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Original Article

Horizontal transfer of *bla*TEM, *bla*CTX-M and class 1 integron genes from uropathogenic *Escherichia coli* to *E. coli* DH5α

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ABSTRACT
Background: Emerging antibiotic resistance in uropathogenic *E. coli* (UPEC) is a global problem that results in treatment failure. Mobile genetic elements are key players in dissemination of antibiotic resistance genes via horizontal gene transfer (HGT).
Objective: This prospective study was designed to investigate the possible role of HGT (via conjugation process) in spread of antibiotic resistance genes from MDR-UPEC strains to sensitive strain of *E. coli* DH5α.
Materials and Methods: This study was performed at the Department of Microbiology, University of Karachi from February to July 2019. Four UPEC strains belonging to different phylogenetic groups A, B1, B2 and D were selected for conjugation assay. Sodium azide resistance was induced in *E. coli* DH5α for the study. Transconjugants were confirmed phenotypically by disc diffusion method and genotypic confirmation was done by PCR method.
Results: *E. coli* DH5α was successfully induced for sodium azide resistance. Conjugation experiment proved that *bla*CTX-M, *bla*TEM and class 1 integron genes were transferred from MDR-UPEC isolates to *E. coli* DH5α.
Conclusion: The current study concludes that drug resistant genes *bla*TEM, *bla*CTX-M and class 1 integron genes may be transmitted from donor MDR UPEC to recipient strain of *E. coli* DH5α via HGT.
Keywords: Multiple drug resistance (MDR), class 1 integron, Uropathogenic *E. coli* (UPEC), horizontal gene transfer (HGT).

Introduction

Escherichia coli is the significant cause of extra intestinal infections such as UTIs [1] and accountable for 90% community acquired as well as 50% nosocomial UTIs.[2, 3] Unfortunately the expansion of antibiotic resistance has threaten human health by the elevated rate of morbidity and mortality worldwide.[4] MDR has become a serious health concern especially in developing countries. It is responsible for 700,000 deaths per year worldwide and about 10 million people will be at danger of death by 2050.[5]

Extended spectrum beta-lactamases (ESBLs) are heterogeneous group of enzymes having more than 1,800 variants.[6] These enzymes are able to hydrolyze the monobactams, penicillins and cephalosporins antibiotics excluding cephamycins and carbapenems and suppressed by beta lactamase inhibitor e.g. clavulanic acid, tazobactam as well as sulbactam.[7] They are further categorized into TEM, SHV and CTX-M ESBLs.

Class 1 integrons are also found to be associated with multidrug resistance, because of possession of various drug resistance genes.[8, 9] The 5´ conserved segment of class 1 integron codes for site-specific recombinase gene, integrase, *intI1* and a strong promoter that warrants the expression of that integrated resistance gene cassettes. The 3´ conserved region carries *qacEΔ1* gene that is responsible for resistance to quaternary ammonium compounds (disinfectant) and *sul1* responsible for sulphonamide resistance.[10]

In Gram-negative bacteria drug resistance is commonly carried on plasmids and/or integrons which can merely be transmitted among other isolates via mechanism of transformation, conjugation or transduction.[8, 11] In Pakistan antibiotic resistance is alarming because of abuse of antibiotics along with inadequate data and the lack of systematic national surveillance program. Studies on the prevalence and resistance to antibiotics have been reported from

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Pakistan but limited data on acquisition of transferable ESBLs and class 1 integron genes emphasizes the need for further studies at molecular level. Therefore, this study sought to get understanding about the nature of drug resistance in UPEC isolates because presence of resistant determinants on plasmids or integron have been a key issue due to its role in the spread of antimicrobial resistance with special reference to ESBL producing UPEC strains.

