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DRUG RESISTANCE AND CURLI FIMBRINATION OF *ESCHERICHIA COLI* ISOLATED FROM BANGLADESHI PATIENTS WITH URINARY TRACT INFECTIONS

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Abstract

A total of 27 isolates from patients with urinary tract infection (UTI) were screened and of these 12 were found to be *Escherichia coli*. All the *E. coli* isolates were multidrug resistant. Among the antibiotics, imipenem and polymyxin B were found to be the best, while oxacillin, cefsulodine and methicillin proved to be worst in effectiveness against the studied *E. coli* isolates. Only 42% of the *E. coli* were susceptible to trimethoprim-sulfamethoxazole (TMP–SMX), the current drug of choice for treating UTI. Middle ranged plasmids were observed in the studied isolates. Five strains expressed curli fimbriae and two elaborated cellulose; two other strains produced both curli and cellulose as extracellular matrix component.

Introduction

Community-acquired urinary tract infections (UTIs) are among the most common bacterial infections especially in women. Uropathogenic *Escherichia coli* (UPEC) strains are responsible for the majority of uncomplicated urinary tract infections.(1) The reasons for the geographic variations in prevalence of antibiotic resistance among *E. coli* causing urinary tract infections are poorly understood.(2) Therapy for these infections is usually begun before results of microbiological tests are known. Furthermore, in women with acute uncomplicated cystitis, empirical therapy without a pre-therapy urine culture is often used. Consequent to this, antimicrobial resistance among uropathogens causing community-acquired UTIs, both cystitis and pyelonephritis, is increasing.

Plasmid borne resistance profile has been a major issue because this extra-chromosomal genetic material plays a role in the spread of antimicrobial drug resistance. But the molecular epidemiologic study of these diverse and mobile elements is complex because of their replication system which dictates plasmid's behavior such as host range and copy number.(3)

UTI strains have been found to have the highest frequency of mutator strains. Mutator strains, having a defective mismatch repair system, are prone to high mutation

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rates.[4] Because most of these isolates are pathogens, it has been hypothesized that mutator and hyper recombination phenotypes may accelerate the evolution of pathogenic strains by, e.g., increasing the variation of surface antigens, as well as by facilitating the acquisition of pathogenic determinants and antibiotic resistance. It has been observed that the levels of resistance to antibiotics were significantly higher in mutator than in nonmutator pathogenic *P. aeruginosa* isolates.[5]

Adhesion to abiotic surfaces (biofilm formation) and expression of curli fimbriae and cellulose as extracellular matrix components is an important property for uropathogens. Curli fimbriae and/or cellulose are expressed by *E. coli*, *Salmonella* spp. and other *Enterobacteriaceae*.[6-7] While the contribution of cellulose to virulence is not well-known, several virulence-associated features have been assigned to curli fimbriae.[8-9]

The present study was conducted to determine antibiotic resistance profile of urine isolates of *E. coli* from urinary tract infected patients; plasmid content of the isolates were also examined to decipher whether their presence correlated with the antibiogram. The current investigation also had a goal to seek the potential of the UPEC isolates to express curli and/or cellulose as extracellular material.

Materials and Methods

Urine samples of patients with urinary tract infection were collected from a hospital in Sylhet, Bangladesh. Samples were streaked on nutrient agar and then selected on MacConkey agar. Colonies that presumptively matched with the appearance of *E. coli* on MacConkey agar were subcultured on nutrient agar. The clinical isolates were then stabbed into T_1N_1 soft agar and transported into the laboratory.

The isolates were resuscitated in nutrient agar and given laboratory codes as identification numbers. The organisms were then identified as *E. coli* following standard microbiological and biochemical methods.[10]

The susceptibility tests of all the 12 isolates to the antimicrobial agents were measured *in vitro* by the modified Kirby-Bauer disk diffusion method.[11] Commercially available antimicrobial discs (Oxoid, In Vitro Diagnosticum) used in this study were Polymyxin B (30 μg), Spectinomycin (100 μg), Oxacillin (1 μg), Ciprofloxacin (5 μg), Kanamycin (30 μg), Sulfomethoxazole/trimethoprim (25 μg), Cephalexin (30 μg), Nalidixic acid (30 μg), Cefsulodine (30 μg), Ceftizidime (30 μg), Rifampicin (30 μg), Ampicillin (10 μg), Methicillin (5 μg), Cefotaxim (30 μg), Imipenem (10 μg), and Ceftriaxone (30 μg). *E. coli* ATCC 25922 was used as control strain for susceptibility studies.

Along with scoring the clear zone for antibiotic susceptibility the strains were also checked for colonies growing inside the growth inhibition zone (squatter colonies).[4] The presence of squatter colonies reflects the high frequency of mutations conferring resistance to antibiotics.

