JMID/
Journal of Microbiology and Infectious Diseases

2021; 11 (2):66-73
doi: 10.5799/jmid.951495

RESEARCH ARTICLE

Molecular Epidemiology of Multidrug-resistant *Escherichia coli* from Urinary Tract Infections

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ABSTRACT

Objectives: The purpose of this study was to investigate the phylogenetic groups, antibiotic resistance, antibiotic resistance genes (ARGs), integrons, extraintestinal virulence genes and genetic diversity of *Escherichia coli* isolates from human urinary tract infection.

Methods: A total of 100 *E. coli* isolates were collected from patients with urinary tract infections in Kerala, South India. Antibiotic susceptibility testing of all *E. coli* isolates against different antibiotics was determined by the disc diffusion method. Phylogenetic groups, extraintestinal virulence genes, ARGs, and integrons were detected by PCR. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) was used to check the genetic relatedness among *E. coli* isolates.

Results: *E. coli* isolates have mainly belonged to phylogenetic group B2. Resistance to ampicillin was most frequent among the *E. coli* isolates followed by resistance to cefoxitin, cefpodoxime, nalidixic acid, trimethoprim, and co-trimoxazole. Among *E. coli* isolates, 96% were multidrug-resistant (MDR), and 86% and 32% harbored ARGs and integrase 1 (*int1*) respectively. Seventy-nine percent of the isolates were extraintestinal pathogenic *E. coli* (ExPEC), and 86% of them (n = 68) harbored ARGs. One extensively drug-resistant ExPEC was obtained in this study. The present study revealed a significant association between the presence of virulence genes and antibiotic resistance. A high degree of genetic diversity was observed among the ARGs-harboring *E. coli* isolates.

Conclusion: Understanding the association between extraintestinal virulence genes and antibiotic resistance genes would result in the proper treatment of urinary tract infections. *J Microbiol Infect Dis 2021; 11(2):66-73.*

Keywords: *Escherichia coli; antibiotic resistance; ExPEC; India*

INTRODUCTION

Antibiotic-resistant bacteria have caused a major public health concern all over the world. The extensive use of antibiotics in hospital settings, particularly when the infection control practices are inadequate, has contributed to an increased prevalence of antibiotic-resistant bacteria. The antibiotics commonly used to treat bacterial infections are cephalosporins, trimethoprim, and quinolones. However, antibiotic resistance is a major problem hindering the treatment of bacterial infections. According to the World Health Organization [1], 3rd, 4th, and 5th generation cephalosporins and quinolones are categorized as critically important with the highest priority, and 2nd, 3rd generation cephalosporins, and trimethoprim are listed as highly important antibiotics.

Escherichia coli is one of the major pathogens that cause common hospital and community-acquired infections across the world. *E. coli* strains could be broadly categorized as (1) commensal strains, (2) intestinal pathogenic strains (diarrheagenic *E. coli*), and (3) extraintestinal pathogenic *E. coli* (ExPEC). ExPEC associates with human and animal infections that occur outside of the intestinal tract, such as urinary tract and bloodstream

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Received: 26 August 2020 Accepted: 05 May 2021

infections. The ExPEC possesses a broad range of virulence genes such as adhesins, iron acquisition systems, polysaccharide coatings, and toxins associated with the extraintestinal disease. Uropathogenic *E. coli* (UPEC), sepsis-associated pathogenic *E. coli* (SePEC), newborn meningitis *E. coli* (NMEC), and avian pathogenic *E. coli* (APEC) are different pathotypes that belong to ExPEC which may cause various infectious diseases in humans and animals [2].

Urinary tract infections (UTIs) caused by multidrug-resistant (MDR) *E. coli* harboring antibiotic-resistant genes pose a serious challenge to clinicians because these bacteria are resistant to a broad range of antibiotics. These MDR *E. coli* have complicated the management of UTIs and limit treatment options. To curb the threat of antibiotic resistance pathogens, knowledge of antibiotic susceptibility, genes encoding resistance, and genetic relatedness are essential. Further, antibiotic resistance in *E. coli* is encoded on mobile genetic elements, thus enabling the rapid dissemination of antibiotic resistance genes among different species of bacteria. Therefore, molecular characterization of the MDR *E. coli* from human urinary tract infection is important in successful infection control, involving the better prediction of the antibiotics for treatment. Thus, the objectives of this study were to determine the phylogenetic groups, antibiotic resistance, antibiotic resistance genes (ARGs), integrons, extraintestinal virulence genes and genetic diversity of *E. coli* isolates from human urinary tract infection.