Materials and methods

This study was performed at Department of Microbiology, University of Karachi from February to July 2019. Four UPEC strains belonging to different phylogenetic groups A (chuA-, yjaA+, TspE4.C2-), B1 (chuA-, yjaA-, TspE4.C2+), B2 (chuA+, yjaA+, TspE4.C2+) and D (chuA+, yjaA-, TspE4.C2+) according to Clermont's phylogenetic scheme [12] were selected for conjugation assay. The isolates were obtained initially from urine specimens with bacterial count ≥10^5 CFU/ml submitted to clinical laboratory of tertiary care hospital in Karachi, Pakistan for routine culture and antimicrobial susceptibility testing. The isolates were purified on MacConkey's agar, confirmed by conventional biochemical tests.[13] Antibiotic susceptibility was performed against 16 different antibiotics by disk diffusion assay [14, 15] as per the criteria specified by the Clinical Laboratory Standards Institute.

E. coli DH5α strain was evaluated for minimum inhibitory concentration (MIC) of sodium azide and continuously exposed to increasing concentration of sodium azide.[16] *E. coli* DH5α strain with MIC greater than 256µg/ml was used as recipient strain. Conjugation experiment was performed between MDR UPEC isolates (donor) and azide resistant *E. coli* DH5α (*E. coli* DH5α ^{AR}) (recipient). *E. coli* DH5α ^{AR} and four MDR isolates of UPEC were grown overnight and suspension was made in normal saline matching to density of McFarland index 0.5. Afterwards, the donor and recipient strains were mixed at a ratio of 1:3 in nutrient broth and incubated at 37˚C overnight. Transconjugants were retrieved on MacConkey's agar plates containing 100µg/ml sodium azide and 4µg/ml cefotaxime.

Transconjugants were confirmed phenotypically by disc diffusion method [14, 15] and genotypic confirmation was done by polymerase chain reaction as described elsewhere.[17, 18] Primers for ESBL (*bla* TEM, CTX-M and class 1 integron genes (*intI1, sul1* and *qacEΔ1*) are given in table 1. PCR reaction mixture was prepared by adding 12.5 µl of Promega green master mix, 1 µl of each forward and reverse primer, 0.5µl of nuclease free water and 10µl of DNA template. Amplification was performed in thermal cycler (Quanta Biotech, S-24) using cycling parameters; initial denaturation at 94□C for 5 min, followed by 30 cycles of 94□C for 1 min (denaturation), 54□C for 1 min (annealing) and 72□C for 1 min (elongation) followed by final elongation at 72□C for 5 min. PCR products were visualized on 1.5% agarose gel containing ethidium bromide. A 100bp plus DNA ladder (Thermofisher Scientific) was used as a size marker. The strains have shown previously to produce CTX-M and TEM beta-lactamase were confirmed by sequencing and used as control for respective beta-lactamase.

Table 1: Primers for amplification of class 1 integron

Gene	Primer	Primer Sequence (5′-3′)	PCR product (bp)	References
intI1	*intI1* F	GGTTCGAATGTCGTAACCGC	248	(Phongpaichit *et al.*,2006)
	intI1 R	ACGCCCTTGAGCGGAAGTATC		
sul1	*sul1* F	ATCAGACGTCGTGGATGTCG	346	
	sul1 R	CGAAGAACCGCACAATCTCG		
qacE1	*qacE1* F	GAGGGCTTTACTAAGCTTGC	200	
	qacE1 R	ATACCTACAAAGCCCCACGC		
*bla*CTX-M	CTX-M F	SCSATGTGCAGYACCAGTAA	544	(Eckert *et al.*, 2004)
	CTX-M R	CCGCRATATGRTTGGTGGTG		
*bla*TEM	TEM-F	ATGAGTATTCAACATTTCCGTG	861	(Jena *et al.*, 2017)
	TEM-R	TTACCAATGCTTAATCAGTGAG		

Results

Four MDR strains were selected for in-vitro conjugation assay that showed resistance phenotype to multiple antibiotics as described in table 2.

E. coli DH5α was susceptible to all tested antibiotics against UPEC strains (Fig 1). Initially *E. coli* DH5α showed 32µg/ml MIC against sodium azide that was successfully induced to sodium azide resistance with

MIC greater than 256µg/ml and subsequently used as recipient for conjugation assay.

