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Original Article

Characterization of uropathogenic *E. coli* from various geographical locations in India

Gulshan Kumar, Ph.D. [a,b,*], Yashwant Kumar, Ph.D. [a], Gaurav Kumar, Ph.D. [b] and Ajay K. Tahlan, MD [a]

[a] *National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh, India*
[b] *Department of Microbiology, Lovely Professional University, Phagwara, Punjab, India*

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المخلص

أهداف البحث: الجرثومة القولونية المسببة لعدوى البول هي العامل المسبب الأكثر شيوعا لإلتهاب المسالك البولية، حيث تشكل أكثر من 80٪ من حالات التهاب المسالك البولية في جميع أنحاء العالم. تقدم هذه الدراسة بيانات حول الأنماط السائدة، ملامح المقاومة، وعوامل شدة الإمراض التي تساعد على التوطين للجرثومة القولونية المسببة لعدوى البول من مناطق جغرافية مختلفة في الهند.

طرق البحث: تم تصنيف الأنماط الجرثومية عن طريق التصقل في لوحات القراءة الصغيرة، تم استخدام الأساليب القياسية لكشف عوامل شدة الإمراض المختلفة، أي الأغشية الحية (باستخدام طريقة زراعة الأنسجة)، إفراز الجزيئات الناقلة للحديد (تم الفحص على وسط الكروم أزورول اس وتم تصنيفها وفقا لطرق كساكي وأرنو)، إفراز الكوليسين (باستخدام تقنية تغطية الأغار)، هدم الجيلاتين (على وسط الجيلاتيناز)، التصاق الخلايا السطحي (باستخدام طريقة تجمع الملح) وملامح مقاومة المضادات الحيوية (باستخدام عشرين مضادا حيويا) والبيتا-لاكتاميز ذات الطيف الواسع تم تقييمها وفقا لمعايير معهد الأمراض السريرية والمختبرية.

النتائج: أظهرت سلالات الجرثومة القولونية المسببة لعدوى البول معدلات مقاومة عالية جدا لمعظم المضادات الحيوية المستخدمة بشكل شائع مع أعلى معدلات مقاومة للأمبيسيلين (63.4٪)، وحامض النالديكسيك (63.4٪)، والسيفوتاكسيم (62.1٪). تم الكشف عن معدلات عالية من مقاومة الأدوية المتعددة (63.36٪)، وإنتاج البيتا-لاكتاميز ذات الطيف الواسع (34.1٪)، والجرثومة القولونية المسببة لعدوى البول المقاومة للكاربابينيم (25.0٪) وكانت من جميع المناطق الجغرافية في الهند. التصاق الخلايا السطحي (61.2٪)، إنتاج الأغشية الحية (62.5٪)، وإنتاج الجزيئات الناقلة للحديد (67.7٪) كانت من أكثر خصائص الخبيثة شيوعا في عزلات الإشريكية القولونية المسببة لعدوى الجهاز البولي. كان تعبير مشترك عن عوامل شدة الإمراض شائع جدا (69.8٪) في سلالات الإشريكية القولونية المسببة لعدوى الجهاز البولي.

الاستنتاجات: توجد في الهند سلالات من الجرثومة القولونية المسببة لعدوى البول مقاومة للأدوية المضادة للميكروبات بمعدلات عالية جدا، وهي متنوعة من حيث الأنماط الجرثومية وعوامل شدة الإمراض.

الكلمات المفتاحية: الجرثومة القولونية المسببة لعدوى البول؛ مقاومة الأدوية المتعددة؛ البيتا-لاكتاميز ذات الطيف الواسع؛ عوامل شدة الإمراض؛ الإشريكية القولونية المسببة للأمراض خارج الأمعاء؛ الجرثومة القولونية.

Abstract

Objectives: Uropathogenic *Escherichia coli* (UPEC) is the most common causative agent of urinary tract infection, accounting for more than 80% of cases worldwide. This study presents data on prevalent serotypes, resistance profiles, and colonization-aiding virulence characteristics of UPEC from different geographical regions in India.

Methods: UPEC were serotyped through microtiter plate agglutination. Standard techniques were used to detect various virulence characteristics, i.e., biofilm formation (tissue culture plate method), siderophore production (screened on Chrome Azurol S agar and categorized with Csaky's and Arnow's methods), colicin release (agar overlay technique), gelatin hydrolysis (on gelatinase agar), and cell surface hydrophobicity (salt aggregation method). Antibiotic resistance profiles (against 20 antimicrobial agents) and extended-spectrum beta-lactamase (ESBL) were evaluated according to Clinical and Laboratory Standards Institute guidelines.

