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A STUDY TO DETECT VIRULENCE MARKERS & ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN UROPATHOGENIC ESCHERICHIA COLI CAUSING UTI AT A TERTIARY CARE HOSPITAL KOTA, SOUTH-EAST RAJASTHAN, INDIA

Microbiology

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ABSTRACT

Background: UTIs are the most commonly encountered bacterial infections both in community and health care settings, mostly which is brought on by Escherichia coli. The development of certain virulence factors that allow UPEC to adhere to uroepithelial cells and cause UTIs sets them apart from non-pathogenic E. coli. **Objectives:** This study was conducted to determine the virulence marker (VMs) of UPEC and to determine its antibiotic susceptibility and resistance pattern. The ultimate goal of this study is to reduce the morbidity, hospital stay and also will provide valid information for effective hospital infection control. **Methodology:** The present study was conducted in microbiology laboratory of Govt. Medical College, kota from the period of 1year (April 2024 to March 2025). All specimens were collected under sterile aseptic conditions in sterile container and specimens were processed for gram staining, culture and biochemical reactions, virulence markers detection & Antibiotic susceptibility pattern. **Results:** Total 170 Urine samples collected as Uropathogenic Escherichia coli causative agent for UTI. Out of 170 UPEC, female:male ratio was 3:2, Among female patients age group of 21 to 45 years (45.54%) was higest affected whereas in male patients, it was above 61 years (46.37%). Out of 170 UPEC hemagglutination, haemolysin production, serum resistance, biofilm formation, and siderophore production, gelatinase production was observed 61.76%, 60.58%, 59.41%, 54.70%, 45.88%, and 11.76% respectively. All the six virulence markers were positive in only 3 isolates, five in 20, four in 40, three in 44, two in 39, only one in 18 isolates and 06 isolatesdon't show positivity for any virulence factor. Highest resistance was observed for Ampicillin followed by Amoxiclav, Ceftriaxone, Cotrimoxazole, whereas the highest sensitivity of the isolates was observed with Imipenem followed by Piperacillin – Tazobactam, Nitrofurantoin, and Amikacin. Out of 170 isolates, 36(21.17%) showed resistance to at least three or more than three group of antibiotics. **Conclusion:** The use of broad-spectrum antibiotics has led to an increase in the emergence of multidrug-resistant strains of Escherichia coli. The virulence markers utilized in this work can be detected quite easily, and routinely screening them in a clinical microbiology laboratory will aid in patient early detection and treatment. It is hoped that this information will definitely help to reduce the morbidity, hospital stay and also will provide valid information for effective hospital infection control.

KEYWORDS

Virulence Marker(VMs), Uropathogenic Escherichia Coli (UPEC), Multidrug Resistant (MDR), Urinary Tract Infection(UTI).

INTRODUCTION

About 150 million individuals worldwide suffer from urinary tract infections (UTIs), which are the most frequent bacterial infection. [1] UTIs are the most commonly encountered bacterial infections both in community and health care settings.[2] Common UTI causing bacterial pathogens belong to family Enterobacteriaceae, Escherichia coli being the most common member isolated. Other Enterobacteriaceae group, Staphylococcus spp., Enterococcus spp., Pseudomonas aeruginosa are the next most common causes. Mycobacterium tuberculosis, Chlamydia trachomatis, Candida species are other rare causes of UTI Rarely UTI may be caused by viruses or fungi. It has been known that certain serotypes of E. coli are consistently associated with uropathogenicity and are designated as uropathogenic E. coli that expresses chromosomally encoded virulence markers. [3] The common virulence factors include surface hydrophobicity, Biofilm formation, colonization factor, capsule, serum resistance, resistance to phagocytosis, haemolysin, enterotoxin and siderophore, fimbriae and haemagglutination[4].

AIM & OBJECTIVES

The study was conducted to determine the virulence marker (VMs) of UPEC and to determine its antibiotic susceptibility and resistance pattern, because there have been few investigations on VMs of UPEC that cause UTI. It was anticipated that this information will undoubtedly contribute to a decrease in hospitalization and morbidity while also offering reliable data for efficient hospital infection control.

MATERIALS AND METHODS:-

Study Type – Observational & cross-sectional study

Study Period – 1year (April 2024 to March 2025) after approval from ethical committee (/2022).