METHODS

Collection and identification of E. coli

Between January 2013 and June 2013, consecutive non-duplicate strains of *E. coli* (n = 100) of human urinary tract infection origin were collected from one hospital (Mar Augustine Jubilee hospital) and two diagnostic centers (Dianova Lab and Hi-Tech Lab, Cochin) in Cochin city, Kerala, South India. The study protocol was approved by the institutional ethics committee. *E. coli* isolates were characterized by biochemical analyses [3] and amplification of the *uidA* gene [4]. *E. coli* DNA was isolated using the Proteinase-K digestion method [5].

Phylogenetic analysis

The phylogenetic group was determined for *E. coli* isolates (n = 100) by the new phylogenetic group assignment polymerase chain reaction (PCR)-based method [6]. Clermont et al. [6] classified *E. coli* into 7 groups such as A, B1, B2, C, D, E, and F. PCR reactions were performed under the following conditions: denaturation 4 min at 94 ^{0}C, 30 cycles of 5 s at 94 ^{0}C and 20 s at 57 ^{0}C (group E) or 59 ^{0}C (quadruplex and group C), and a final extension step of 5 min at 72 ^{0}C, on a ProFlex™ 3x32-Well PCR system (Applied Biosystems, United States).

Detection of extraintestinal virulence factor genes

The PCR detection of five key virulence genes was performed as described by Johnson and Stell [7]. Based on PCR results, *E. coli* isolates positive for two or more virulence genes (*papAH*, *papC*, *sfa/focDE*, *iutA*, and *kpsMT* II) were classified as ExPEC [7]. PCR conditions were used in the following way: 1 cycle of initial denaturation (94 ^{0}C for 5 min), followed by 30 cycles of denaturation (94 ^{0}C for 30 s), annealing (64 ^{0}C, 30 s), extension (68 ^{0}C, 3 min) and final extension (72 ^{0}C, 10 min) on a ProFlex™ 3x32-Well PCR system (Applied Biosystems, United States).

Antibiotic susceptibility test

Antibiotic susceptibility testing of all *E. coli* isolates against different antibiotics was determined by the disc diffusion method [8] on Mueller-Hinton agar (Hi-Media, India). The antibiotics and concentration used were as follows; ampicillin (Amp, 10 mcg), cefotaxime (Ctx, 30 mcg), cefoxitin (Cx, 30 mcg), cefpodoxime (Cpd, 10 mcg), ceftazidime (Caz, 30 mcg), ceftriaxone (Ctr, 30 mcg), cefuroxime (CMX, 30 mcg), chloramphenicol (C, 30 mcg), ciprofloxacin, (Cip, 30 mcg), co-trimoxazole (Co, 25 mcg), gentamicin (Gen, 10 mcg), nalidixic acid (Na, 30 mcg), streptomycin (S, 10 mcg), tetracycline (Te, 30 mcg) and trimethoprim (Tr, 5 mcg). The results were interpreted according to the clinical laboratory standards institute guidelines [9]. MAR index or Multidrug resistance (MDR, resistance to 3 or more antibiotic classes) was calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics used [10].

Detection of antibiotic resistance genes and integrase genes

PCR was used for the detection of gene encoding resistance against clinically relevant antibiotics including bla_{TEM} [11], bla_{SHV} [12] bla_{CTX-M} [13], *tetA* and *tetB* [11], *aphA2* [11], *strA* [14], *sul1* and *sul2* [11], *catI* [11] *dhfr1*, *dhfr7* [15], and *qnrA* [16], *qnrS* [17], and *aac(6')-Ib-cr* [18]. The integrons were detected through PCR amplification of *int1* [19], *int2* and *int3* integrase genes [20]. The PCR reactions were carried out using ProFlex™ 3x32-Well PCR system (Applied Biosystems, United States) under conditions as follows: initial denaturation at 94 ^{0}C for 5 min, followed by 30 cycles of 94 ^{0}C for 30 s, annealing (50 ^{0}C for bla_{TEM}, bla_{SHV}, *tetA*, *tetB*, *aphA2*, *strA*, *sul1*, *sul2*, *qnrA*, *qnrS*, *aac(6')-Ib-cr*, *int2*, *int3*; 55 ^{0}C for *dhfr1*, *dhfr7*, *int1*; 60 °C for bla_{CTX-M}, *catI*) for 30 s, and 72 ^{0}C for 1.5 min, with a final extension at 72 ^{0}C, 5 min.