* Corresponding address: National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh, 173204, India.
E-mail: gulshankumar3@gmail.com (G. Kumar)

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Results: UPEC strains exhibited very high drug resistance rates to most of the commonly used antimicrobial agents; the highest resistance rates were observed for ampicillin (63.4%), nalidixic acid (63.4%), and cefotaxime (62.1%). High rates of multi-drug resistance (63.36%), ESBL-production (34.1%), and carbapenem-resistance (25.0%) were detected in UPEC strains from all geographical regions of India. Hydrophobicity (61.2%), biofilm production (62.5%), and siderophore production (67.7%) were the most common virulence characteristics of UPEC isolates. Co-expression of virulence characteristics was common (69.8%) in UPEC strains.

Conclusion: UPEC strains with very high antimicrobial-resistance are in circulation in India, and have diverse serotypes and virulence characteristics.

Keywords: ESBL; MDR; UPEC; Virulence factors

Introduction

Uropathogenic *Escherichia coli* (UPEC) is considered the primary cause of community-acquired urinary tract infections (UTIs). Approximately 20% of women above 18 years of age have been estimated to have a UTI during their lifetimes.[1] Approximately 70–95% of community-onset UTI cases and 50% of nosocomial UTIs are attributed to UPEC. More than 80% of UTIs have been estimated to be attributable to UPEC as the causative agent worldwide.[2–5] Women, because of anatomical and biological differences from men, are the most affected group; as many as 72% of all UPEC infections occur in women.[6] In India, UTIs are quite common, and affect more women (approximately 43%) than men (approximately 31%) in all geographical regions of the country; moreover, UPEC is the most common (approximately 50%) causative agent.[7] UPEC pathogenesis includes colonization in the peri-urethral, urethral, and vaginal areas; penetration into the lumen of the bladder; *E. coli* planktonic cell growth in the urine; bacterial adherence to the cell surface; interaction with the defense mechanism of the epithelium of the bladder, thus leading to biofilm formation; and further invasion and replication in bladder cells, thus causing renal damage and an increased risk of bacteremia/septicemia.[8] Various virulence factors are involved in the pathogenesis of UPEC, including toxins, adhesions, secretions, and invasins, which facilitate attachment, colonization, and lesion formation in the infected host. Understanding these virulence factors present in various geographical locations aids in determining the best intervention strategies for treatment and avoiding infections.[9,10] Treatment of UPEC infections is complicated by the emergence of highly drug-resistant strains, i.e., multi-drug resistant (MDR), extended-spectrum beta-lactamase (ESBL)-producing, and carbapenem-resistant strains.[11,12] The worldwide emergence of such MDR "superbugs" is a growing public health concern.[13] Surveillance to detect UPEC, their virulence characteristics, and local resistance profiles is critical in planning effective infection control and management strategies. India is a large and geographically diverse country. In the present study, we determined the drug resistance profiles of 232 UPEC isolates from various geographical locations in India against 20 different antimicrobial agents, and their ESBL production. Common virulence factors, such as cell surface hydrophobicity, colicin production, gelatinase activity, biofilm formation, and siderophore production, were also studied in these strains.

Materials and Methods

Study samples

A total of 346 UTI bacterial isolates suspected to be *E. coli*, submitted from different parts of India to the National Salmonella & *E. coli* Center (NSEC), Central Research Institute, Kasauli, between January 2016 to January 2018, were examined in the present study.

Biotyping

All bacterial isolates were tested with a set of biochemical tests (indole, methyl red, Voges Proskauer, and citrate utilization; triple sugar iron; urea utilization and nitrate reduction; fermentation of glucose, lactose, and sucrose; catalase; oxidase; and ortho-nitrophenyl beta D-galactopyranoside), and their culture characteristics on MacConkey agar, nutrient agar, and nutrient broth were studied. Bacterial samples with biochemical and culture characteristics consistent with those of *E. coli* were considered to be confirmed to be *E. coli* and evaluated in further testing.[14]

Serotyping

All biochemically confirmed *E. coli* isolates were serotyped with standard anti "O" *E. coli* antiserum, as described by Orskov and Orskov.[15] *E. coli* antisera for serotyping were provided by NSEC.

