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Multiresistant Uropathogenic *Escherichia coli* from a Region in India Where Urinary Tract Infections Are Endemic: Genotypic and Phenotypic Characteristics of Sequence Type 131 Isolates of the CTX-M-15 Extended-Spectrum-β-Lactamase-Producing Lineage

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***Escherichia coli* sequence type 131 (O25b:H4), associated with the CTX-M-15 extended-spectrum beta-lactamases (ESBLs) and linked predominantly to the community-onset antimicrobial-resistant infections, has globally emerged as a public health concern. However, scant attention is given to the understanding of the molecular epidemiology of these strains in high-burden countries such as India. Of the 100 clinical *E. coli* isolates obtained by us from a setting where urinary tract infections are endemic, 16 ST131 *E. coli* isolates were identified by multilocus sequence typing (MLST). Further, genotyping and phenotyping methods were employed to characterize their virulence and drug resistance patterns. All the 16 ST131 isolates harbored the CTX-M-15 gene, and half of them also carried TEM-1; 11 of these were positive for bla_{OXA} groups 1 and 12 for *aac(6′)-Ib-cr*. At least 12 isolates were refractory to four non-beta-lactam antibiotics: ciprofloxacin, gentamicin, sulfamethoxazole-trimethoprim, and tetracycline. Nine isolates carried the class 1 integron. Plasmid analysis indicated a large pool of up to six plasmids per strain with a mean of approximately three plasmids. Conjugation and PCR-based replicon typing (PBRT) revealed that the spread of resistance was associated with the FIA incompatibility group of plasmids. Pulsed-field gel electrophoresis (PFGE) and genotyping of the virulence genes showed a low level of diversity among these strains. The association of ESBL-encoding plasmid with virulence was demonstrated in transconjugants by serum assay. None of the 16 ST131 ESBL-producing *E. coli* strains were known to synthesize carbapenemase enzymes. In conclusion, our study reports a snapshot of the highly virulent/multiresistant clone ST131 of uropathogenic *E. coli* from India. This study suggests that the ST131 genotypes from this region are clonally evolved and are strongly associated with the CTX-M-15 enzyme, carry a high antibiotic resistance background, and have emerged as an important cause of community-acquired urinary tract infections.**

Escherichia coli is a universal commensal bacterium causing infections in humans and animals and serves as a common cause of urinary tract infections (UTI) and bacteremia in humans (43). In addition, this group of strains, designated extraintestinal pathogenic *E. coli* (ExPEC), causes a variety of infections at extraintestinal sites ranging from the biliary system to the central nervous system. These infections are prevalent both in nosocomial and in community settings (46). UTI, although treatable, is now becoming increasingly tough to control because of rampant antimicrobial resistance in the *Enterobacteriaceae* family, particularly in *E. coli* (43, 52). As a result, these organisms are responsible for significant social and economic burdens for the communities and public health departments (24).

In the past decade, there has been a dramatic increase in the identification of *E. coli* strains with CTX-M enzymes, a new group of plasmid-mediated extended-spectrum beta-lactamases (ESBLs) that have replaced classical TEM- and SHV-type ESBLs in many countries (38). There are more than 80 variants described in the CTX-M group of enzymes that are the primary cause of resistance to expanded-spectrum cephalosporins (8). Currently, the most widely distributed CTX-M enzyme is CTX-M-15, which was first detected in *E. coli* from India in 2001 (34, 46). One of the reasons for widespread occurrence of antibiotic-resistant *E. coli* in communities from multiple locales is thought to be due to the dissemination of clonal organisms harboring antimicrobial resistance genes (13, 30, 37).

Recent studies using MLST explored the population biology of ESBL-producing *E. coli* and uncovered emergence of an apparently dominant clone of CTX-M-15-producing *E. coli* carrying high levels of virulence-associated genes (VAGs); this was designated sequence type 131 (ST131), occurring in many different countries, and was thus recognized as a pandemic ExPEC clone (51). It has been shown that this group (ST131) of ESBL-producing *E. coli* strains, in addition to being resistant to most beta-lactam antibiotics, is frequently resistant to aminoglycosides and fluoroquinolones (36). Also, their spread posed a significant threat to human health, as they entail serious therapeutic challenges due to their ability to withstand the effect of different classes of antimicrobial agents. Moreover, the prevention and control of

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the transmission of uropathogenic *E. coli* infections are limited by poor understanding of the population genetics and virulence/resistance genotypes of these pathogens (28).

The endemic potential and ability of particular lineages of antibiotic-resistant *E. coli* to disseminate and cause disease are seldomly studied in countries such as India, where recent surveys have identified prevalence of ESBL producer groups to be up to 70 to 90% of the total *Enterobacteriaceae* reported, although this figure may be based on studies with biased sampling; nevertheless, they indicate a serious problem (26, 27). Moreover, it was demonstrated that there exists a great propensity of transmission of multiresistant clones from humans to animals and vice versa (19, 20).

