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RESEARCH ARTICLE

STUDY OF VIRULENCE FACTORS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF UROPATHOGENIC ESCHERICHIA COLI IN A TERTIARY CARE HOSPITAL

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Abstract

Introduction:-Escherichia coli is the major causative agent of urinary tract infections. UPEC possess a variety of virulence factors that enable it to colonize, invade and persist within the urinary tract, leading to both complicated and uncomplicated UTIs. Knowledge of virulence factors of Escherichia coli is responsible for pathogenesis of UTIs and their antimicrobial susceptibility pattern will help in better understandin g of the treatment of UTI. Emergence and spread of multi drug resistant strains of Escherichia coli have raised considerable interest in understan ding their diversity and epidemiology of infections in humans.

Aim: To determine the virulence factors and antimicrobial susceptibility pattern of Uro Pathogenic Escherichia coli.

Materials and Methods:-A prospective study is done on urine samples over a period of 3 months from May 2025 to July 2025 in a tertiary care hospital to detect virulence factors like Hemolysis, Haemagglutination and Gelatin hydrolysis. Antibiotic susceptibility test was done by Kirby Baur disc diffusion method as per the CLSI guidelines.

Results:-Out of total 350 urine samples tested 114 were Escherichia coli isolates. Out of the 114 Escherichia coli isolates 69 were females and 45 males. Among the 114 isolates -43 isolates showed hemolysis virulence factor, 31 isolates showed haemagglutination factor- 22 were mannose resistant and 9 mannose sensitive haemagglutination and, 11 isolates showed gelatin hydrolysis and 29 isolates did not show any of the virulence factor.Antibiotic susceptibility testing revealed that UPEC strains showed maximum resistance to Ampicillin (55.2%), followed by Cotrimoxazole (48.2%) and Norfloxacin (44.7%). Most isolates were sensitive to Meropenem (82.4%), Amikacin (79.8%) and followed by Nitrofurantoin (74.5%).

Conclusion:-Detection of virulence factors of Uro-pathogenic Escherichia coli shows a strong association to urinary tract infection. And presence of multiple virulence factors leads to drug resistance.

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Introduction:-

One of the most prevalent bacterial illnesses that affect people throughout their lives are urinary tract infections (UTIs). They may exhibit symptoms or not. Escherichia coli is the most frequent cause of UTIs, accounting for over 85% of infections acquired in the community and 50% of infections acquired in hospitals. It is highly prevalent at all ages. Approximately 80–90% of community-acquired UTIs are caused by UPEC. Escherichia coli is a commensal in the human digestive tract that can cause a number of illnesses, including UTIs, sepsis, pyelonephritis, and others, when it penetrates unnatural locations. Uropathogenic Escherichia coli (UPEC)[1] is the term used to describe serotypes that cause UTIs[1].Certain E. Coli serotypes that exhibit chromosomally encoded virulence markers are known to be uropathogenic because they are consistently linked to uropathogenicity[3]. Up to 50% of nosocomial UTIs and 90% of UTIs in ambulatory patients are caused by uropathogenic strains[2].The common virulence factors include surface hydrophobicity, colonization factor, capsule, serum resistance, resistance to phagocytosis, hemolysin production, enterotoxin and siderophore, fimbriae and haemagglutination. The ability of E. coli to adhere to the uroepithelium is mediated by fimbriae, thereby resisting elimination by the flow of urine. Adhesion is therefore measured to be important step in the pathogenesis of UTI[4].

During UTIs, UPEC pathogenesis includes:

(a) UPEC colonization of the periurethral and vaginal areas with colonization of the urethra;
(b) ascending into the bladder lumen and growth as plantktonic cells in urine;
(c) adherence to the surface and interaction with the bladder epithelium defense system (see below);
(d) biofilm formation;
(e) invasion and replication by forming bladder Intracellular Bacterial Communities (IBCs) where quiescent intracellular reservoirs (QIRs) form and reside in the underlying urothelium;
(f) kidney colonization and host tissue damage with increased risk for bacteremia/septicemia.

Considering the high degree of morbidity and mortality due to UTIs caused by uropathogenic E. coli and also the drug resistance among strains has further aggravated the problem of UTI's.

