Characterization of Some Virulence Factors Associated with Enterbacteriaceae Isolated From Urinary Tract Infections in Mansoura Hospitals

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ABSTRACT

Purpose: The aim of the present study was to determine the role of some virulence determinants and biofilm production in bacteraemia of urinary tract origin by phenotypic detection of these virulence factors. In addition, this research was done to characterize and compare, using genetic techniques, bacterial genes that encode virulence determinants. Methods: A total of 111 strains of Enterobacteriaceae isolated from urine of patients with clinically diagnosed urinary tract infection in Urology and Nephrology Center, Mansoura University, were included in this study. The isolated strains were phenotypically screened for virulence factors, namely mannose-resistant, mannose-sensitive haemagglutination (MRHA, MSHA), hemolysin production, biofilm formation, serum resistance, and lipase, protease and lecithinase production. They were also genotypically examined by PCR for the presence of genes for the virulence factors MRHA (papC), MSHA (fimH), serum resistance (iss) and biofilm formation (biofilm). Results: Among 111 urinary isolates of Enterobacteriaceae, 40 isolates of E. coli, 39 isolates of K. pneumonia and 32 isolates of Enterobacter were identified. The antibiotic sensitivity test showed that amikacin and imipinem were the most active antibiotic against all isolates (90%-100%). While most isolates were resistant to ampicillin (95%-100%). The phenotypic detection results revealed that 29(72%) of E. coli, 32(82%) of K. pneumoniae and 22(69%) of Enterobacter isolates showed MRHA. MSHA was detected in 11(28%) isolates of E. coli, 7(18%) of K. pneumoniae and in 10(31%) of Enterobacter isolates. Serum resistance was seen in 15(37%) of E. coli, 13(33%) of K. pneumoniae and 7(21%) of Enterobacter isolates. Biofilm formation was observed in 27(67%) of both E. coli and K. pneumoniae and in 30(94%) of Enterobacter isolates. All E. coli isolates were negative lipase and protease producers, while 16(33%) of K. pneumoniae isolates showed lipase production and one isolate showed protease production. For Enterobacter, none of isolates produce lipase, while 4(12%) of isolates were protease producers. All isolates showed no lecithinase production. For genotypic detection, it was observed that among E. coli isolates, 69% were positive for papC gene, 31% were positive for fimH gene, 30% were positive for iss gene and 77% were positive for biofilm gene. For K. pneumoniae, papC gene was detected in 80% of isolates, 20% were positive for fimH gene, 8% were positive for iss gene and 85% contained the biofilm gene. Results of Enterobacter isolates were 89%, 11%, 5% and 95% positive for genes of papC, fimH, iss and biofilm respectively. Conclusion: The present study shows the significance role of virulence factors in urinary tract infections caused by Enterobacteriaceae and the genotypic characterization was more prominent compared to the phenotypic detection of these virulence factors.

INTRODUCTION

Urinary tract infections (UTI) are among the most common human infections and may have serious sequelae. Most UTI are caused by members of the bacterial family Enterobacteriaceae. Urinary tract infections (UTI) are probably the most common bacterial infections(1). Under normal circumstances, these bacteria are cleared from the urinary system by effective protective mechanisms. If, however, they overcome these mechanisms, they can colonize the lower urinary tract. Subsequent progress is determined by the host susceptibility and bacterial virulence factors. Manifestations can vary from asymptomatic bacteriuria to symptomatic cystitis, pyelonephritis and bloodstream infection(2).

The family Enterobacteriaceae is composed of a large number of closely related bacterial species that inhabit the large bowel of man and animals, soil, water, and decaying matter. Because of their normal habitat in man, they have often been referred to as the “enteric bacilli”. The organisms of this family are responsible for the majority of nosocomial infections, causing urinary tract and wound infections, pneumonia, meningitis and septicemia(3).
Virulence (from the Latin word for poisonous) is defined as the ability of an organism to cause disease in a particular host. Virulence results from the cumulative impact of one or several special properties, or virulence factors (VFs), which serve to distinguish potential pathogens from harmless intestinal strains. The virulence factors include different adhesins, hemolysin production, serum resistance and biofilm formation.