ERIC fingerprinting

Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) was used to check the genetic relatedness among *E. coli* isolates [21]. The cycling conditions were as follows: 1 cycle of initial denaturation at 94 ^{0}C for 5 min, followed by 30 cycles of denaturation (94 ^{0}C, 30 s), annealing (60 ^{0}C, 1 min), extension (72 ^{0}C, 1 min) and final extension (72 ^{0}C, 5 min) on a ProFlex™ 3x32-Well PCR system (Applied Biosystems, United States). ERIC-PCR fingerprints of the *E. coli* isolates were analyzed using Fingerprint data analysis Gelcompare II version 6.0 software (Applied Maths, Texas).

Statistical analysis

A Pearson's Chi-squared test was used (i) to test the distribution of *E. coli* in different phylogenetic groups, (ii) to compare the association of virulence genes (VGs) and resistance to antibiotics, (iii) to compare the association of VGs and ARGs, and (iv) to compare the association of ARGs and *int1* in *E. coli* isolates. Statistical analysis of the results was carried out using IBMSPSS version 22 (IBM Corporation, Armonk, New York, USA). Statistical significance was set at a p-value of <0.05.

RESULTS

Phylogenetic groups

Phylogenetic analysis of *E. coli* isolates recovered from UTIs, showed that such strains mainly belonged to phylogenetic group B2 (61%), followed by A (10%), Unknown (9%), D (6%), F (6%), E (4%), B1, C (2%) (Figure 1). *E. coli* isolates were significantly more belonged to B2 than all other groups ($p < 0.05$). Phylogenetic group A isolates were significantly more than group E, B1, and C isolates ($p < 0.05$).

Prevalence of ExPEC

ExPEC associated virulence genes were detected in 84 *E. coli* isolates. Seventy-nine percentages of *E. coli* isolates carried two or more extraintestinal virulence genes and termed as ExPEC. *iutA*, *kpsMT* II, and *papC* genes were detected in 67%, 63%, 57% of isolates respectively. *papAH* and *sfa/focDE* were detected in 15% and 14% of the isolates, respectively. The *iutA* and *kpsMT* II were the most frequent combination of virulence genes detected. Fifty-four percent of *E. coli* isolates showed this combination. Forty percent of *E. coli* isolates showed *iutA* + *papC* combination.

Antibiotic resistance

In the present study, we found that out of 100 *E. coli* isolates, 96% were multidrug-resistant (resistant to 3 or more antibiotics). Among the various antibiotics, resistance to ampicillin (98%) was most frequent among the *E. coli* isolates followed by resistance to cefoxitin (92%), cefpodoxime (82%), nalidixic acid (78%), trimethoprim (63%), and co-trimoxazole (56%). *E. coli* isolates showed moderate resistance (47% - 38%) against ciprofloxacin (47%), tetracycline (46%), cefuroxime (45%), cefotaxime (41%), and streptomycin (38%). Resistance to ceftazidime, ceftriaxone, gentamicin, and chloramphenicol, were lower, with percentages of 26%, 23%, 22% and 13%, respectively (Suppl. Fig. 2). Seventy percentages of isolates showed resistance against more than 5 antibiotics. Multiple antibiotics resistance (MAR) index of individual isolates showed that 96% of the urinary isolates had MAR index >0.2 with the highest being 1 (Suppl. Table 2). Multidrug-resistant *E. coli* isolates have significantly more belonged to the B2 group than that of other groups ($p < 0.05$).