Study Population – Urine samples of all Patients attending OPD/IPD in Tertiary care hospital, Kota (Raj)

Type of Sample – Urine samples

Sample Size – Total number of Uropathogenic E. coli isolated from urine samples during the study period is 170. Sample size was calculated by using formula as follows: $n=Z^2pq/d^2$

Inclusion Criteria– Urine samples of all age group in which Escherichia coli was isolated as Uropathogen.Urine samples of both OPD & IPD patients in Tertiary care hospital.

Exclusion Criteria – Urine samples in which Escherichia coli was not a causative agent.

Sample Processing:- The urine sample was collected according to standard guideline and sample was transported to the laboratory and processedimmediately to ensure maximum recovery of the pathogen and also to minimize the multiplication of commensal.The clinical specimens were processed as per standard guidelines using appropriate culture media and incubation conditions. AST will be performed on Mueller-Hinton Agar using Kirby-Bauer disk diffusion method. Antimicrobial sensitivity testing (AST): As per CLSI 2024 guidelines,

Statistical Analysis:- The Interpretation and analysis of the data were done by using Microsoft excel spread sheet. The quantitative data were expressed as numbers and percentage in a tabular form.

OBSERVATIONS AND RESULTS

The observations of the present study was presented in the following pages with regard to microbiological and its clinical association.Out of 170 UPEC, the maximum number of isolates were recovered from the female patients (n=101, 59.41%) as compared to males (n=69, 40.58%).

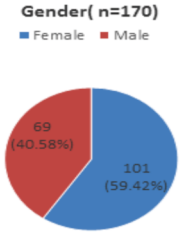

Fig 1: Female: Male Ratio(n=170)

Age and Gender Distribution in UPEC Infection:-

The incidence of UTI in females was higher in the age group of 21 to 45 years (45.54%) followed by the age group of 46 to 60 years (26.73%) and the least percentage (7.92%) was seen below 20 years of age group. Among the male patients, the highest prevalence of UTI was observed above 61 years (46.37%) and the lowest was less than 20 years (5.79%).

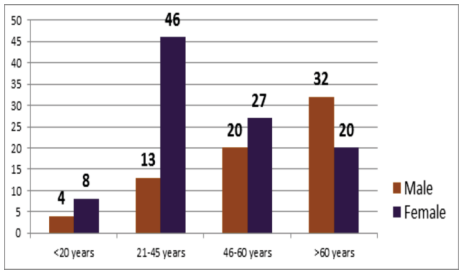

Fig-2: Age and Gender Wise Distribution of UPEC (n=170)

Screening and Rate of Detection of Virulence Markers in UPEC:-

In the present study, among the various virulence markers detected in all 170 UPEC, hemagglutination was observed in highest number 105 (61.76%), followed by of haemolysin production in 103 isolates (60.58%), serum resistance in 101 (59.41%), biofilm formation in 93 (54.70%) and siderophore production in 78 isolates (45.88%). The least positivity was seen with gelatinase production which was observed in 20 isolates (11.76%).

Table-1:Frequency Virulence markers in UPEC (n=170)		
Virulence markers	Positive	Negative
Haemolysin production	103(60.58%)	67(39.41%)
Gelatinase production	20(11.76%)	150(88.23%)
Haemeagglutination	105(61.76%)	65(38.23%)
Serum resistance	101 (59.41%)	69(40.58%)
Siderophore production	78(45.88%)	92(54.11%)
Biofilm formation	93 (54.70%)	97(45.29%)

Frequency of Virulence Markers in UPEC Isolates:-

All the six virulence factors were positive in only 3 isolates (1.76%), five were detected in 20 isolates (11.76%), four in 40 (23.52%), three in 44 (25.88%), two in 39 (22.94%), only one virulence factor was found to be positive in 18 isolates (10.58%) and 06 isolates (3.52%) did not show positivity for any virulence factor.

Table 2- Strains carrying the number of Virulence factors of UPEC (n=170)	
Number of Virulence factors	Number of UPEC Isolates
Zero	06(3.52%)
One	18(10.58%)
Two	39 (22.94%)
Three	44 (25.88%)
Four	40 (23.52%)
Five	20 (11.76%)
Six	3 (1.76%)

Antimicrobial Susceptibility Testing of Uropathogenic E.coli Isolates:-

Antimicrobial susceptibility pattern of all UPEC isolates were tested against the following antibiotic discs by Kirby Bauer disk diffusion method. The results were interpreted as recommended by CLSI 2024 guidelines.