Materials and Methods:-

This is a prospective study which was conducted in the Department of Microbiology in a tertiary care hospital over a period of 3 months i.e from May 2025-July 2025. Patients of all age group were included. A total of 350 urine samples were tested out of which 114 were Escherichia coli were isolates.The samples were processed immediately as per the standard guidelines in the lab. The isolates were taken for the detection of virulence factors. The virulence factors tested were

1.Hemolysin production:
2.Haemagglutination
3.Gelatin hydrolysis

1.Hemolysin production

The Escherichia coli isolates were inoculated onto 5 %sheep blood agar and incubated overnight at 37degree Celsius and observed for a zone of complete lysis around the colony. Escherichia coli ATCC 25922 was used as a negative control.Presence of clear zone of complete hemolysis indicates hemolysin production.

Fig 1: Hemolysis

Haemagglutination:-

The test was carried out as per the direct bacterial haemagglutination test-slide method. One drop of red blood cell (RBC) suspension was added to a drop of broth culture and the slide was rocked at room temperature for 5 min. Presence of clumping was taken as positive for haemagglutination. Mannose-sensitive haemagglutination was detected by the absence of haemagglutination in a parallel set of test in which a drop of 2% W/V D-mannose was added to the red cells and a drop of broth culture. Mannose resistant haemagglutinating (MRHA) was detected by the presence of haemagglutination of 3% 'O' blood group human RBCs in the presence of 2% W/V D-mannose.