Antibiotic resistance genes

In our study 86 ARGs-harboring *E. coli* were detected. Seventy-seven percentages of ARGs-harboring isolates possessed two or more genes conferring resistance to multiple antibiotic classes. Among ampicillin-resistant isolates (n = 98) bla_{TEM} and bla_{SHV} was detected in 71.4% and 38.7% of isolates respectively. bla_{CTX-M} gene was detected in 15.7% of cefotaxime resistant isolates. Sulphonamide resistance was mainly associated with the presence of the *sul1* gene (91%), followed by *sul2* (69.6%). Among tetracycline-resistant strains (n=46), *tetA* (91.3%) was the most frequently detected resistance determinant, than *tetB*. Regarding aminoglycosides, the *strA* (84.2%) was widely present in streptomycin-resistant isolates, and *aphA2*, involved in the gentamicin resistance was detected in 81.8% of the isolates. *cat1* gene was detected in 84.6% of chloramphenicol resistant isolates. *dhfr1* and *dhfr7*, involved in the trimethoprim resistance were detected in 19% and 7.9% of the isolates respectively. Among quinolone-resistant isolates, plasmid-mediated quinolone resistance (PMQR) genes such as *qnrA*, *qnrS*, and *aac(6')-Ib-cr* were detected in 15.3%, 28.2%, and 12.8% respectively.

Prevalence of integrase

The percentage of *int1*- positive *E. coli* was 32%. All the *int1*-positive *E. coli* isolates were multidrug-resistant. Apart from *cat1* and *qnrS* genes, all the ARGs showed a statistically significant ($p < 0.05$) association with *int1* among *E. coli* isolates. *Int2* and *int3* were absent in all the isolates tested.

Co-occurrence of virulence genes and antibiotic resistance genes

Concerning antibiotic resistance, the results showed that 98.7% (78/79) of the ExPEC isolates were resistant to two or more antibiotics; and that 86.0% (68/79) were ARG-harboring ExPEC. There was a statistically significant association between the presence of the *iutA* gene and bla_{CTX-M}, *tetB*, *strA*, *dhfr7*($p > 0.05$). bla_{CTX-M} harboring isolates were showed a negative association with *iutA* gene. *papC* was not significantly ($p > 0.05$) associated with ARGs, except bla_{SHV} and *qnrA*. *kpsMT* II gene was associated with bla_{TEM} and bla_{SHV}. *papAH* was significantly associated with bla_{TEM}, bla_{SHV}, bla_{CTX-M}, *su1I*, *tetB*, *strA*, *cat1*, *dhfr1*, *dhfr7*, and *qnrA* (Table 1). Integrase 1 (*int1*) was significantly more associated with *papAH* and *kpsMT* II.

ERIC fingerprints

E. coli isolates harboring two or more ARGs were characterized by ERIC PCR to determine the genetic diversity and phylogenetic relationship among the strains. In ERIC PCR, all the isolates were typeable and produced amplicon sizes ranging from 150 to 1400 bp. ERIC fingerprint analysis showed that there was great genetic diversity among multi-drug-resistant *E. coli* isolates, with 59 isolates divided into fourteen clusters (Figure 2).