Furthermore, it is possible that the virulence genotypes and phylogenetic background of Enterobacteriaceae differs in different geographical regions. While some previous studies have shown that bacteremia in adults is caused by E. coli strains predominantly from phylogroups B2 followed by D, A and B1, demonstrated a predominance of strains of the D phylogroup. The authors postulated that the relative predominance of different phylogroups may be subject to geographical variations. In addition, fully pathogenic group D strains, such as those of serotype O15:K52:H1, in certain geographical areas are endemic and exhibit an increasing involvement in bloodstream infections. Therefore, the characteristics of isolates from various regions need to be assessed.

The present study shows the role of virulence factors in urinary tract infections caused by Enterobacteriaceae and comparing between phenotypic and genotypic characterization of these virulence factors.

MATERIALS & METHODS

I- Clinical strains:
Forty isolates of E. coli, thirty-nine isolates of K. pneumonia and thirty-two isolates of Enterobacter were isolated and identified from Urology and Nephrology Center (UNC), Mansoura, Dakahlia governorate, Egypt. All the bacterial isolates were obtained from urine clinical specimens. The specimens were processed immediately using standard procedures and were identified according to Barrow and Feltham, 1993 and Collee et al., 1996.

II- Antibiotic Susceptibility Testing:
The antimicrobial susceptibility tests of the identified bacterial isolates were determined according the Kirby-Bauer disc diffusion test and the following drugs were tested: ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftazidime, cefotaxime, imipenem, amikacin, gentamicin, netilmicin, tobramycin, ciprofloxacin, norfloxacin, ofloxacin, nitrofurantoin and trimethoprim/sulphamethoxazole. The results were interpreted as susceptible, intermediate, or resistant, based on the criteria listed in the National Committee for Clinical Laboratory Standards.

III- Determination of the Minimum Inhibitory Concentrations (MICs):
The MICs of the selected antibiotics were made by using two fold serial dilution methods in nutrient broth. The MIC value was calculated as the lowest concentration of the tested agent that inhibits any visible growth of the organism. Control experiments were simultaneously carried out.

IV- Phenotypic Detection of Virulence Factors:
1) Plate Hemolysis
Isolates were tested for the production of a hemolytic phenotype on blood agar plates containing 5% (vol/vol) blood. Production of hemolysis was read after overnight incubation at 37°C.

2) Haemagglutination
The haemagglutination was detected by clumping of erythrocytes by fimbriae of bacteria in the presence of D-mannose. This test was carried out as in the direct bacterial haemagglutination test – slide method. Isolates were inoculated into 1% nutrient broth and incubated at 37°C for 48 hours for full fimbriation. A panel of red blood cells was selected by obtaining blood from human (blood group ‘O’). The red blood cells were then washed three times in normal saline and made up to a 3% suspension in fresh saline. They were used immediately or within a week when stored at 3-5°C. On a glass slide, one drop of the RBC suspension was added to a drop of the broth culture and slide was rocked at room temperature for 5 minutes. Presence of clumping was taken as positive for haemagglutination. Mannose-sensitive haemagglutination was detected by the absence of haemagglutination in a parallel set of test in which a drop of 2% w/v D-mannose was added to the red cells and a drop of broth culture. Mannose-resistant haemagglutination was detected by the presence of haemagglutination of 3% ‘O’ group human RBC suspension in the presence of 2% D-mannose.