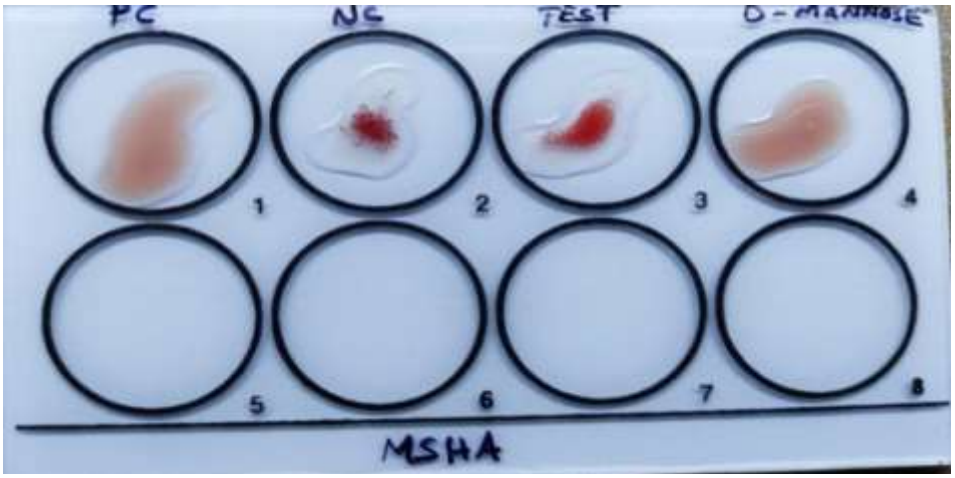

Figure 2: Mannose Sensitive Haemagglutination

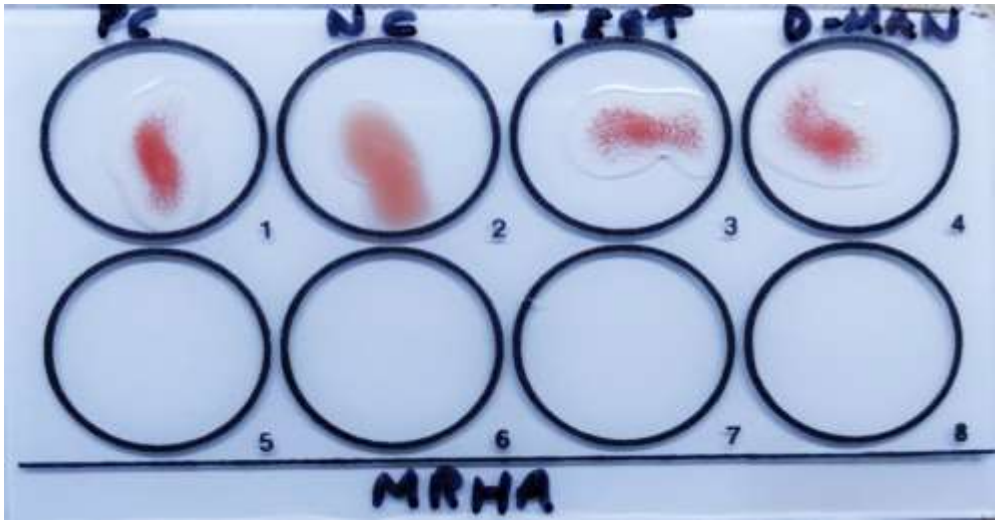

Figure 3: Mannose Resistant Haemagglutination

Gelatin Hydrolysis:-

Gelatinase production was tested using gelatin agar. Escherichia coli isolated was inoculated on gelatin agar and incubated overnight at 37 degrees celsius for 24 hrs. Later the gelatin agar plate was flooded with 1%tannic acid. Development of opacity around colonies shows gelatinase production.

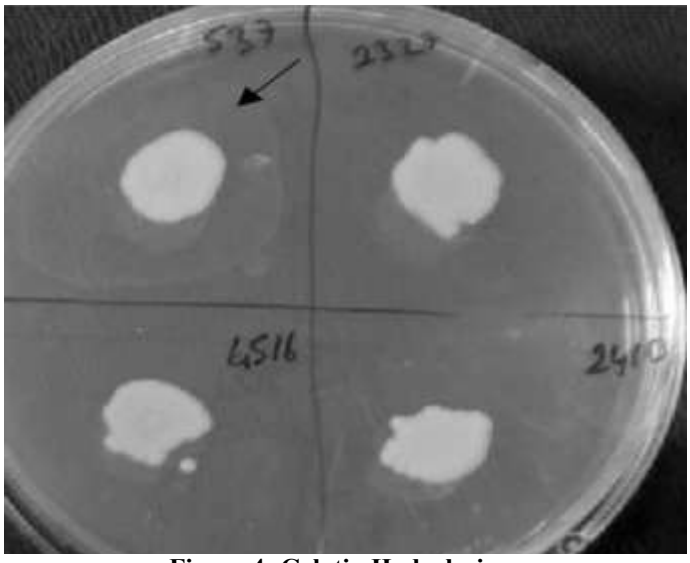

Figure 4: Gelatin Hydrolysis:-

Antimicrobial susceptibility:-
Antimicrobial susceptibility testing was done on Mueller Hinton Agar by Kirby Bauer disc diffusion method as per CLSI guidelines.

Results:-

A total of 350 urine samples received from symptomatic cases of urinary tract infection with significant bacteriuria were taken. Out of these, 114 Escherichia coli isolates were processed and studied for the virulence factors and their antibiotic susceptibility pattern. Out of the 114 Escherichia coli isolated samples, 60.5% were from females and 39.47% were from males. Among 114 isolates, 37.7% showed hemolysis, 19.3% showed mannose resistant haemagglutination, 7.9% showed mannose sensitive haemagglutination, 9.7% showed gelatin hydrolysis and 25.4% did not show any of the virulence factors.Antibiotic susceptibility testing revealed that UPEC strains showed maximum resistance to Ampicillin (55.2%)**,** followed by Cotrimoxazole (48.2%) and Norfloxacin (44.7%)**.** Most isolates were sensitive to Meropenem (82.4%)**,** Amikacin (79.8%) and followed by Nitrofurantoin (74.5%)**.**

Table 1: Gender Wise Distribution Of Escherichia Coli Isolates

GENDER	TOTAL	PERCENTAGE
FEMALES	**69**	**60.5%**
MALES	**45**	**39.4%**
TOTAL	**114**	**100%**

Table 2: Virulence Factors Among The Uropathogenic Escherichia Coli Isolates(N=114)

Virulence Factor	Number	Percentage
Hemolysis	43	37.7%
Haemagglutination	(31)	
Mannose Resistant Haemagglutination	22	19.3%
Mannose Sensitive Haemagglutination	9	7.9%
Gelatin Hydrolysis	11	9.7%
Did Not Exhibit Any Of The Virulence Factors	29	25.4%