Table3- Antimicrobial susceptibility pattern of UPEC(n=170)				
S.No	Drugs	Sensitive	Intermediate	Resistance
1	Ampicillin	34(20.00%)	5(2.94%)	131(77.05%)
2	Amoxclav	27(15.88%)	15(8.82%)	128(75.29%)
3	Amikacin	146(85.88%)	2(1.17%)	22(12.94%)
4	Ciprofloxacin	51(30%)	12(7.05%)	107(62.94%)
5	Ceftriaxone	33(19.41%)	12(7.05%)	125(73.53%)
6	Cefotaxime	49(28.82%)	-	121(71.17%)
7	Cefepime	137(80.58%)	-	33(19.41%)
8	Ceftazidime	52(30.58%)	-	118(69.41%)
9	Cotrimoxazole	34(20%)	13(7.64%)	123(72.35%)
10	Gentamycin	39(22.94%)	7(4.11%)	124(72.94%)
11	Imipenem	159(93.52%)	-	11(6.47%)
12	Piperacillin – Tazobactam	154(90.58%)	-	16(9.41%)
13	Nitrofurantoin	153(90%)	-	17(10%)

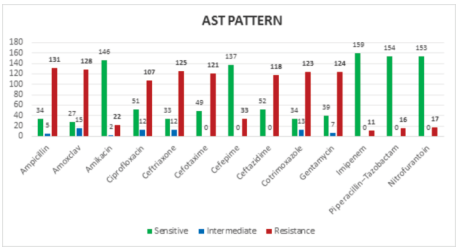

Fig 3- Antimicrobial Susceptibility Pattern of UPEC(n=170)

In this study, the highest resistance was observed for all isolates with Ampicillin (77.05%) followed by Amoxiclav (75.29%), Ceftriaxone (73.53%), Cotrimoxazole (72.35%), whereas the highest sensitivity of the isolates was observed with Imipenem (93.52%) followed by Piperacillin – Tazobactam (90.58%), Nitrofurantoin (90%), and Amikacin (85.88%).

Association of Virulence Factors and Multi Drug Resistant Pattern:- Out of 170 isolates, 36(21.17%) showed resistance to at least three or more than three group of antibiotics. MDR strains of UPEC were compared with various virulence factors and furnished in the below table.

Table:4-Virulence factorsinMDRandNon-MDRisolates (n=170)		
Virulencefactors(VF)	MDR positive isolates (36)	MDR negative isolates (134)
Hemolysis(103)	82(79.61%)	21(20.38%)
Gelatinase(20)	14(70.0%)	6(30.0%)
Hemagglutination (105)	78(74.28%)	27(25.71%)
Serumresistance(101)	66(65.34%)	35(34.65%)
Siderophore(78)	44(56.41%)	34(43.58%)
Biofilm(93)	75(80.64%)	18(19.35%)

DISCUSSION

The primary pathogen group responsible for UTIs is UPEC. The high prevalence of UTI morbidity and death brought on by the development of virulent and resistant strains in hospitals and the community calls for greater emphasis and attention on UPEC. As a result, the present study was conducted to ascertain the pattern of antimicrobial susceptibility and the expression of virulence markers of UPEC. Our study showed that the UTI in females (59.42%) was higher than males (40.58%). KasiMurugan et al[5] and Manikandan C et al[6] observed a prevalence of 69.8% and 52.10% in females when compared to 30.2% and 47.9% in males respectively. In the present study, UPEC was the most frequent pathogen seen in females in the age group of 21 to 45 years (45.54%) due to Female are sexually active in this age group. Kalal et al[7] observed that 31.7% of females were affected in the age group of 15- 59 years. The study Savitha T et al[8]observations are similar to our study and proved that UTI is more common in older age group 41- 50 years. Irene Eriksson et al[9] reported that UTI is common in older age due to associated risk factors such as urinary incontinence etc. Males above 61 years (46.37%) were found to be affected in the present study. it is strongly supported by the study Dev Anand Prakash et al[10] where 30.30% of males were affected after 48 years. In this study, it was observed that 42.94% of UPEC produced strong biofilm whereas in the study done by Poovendran Ponnusamy et al[11] (88.2%) produced strong biofilm and in Tabasi M et al[12] studies where only 17.3% were strong biofilm producers. In this study also, it has been found that 32 out of 73 UPEC isolates among catheterized samples showed biofilm formation. Of these, 32 (43.83%) were found to be strong biofilm producers. In the present study, it was observed that 60.58% of isolates produced hemolysin. The prevalence of hemolysin production in most of the studies from India which was similar to the current data.RebeccaNaveen et al[13], Seigfried et al[14]) had reported that 40.7% , 59.6% of isolates expressed hemolysin production respectively. It was similar to the results of other studies performed by Mittal et al[15]and Vagarali et al[16] who showed that 88%, and 98% isolates produced siderophore respectively. In the current study, 59.41% of isolates showed resistance to serum bactericidal action. It shows similarity with the study conducted by Kauser et al[17] in which 49.5% of UPEC isolates showed serum resistance. Hughes et al[18] stated that high degree of serum resistance was correlated with increased virulent property of bacteria.The present study showed that only 11.76% UPEC isolates produced gelatinase. It is in contrast to the studies done by Sharma et al[19] where only 6.9% isolates produced gelatinase.In the present study 40 isolates (23.52%) isolates showed Multiple Virulence Factors MVFs (>4). It was supported by the study by Sharma S et al[19]