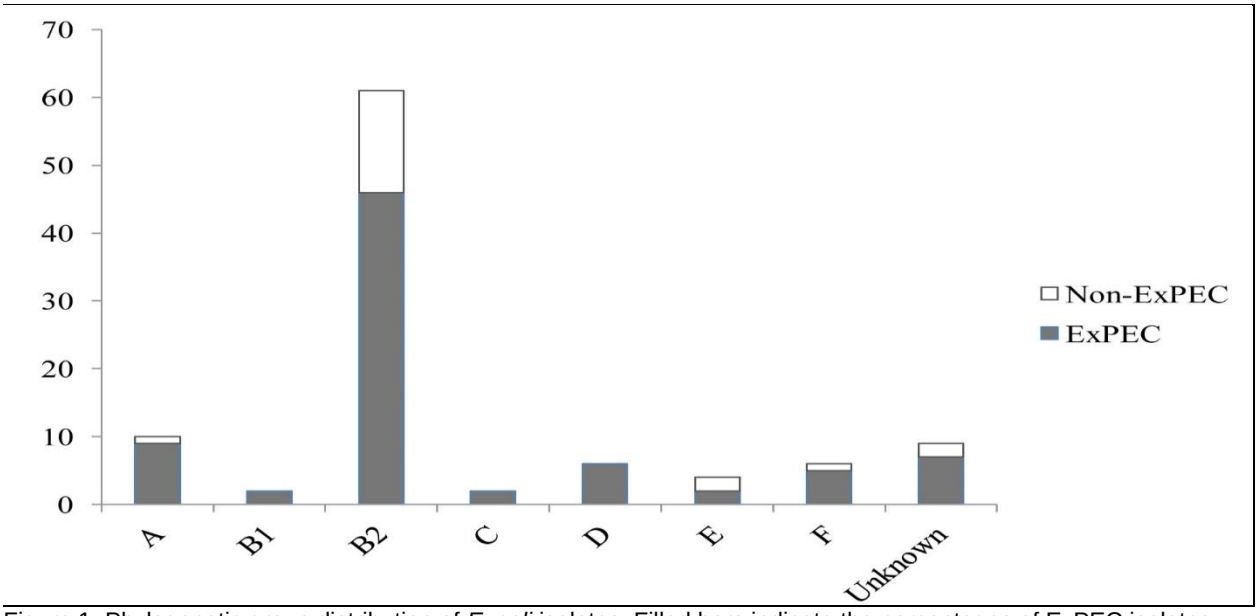

Figure 1. Phylogenetic group distribution of *E. coli* isolates. Filled bars indicate the percentages of ExPEC isolates.

