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Osmotolerance studies of uropathogenic *Escherichia coli*

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ABSTRACT

E.coli isolates from urine samples of patients suffering from Urinary Tract Infections caused by *E.coli* was collected from various private diagnostic centers in Davangere, Karnataka, India. Isolates were cultured and maintained using specific media. Urea and Salinity tolerance of samples was checked by observing bacterial growth in artificial urine with varying urea and salt concentrations. Biochemical assays were carried out to identify virulence factors of Uropathogenic *E.coli* in samples showing high salt and urea tolerance.

KEYWORDS

UPEC;
Osmotolerance studies;
Biochemical tests.

INTRODUCTION

E.coli is the head of the large bacterial family, *Enterobacteriaceae*, the enteric bacteria, which are facultative anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease. The *Enterobacteriaceae* are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella, Shigella, Yersinia*). Several others are normal colonists of the human gastrointestinal tract (e.g. *Escherichia*, *Enterobacter*, *Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of humans.

Of the different organisms known to cause Urinary Tract Infections(UTI), Escherichia coli is the most predominant pathogen being isolated in 70-90% of cases. It has been accepted that UTI caused by *E.co*li is an ascending infection caused by the strains originating in the intestinal tract, because a high similarity exists between *E.coli* strains from urine and faeces of infected individuals.

Uropathogenic *E. coli* cause 90% of the urinary tract infections (UTI) in anatomically-normal, unobstructed urinary tracts. The bacteria colonize from the feces or perineal region and ascend the urinary tract through the bladder. With the aid of specific adhesins they are able to colonize the bladder.

Different virulence factors of *E.coli* which are thought to have a role in the pathogenesis of Urinary Tract Infections, some of them are O Antigens, K Antigens, Serum resistance, Adhesins, Colicins, Invasins etc.

Many studies (Fowler et al 1977, Kallenius et.al 1978) have shown that bacterial adherence is an essential virulence factor in the pathogenesis of community acquired Urinary Tract Infections. Duguid et al (1966) studied fimbriae of *E.coli* in great detail and classified them into three groups depending upon their haemagglutinating properties as the MSHA(Mannose Sensitive Haemagglutinating) type, MRHA(Mannose

Resistant Haemagglutinating) type and non-fimbrial haemagglutinin type.There is considerable evidence to support the use of cranberries for the prevention of urinary tract infections (Bodel et al., 1959; Moen, 1962; Sternlieb,1963; Papas et al., 1968; Avorn et al., 1994).

Colicins belong to a general class of natural antimicrobials called bacteriocins. These bacteriocins may be defined as the antimicrobial substances or complexes of antibiotic substances, which are highly specific and are produced by certain strains of intestinal bacteria act upon other related strains i.e. same or related species .

To assign *E.coli* from significant bacteriuria cases as 'uropathogenic' is not possible because there is no specific identification test. The virulence factors are several and each UTI-pathogen may possess any or all of these virulence factors. From another angle, the primary requirement of *E.coli* causing UTI is to be able to multiply in urine, under the prevailing urea and salt concentrations. Also, it is now known that they possess osmoregulatory genes that may play a role in countering these conditions. Our objective therefore, was to find out whether the ability of UTI causing *E.coli* to survive and multiply in urine is a property unique to them and if so, whether this itself could become the test for identifying them as uropathogens.

MATERIAL AND METHODS

Urine samples of patients suspected to be suffering from Urinary Tract infection was collected from various private diagnostic centers in Davangere, Karnataka, India. These samples were then swabbed on Mac conkey's media . Media was left for incubation period of 24 hours at 35^0C. Since this is a selective media for *E.coli*, it will inhibit the growth of other form of bacteria and supports the growth of only *E.coli* strains. This procedure was employed for selection of *E.coli* strains. Strains were labelled for convenience.

Osmotolerance studies

Various molar concentration solutions of Urea and NaCl were prepared in nutrient broth and 25 ml of each sample were taken in separate conical flasks. Starter culture of each strain prepared initially with overnight incubation of few colonies of *E.coli* in nutrient broth .2 % of starter culture was inoculated onto various molar concentration of urea and NaCl prepared in nutrient broth. Growth of *E.coli* in various concentrations (15.625Mm-1M) of NaCl and urea was noted by measuring absorbance of the samples at 600nm. E.coli K-12 and MTCC –729 were used as positive and negative controls

Biochemical assays

Following Biochemical assays were carried out to identify certain virulence factors.