In appreciation of the above-described issues, we designed a pilot study to investigate the prevalence and to determine the virulence and antimicrobial properties of the ST131 clones present among clinical *E. coli* isolates cultured from the urine of infected patients attending a tertiary care hospital in Pune, India. We believe this study is important in the backdrop of increased occurrence of carbapenem resistance genes in *Enterobacteriaceae*, especially $bla_{\text{NDM-1}}$ in the Indian subcontinent (35), and for looking into the possibility of their dissemination being associated with highly virulent/resistant clones.

MATERIALS AND METHODS

Bacterial isolates, O typing, and antimicrobial susceptibility testing. A total of 100 clinical isolates of *E. coli* from patients with UTI were initially used that were recovered from urine samples of human patients giving a viable count of $>10^5$ CFU/ml. These isolates were received from the microbiology department of a hospital in Pune. Seven European ST131 ESBL *E. coli* isolates archived at the Institute of Microbiology and Epizootics (IMT), Free University Berlin, were also obtained for pulsed-field gel electrophoresis (PFGE). The ESBL production was confirmed phenotypically using the clinical and laboratory standards institute (CLSI) criteria for ESBL screening (16). O typing of ESBL-positive *E. coli* strains was done by a recently described molecular approach based on allele-specific PCR, targeting the *rfb*O25b subgroup gene locus (14). After this stage, only 16 out of the above-described 100 *E. coli* strains were used for further assays, described below. Susceptibility to the following non-beta-lactam molecules was assessed by the disc diffusion procedure: ciprofloxacin, chloramphenicol, gentamicin, sulfamethoxazole-trimethoprim, and tetracycline. Isolates were defined as resistant or susceptible according to CLSI guidelines (16).

MLST and phylogenetic grouping. Identification of *E. coli* phylogenetic groups was performed using the multiplex PCR-based method of Clermont et al. (13). Multilocus sequence typing (MLST) was performed as described previously (54). Gene amplification and sequencing were performed by using primers specified at the *E. coli* MLST website (http://mlst.ucc.ie/mlst/mlst/dbs/Ecoli). Sequences were analyzed by the software package Ridom SeqSphere 0.9.19 (http://www3.ridom.de/seqsphere), and sequence types were determined accordingly.

Antimicrobial resistance gene detection. PCR amplification and sequencing were used to test for the presence of bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, and bla_{OXA} genes among the phenotypically ESBL-positive strains (49). PCR was used to determine the presence of other antibiotic resistance genes, such as those conferring resistance to tetracycline [namely, *tet*(A), *tet*(B), *tet*(C)], to sulfonamides (*sul1*, *sul2*), to streptomycin (*strA*, *strB*), to aminoglycosides (*aadA1*-like, *aac4*), and to some other plasmid-mediated quinolone resistance determinants, such as *aac*(6′)-*Ib*, *qnrA*, *qnrB*, and *qnrS* (4, 3, 7, 23, 32, 42, 48). The 16 ESBL-producing ST131 *E. coli* isolates were screened by PCR for the presence of *intl1* and *intl2* genes, encoding class 1 and 2 integrases with the help of primers described elsewhere (53). To identify the gene cassettes, a PCR and subsequent sequence analysis were performed on the isolates that were positive for *intl1*, using primer pairs defined earlier (39).

Carbapenemase detection. Imipenem and meropenem susceptibility testing was performed using the disk diffusion method in accordance with the CLSI criteria (17). The modified Hodge test was performed on all the 16 isolates on Mueller-Hinton agar (1). After a 12-h incubation, the plates were observed for clover leaf-type pattern at the junction of the test organism and the standard strain within the zone of inhibition of the carbapenem disc. Briefly, the Mueller-Hinton agar plate was inoculated with a dilution of a 0.5 McFarland suspension of *E. coli* ATCC 25922 and streaked with a swab. A 10-μg ertapenem disk was placed in the center of a petri dish, and each test isolate was streaked from the disk to the edge of the dish. *Klebsiella pneumoniae* (ATCC BAA-1705) and *E. coli* (ATCC 25922) were used as positive and negative controls, respectively. Four carbapenemase genes, $bla_{\text{OXA-48}}$, bla_{KPC}, bla_{VIM}, and $bla_{\text{NDM-1}}$, were amplified as described previously (4, 44).

PFGE analysis. XbaI PFGE analysis was used to explore the possible clonal nature of the 16 Indian ST131 isolates in comparison with seven European ST131 strains to construct a dendrogram. The analysis was performed using a CHEF DRIII system (Bio-Rad, Munich, Germany). Cluster analysis of dice similarity indices based on the unweighted pair group method with arithmetic mean (UPGMA) was used to generate a dendrogram describing the relationships among PFGE profiles. Isolates were considered to be belonging to the same PFGE cluster if their Dice similarity index was ≥85% (10, 40).

