanced genotyping techniques to clarify the genetic complexity of *E. coli's* pathogenicity (9).

The genome of *Escherichia coli* is extremely flexible, with several virulence factors encoded in chromosomal regions, plasmids, and mobile genetic elements (10). Recent research has shed light on the complex relationship between genetic factors and the development of virulence factors in *E. coli*, demonstrating how this process is constantly evolving (11).

The development of more accurate genotyping methods has made it possible to classify and characterize virulence factors with remarkable precision. Whole-genome sequencing (WGS) and other high-throughput sequencing methods have changed the game when it comes to *E. coli* genotyping (12). Research has demonstrated that WGS is an effective tool for understanding the genetic factors that control *E. coli* virulence, which in turn has led to the discovery of new genes linked with virulence and the dynamics of their evolution (13).

The identification of genetic markers associated with pathogenicity, thorough analysis of virulence factor profiles, and phylogenetic relationships have all been made possible by advances in bioinformatics approaches (14). The genotype-phenotype correlations of *E. coli* virulence factors have been better understood by the integration of machine learning algorithms and comparative genomic investigations. This has opened up new possibilities for predictive modelling and targeted therapeutic interventions (15).

Additionally, new opportunities for virulence factor gene manipulation have emerged with the development of CRISPR-based technologies. This innovative method has the potential to shed light on the functions of specific virulence factors and pave the way for the creation of new antimicrobial treatments that specifically target the pathogenicity of *E. coli* (16).

Method.

Patients at the Al Zahraa hospital in the Wasit province provided 90 samples. Where 50 were inpatients (not including outpatients) and 40 were inpatients. In order to diagnose a UTI, the medical team at the hospital used a combination of clinical symptoms and laboratory tests. Testing for a urinary tract infection (UTI) caused by *Escherichia coli* required a positive culture containing at least 105 CFU off the bacteria / millilitre. Using established protocols, we were able to identify *Escherichia coli*. We kept the strains at -70°C in TSB/glycerol until we could analyze them more. In TSB at 37°C, *Escherichia coli* isolates were subcultured for 18 hours. A DNA extraction kit from the Bionner Company in South Korea was used in accordance with the manufacturer's instructions to extract DNA. Table 1 shows the specific primers used to amplify the fimH, pap, sfa, and hly genes' sequences. The sizes of the amplified products were

anticipated by primer sequences, and the reference provided explicit details about the conditions (3).

Table (1). The primer which used to detection of virulence genes.

primer	gene	sequence	Product size
PAP3 PAP4	papE\F	GCAACAGCAACGCTGGTTGCATCAT AGAGAGAGCCACTCTTATACGGACA	336
fim1 fim2	fimh	GAGAAGAGGTTTGATTTAACTTATTG AGAGCCGCTGTAGAACTGAGG	508
sfa1 sfa2	sfaD\E	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	410
hly1 hly2	hly4	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177

Result

In this research, virulence factors were found in as low as 32.0% of cases for sfa and as high as 73.0% for fimH; for pap and hly, the corresponding rates were 46% and 47%. Table 2 shows that out of all the adhesins, type fimH fimbriae and hly fimbriae were the most common, accounting for 73 and 47 strains, respectively. Authenticity was confirmed through sequencing of the PCR results. The figure 1 show the PCR result where the pap gene has size 336 bp, while hly gene has product size 1177 bp.

Table 2: - The redundancy of uropathogenic *E. coli* virulence factor isolated from patients.

Number of Sample	Negative	hly	sfa	pap	fimH	Method / PCR
90	5	47	32	46	73	

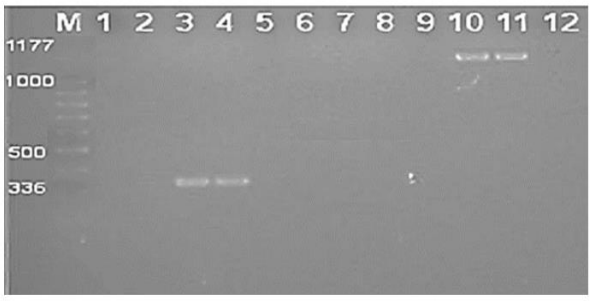

Figure (1) shows the presence of M (DNA ladder 100 bp). 3 and 4 indicating positivity for pap (336 bp) while 10 and 11 indicating positivity for hly (1177bp).

Discussion

Both the outpatient and the inpatient samples, *Escherichia coli* is the primary pathogen responsible for UTIs. It is possible for these infections to progress from cystitis to pyelonephritis (17). Virulence of the infecting bacteria and host vulnerability determine the level of severity. A greater understanding of the infectious microbe's virulence factors will help the doctor foresee how the infection will progress in the host (5). On the other hand, mutations at the gene level could cause it to go undetected sometimes. Accordingly, virulence genes can be detected by a positive PCR, but the lack of the matching operon cannot be inferred from a negative PCR (6,7). Based on our findings, the virulence genes play a crucial role in UTI-causing *Escherichia coli*, since the frequency of fimH was higher than the other genes. Published publications highlight the predominance of fimbriae type 1 among the UPEC strains (8), which is in agreement with the prevalence data. Our findings corroborated those of several studies that have looked at the prevalence of P fimbriae; specifically, we found that 30% of cystitis patients and 80% of patients with acute pyelonephritis exhibit this parasite. The presence of both sfa and pap at the same time was strongly correlated (9,10), and this was observed in 15% of the strains. Research has also shown that pap adhesion genes have a significant role in the development of pyelonephritis due to *Escherichia coli* (11). There is a correlation between the phylogenetic groupings, host clinical conditions, and geographic location and the various gene prevalence estimates (9,11). The study employed polymerase chain reaction (PCR) to demonstrate that UPEC strain adhesins, fimH, and pap were more prevalent and hly, sfa comparable too previous research but lower than others (12,13). Patients with pap smears likely experienced pyelonephritis and ascending infections or were at least infected in a similar setting. Patients with both sfa and hly are likely to have primary sepsis. It is reasonable to assume that all UPEC strains lacking virulence factors are either anaerobes or belong to the natural flora of the gastrointestinal tract (ABU). We found that UPEC strains found in Iran differ in their virulence profile from those found in other research; furthermore, it appears that the virulence of UPEC viruses is climate and geographical dependent. Perhaps the frequency of virulence genes in UPEC strains is greatly influenced by a number of factors, including but not limited to public health, food habits, customs, and sampling methodologies.

Conclusion

We demonstrated that elucidating the genetic factors that determine the virulence of *Escherichia coli* strains isolated from urinary tract infections (UTIs) is an essential part of this process. In order to contemplate potential preventative interventions, additional research is required to establish the physiopathology of UTIs caused by *Escherichia coli* and to discover the virulence components of this infection. Serotyping is helpful for studying the link between virulence factors and for detecting UPEC strains' virulence factors. The validity of these findings will also necessitate in vivo investigations.

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