Cell surface hydrophobicity

Cell surface hydrophobicity was assayed with salt aggregation tests.[16] Ammonium sulfate in various concentrations (5 M, 2.5 M, 1.25 M, 0.625 M, and 0.3125 M) was used to determine the hydrophobicity of the test isolates. Isolates showing aggregation with an ammonium sulfate concentration $\leq$1.25 M were considered to exhibit cell surface hydrophobicity.

Colicin production

Colicin production by DEC isolates was tested with the phenotypic soft agar overlay technique.[17] *E. coli* K12 was used as the colicin-sensitive strain, to observe colicin production by the test organisms.

Gelatin hydrolysis

Gelatin hydrolysis was detected on gelatinase agar plates.[18] Overnight cultures of the isolates on gelatinase plates (containing 1% extra-pure gelatin) were flooded with acidic mercuric chloride solution (15 g $HgCl_2$, 20 ml conc. HCl, and 100 ml water for injection). The appearance of a zone of clearance around bacterial growth and cloudiness of the gelatinase medium was considered to indicate gelatinase production.

Siderophore production

Isolates were screened for siderophores production on Chrome Azurol S agar[19] and chemically characterized as hydroxamate and catecholate siderophores with Csaky's and Arnow's methods, respectively.[20]

Biofilm formation

Biofilm formation was detected with the tissue culture plate method, as described by Christensen,[21] and scored as weak, moderate, or strong according to the criteria of Stepanovic.[22]

Antimicrobial resistance profiles

Antibiotic sensitivity testing was performed on Muller Hinton Agar with the Kirby Bauer disc diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines,[23] with 20 drugs representing different antimicrobial groups: amoxicillin/clavulanate (30 μg), ampicillin (10 μg), amikacin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), ceftazidime (30 μg), cefotaxime (30 μg), cotrimoxazole (25 μg), ceftriaxone (30 μg), cefepime (30 μg), cefuroxime (30 μg), gentamicin (10 μg), kanamycin (30 μg), imipenem (10 μg), meropenem (10 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), nalidixic acid (30 μg), trimethoprim (5 μg), and piperacillin-tazobactam (100/10 μg). The antibiotic discs were purchased from Hi-Media Laboratories Pvt. Ltd., Thane, India. Isolates were categorized as resistant, intermediate, or sensitive, according to CLSI guidelines. Resistance to an antimicrobial class was characterized by isolates showing resistance to at least one of the antimicrobial agents in a class. Isolates showing resistance to more than two classes of antimicrobial agents were considered to be MDR. An isolate showing characteristics of MDR, ESBL production, and resistance to the carbapenem class of antimicrobial agents was considered highly resistant.

ESBL production

ESBL production was detected with disk diffusion assays on Muller Hinton agar, according to the CLSI guidelines,[23] with cefotaxime and ceftazidime alone or in combination with clavulanic acid. The difference in the zone diameter of the drug with clavulanic acid versus the drug alone was used for scoring isolates as ESBL positive and negative. A $\geq$5 mm difference was considered positive, and a $<$5 mm difference was considered negative.

Statistical analysis

SPSS version 22.0 was used for the calculation of frequencies, percentages, associations, and significance. Associations between various characteristics of the study isolates were evaluated through calculation of Pearson's correlation coefficient at a 95% confidence level. The significance of regional differences in resistance rates to different drug classes was evaluated with chi square test at a 95% confidence level.

Results

Of 346 UTI isolates, only 232 were confirmed to be *E. coli* after biotyping; the remainder were *Klebsiella* spp. (69), *Pseudomonas* spp. (27), *Proteus spp.* (8), *Citrobacter* spp. (6), and *Staphylococcus* spp. (4). Hydrophobicity (61.2% of isolates), biofilm formation (62.5% of isolates), and siderophore production (67.7% of isolates) were the most common virulence characteristics exhibited by UPEC isolates, whereas colicin production and gelatinase were detected in relatively fewer isolates (31.0% and 1.29% of isolates, respectively). These virulence factors were expressed by UPEC isolates in different combinations. None of the isolates from northern India produced colicin, and none of the isolates from northern and western India produced gelatinase (Table 1). Most isolates (69.8%) showed more than one virulence characteristic. The different combinations of virulence factors expressed by UPEC isolates are presented in Figure 1. Three virulence traits—cell surface hydrophobicity, biofilm formation, and siderophore production—were significantly co-expressed in a substantial

Table 1: Virulence factors expressed by UPEC isolates from various geographical areas.