Conjugal transfer and plasmid analysis. Conjugative transfer of ESBL genes was tested by broth mating experiments by using a plasmid-free, sodium azide-resistant *E. coli* J53Azi$^{\text{R}}$ strain as a recipient. The strains were grown to the exponential phase and then mixed in the ratio of 1:2 (donor:recipient). Transconjugants were selected on Endo agar containing 100 μg/ml sodium azide and 4 μg/ml cefotaxime. Potential transconjugants were verified by PCRs, and the presence of relevant *bla* genes and cotransfer of resistance determinants were confirmed by amplifying the respective genes in the transconjugants as described above (22). The number and approximate sizes of the plasmids in each of the 16 ST131 isolates and the respective transconjugants were determined along with four reference plasmids of known sizes (*E. coli* [RS478], 272 kb; *E. coli* [x-109/97], 19 kb; *Salmonella enterica* serovar Typhimurium, 91.2 kb; *E. coli* [RS478], 170.24 kb) by using a modified version of the methodology previously described by Kado and Liu (33). This was followed by separation of plasmids by electrophoresis in 0.4% agarose gels made with 1× Tris-EDTA (TE) buffer. Gels were run at 20 V/cm for 8 h, stained with ethidium bromide, and photographed. Plasmids were assigned to major plasmid families by PCR-based replicon typing (PBRT) (9).

Serum sensitivity and biofilm formation assay. Serum sensitivity assay was performed on three clinical strains and their transconjugants as previously described (18). Briefly, the overnight bacterial cultures were washed and suspended in 1 ml phosphate-buffered saline (PBS); 50 μl of this suspension was added to 450 μl of serum, and 100 μl from each well was taken out for CFU determination before and after incubation at 37°C in a shaker incubator. Strains IMT10740 and IMT5053 were used as positive and negative controls, respectively. Biofilm formation assays were performed on 15 clinical strains and their conjugant strains by using a modified version of a previously described method (41). Strains were grown overnight in LB broth, and the cultures were diluted to an optical density at 600 nm (OD_{600}) of 0.05 in fresh M63 medium. Then, 200 μl of aliquots was placed in wells of polystyrene microtiter plates and incubated for 48 h at 28°C without shaking. Afterward, ODs were read at 600 nm, the wells were washed thrice, and the staining of adhered bacteria was performed with 0.1% crystal violet (solubilized in ethanol) followed by reading the OD at 570 nm. Biofilm measurements were calculated using the formula as previously described (41).

Virulence gene typing. All the 16 ST131 isolates were investigated by multiplex PCR for the presence of 38 virulence-associated genes (VAGs) corresponding to the extraintestinal pathogenic *E. coli* based on the targets

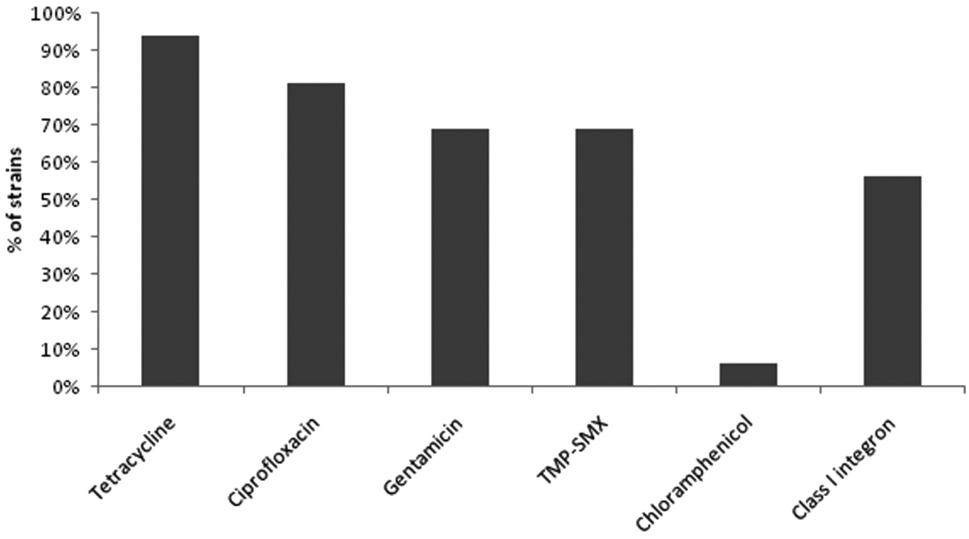

FIG 1 The percentages of 16 ST131 CTX-M-15-producing *E. coli* isolates resistant to five different non-beta-lactam antibiotics and the percentages of isolates positive for class 1 integron. Antimicrobial susceptibility testing was performed using the disk diffusion method according to CLSI criteria.

that have been previously described (49, 31, 5, 20). The genes tested correspond to the main classes of extraintestinal VAGs; adhesin, toxin, iron capturing system, and protectin/invasin.