Tansconjugants were recovered successfully on plates containing 100µg/ml sodium azide and 4µg/ml cefotaxime while on control plates no growth was observed for MDR-UPEC strains containing 100µg/ml sodium azide alone and for *E. coli* DH5α 4µg/ml cefotaxime alone.

All four transconjugants showed resistance phenotypes for 3rd generation cephalosporins phenotypically and showed positive genotypes of CTX-M, TEM beta-lactamases and class 1 integron genes (*intI1, sul1* and *qacEΔ1*) except for UPEC strain belonging to phylogenetic group A that was unable to transfer *intI1*gene in transconjugants (Fig. 2).

Table 2: UPEC strains of *E. coli* showing resistance to different antibiotics

Bacterial Strains	NA	OFL	CIP	LEV	ATM	CTX	CRO	FOX	AMC	SXT	CN	AK	FOS	F	IPM	CT
E. coli DH5α	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
1078	R	R	R	R	R	R	R	R	R	R	R	S	S	R	S	S
1069	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
1098	R	R	R	R	R	R	R	R	R	R	R	S	S	R	S	S
1079	R	R	R	R	R	R	R	R	R	R	R	S	S	R	S	S

NA = nalidixic Acid, OFL = ofloxacin, CIP = ciprofloxacin, LEV = levofloxacin, ATM = aztreonam, CTX = cefotaxime, CRO = ceftriaxone, FOX = cefoxitin, AMC = amoxicillin/Clavulanic acid, SXT = trimethoprim /salfamethoxazole, CN = gentamicin, AK= amikacin, FOS = Fosfomycin, F= nitrofurantoin, IMP = imipenem, CT = colistin, S= sensitive, R= resistant

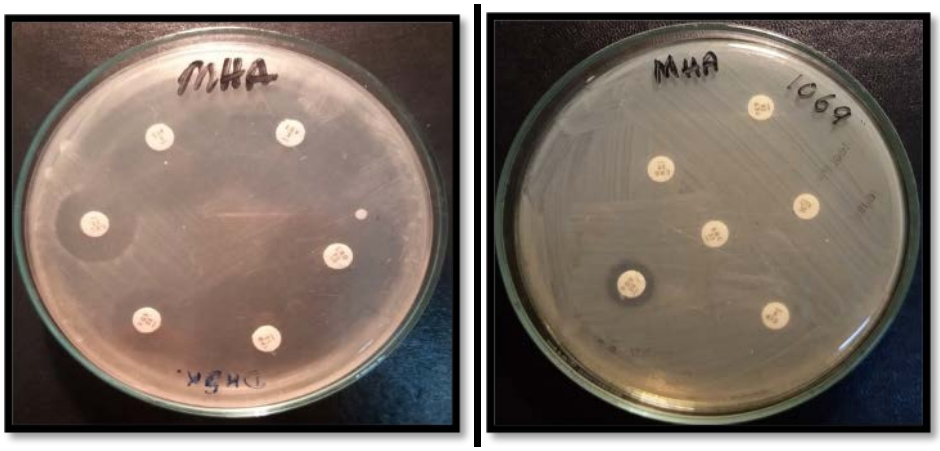

Figure 1. *E. coli* DH5α showing susceptibility to all tested antibiotics and MDR Strain (1069) showing resistance to antibiotics used in this study