Geographical Area	Expression Rates of Different Virulence Factors n (%)				
	Cell surface hydrophobicity	Biofilm formation	Siderophore production	Colicin	Gelatin hydrolysis
Central India (N = 30)	20 (66.7)	19 (63.3)	26 (86.7)	11 (36.7)	1 (3.3)
Eastern India (N = 37)	26 (70.3)	22 (59.5)	26 (70.3)	20 (54.1)	1 (2.7)
Northern India (N = 23)	11 (47.8)	4 (17.4)	6 (26.1)	0 (0)	0 (0)
Southern India (N = 124)	75 (60.5)	83 (66.9)	91 (73.4)	37 (29.8)	1 (0.8)
Western India (N = 18)	10 (55.6)	17 (94.4)	8 (44.4)	4 (22.2)	0 (0)
Total (N = 232)	142 (61.2)	145 (62.5)	157 (67.7)	72 (31)	3 (1.3)

Note: The numbers in the table are frequencies (and percentages).

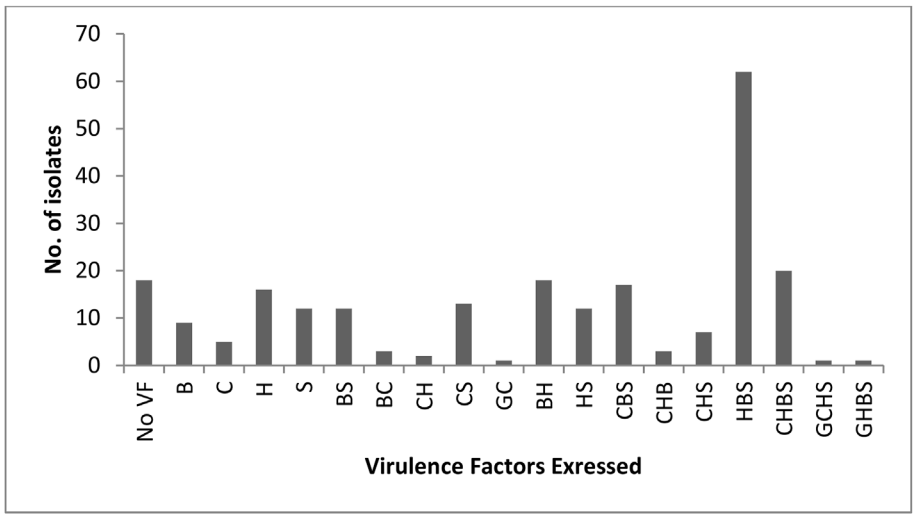

Figure 1: Virulence factor patterns of UPEC isolates.

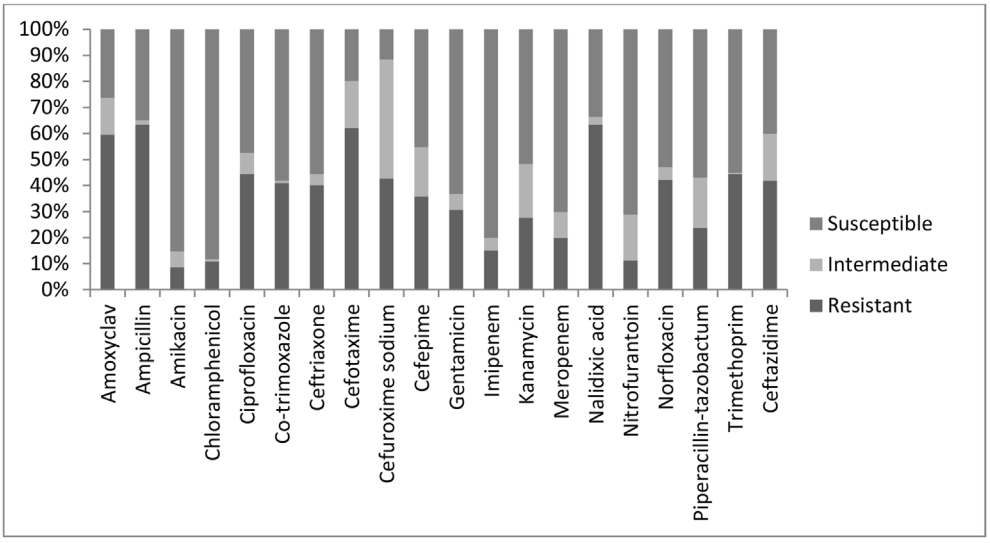

Figure 2: Antimicrobial resistance patterns of UPEC isolates.

fraction of isolates (35.8%), with or without other factors. Positive biofilm formation-hydrophobicity ($p = 0.000$), biofilm formation-siderophore production ($p = 0.000$), and hydrophobicity-siderophore production ($p = 0.044$) trait correlations were observed.