Statistical analysis. The student's *t* test was performed using GraphPad Prism 5.0 software. The *P* value was calculated for each experiment conducted in triplicates.

RESULTS

Isolation and antimicrobial resistance profiling. One hundred *E. coli* samples were obtained between January 2009 and October 2009 (28). A total of 23 (23%) ESBL-producing isolates were detected phenotypically using the CLSI criteria for ESBL screening and disk confirmation test. These 23 isolates were then subjected to O typing that revealed 16 (70%) out of 23 ESBL-producing strains to be positive for the *rfbO25b* subgroup, indicating the presence of 16 CTX-M-15-O25b-ST131 group strains. Of these 16 strains, 10 were from male patients with an average age of 40 years who were diagnosed with prostatitis and pyelonephritis. To assess multidrug resistance of the 16 ST131 isolates, susceptibility to non-beta-lactam antimicrobials was tested. Ninety-four percent of the isolates were resistant to tetracycline, 81% to ciprofloxacin, and 69% to gentamicin and sulfamethoxazole-trimethoprim (TMP-SMX), but only 6% to chloramphenicol. Out of the16 isolates, 12 (75%) were resistant to at least three classes of antimicrobials, and therefore a total of 12 multidrug-resistant (MDR) ESBL ST131 strains were finally identified (Fig. 1).

Genetic relationships and plasmid profiles. All of the 16 ESBL strains positive for the *rfbO25b* locus were allocated to phylogenetic group B2 on the basis of triplex PCR. MLST analysis of these 16 strains showed that they belonged to the ST131 clone. To obtain a finer resolution of clonal relationships among the 16 Indian ST131 *E. coli* strains and seven ST131 strains from European origin, a PFGE analysis was performed (Fig. 2). Owing to their common genetic background, the ST131 isolates exhibited more homogenous PFGE profiles. All the ST131 strains tested by us formed five small clonal groups (groups 1 to 5) as documented at the 85% similarity level. Three of the five PFGE groups (groups 1, 4, 5) corresponded consistently to the geographic/host origin, whereas two groups (groups 2 and 3) carried strains both from the Indian and European origins. Overall, 10 out of the16 $bla_{\text{CTX-M}}$ (Indian) ST131 isolates were found to be relatively clonal, and also three out of seven European strains clustered closely with the Indian strains, indicating their genetic affinities despite geographic barriers and different host origins. This highlights the fact that they are an epidemiologically important clone appearing/emerging in different continents.

From a total of 16 CTX-M-producing isolates, conjugation to *E. coli* J53Azi^{R} was demonstrated in 15 strains, wherein larger plasmids ranging in sizes from 120 kb to 272 kb were transferred or exchanged. In these strains, all the antimicrobial resistance genes as reported in the parental strains along with the $bla_{\text{CTX-M-15}}$ gene were detected on IncFIA conjugative plasmid by PCR. The parental clinical strains carried up to six plasmids, most of them displayed two larger plasmids of around 120 kb and/or 272 kb (data not shown); plasmid analysis among transconjugants revealed larger plasmids of >100 kb, except for one strain which revealed an identical profile. The plasmids belonged to the narrow host range incompatibility group IncF; 15 isolates contained the FIA replicon, 13 isolates had the IncFIB replicon, while one isolate harbored P, Y, and A/C replicons in addition to FIA and FIB replicons.

All the 16 ST131 isolates were positive for $bla_{\text{CTX-M}}$ genes (CTX-M-15); additionally, eight (50%) of these isolates also produced TEM-1. bla_{SHV} was absent in all, and the bla_{OXA} group 1 was present in 11 (68.7%) of the 16 Indian isolates. Only one strain (6.3%) was positive for bla_{OXA} group 2. The gene variant *aac(6′)-Ib-cr*, which has been associated with fluoroquinolone resistance, was detected in 12 (75%) ST131 strains as determined by digestion of the amplicons with BsegI. No other plasmid-mediated quinolone resistance genes (*qnrA*, *qnrB*, and *qnrS*) were detected. Other resistance genes detected among ST131 isolates are shown in Table 1. Screening of integron classes revealed that 9 (56.3%) out of 16 ESBL producers contained the *intl1* gene, whereas the *intl2* gene, which is specific for class 2 integron, was absent in all 16 isolates. The gene cassette arrangement detected among the *intl1*-