3) Serum Resistance
Serum resistance was analyzed using a turbid metric assay. One hundred and fifty microliters of serum was mixed with 50 µl of bacterial suspension in a 96 well microplate. Each isolate was tested in duplicate and negative controls (0.9% NaCl instead of serum) were included. The initial absorbance at 620nm...
**RESULTS**

I- Antibiotic Susceptibility Testing:

I- Antibiotic Susceptibility Testing: Table (2) represents the antibiotic susceptibility pattern of the three isolates *E.coli*, *K.pneumoniae* and *Enterobacter*. It was shown that most isolates were sensitive to amikacin and imipenem (90%-100%). Maximum resistance was recorded for ampicillin 95% for *E. coli* and 100% for both *K. pneumonia* and *Enterobacter*. The range of sensitivity of amoxicillin/clavulanic acid against all isolates was (45%-82%) while for cephalosporines (cefoxitin, cefazidime, and cefotaxime) was (35%-62%). Aminoglycosides (gentamicin, netilmicin, and tobramycin) were more active

was measured, and compared with the absorbance after 3 hours of incubation, using a micro plate reader. The final absorbance was determined as the average of the two replicates, and the percentage remaining absorbance relative to the initial absorbance was calculated. If the remaining absorbance after 3 hours (OD620, 3 h) was higher than 100% (relative to the initial absorbance), isolates were designated serum resistant and less than 100% were considered sensitive\(^{(14)}\).

4) Biofilm Formation

A qualitative assessment of biofilm formation was determined by the tube method\(^{(18)}\). TSBglu (10mL) was inoculated with loopful of microorganism from overnight culture plates and incubated for 24 hours at 37°C. The tubes were decanted and washed with PBS (pH 7.3) and dried. Dried tubes were stained with crystal violet (0.1%). Excess stain was removed and tubes were washed with deionized water. Tubes were then dried in inverted position and observed for biofilm formation. Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation. Tubes were examined and the amount of biofilm formation was scored as 0-absent, 1-weak, 2-moderate or 3-strong.

5) Lipase Production

Isolates were spotted on Tween 80 agar as a substrate at a final concentration of 1% and were incubated at 37°C until 7 days. An opaque (precipitation) zone around zone was registered as positive reaction\(^{(12)}\).

6) Protease Production

Protease production was determined using 15% soluble casein agar as a substrate according to Panus *et al.*, 2008 (12). After incubation at 37°C for 24 hr., a clearing zone surrounding the growth indicated casein proteolysis.

7) Lecithinase Production

The cultures were spotted into 2,5% yolk agar and incubated at 37°C for 7 days. An opaque (precipitation) zone around zone was registered as the lecithinase production\(^{(12)}\).

V- Genotypic Detection of Virulence Factors:

1) Genomic DNA Extraction

The genomic DNA of 65 isolates (22 *E.coli*, 24 *Klebsiella* and 19 *Enterobacter*) were prepared using QIA amp® DNA mini kit Cat. no. 51304 supplied by Qiagen Inc. according to the manufacturer's instructions for bacteria 2003. DNA was eluted by adding 50 µl Qiagen EB buffer (10 mM Tris-Hcl, pH 8.5) and visualized by electrophoresis on horizontal gels containing 1% agarose and Fermentas 100-bp plus DNA ladder.

2) PCR Detection of Specific Gene Sequences

The primers used in this study were specifically designed before in Biodiversity Institute of Ontario, University of Guelph, Canada (Table 1). Amplification was performed in a total volume of 25 µl containing 1µl DNA extract, 1µl of forward primer (10 µM), 1 µl of reverse primer (10 µM), 12.5 µl of DreamTaq™ Green PCR master mix and 9.5 µl of nuclease-free water. PCR was performed in Mastercycler egradient S thermacycler (Epindorf, Mississauga, ON, Canada) including a negative control reaction with each primer set.

PCR detection of *pap*-specific gene sequences (328-bp product) was performed with primers *pap1* and *pap2* selected from the *papC* gene sequence. PCR was performed at 94°C for 1 min, followed by 30 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 5 min.

PCR detection of *fimH*-specific gene sequences (640-bp product) and *iss*-specific gene sequences (260-bp product) were performed at 94°C for 2min, followed by 40 cycles of 94°C for 40 sec, 50°C for 1min and 72°C for 1min, followed by final extension of 72°C for 5min.