Very high drug resistance was observed: the resistance rates to 11 of the 20 antimicrobial agents evaluated in this study exceeded 40%. Maximum resistance, with a resistance rate exceeding 60%, was observed for ampicillin (63.4%), nalidixic acid (63.4%), and cefotaxime (62.1%). Chloramphenicol, amikacin, imipenem, nitrofurantoin, and meropenem were the most effective drugs, with susceptibility rates ranging from 70.3% to 88.4% (Figure 2). Seventeen isolates were resistant to more than 15 antimicrobial agents tested; one of the isolates was resistant to 18 antimicrobial agents.

The UPEC isolates showed very high resistance to the penicillin, cephalosporin, quinolone, and sulfonamide classes of drugs. Resistance to carbapenems was observed in 25.0% of the isolates. Resistance rates to some drug classes, i.e., cephalosporins ($p = 0.074$), aminoglycosides ($p = 0.210$), and nitrofurantoins ($p = 0.119$), showed no significant differences among regions. However, significant differences in resistance rates to the penicillin ($p = 0.007$), carbapenem ($p = 0.000$), sulfonamide ($p = 0.011$), quinolones ($p = 0.000$), and chloramphenicol ($p = 0.035$) classes of drugs were observed among regions (Figure 3).

MDR *E. coli* strains were frequently detected among the studied UPEC isolates from all geographical areas, ranging from 56.5% in northern Indian isolates to 88.9% in western Indian isolates (Figure 4). A total of 79 (34.1%) of the UPEC isolates were detected to be ESBL-producing. These ESBL-producing UPEC isolates were detected from all geographical areas with varying percentages (from 8.70% in northern India to 55.6% in western India; Figure 5). A total of 32 (13.8%) UPEC isolates were highly resistant, i.e., MDR, ESBL-producing, and resistant to the carbapenem class of drugs.