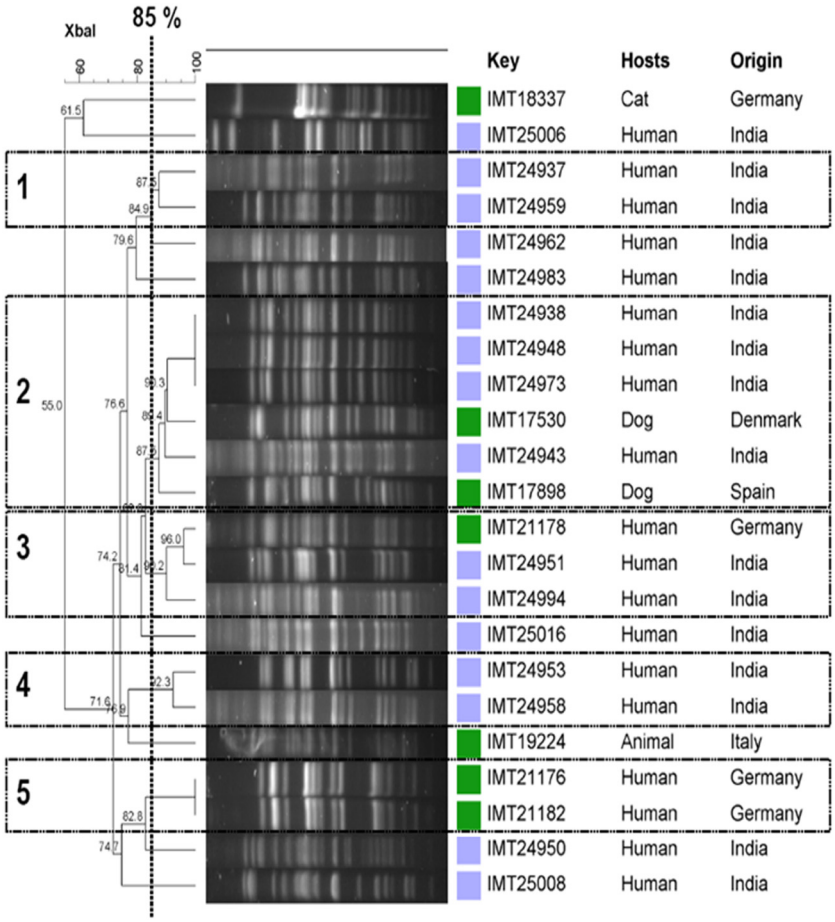

FIG 2 Dendrogram based on PFGE profiles of 16 Indian and 7 European ESBL-producing ST131 *E. coli* isolates. The dendrogram for the 23 isolates as produced by the UPGMA algorithm based on the Dice similarity coefficient included five PFGE groups as defined based on ≤85% similarity of PFGE profiles. Different geographically distinct isolates are accordingly labeled. The blue boxes indicate strains from India; all European origin strains are labeled with green boxes.

positive isolates was *dfrA12-aadA2* (for two isolates) and *dfrA17-aadA5* (for six isolates). All the ST131 isolates were found to be carbapenem sensitive by the disc diffusion method. The four carbapenemase genes, bla_{OXA-48}, bla_{KPC}, bla_{VIM}, and bla_{NDM-1}, were also absent.

Profiling of virulence genotypes/phenotypes. Serum resistance assay of the three clinical strains (IMT24943, IMT24951, IMT24973) versus their transconjugants along with the strain J53 revealed remarkable increase in serum resistance (Fig. 3) by the laboratory strain J53 alone and J53 with ESBL plasmids from clinical strains ($P = 0.0001$ for each pair). J53 with ESBL plasmids (transconjugants) displayed almost similar or increased resistance compared to that of their parental strains. The pattern of biofilm formation observed in cases of the clinical isolates, transconjugants, and the empty host J53 is shown in Fig. 4. Basically, there were no prominent differences observed (with respect to biofilm formation) between the empty J53 host strain and J53 with ESBL plasmids. It may be concluded that there is reduced influence of plasmids on biofilm formation; alternatively, the basic capacity of the wild-type strains to produce a biofilm is simply too low to identify significant differences in transconjugants.

Virulence profiles were determined (Fig. 5) for the 16 ST131 clinical isolates to get an idea of the extent of within-group diversity and the virulence potential of this clonal group. Out of 38 extraintestinal VAGs, 23 were detected at least once, with their prevalence ranging from as low as 6.3% with respect to *iroN* (catecholate siderophore) to 100% presence of the following genes: *chuA* (gene for heme transport), *traT* (serum resistance), *sitD* (salmonella iron transport gene), *ompA* (outer membrane protein), *iucD* (aerobactin), *sat* (secreted autotransporter toxin), *fyuA* (*Yersinia* siderophore receptor), *mat* (meningitis-associated fimbriae), and *feoB* (iron transporter). In general, the virulence gene profile similarity was high among the 16 ST131 isolates. The three identical (IMT24938, IMT24948, IMT24973) isolates seen in group 2 of the PFGE dendrogram exhibited an almost identical virulence profile. Relatively, the isolates within the clusters were more similar in their virulence profiles than the strains from adjacent clusters and singleton strains.