Plasmid DNA was extracted according to the procedure as described by Birnboim and Doly[12] with slight modification. The isolated plasmid DNA thus prepared was electrophoresed in agarose gel followed by staining for 10 min in ethidium bromide (0.5 μg/ml) solution at room temperature. DNA bands were visualized and photographed using UV transilluminator (Gel Doc, Bio-Rad, USA). The molecular weight of the unknown plasmid DNA was determined on the basis of its mobility through agarose gel and was compared with the mobility of the known molecular weight plasmids (1 kb supercoiled plasmid marker, Invitrogen).

To examine whether the studied isolates express their genes for formation of curli fimbriae, the cells were inoculated on Congo red (CR) medium (composition: 1% casamino acids, 0.15% yeast extract, 0.005% $MgSO_4$ and 2% agar with 0.004% congo red plus 0.001% coomassie blue).[13-14] After incubation at 28ºC for 48 hrs colony morphology was inspected on CR plates. Colony morphologies on CR plates were scored according to the basic morphotypes[15] previously detected in *S. typhimurium*: these were categorized as, rdar (violet colony, expresses curli fimbriae and cellulose), pdar (pink colony, expresses cellulose), bdar (brown colony, expresses curli fimbriae) and saw (no expression of curli fimbriae or cellulose).

Results and Discussion

High potential for developing drug resistance among pathogenic isolates of *E. coli* has been reported earlier.[3] The clinical isolates of *E. coli* are continuously exposed to the hospital environment where they acquire resistance to numerous antibiotics. Resistance properties accrue through different routes, such as natural or intrinsic resistance (inaccessibility of the target, multidrug efflux systems and drug inactivation), mutational change, and attainment of extra-chromosomal materials. Thus intensive analysis of several factors especially molecular characterization is required.

In the present study, 12 strains were identified as *E. coli* from 27 urine specimens collected from urinary tract infected patients. Of the 12 isolates, all were found to be resistant to oxacillin, cefsulodine and methicillin. Trimethoprim-sulfamethoxazole (TMP–SMX), the current drug of choice[16] for treatment of acute uncomplicated cystitis in women was found to be effective against only 42% of the isolates. The most successful drugs appeared in this study were polymyxin B and imipenem (sensitivity 100%) (Fig.1). Ceftazidime, cefotaxim and ceftriaxone were found to be the next most efficient drug in this investigation. All of the studied isolates were found to be multidrug resistant. Among the isolates, MCL 15, 27, 28, 29 and 30 were resistant to ≥ 10 of the 15 antibiotics (Table 1).

Almost all of the strains contained plasmids of mid-range sizes (Fig. 2) which could be easily transferred between bacterial floras; if such transferrable elements carry the resistance (R) factor, drug resistance can spread quickly. However, we found no

correlation between the presence of plasmids and the number of antibiotic resistance among the isolates. Plasmid curing from the resistance isolates could be done in future to track whether the antibiotic resistance property was retained or not in the cured strain. Additionally, each plasmid could be transferred in a plasmid-free bacterium to see whether the transformed bacterium shows resistance against any specific antibiotic or not.

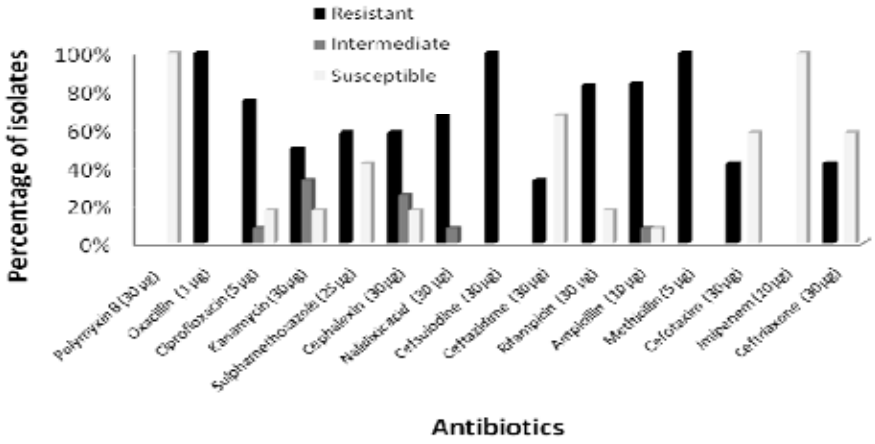

Fig. 1. Antimicrobial resistance of the studied UPEC isolates.

Table 1. Antibiotic susceptibility and presence of squatter colonies in inhibition zone.