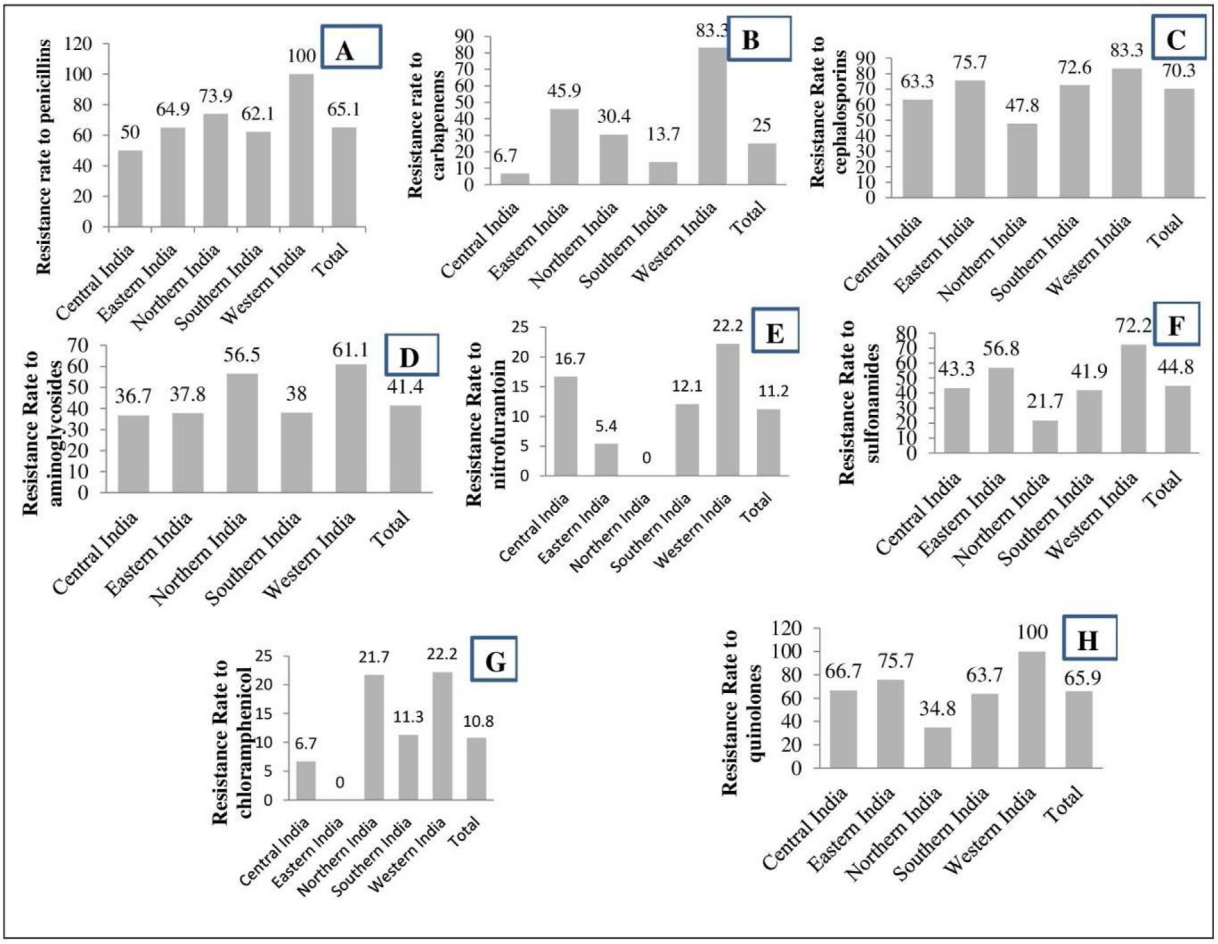

Figure 3: UPEC resistance rates to different classes of antimicrobial agents.

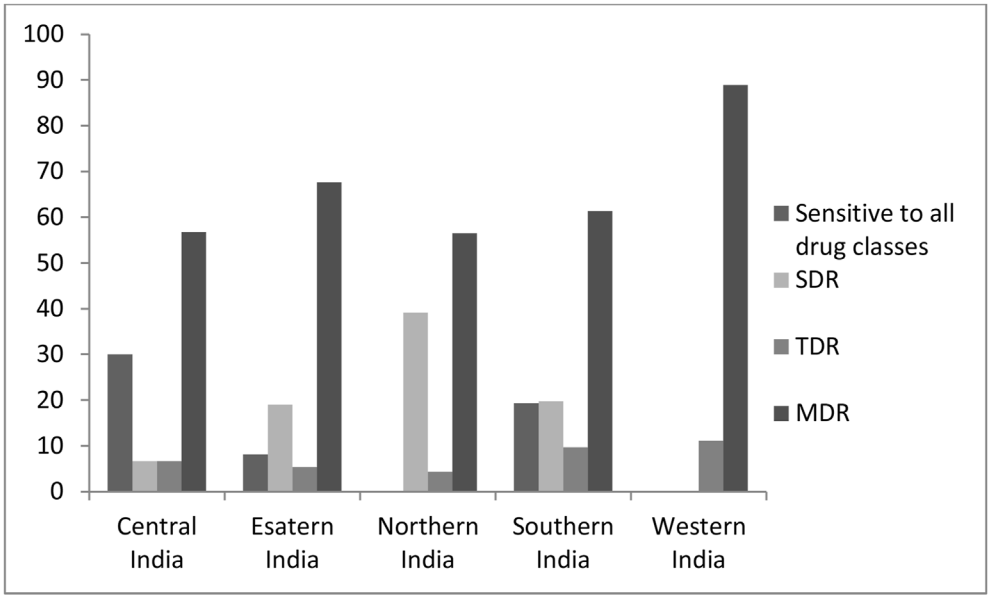

Figure 4: Antimicrobial resistance/sensitivity rates to antimicrobial agents by region.