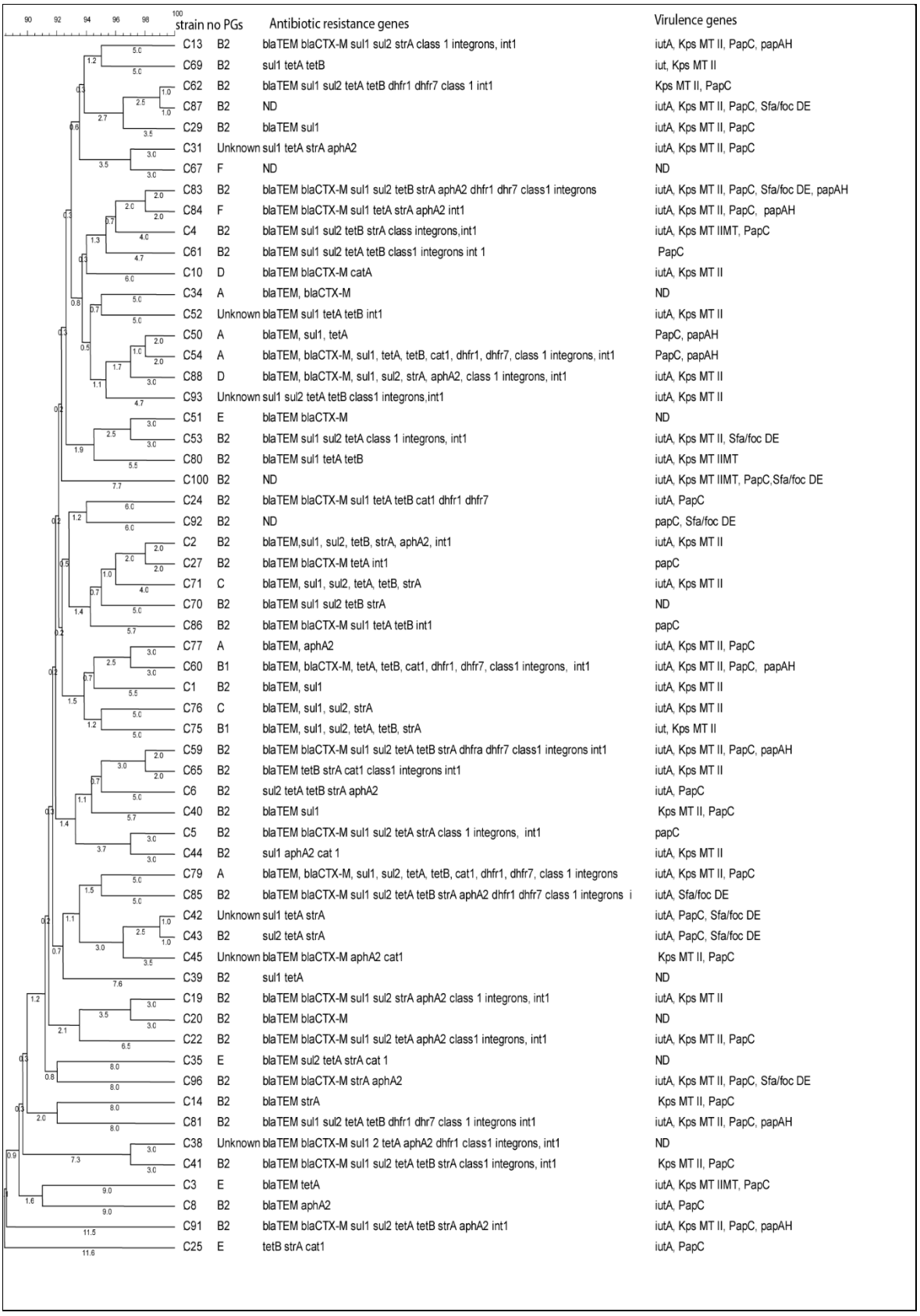

Figure 2. Genetic relatedness of ERIC-PCR profiles of antibiotic resistant *E. coli* isolates. PGs (Phylogenetic groups); ND: Not detected; A, B1, B2, C, D, and F denotes different phylogenetic groups of *E. coli*.

Table 1. Prevalence of virulence genes in ARG-harboring and Non-ARG harboring *E. coli* isolates.

ARG	*iutA* (n=67)	*kpsMT II* (n=63)	*papC* (n=57)	*papAH* (n=15)	*sfa/focDE* (n=14)
bla_{TEM} (n=70)	67.1% (45)	76.1% (48)*	68.4% (39)	93.3% (14)*	35.7% (5)*
bla_{SHV} (n = 38)	40.3% (27)	42.8% (27)*	43.8% (25)*	73.3% (11)*	21.4% (3)*
bla_{CTX-M} (n =26)	20.8% (14)*	23.8% (15)	29.8% (17)	46.6% (7)*	21.4 % (3)
sul1 (n=51)	55.2% (37)	57.1% (36)	45.6% (26)	66.6% (10)*	42.8% (6)
sul2 (n=39)	41.7% (28)	39.6% (25)	35.0% (20)	46.6% (7)	42.8% (6)
tetA (n = 42)	43.2% (29)	38.0% (24)	43.8% (25)	53.3% (8)	42.8% (6)
tetB (n = 32)	36.8% (24)*	33.3% (21)	31.5% (18)	46.6% (7)*	21.4 % (3)*
strA (n = 32)	40.2% (27)*	31.7% (20)	36.8% (21)	46.6% (7)*	35.7% (5)
aphA2 (n = 28)	20.8% (14)	19.0% (12)	19.2% (11)	20% (3)	21.4 % (3)
cat1 (n = 11)	11.9% (8)	11.1% (7)	10.5% (6)	20% (3)*	0% (0)*
dhfr1 (n = 12)	13.4% (9)	12.6% (8)	14.0% (8)	26.6% (4)*	14.2% (2)
dhfr7 (n = 10)	11.9% (8)	11.1% (7)	12.2% (7)	20% (3)	7.1% (1)
qnrA (n = 12)	10.4% (7)	12.6% (8)	17.5% (10)*	33.3% (5)*	14.2% (2)
qnrS (n=22)	25.3% (17)	23.8% (15)	19.2% (11)	13.3% (2)*	14.2% (2)
aac(6')-Ib-cr (n = 10)	10.4% (7)	9.5% (6)	12.2% (7)	20% (3)*	0 (0)*

Percentages in the parenthesis were calculated with the column values. *Significant, p value; ($p < 0.05$).

DISCUSSION

Urinary tract infections (UTIs) are one of the most common infections worldwide and *E. coli* is the main causative agent. In the present study, 100 urinary *E. coli* isolates from human urinary tract infection were analyzed for their phylogenetic background, resistance to various antibiotics, presence of ARGs, and presence of virulence factors.

Interestingly, 61% of UTI isolates belonged to phylogenetic group B2, which is inconsistent with other studies [22]. More than seventy percentages of ExPEC isolates belonged to pathogenetic phylogenetic groups (B2, D, E, and F) than non-pathogenetic groups. *E. coli* responsible for extra-intestinal infection were far more likely to be members of phylogenetic groups B2 or D than A or B1 [7].

Regarding the prevalence of ExPEC, we identified 79 ExPEC harboring 2 or more virulence genes. Our results show a higher frequency of *iutA*, *kpsMT* II, and *papC* compared with the rest of the genes, which may indicate a crucial role of these virulence genes in the pathogenesis. *iutA*, *kpsMT* II, and *papC* gene encode aerobactin receptor, group II capsular polysaccharide synthesis, and P fimbrial assembly systems, respectively. Genes coding for fimbrial adhesive systems represent the most common factors for the virulence of *E. coli* in UTIs. *papC* and *sfa* genes encoding adhesins are known to be involved in binding to urinary tract epithelial cells [23].