DISCUSSION

The worldwide occurrence of *E. coli* with CTX-M extended-spectrum beta-lactamases partly reflects the spread of clonal lineages, notably ST131, and hence we sought to identify the ST131 clone of bacteria among the ESBL-positive *E. coli* from the peri-urban areas of Pune, India, and to compare them with the isolates of European origin. The emergence of new MDR (extraintestinal)

TABLE 1 Results of different screenings of antimicrobial resistance genes other than ESBL genes[a]

Strain no.	Uniform code	Result						
		sul1	*sul2*	*tet*(A)	*str*(A)	*str*(B)	bla_{OXA} group 1	*acc(6′)-Ib-cr*
IMT24938	2	+	+	+	+	−	−	+
IMT24948	3	−	−	+	+	−	−	−
IMT24958	8	+	+	+	+	+	+	+
IMT24962	14	+	+	+	+	+	+	+
IMT24983	17	+	+	+	+	−	+	−
IMT25006	18	+	+	+	+	+	+	+
IMT24937	20	+	−	+	+	−	+	+
IMT24943	25	+	+	−	+	−	+	+
IMT24950	26	−	−	+	+	+	+	+
IMT24951	29	+	+	+	+	−	+	+
IMT24953	41	+	−	+	+	−	+	+
IMT24959	51	+	+	+	+	−	−	+
IMT24973	62	−	−	+	+	+	−	−
IMT24994	72	+	+	+	+	−	+	+
IMT25008	75	+	+	+	+	−	−	+
IMT25016	83	+	−	+	+	−	+	−

[a] The 16 ESBL ST131 strains were screened for the *tet*(B), *tet*(C), *sul3*, *qnrA*, *qnrB*, *qnrS*, *aadA*, and *aaC4* antimicrobial resistance genes, which were found to be absent in all 16 strains. +, present; −, absent.

strains of *E. coli* spreading through populations with the capacity to evolve continuously appears to pose a significant public health threat. A great deal of attention is required in this direction. To get a deeper understanding of the underlying resistance genotypes and the mechanisms thereof, it was intended to characterize these clinical isolates in detail with regard to their ESBL production capacity and other antimicrobial resistance features. We also sought to find out whether there is any relationship between ESBL plasmids and virulence.

Our analysis of 100 rigorously selected *E. coli* isolates found that the clonal group ST131 was prevalent particularly among the ESBL isolates. We could confirm the presence of a total of 16 ST131 isolates by MLST in just a small collection of 100 isolates, suggesting a moderately high level of abundance. They were all affiliated to the phylogenetic group B2, which extends to most of the extraintestinal pathogenic *E. coli*. The representation of ST131 isolates was found to be more among male patients; this explains the enhanced capability of ST131bacteria to establish ascending urinary tract infections (pyelonephritis and prostatitis) despite the male anatomical barriers.

In agreement with other reports, most of the ST131 ESBL producers from this study were determined to be multidrug resistant and displayed high coresistance rates toward tetracycline, fluoroquinolones, sulfamethoxazole-trimethoprim, and gentamicin. Most of the isolates remained susceptible to chloramphenicol, which is effective against a wide variety of Gram-positive and

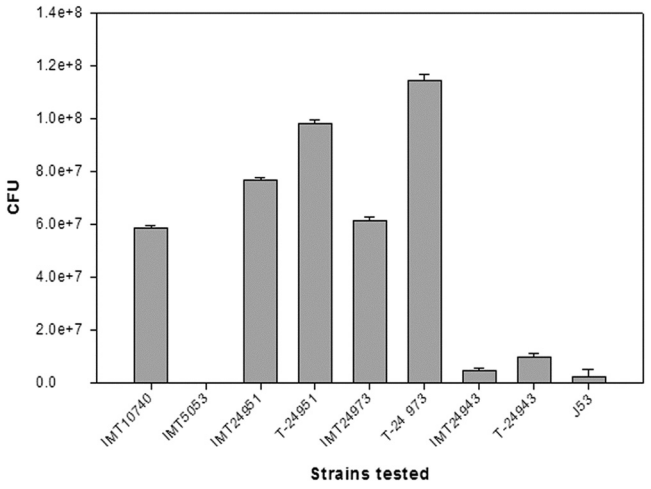

FIG 3 Serum resistance assays of three parental (IMT) ST131 ESBL-producing *E. coli* isolates and each transconjugant (T). The assays were performed for 3 h. Samples were taken in triplicates at time zero and at each hour. The graph is based on the results of three assays for each strain. Results are shown as CFU viability. Error bars indicate standard deviations.