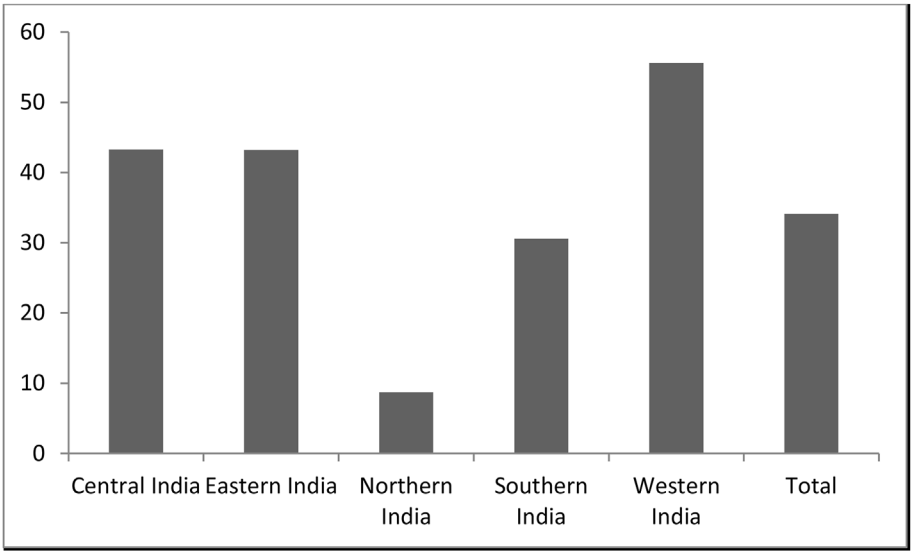

Figure 5: Rates of ESBL-producing isolates from different regions.

Table 2: Serotypes predominantly associated with different UPEC isolates.

Predominantly associated serotypes	
O Serogroup	Occurrence N (%)
UT (untypeable)	43 (18.5)
O7	16 (6.9)
O8	22 (9.5)
O11	28 (12.1)
O35	16 (6.9)
O83	10 (4.3)
O88	16 (6.9)
O89	7 (3.0)
O141	13 (5.6)
O149	8 (3.4)

Note: The numbers in the table are frequencies (and percentages).

UPEC isolates expressed a wide variety of serotypes. The most commonly detected serogroups in study isolates are shown in Table 2. No significant associations were observed between serogroups and the different virulence characteristics, i.e., biofilm formation ($p = 0.888$), siderophore production ($p = 0.351$), hydrophobicity ($p = 0.592$), colicin ($p = 0.999$), gelatinase hydrolysis ($p = 0.846$), drug resistance pattern ($p = 0.770$), MDR ($p = 0.446$), and ESBL production ($p = 0.943$).

Discussion

E. coli is a ubiquitous microorganism and the most common cause of UTI, a condition affecting young women more than men, owing to anatomical differences. During infection, the first and most critical step in UPEC virulence is the attachment of the bacterium to the urinary tract, and its colonization and spread in the ascending or descending direction of the urinary tract. Various adhesins and colonization mechanisms are used by virulent *E. coli* strains to accomplish this important virulence initiation step. In this study, UPEC isolates from multiple geographical locations in India were evaluated, to study some common virulence characteristics (cell surface hydrophobicity, siderophore production, biofilm formation, gelatin hydrolysis, and colicin production) facilitating this first virulence step. These virulence factors support pathogenic *E. coli* adhesion to host cells, survival in host systems by evading host defense mechanisms, colonization, persistence, spread, and competitive advantage over other pathogens and commensal organisms in the host's body.[24,25]

The expression of multiple virulence factors enables a pathogen's evasion of host defenses and increases its virulence. Many UPEC isolates from all geographical locations exhibited multiple virulence factors, and the majority simultaneously had hydrophobicity, siderophore production, and biofilm-forming abilities. These three abilities were also individually the most common virulence characteristics among UPEC isolates from all regions of the country. Cell surface hydrophobicity, siderophore production, and biofilm formation play important roles in medical device-associated nosocomial infections, which usually result in recurrent and persistent drug-resistant UTIs.[10,26–28] Colicin and gelatinase production, which are associated with bacterial colonization and dissemination, were also detected in isolates from all regions except those from northern India, in which neither of these virulence factors was detected, and in western India, in which no gelatinase was detected.