in which 44% of isolates were positive for MVFs. The Highest percentage of resistance was observed with Ampicillin (77.05%) followed by Amoxicillin–clavulanate (75.29%), Ceftriaxone (73.52%), Gentamycin (72.94%) and Cotrimoxazole (72.35%). The resistance pattern varies from place to place. It was similar to the study conducted by Mohammad Reza Shakibaie et al[20] suggested that E. coli was extremely resistant to Ampicillin (100%). Emergence of the resistance to the fluoroquinolones was very high. 62.94% were resistant to Ciprofloxacin in the current study. The incidence of multiple virulence factors in UPEC establishes the uropathogenicity of urinary tract. In the present study multiple virulence factors (>4) were present in 40 isolates (23.52%) and they had strong correlation with MDR pattern. It was supported with the observation of studies by Sharma S et al[19]where 44% of UPEC expressed Multi Virulent factors.

Limitation of Study- Clinical characteristics of the subjects were not taken into consideration in this study. Treatment outcome was not followed up in this study. Comorbidities and vulnerability of the patients were not assessed.

SUMMARY & CONCLUSION

Bacterial isolates susceptibility to antibiotics fluctuates over time and in different environments. The use of broad-spectrum antibiotics has led to an increase in the emergence of multidrug-resistant strains of Escherichia coli. Antibiotic resistance and the phenotypic pathogenicity characteristics of the UPEC isolates are correlated. The virulence markers utilized in this work can be detected quite easily, and routinely screening them in a clinical microbiology laboratory will aid in patient early detection and treatment. It is recommended that more research be done to determine the relationship between the production of virulence factors, the clinical course, and the treatment response. It is recommended that the doctor consult with the microbiologists when selecting antibiotics since resistant E. coli is the new uropathogen that is present everywhere. There are currently no vaccinations for it, despite the fact that it is still widespread. The development of vaccines against UPEC still has a long way to go. Therefore, the following advice can be used to stop resistance. This study will assist medical professionals in treating patients in healthcare settings promptly and affordably. The initial choice of empiric treatment should be based on Gram stain, urine culture and antibiogram. Treatment with broad spectrum antibiotics i.e. quinolones, aminoglycosides and 3rd generation cephalosporin should be avoided.

DECLARATION

Conflict of Interest: The authors have no conflict of interest.

Human and Animal Rights and Informed Consent: This article does not contain any studies with human or animal subjects performed by authors.

Bio-Safety: All standard precautions, bio-safety measures & Biomedical Waste Management in our study according to Biological Waste Management's Rules 2016 and it`s new amendment were observed.

Data Availability: All datasets generated of analyzed during this study are included in the manuscript.