Salt aggregation test (SAT) for detection of adhesins

This test was performed as described by lindhal et al. (1981). The test strains have to be grown on CFA-agar plates at 37^0/18 hrs to enhance the production of fimbrial antigens. 4M Solution of $(NH_4)_2 SO_4$ was prepared in 0.002M sodium phosphate ($Na_2 PO_4$) buffer solution pH 8.6. Two fold dilutions of ammonium sulphate with sodium phosphate buffer solution was done to obtain 2M, 1M, 0.5M, 0.25M and 0.125 dilutions.

One drop of each dilution over (from 4M, 2M, 1M, 0.5M, 0.25M upto 0.125M) was taken over a clean glass slide. The bacterial growth from the growth of the test strain was taken from CFA agar plate (after incubation at 37^0C for 18 hours). One drop (50 ul) of each type of 1% PBC was taken separately for MHA and MSHA activity and the growth was emulsified in it and then examined for 1 minute by gently rotating the suspension over the slide. Positive and negative controls were also put simultaneously to compare the results of test strains. *E.coli* K-12 and MTCC –729 were used as positive and negative controls for the presence and absence of CFAI.

The interpretation of the results was made as follows:

1. Mannose resistant haemagglutination (MRHA) (If HA occurred in presence of 2% D-mannose).
2. Mannose sensitive haemagglutination (MSHA) if agglutination of RBCs occurred in absence of mannose but was inhibited in the presence of D-mannose.
3. No haemagglutination-if there is no agglutination in both.

Hemolysin production

Hemolysin production by *E.coli* strains can be detected using 2 methods:

1. Tube method and

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2. Plate method

RBCs of different species which were Human type A (Hu), Sheep (Sp), Guinea-pig (Gp).

Tube method was used for experimentation.

Tube method

This test was done as described by Feeley and Pittman (1963), with suitable modifications. E.coli test strains were inoculated in the meat extract broth (Difco) (pH 7.4) 0.4 ml per tube. Each test strain was inoculated in the 3 tubes to test against the RBCs from 3 different species. The tubes were incubated at 37^0C for 24 hrs and 1% suspension of the RBCs (0.4 ml/ tube) of different types was added to the respective tubes. These were again incubated at 37^0C for 2 hrs and examined for the lysis of RBCs. Then without disturbing, the tubes were kept in the cold room at 4^0C overnight to observe for hot and cold phenomenon and for further lysis of RBCs.

Test for colicin production

This test was performed as described by frederiocq (1951) using colicin agar. The plates were incubated at 37^0C/48 hrs by wrapping the plates in polythene bag. After incubation, the plates were exposed to Chloroform for 10-15 minutes to kill the bacterial growth. The semisolid agar gel (0.5% noble agar) was prepared and 4 drops (0.2 ml) of overnight broth culture of *E.coli* K12 colicin sensitive strains were added to 8 ml of this agar gel while it was still molten (45^0- 50^0C). This was mixed thoroughly and overlayed on the surface of 'Chloroform treated' plates. The agar was allowed to set and reincu bated at 37^0C/24 hours. This sensitive strain was examined for zone of inhibition by the test strains. Clear zone of inhibition indicated the production of colicin.

RESULTS

Note:- Samples were labeled with numbers for convenience.

Osmotolerance studies were carried out in batches

TABLE 1

Batch 1 : Growth of *E.coli* in various concentration of NaCl noted by measuring the absorbance of the samples at 600nm

Strains	1M	500mM	250mM	125mM	62.5mM	31.25mM	15.625mM
K12	0.112	0.194	0.287	0.364	0.472	0.541	0.612
MTCC-729	0.051	0.101	0.152	0.210	0.278	0.341	0.374
1405	0.064	0.112	0.156	0.210	0.286	0.310	0.350
558	0.089	0.124	0.189	0.210	0.265	0.301	0.320
CD1	0.041	0.101	0.134	0.200	0.274	0.300	0.340
Y1	0.062	0.104	0.142	0.190	0.246	0.290	0.310
552	0.101	0.184	0.262	0.350	0.452	0.530	0.660
553	0.090	0.172	0.24`	0.340	0.421	0.500	0.650
526	0.032	0.110	0.132	0.210	0.246	0.310	0.320
540	0.051	0.110	0.131	0.210	0.230	0.270	0.300
522	0.110	0.161	0.251	0.320	0.414	0.541	0.610
533	0.101	0.142	0.240	0.310	0.400	0.550	0.590
567	0.070	0.120	0.160	0.210	0.230	0.283	0.310
1397	0.056	0.100	0.140	0.180	0.210	0.258	0.320