Historically, serotypes were believed to be closely associated with pathogenesis.[29,30] Some classical UPEC serotypes, i.e., O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75, and O83, have frequently been associated with UPEC infection.[31] A wide range of "O" serogroups was detected among UPEC in the present study. The classical serogroups O2, O8, O22, and O83 were also among the serogroups most frequently detected. However, the most frequently detected serogroups were the non-classical serogroups. No significant association between serotypes and UPEC was observed ($p > 0.05$). Other studies

have also suggested that a wide range of serotypes are associated with UPEC, but this association is not strict,[32] thus indicating that, although serotyping is important and is the most widely used epidemiological marker for the characterization of clinical *E. coli* isolates, phenotypic or genotypic characterization of UPEC isolates is necessary for correct identification.

Drug resistance and increasing incidence of MDR infections have emerged as major problems in the management of community and hospital-acquired infections, and thus are considered a growing public health concern.[13,33] In India, 58,000 deaths due to drug-resistant infections have been estimated to occur in newborns every year.[34] MDR *E. coli* strains have been widely reported; some studies have reported the migration of drug-resistant strains from the Indian subcontinent to the rest of the world.[35,36] The increasing emergence of carbapenem-resistant strains worldwide has led various agencies to include strains of *E. coli* among the most critical/highest priority microorganisms in their priority/threat lists, along with members of family Enterobacteriaceae, mainly *K. spp*. Moreover, these strains of *E. coli* are under surveillance in the national antimicrobial program in India.[37–40] A very high rate of drug resistance was observed among the UPEC isolates from all geographical regions dominated by MDR strains. No association between resistance rates and virulence factors was observed ($p > 0.05$). Similar observations have also been reported in other studies.[41,42] Many isolates were resistant to most classes of antimicrobial agents, including sulfonamides, fluoroquinolones nitrofurantoin, cephalosporins, and carbapenems, which are commonly used in UTI treatment. An increase in the resistance rates over the past few years to third-generation cephalosporins (70%–83%), fluoroquinolones (78%–85%), and carbapenems (10%–13%) has been reported for *E. coli* in India.[43] Geographical variations in drug resistance profiles have been observed, and these drugs are usually effective in ≥80% of UTI infections in developed countries.[44] However, the emergence of resistant strains to quinolones, newer generation cephalosporins, and carbapenems worldwide is a matter of serious concern.[45,46] A worldwide increase in the incidence rates of ESBL-producing UPEC infections has also been reported.[47] Resistance to newer 3rd and 4th generation cephalosporins and ESBL has been observed in many isolates in a recent study from all geographical locations of India. Some isolates (32) were very highly resistant, i.e., MDR, ESBL producing, and carbapenem resistant. In this study, isolates resistant to as many as 15–18 drugs were detected, and UPEC with very high drug resistance characteristics was found to be in circulation throughout the country. Because of a lack of information on patient status, predicting the reasons for the higher rates in isolates of western India in this study is difficult. However, samples from this area were from densely populated cities, and resistant organisms can easily spread to many hosts in densely populated areas. Other studies from different geographical locations in India have reported UPEC with a very high rate of drug resistance.[48,49] Irrational use of antimicrobial agents in developing countries is considered a major factor responsible for the emergence of MDR strains.[50] The management of UPEC infections is complicated by the occurrence of diverse UPEC with multiple virulence characteristics and resistance to multiple drugs, and by poor socioeconomic conditions in developing countries including India. *E. coli* is a ubiquitous bacterium that co-exists with other non-pathogenic and pathogenic microorganisms, and poses a threat of transfer of resistant genes. The One Health approach, suggested by the World Health Organization to manage drug resistance problems, must be strictly implemented worldwide in full spirit. Regular surveillance programs for the characterization and drug resistance profiles of UPEC under improved socioeconomic conditions would help decrease UPEC UTIs in developing countries.

The present study highlighted the virulence characteristics and antimicrobial profiles of pathogenic *E. coli* isolates in all geographical locations of India. The findings provide preliminary information on the diversity of pathogenic *E. coli* strains circulating in India. However, to collect more comprehensive data on the prevalence and epidemiology of pathogenic strains, more detailed studies including samples from large populations with diversity in terms of sex, age groups, and religious groups, etc., is required.

The observations from this study indicate that pathogenic *E. coli* strains with diverse virulence traits and drug resistance characteristics are in circulation throughout India, thus posing a major public health concern.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

Not applicable, because no animal or human participants were involved in the study.

Authors' contribution

GK: Analysis, data collection, interpretation, drafting, editing. YK and GA: Design, supervision, data analysis, interpretation, and critical review. AK: Resource allocation, analysis, interpretation, and critical review. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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