Antibiotic resistance is becoming a major concern all over the world with reported rates of multidrug-resistant *E. coli* in the urinary tract. In our study, 94% of isolates were multidrug-resistant. A high percentage of *E. coli* isolates exhibited resistance to β-lactam antibiotics, such as cefoxitin, cefpodoxime, cefuroxime, cefotaxime. This is a reason for concern because most of these antibiotics continue to be the first-line treatment option for UTIs. In the present study, 56% and 63% of *E. coli* isolates showed resistance against co-trimoxazole and trimethoprim, respectively. Trimethoprim is one of the main antibiotics used for treating patients with uncomplicated UTIs and normal kidney function. Quinolones are among the most common therapeutic agents used in treating UTIs. In this study, the prevalence of nalidixic acid and ciprofloxacin-resistant *E. coli* was also high.

In our study, a high percentage of *E. coli* were harbored multiple ARGs. The most frequent resistance genes were bla_{TEM} followed by *sul1*, *tetA*, and *sul2*. bla_{TEM} is the prevalent ARGs among clinical *E. coli* strains [25]. Our data showed that *tetA* was the most frequently detected resistant determinant among tetracycline-resistant isolates. The prevalence of bla_{CTX-M} harboring *E. coli* was low in the current study. The prevalence of bla_{SHV} was higher than those previously reported by many studies [25,26]. In sulfonamide-resistant *E. coli*, *sul1* was predominant. Aminoglycosides resistant determinant, *strA*, *aphA2* were frequent among streptomycin-resistant and gentamicin-resistant isolates respectively. Our results showed that *dhfr1*, *dhfr7*, and *cat1* genes were detected less frequently. Similar to our study, several studies reported the presence of PMQR genes among clinical *E. coli* isolates [25,26].

The virulence genes *iutA*, *kpsMT* II, *sfa/focDE*, and *papAH* showed an association with ARGs. Whereas adhesion-related gene, *papC* did not show a positive association with any of the ARGs. *iutA* and *papAH* showed an association with trimethoprim resistance genes (*dfhr*). *sfa/focDE* and *iutA* showed a negative association with some of the ARGs. The prevalence of bla_{TEM}, *tetB*, and *cat1* genes was high among *sfa/focDE* negative isolates. The prevalence of ESBL gene, bla_{CTXM} was high in *iutA* negative isolates, which is in contrast to the findings of Chakraborty et al. [22]. *papAH* was the virulent gene that showed association with multiple ARGs (n = 8).

Apart from *cat1* gene, all the tested ARGs showed a significant positive association with *int1* among *E. coli* isolates. *Int1* was more prevalent in *sfa/focDE* negative isolates, whereas *papAH* and *kpsMT* II correlates with a high incidence of *int1*. Integrons are genetic elements able to integrate and express diverse open reading frames included in gene cassettes. Integrons carry antibiotic resistance genes, being frequently associated with multidrug resistance in Gram-negative bacteria.

Conclusion

The present study revealed a significant association between the presence of virulence genes and antibiotic resistance. The high prevalence of multidrug-resistant *E. coli* is of great concern; it may result in treatment failure and reducing therapeutic choices. Understanding the association between extraintestinal virulence genes and antibiotic resistance genes would result in the proper treatment of urinary tract infections.

ACKNOWLEDGMENTS

Dr. Divya would like to thank the Council of Scientific and Industrial Research (CSIR), Govt of India for the research fellowship to carry out the work. The authors are also thankful to the Head, Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology for providing the facilities.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

Funding: Not applicable.

REFERENCES

1. WHO. Critically important antimicrobials for human medicine 5th revision. World Health Organization, Geneva, Switzerland. http://who.int/foodsafety/ publications/antimicrobials-fifth/en/. 2017. (accessed 21.06.20).

2. Ewers C, Li G, Wilking H, Kiessling S, et al. Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: How closely related are they? Int J Med Microbiol 2007;297: 163–176.

3. Barrow GI, Feltham RKA. Cowan and Steel's manual for the identification of medical bacteria.1993:317.

4. Bej AK, Dicesare JL, Haff L, et al. Detection of *Escherichia coli* and Shigella spp. in water by using the polymerase chain reaction and gene probes for uid. Appl Environ Microbiol 1991;57: 1013–1017.