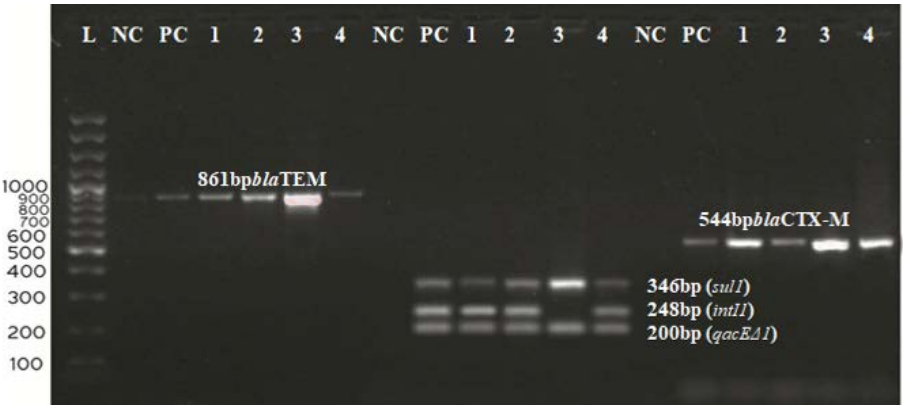

Figure2. PCR amplification of *bla* TEM, class 1 integron and *bla* CTX-M in transconjugants L=1Kb size marker, NC= negative control, PC= positive control, Lanes 1, 2, 3, 4 showed transconjugants 1098 (B2), 1069 (B1), 1078 (A) and 1079 (D) respectively.

Discussion

E. coli constitutes major invasive Gram-negative pathogens with worrisome increasing reports of multiple drug resistance and rapid spread of drug resistance. Plasmids and integrons are more frequently involved in phenomenon of HGT and the emergence of drug resistance in bacteria. To verify the acquisition and horizontal dissemination of drug resistance genes we selected 4 UPEC isolates belonging to four phylogenetic groups (A, B1, B2 and D). Interestingly, the resistance genes for beta- lactamase and class 1 integron were successfully transferred from donor (MDR UPEC) to recipient (*E. coli* DH5α) suggesting that drug resistance genes could be present on conjugative plasmid (s). Acquisition of resistance as well as virulence features provide survival benefit to bacteria that can co transfer under selective pressure of antibiotic.[19]

In current study UPEC strains have transferred resistance genes irrespective of their phylogeny. Our results agree to previous study that also reported the transfer of integron from int1+ UPEC strain to *E. coli* K12 strain via conjugation process and also reported no correlation between phylogenetic background and resistance gene transfer.[9]

Occurrence of ESBL genes together with class 1 integron genes conferring MDR features is extremely worrisome. The presence and transfer of resistant determinants irrespective of pathogenic and commensal isolates of UPEC showing commensal strains may serve as reservoir for these resistance genes as well as virulent traits.

Plasmids harbored drug resistance genes have great contribution in transfer of drug resistance that cause emergence of multiple drug resistance in pathogens moreover; plasmids also favor co-resistance to other antibiotics that ultimately results to serious consequences. Indeed, plasmids carry genetic determinants to encourage bacteria in planktonic form favours not only transfer of infectious plasmid but also resistant determinants in bacteria. This phenomenon suggests that clinically bacterial strains bearing plasmids are more prone to form biofilms that effect likelihood of risk of biofilm associated infections, dissemination of virulence factors and resistant determinants.[20]

Resistant *E. coli* strains are emerging globally as a threat to favorable outcome of common infections in community and hospital settings. These conditions particularly are increasing in developing countries that frequently use antibiotic agents in both animals and humans, where people without supervision and without prescription of physician consume antibiotics.[21,22] Inappropriate antibacterial treatment and abuse of antibiotics have contributed to the emergence of antibacterial resistance in bacteria.[23] The wide spread of multidrug resistant *E. coli* in UTIs gave an eye opening all over the world particularly in Pakistan.

Conclusion

The current study concludes that drug resistant genes ^{bla}TEM and ^{bla}CTX-M and class 1 integron genes may be transmitted from donor MDR UPEC to recipient strains of *E. coli* DH5α. This study showed significant role of HGT in rapid spread of drug resistance in UPEC strains irrespective of their phylogenetic background. This study also contributes to the existing pool of knowledge regarding HGT which indicates that horizontal transfer of resistant genes could be responsible for emergence of new antibiotic resistant bacterial strains.

Conflicts of interest

All authors declared that there is no conflict of interest.

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CONTRIBUTION OF AUTHORS:

- Saima Javed and Zaid Ahmed Pirzada contributed to the experimental work and manuscript writing.
- Saima Javed and Zulfiqar Ali Mirani contributed to data analysis.
- Saima Javed and Zaid Ahmed Pirzada contributed to study design and reviewed the article.
- All authors of this paper have read and approved the final version submitted.