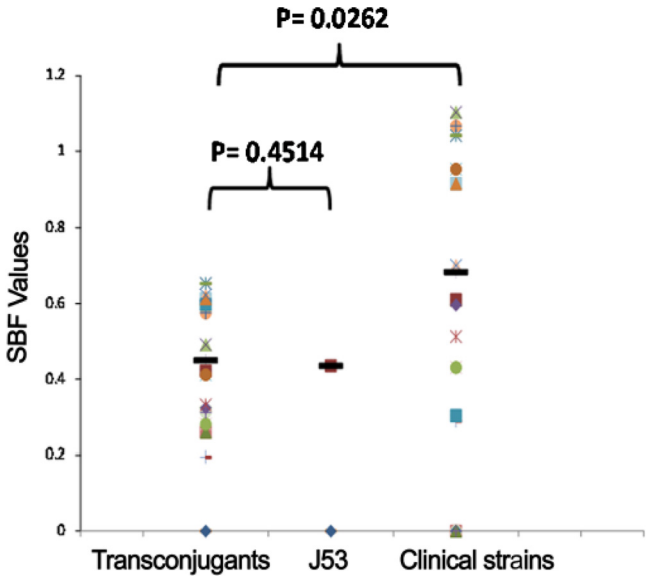

FIG 4 *In vitro* biofilm formation characteristics of transconjugants, clinical strains, and the plasmid-free host J53. The black bar indicates mean values. The *t* test analysis represents the differences between the transconjugants and clinical strains to be highly significant, whereas the difference between transconjugants and J53 was found not to be significant.

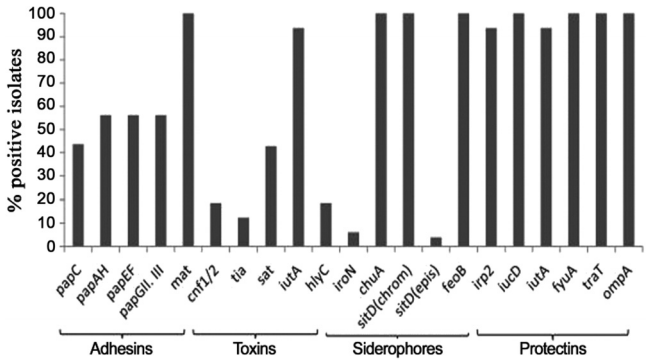

FIG 5 Virulence scores of 16 ST131 UPEC isolates for 23 genes out of 38 extraintestinal virulence-associated genes assessed by multiplex PCRs.

Gram-negative bacteria. Consistent with most of the studies, our ST131 isolates were also highly resistant to fluoroquinolones (81.3%) and trimethoprim-sulfamethoxazole (68.8%) (4).

Transconjugants were created in *E. coli* J53 from 15 (93.8%) out of the 16 strains. It was demonstrated that all of the transferred plasmids were ESBL plasmids. This proved their readily transferable nature with a strong potential for spreading of the ESBL and antimicrobial-resistant genes among bacterial populations. An antimicrobial resistance phenotype similar to the clinical strains was seen in transconjugants, and hence, this observation validates that the genes encoding ESBLs were located on transferable plasmids that harbored genes encoding resistance to several other classes of antimicrobials. Further, we sought to find out whether there was any relationship between the ESBL plasmids and virulence, and surprisingly, we found that the ESBL plasmids not only carried antimicrobial resistance genes but also conferred virulence and survival advantage to the recipient bacteria against the bactericidal activity of sera. However, when we further investigated these plasmids for their role in another virulence attribute, the biofilm formation, we didn't observe any positive influence of ESBL plasmids on biofilm formation. This could simply be due to the regulatory mechanisms involved in biofilm formation that were active on the chromosomal part of the bacteria. Further study of biofilm formation and other virulence features of these plasmids with the plasmid-cured strains and their comparison with parental clinical strains will be important for understanding the relationship between virulence phenotypes and ESBL plasmids.

The CTX-M beta-lactamases have been recognized worldwide as an important mechanism of resistance to cephalosporins (cefotaxime and ceftriaxone) used for Gram-negative pathogens (8). We found that all of the 16 ST131 strains harbored the CTX-M-15 allele. Our finding was in agreement with most of the previously published works reporting a strong association of ST131 strains with CTX-M-15-type enzymes, indicating that they are frequently carried in "aggressive" lineages with high virulence genotypes (8). Similar to the earlier studies, the CTX-M-15 allele was located on plasmids with replicon type FII-FIA, whereas the other replicon types, such as FIB, A/C, P, and Y, which were detected in the clinical wild-type strains, were absent in the transconjugants obtained from them (15, 21).

Plasmid-mediated quinolone resistance (PMQR) has become an important emerging issue. The most common PMQR gene observed in this study was *aac(6′)-Ib-cr* (81%). The association of *aac(6′)-Ib-cr* with CTX-M-producing *E. coli* is not surprising, as many studies have shown that it is often harbored on the same plasmids as the $bla_{\text{CTX-M}}$ gene (42, 45, 48). We did not detect any other PMQR genes, like *qnrA*, *qnrB*, and *qnrS*, which have also been shown to be associated with CTX-M-producing *E. coli* in recent studies. However, a number of other studies reported that these genes are rather uncommon among CTX-M-producing *E. coli*, and hence our study is in agreement with the observations reported elsewhere (42, 45, 48). The fluoroquinolone resistance phenotype among these 16 ST131 strains also could be due to mutations within the genes, such as *gyrA* and *parC* (11), which has not been determined by us.