Batch 2

Strains	1M	500mM	250mM	125mM	62.5mM	31.25mM	15.625Mm
K12	0.121	0.182	0.264	0.334	0.461	0.521	0.562
MTCC-729	0.0410	0.190	0.142	0.201	0.261	0.321	0.374
555	0.080	0.162	0.212	0.331	0.412	0.510	0.550
571	0.060	0.110	0.167	0.210	0.250	0.290	0.340
579	0.410	0.121	0.171	0.212	0.260	0.294	0.328
697	0.101	0.150	0.210	0.313	0.421	0.550	0.602
702	0.099	0.172	0.230	0.285	0.340	0.410	0.501
1370	0.061	0.121	0.170	0.213	0.241	0.311	0.354
1371	0.082	0.182	0.241	0.322	0.413	0.521	0.584

E.coli with Samples numbers 522,533,552,553, 555,697,702 have shown growth rates even in higher

TABLE 2

Batch 1: Growth of *E.coli* in various concentration of urea noted by measuring the absorbance of the samples at 600nm

Strains	1M	500mM	250mM	125mM	62.5mM	31.25mM	15.625mM
K12	0.130	0.201	0.290	0.355	0.497	0.553	0.672
MTCC-729	0.051	0.102	0.163	0.221	0.280	0.354	0.411
1405	0.042	0.122	0.143	0.211	0.276	0.344	0.393
558	0.061	0.109	0.155	0.234	0.281	0.322	0.305
CD1	0.052	0.111	0.132	0.223	0.264	0.311	0.389
Y1	0.049	0.125	0.161	0.233	0.258	0.291	0.322
552	0.117	0.215	0.279	0.351	0.465	0.514	0.620
553	0.101	0.211	0.285	0.324	0.447	0.531	0.592
526	0.038	0.103	0.153	0.200	0.266	0.332	0.494
540	0.049	0.112	0.144	0.186	0.211	0.267	0.291
Batch 2							
Strains	**1M**	**500mM**	**250mM**	**125mM**	**62.5mM**	**31.25mM**	**15.625mM**
K12	0.110	0,215	0.265	0.321	0.514	0.557	0.609
MTCC-729	0.044	0.113	0.172	0.233	0.285	0.336	0.390
697	0.123	0.211	0.246	0.319	0.522	0.589	0.616
702	0.148	0.206	0.241	0.312	0.511	0.574	0.596
1370	0.056	0.099	0.143	0.176	0.234	0.296	0.301
1371	0.062	0.101	0.133	0.169	0.210	0.234	0.287
555	0.114	0.218	0.276	0.321	0.552	0.548	0.613
571	0.032	0.095	0.114	0.158	0.211	0.256	0.302
579	0.041	0.074	0.110	0.137	0.165	0.193	0.245
522	0.123	0.211	0.286	0.345	0.477	0.529	0.663
533	0.116	0.200	0.293	0.331	0.485	0.543	0.667
567	0.032	0.089	0.124	0.167	0.201	0.233	0.257
1397	0.062	0.161	0.136	0.189	0.211	0.285	0.332

concentration of salt and urea.

Results of biochemical tests

Test	Samples showing positive result
Hemolysin Test	553,697
Test for colicin production	552,702
Salt Aggregation Test (SAT)	555,522,533

CONCLUSION

We have used Urea and salt in the concentration range corresponding to normal physiological levels in our experiments. We have seen a dose-dependent decrease in growth of the *E.coli* tested concomitant with increasing concentration of Urea/salt indicating sensitivity. However, we see that they are able to survive at concentrations of Urea which represents normal levels in urine. We believe that this approach warrants further work and would yield a rapid method of differentiating Uropathogenic bacteria.

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