5. Sambrook J, Fritsch E, Maniatis V. Molecular Cloning. A Laboratory Manual. New York: Cold spring Harbor Laboratory Press.1989.

6. Clermont O, Christenson JK, Denamur E, et al. The Clermont *Escherichia coli* phylo-typing method revisited: Improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 2013;5: 58–65.

7. Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis 2000; 181:261–272.

8. Bauer AW, Kirby WMM, Skerris JC, et al. Antibiotic susceptibility testing by a standardized single diffusion method. Am J Clin Pathol 1966;454: 493–496.

9. CLSI. Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI Document M100-S22, Clinical and Laboratory Standard Institute, Wayne, 2012.

10. Krumperman PH. Multiple Antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol 1983; 46:165–170.

11. Maynard C, Fairbrother JM, Bekal S, et al. Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* O149:K91 isolates obtained over a 23-year period from pigs. Antimicrob Agents Chemother 2003; 47:3214–3221.

12. Dallenne C, da Costa A, Decré D, et al. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J Antimicrob Chemother 2010; 65:490–495.

13. Batchelor M, Hopkins K, Threlfall EJ, et al. $bla_{CTX\text{-}M}$ genes in clinical Salmonella isolates recovered from humans in england and wales from 1992 to 2003. Antimicrob Agents Chemother 2005; 49:1319–1322.

14. Rosengren LB, Waldner CL, Reid-Smith RJ. Associations between antimicrobial resistance phenotypes, antimicrobial resistance genes, and virulence genes of fecal *Escherichia coli* isolates from healthy grow-finish pigs. Appl Environ Microbiol 2009; 75:1373–1380.

15. Navia MM, Ruiz J, Sanchez-Cespedes J, et al. Detection of dihydrofolate reductase genes by PCR and RFLP. Diagn Microbiol Infect Dis 2003; 46:295–298.

16. Cattoir V, Poirel L, Rotimi V, et al. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. J Antimicrob Chemother 2007; 60:394–397.

17. Benaicha H, Barrijal S, Ezzakkioui F, et al. Prevalence of PMQR genes in *E. coli* and Klebsiella spp. isolated from North-West of Morocco. J Glob Antimicrob Resist 2017; 10:321–325.

18. Kim KY, Park JH, Kwak HS, et al. Characterization of the quinolone resistance mechanism in foodborne Salmonella isolates with high nalidixic acid resistance. Int J Food Microbiol 2011; 146:52–56.

19. Kraft CA, Timbury MC, Platt DJ. Distribution and genetic location of Tn7 in trimethoprim-resistant *Escherichia coli*. J Med Microbiol 1986; 22:125–131.

20. Goldstein C, Lee MD, Sanchez S, et al. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob Agents Chemother 2001; 45:723–726.

21. Meacham KJ, Zhang L, Foxman B, et al. Evaluation of genotyping large numbers of *Escherichia coli* isolates by enterobacterial repetitive intergenic consensus-PCR. J Clin Microbiol 2003; 41:5224–5226.
http://jcm.asm.org/content/41/11/5224.full.

22. Chakraborty A, Adhikari P, Shenoy S, et al. Molecular characterization and clinical significance of extraintestinal pathogenic *Escherichia coli* recovered from a south Indian tertiary care hospital. Microb Pathog 2016; 95:43–48.

23. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. Int J Med Microbiol 2005; 295:383–404.

24. van Hoek AHAM, Stalenhoef JE, van Duijkeren E, et al. Comparative virulotyping of extended-spectrum cephalosporin-resistant *E. coli* isolated from broilers, humans on broiler farms and in the general population and UTI patients. Vet Microbiol 2016; 194:55–61.

25. Shahbazi S, Asadi KMR, Habibi M, et al. Distribution of extended-spectrum β-lactam, quinolone and carbapenem resistance genes, and genetic diversity among uropathogenic *Escherichia coli* isolates in Tehran, Iran. J Glob Antimicrob Resist 2018; 14:118–125.

26. Al-Agamy MH, Aljallal A, Radwan HH, et al. Characterization of carbapenemases, ESBLs, and plasmid-mediated quinolone determinants in carbapenem-insensitive *Escherichia coli* and Klebsiella pneumoniae in Riyadh hospitals. J Infect Public Health 2018; 11:64–68.