Several studies have investigated the distribution of integrons in uropathogenic *E. coli* (UPEC) and have established a strong association between the presence of integrons and antimicrobial resistance in MDR and single-drug-resistant *E. coli* strains (6, 47). While the analysis of integron-encoded integrases indicated that class 1 integron was the principal integron class in the 16 Indian ESBL strains, no class 2 integron-encoded integrases were detected; these integrons were plasmid encoded and transmissible, as indicated by conjugation experiments. These results are similar to a recent report on antimicrobial resistance in uropathogenic *E. coli* from Europe and Canada (6). We observed a higher frequency of *sul1* than *sul2*. Sulfonamides are regarded as highly important antimicrobial agents for the treatment of *E. coli* infections, and the presence of sulfonamide resistance can lead to treatment failure in cases of UTI (6). The association of ESBL genes with other resistance determinants (such as genes conferring resistance to sulfonamides, tetracycline, and aminoglycoside) were observed in this study, as the candidate resistance genes are often carried on the same plasmid, as demonstrated by similar antimicrobial profiles of transconjugants (30, 50, 51). These results prove that the ESBL genes continue to evolve and harbor an increasing range of resistance determinants (25).

Our isolates were checked for susceptibilities to carbapenems, and it was observed that all 16 ST131 ESBL producers were highly susceptible. However, a recent study has highlighted the emergence and widespread distribution of NDM-1 in MDR *Enterobacteriaceae* in different parts of India, Pakistan, and the United Kingdom (35). The reason for this contrasting observation may be the geographic variation that is subject to carbapenem usage, biased sampling, or the small sample size of our study. Nevertheless, it can be said that the association of carbapenemase enzymes with ST131 strains is not widespread, as yet. Plasmids that were isolated from transconjugants were found to be larger than 100 kb, implying that the ESBL plasmids in clinical strains are >100 kb in size. However, the donor strains harbored more than one plasmid, ranging in size from 8.5 kb to 272 kb. Genotyping by PFGE showed that the clinical strains were genetically clonal, as they were sourced from a single hospital and from one geographical area. The three indistinguishable Indian strains on PFGE fingerprints were isolated 1 month apart; two strains were from the same ward, and the third one came from a different ward. This possibly suggests a nosocomial spread. The clustering of three European strains with the Indian strains also supports a possible clonal nature of ST131 strains. This observation is in corroboration with two recent reports (12, 29) that have shown sequence similarity of one of our ST131 isolates, NA114, from India to the European isolates (2); this perhaps points to a somewhat conserved genomic

structure of a few ST131 strains of European origin. However, on the other hand, we saw some degree of heterogeneity in the PFGE profiles among the Indian strains and between the European ST131 strains which could possibly be due to the high rates of recombination within the accessory genome of this species. Another worrying fact is that these ESBL-producing strains have a high background of virulence. This resistance-virulence combination might impart ST131 a competitive advantage over other *E. coli* strains.

In conclusion, our study is perhaps the first one to identify and characterize ST131 *E. coli* in UPEC populations from an Indian setting. This study demonstrates the complexity of the acquisition and spread of MDR phenotypes that could pose difficulties in treating serious Gram-negative infections. The observation that the ESBL plasmids are readily transferrable might present another problem of rapid acquisition of MDR genes by the docile strains. The PFGE profiles, virulence factors, and antimicrobial resistance pattern of these ST131 isolates exhibit that the strains from this geographic location were homogenous pathogens. The transfer of virulence phenotype due to ESBL plasmids might lead to an increase in acquisition of virulence among environmental and commensal bacteria. Therefore, the emergence and dissemination of this particular lineage of *E. coli* in Indian infection settings is a cause of concern and points to a need to identify their origins, reservoirs, and transmission pathways so that better prevention strategies are designed. Having said this, we would like to carry out further studies on a large number of *E. coli* isolates from different places within India to have an exhaustive opinion on epidemiology, evolution, and pathogen biology. Whole-genome sequencing of these strains, including their plasmids, would be instrumental to gain more insights into the molecular mechanisms imparting fitness or survival advantage to these ST131 lineages over other pathogens. Finally, on the public awareness and policy front, while antimicrobial resistance is projected as an important public health concern in China (27), India also deserves such attention given the widespread occurrence of new beta-lactamase genes, such as $bla_{\text{CTX-M}}$ and $bla_{\text{NDM-1}}$, amidst the absence of a proper antibiotic policy based on epidemiological knowledge entailing socioeconomic and bio-geo-climatic factors that favor rapid emergence of antimicrobial resistance.

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