Antibiotic Susceptibility Pattern Of Upec Isolates:-

The antibiotic susceptibility pattern of UPEC isolates showed varying levels of sensitivity and resistance.Ampicillin demonstrated low effectiveness,with only 51 isolates (44.8%) being sensitive while 63(55.2%) were resistant. Trimethoprim-sulfamethoxazole showed moderateactivity,with 59 isolates (51.8%) sensitive and 55(48.2%) resistant. Norfloxacin exhibited slightly better efficacy,with 63 isolates (55.3%) sensitive and 51(44.7%) resistant.Ceftriaxone showed improved activity,with 67 isolates(58.8.5%) sensitive and 47(41.2%)resistant.Higher sensitivity rates were observed with meropenem and amikacin,where 94(82.4%) and 91(79.8%) isolates were sensitive,respectively.Nitrofurantoin also demonstrated good effectiveness,with 85 isolates (74.5%) sensitive and 29(25.5%) resistant.Overall meropenem and amikacin were the effective antibioitcs against UPEC isolates,while ampicillin showed the highest resistance rate.

Discussion:-

UPEC are the most important group of micro-organisms responsible for UTI. UPEC differ from non-pathogenic E. coli by the production of specific virulence factors which enable the bacteria to adhere to uroepithelial cells and to establish UTI[5]. UTIs which are not properly treated from their onset can become a renal threat in time, finally leading to renal failure[8].Incidence of UTI was more common in females 60.5% than in males in our study. Piatti et al[5,6]. also reported a higher prevalence of UTI in female (77%).Priscilla et al[1] also reported a higher incidence of UTI was more common in females (66.72%) than in males (33.27%).The present study also correlates with Sanjay Singh Kaira et al [4] who reported 56.09%, Mittal et al[1,8] (53.3%) and Chhaya et al[8,9](53%).

Virulence factors:-

Hemolysin production is associated with human pathogenic strains of E. coli, especially those causing more clinically severe forms of UTI[5,10]. It is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury and impaired host defenses[1,11]. In the present study, 37.7% E. coli isolates produced hemolysin. In other studies conducted by Raksha et al[5,12], Siegfried et al[5,13] Hughes et al[5,14], Shruthi et al [1,15]hemolysin production was detected in 41.36% and 59.6%, 59.7% and 41.9% isolates respectively.The possession of MRHA by UPEC can be considered as one of the important virulence factor in the pathogenesis of UTIs. The present study correlates with Seigfried et al [1,13], Vagarali et al [13] , Raksha et al[1,12], Kauser et al [3,16] have reported the incidence of MRHA E.coli isolates as 23%, 25%, 30.9%, 30% respectively. In the present study also the rate of MRHA positive E. coli isolates was 19.3 % Gelatinase, an important virulence factor which is capable of hydrolyzinggelatin, collagen, and is associated with inflammation. In the present study Gelatinase production was seen in 25.4 % of isolates which is similar to Mittal et al [5] where it was 67.5%. But Vaish et al [18,19]& Jayanthi et al [18,20] showed lesser production of gelatinase which was 2% & 6% respectively.

Antibiotic resistance:

Antibiotic susceptibility pattern was studied for all E. coli isolates. These isolates were most commonly resistant to Ampicillin, Cotrimoxazole and Norfloxacin. The maximum sensitivity was shown to Meropenem, Amikacin and then followed by Nitrofurantoin.The antibiotic susceptibility pattern observed in the present study correlates with Tabasi et al [8,21], Karam et al [8,22] and Chhaya et al[8,9].The present study also correlates with Kauser Y et al [16,18], Vaish et al [18,19]& Jayanthi et al [18,20].

Clinical implications:

The coexistence of multiple virulence factors and antimicrobial resistance provides UPEC with a significant survival advantage within the host. Rising resistance rates necessitate the judicious selection of antibiotics based on culture and susceptibility testing to ensure effective treatment and to curb the emergence of further resistance. The widespread misuse and overuse of antibiotics in healthcare settings contribute significantly to resistance among hospital strains[18,23,24].Therefore, continuous surveillance of virulence factors and antibiotic resistance patterns is essential for guiding empirical therapy, improving patient outcomes, and strengthening antimicrobial stewardship practices.

Conclusion:-

Detection of virulence factors of Uro-pathogenic Escherichia coli shows a strong association to urinary tract infection. And presence of multiple virulence factors leads to drug resistance. So this helps in better understanding and treatment of Urinary tract infection.Since most Urovirulent strains express multiple virulent factors simultaneously, further studies at molecular level are necessa

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