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REFERENCES

1. Nicolle LE. Epidemiology of urinary tract infections. Infect Med. 2001;18: 153–162.
2. Ranjan KP, RanjanN.Complicated urinary tract infection caused by extended spectrum β-lactamase-producing Escherichia coli. Urol Ann 2014;6:112-3.
3. Vagarali MA, Karadesai SG, Patil CS, Metgud SC, Mutnal MB. Haemagglutination and Siderophore production as the urovirulence markers of Uropathogenic Escherichia coli. Indian J Med Microbiol. 2008;26(1):68-70
4. Soto SM, Smithson A, Martinez JA, Horcajada JP, Mensa J, VilaJ,et al. Biofilm formation in uropathogenic Escherichia coli. strains: Relationship with prostatitis, urovirulence factors and antimicrobial resistance. J Urol 2007;177:365-8.
5. Kasi Murugan ,Savitha T, S. Vasanthi "Retrospective study of antibiotic resistance among uropathogens from rural teaching hospital, Tamilnadu, India" Asian Pacific Journal of Tropical Disease. 2012;375-380.
6. C.Manikandan, A.Amsath "Antibiotic susceptibility pattern of Escherichia coli isolated from urine samples in Pattukkottai, Tamilnadu" Int.J.Curr.Microbiol.App.Sci. 2014; 3(10) 449-457.
7. Kalal B S, Nagaraj S. Urinary tract infections: a retrospective, descriptive study of causative organisms and antimicrobial pattern of samples received for culture, from a tertiary care setting, Germs 2016;6(4):132 – 138
8. Savitha, T et al "Surveillence study on emergence of urinary tract infection in Erode District, Tamilnadu, India" International Journal of Current Research, 2011;2(1)67-72.
9. Irene Eriksson et al., "Prevalence and factors associated with urinary tract infections (UTIs) in very old women" Archives of Gerontology and Geriatrics 2010;50(2):132-135.
10. Prakash D and Saxena R S. Prevalence and distribution of gram negative bacteria of Enterobacteriaceae causing Urinary tract infections among hospitalized patients. J Acad Indus Res. 2013;1(11).650-654.
11. PonnusamykonarPoovendran ,NatarajanVidhya and SevananMurugan. Antibiotic Susceptibility Patterns of Different Uropathogenic Escherichia coli (UPEC) Strains at a Tertiary Care Hospital. IJPBA, 2012;3(5) 1213-1216.
12. Tabasi M, Mohammad Reza AsadiKaram M R, Habibi M, Yekaninejad M S and Bouzari S. Phenotypic Assays to Determine Virulence Factors of Uropathogenic Escherichia coli (UPEC) Isolates and their Correlation with Antibiotic Resistance Pattern. Osong Public Health Res Perspect 2015;6(4), 261-268.
13. Rebecca Naveen, Elizabeth Mathai. Some Virulence Characteristics OfUropathogenic Escherichia Coli In Different Patient Groups. Indian J Med Res 2005;122:143-147.
14. Siegfried L, Marta Kmetova, Hana Puzova, Et Al. Virulence Associated Factors In Escherichia Coli Strains Isolated From Children With Urinary Tract Infections. J Med Microbiol 1994; 41:127-132.
15. Mittal S, Sharma M, Chaudhary U. Study of virulence factors of uropathogenic Escherichia coli and its antibiotic susceptibility pattern. Indian J PatholMicrobiol 2014;57(1):61-64.
16. Vagarali MA, Karadesai SG, Patil CS, Metgud SC, Mutnal MB. Haemagglutination and siderophore production as the urovirulence markers of uropathogenic Escherichia coli. Indian J Med Microbiol 2008;26(1):68-70
17. Kauser Y, Chunchanur SK, Nadagir SD, Halesh LH, Chandrashekhar MR. Virulence factors, serotypes and antimicrobial suspectibility pattern of Escherichia coli in urinary tract infections. Al Ameen J Med Sci 2009;2(1):47-51.
18. Hughes C, Hacker J, Duvel H, Goebel W. Chromosomal deletions and reaarangements cause coordinate loss of hemolysis, fimbrination and serum resistance in auropathogenic strain of Escherichia coli. Microbiol Pathogenesis. 1987;2:227-230.
19. Sharma S, Bhat GK, Shenoy S. Virulence factors and drug resistance in E. coli isolated from Extra intestinal infections. Indian J Med Microbiol. 2007;25 (4): 369-373.
20. Mohammad Reza Shakibaie, Adeli S and Salehi M H "Antimicrobial Susceptibility Pattern and ESBL Production among Uropathogenic Escherichia coli Isolated from UTI Children in Pediatric Unit of a Hospital in Kerman, Iran" British Microbiology Research Journal 2014;4(3)262-271.