Isolate	No. of antibiotics			Squatter colonies found in inhibition zones
	Resistant to	Intermediate to	Sensitive to	
MCL 2B	7	2	6	CL, RD
MCL 4A	5	2	8	CL, RD, AMP, CAZ, CTX
MCL 7	7	0	8	SH, CFS
MCL 10B	8	1	6	CFS, AMP
MCL 13	9	1	5	CL, CFS, CRO
MCL 14	8	2	5	None
MCL 15	13	0	2	CAZ
MCL 26B	7	1	7	CTX
MCL 27	11	0	4	CTX
MCL 28	13	0	2	None
MCL 29	10	1	4	None
MCL 30	12	0	3	None

Presence of squatter colonies inside the growth inhibition zone (around fosfomycin or rifampicin disc) has been used to differentiate between mutator and nonmutator strains.[17] Denamur[4] reported the exhibition of colonies inside the growth inhibition

zone (squatter colonies) by the majority of mutator strains, but not by nonmutator strains. However the same authors[17-18] reported the absence of the correlation between high mutation rates and antibiotic resistance; they explained this by the dynamics of selection and counter-selection of mutator alleles. In our study we observed squatter colonies inside the growth inhibition zone around different antibiotic discs in cases where isolates were resistant to lower number of antibiotics (Table 1).

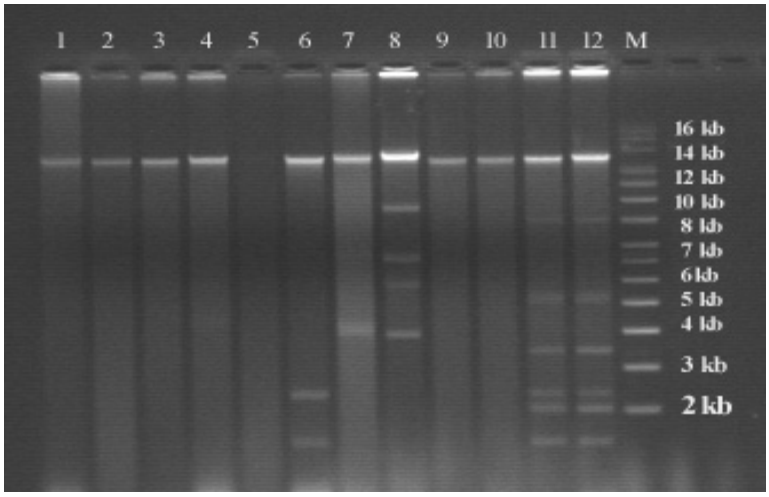

Fig. 2. Agarose gel electrophoresis of plasmid DNA from the studied uropathogenic *E. coli* isolates. Lane: M, supercoiled plasmid marker; 1, MCL 2B; 2, MCL 4A; 3, MCL 7; 4, MCL 10B; 5, MCL 13; 6, MCL 14; 7, MCL 15; 8, MCL 26B; 9, MCL 27; 10, MCL 28; 11, MCL 29; 12, MCL 30.

In the setting of urinary tract with many unfavorable factors along with frequent urine flushing, colonization and long-term persistence are advantageous to the uropathogenic organisms. Extracellular matrix components play important role in such ability of these bacteria. Adhesins can contribute to virulence by promotion of colonization, invasion and replication within uroepithelial cells. Tiba *et al.* [19] reported that the virulent strains of UPEC that cause cystitis typically produce, at least, one adhesion system.

We investigated curli and/or cellulose expression and observed that nine out of 12 (75%) isolates expressed at least one of the biofilm extracellular matrix components (Table 2). The other three isolates, which were negative for expression in both components might possess systems for other adhesions or might not have expressed these components under the present experimental condition. When considered individually, seven of the 12 expressed curli fimbriae and four of the 12 elaborated cellulose as the extracellular material. Beforehand, cellulose was not known to contribute to the pathogenic behavior; but curli fimbriae have been ascribed to have relation with different

virulent-associated features. However, a concrete idea about the role of curli fimbriae in pathogenicity is still not clear.[15]

Table 2. Expression of curli fimbriae in uropathogenic *E. coli* isolated from UTI patients.

Isolate	Color (morphotype)	Expression	
		Curli	Cellulose
MCL 2B	Violet (rdar)	+	+
MCL 4A	Brown (bdar)	+	-
MCL 7	Pink (pdar)	-	+
MCL 10B	Grayish white (saw)	-	-
MCL 13	Brown (bdar)	+	-
MCL 14	Brown (bdar)	+	-
MCL 15	Violet (rdar)	+	+
MCL 26B	Brown (bdar)	+	-
MCL 27	Brown (bdar)	+	-
MCL 28	Pink (pdar)	-	+
MCL 29	Grayish white (saw)	-	-
MCL 30	Grayish white (saw)	-	-

Only two antibiotics were found effective, while the rest were found to be ineffective under the present study condition. The abuse of antibiotics may be responsible for the occurrence of drug resistance trait among these pathogens. The squatter colonies may be of interest in determining which mutation contributed to the drug resistance property. The expression of curli and/or cellulose is also of interest in elucidating the mechanism of biofilm formation which increases the capacity of the organisms to survive in a hostile environment.

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