Amplification cycling of *Biofilm*-specific gene sequences (225-bp product) was performed as follows, primary initial activation step 94°C for 2min, followed by 35 cycles of 94°C for 40 sec, 48°C for 1min and 72°C for 1min, followed by final extension of 72°C for 5min. The generated amplicons were visualized on 1.5% agarose gel electrophoresis stained with ethidium bromide and illuminated under UV transilluminator.

I- Antibiotic Susceptibility Testing:

Table (2) represents the antibiotic susceptibility pattern of the three isolates *E.coli*, *K.pneumoniae* and *Enterobacter*. It was shown that most isolates were sensitive to amikacin and imipenem (90%-100%). Maximum resistance was recorded for ampicillin 95% for *E. coli* and 100% for both *K. pneumonia* and *Enterobacter*. The range of sensitivity of amoxicillin/clavulanic acid against all isolates was (45%-82%) while for cephalosporines (cefoxitin, cefazidime, and cefotaxime) was (35%-62%). Aminoglycosides (gentamicin, netilmicin, and tobramycin) were more active...
against isolated strains (55%-71%) than quinolones (ciprofloxacin, norfloxacin and ofloxacin) with range of (32%-67%). The range of nitrofurantoin was (25%-67%) and for trimethoprim/sulphamethoxazole was (25%-31%).

Table (1): PCR primers and conditions for amplification of virulence genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Oligonucleotide sequence (5’-3’)</th>
<th>Amplicon size (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap C</td>
<td>F</td>
<td>GACGGGCTGTACTGCAGGGTGCG</td>
<td>328</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>ATATCTTTTCTGCAAGGATGCAATA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fim H</td>
<td>F</td>
<td>TACTGCTGATGAGGCTGGTGC</td>
<td>640</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GCCGAGAGGTAAATACCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iss</td>
<td>F</td>
<td>GGCAATGTCTTATTACAGGATGTGC</td>
<td>260</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GAGCAATATACCCGGGGCTTCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biofilm</td>
<td>F</td>
<td>GATTCGAATTTGCGATTCCCTGC</td>
<td>225</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TAAATGAAGTCATTCAGACTCATCC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A total of 111 isolates of Enterobacteriaceae were isolated from specimens collected from urinary tract infections. These were investigated for their possession of virulence factors such as hemolysin, haemagglutination, serum resistance, biofilm formation and enzyme production (lipase, protease and lecithinase) (Table 3).

The most common virulence factor identified was biofilm formation in 27(67%) of E.coli and K.pneumoniae and in 30(94%) of Enterobacter isolates.

In the present study, few isolates produced hemolysin. It was observed in 7(17%) of E.coli, 3(7%) of K.pneumoniae and 6(18%) of Enterobacter isolates.

Twenty nine (72%) isolates of E.coli, 32 (83%) of K.pneumoniae and 22 (69%) of Enterobacter isolates showed serum resistance.

Serum resistance was seen in 15(37%) of E.coli, 13(33%) of K.pneumoniae and 7(21%) of Enterobacter isolates.

All E.coli isolates were negative lipase and protease producers, while 16(33%) of K.pneumoniae isolates showed lipase production and one isolate showed protease production. For Enterobacter, none of isolates produce lipase, while 4(12%) of isolates were protease producers. All isolates showed no lecithinase production. It was observed that the isolates scarcely expressed soluble enzymatic virulence factors.

III- Genotypic detection of virulence factors:

PapC, fimH, iss and biofilm genes were amplified from genomic DNA of 65 isolates (22 E.coli, 24 K.pneumoniae and 19 Enterobacter). PCR detection of papC, biofilm gene showed a 100% correlation between the results of phenotypic and genotypic detection in all isolates (Fig. 1, 4)

Detection of fimH gene showed also 100% correlation with the phenotypic detection in E.coli, 70 % of K.pneumoniae and 40% of Enterobacter isolates were positive for fimH gene (Fig. 2).

Iss genes were found less frequently than other genes, where 54%, 15% and 16% were positive for E. coli, K. pneumoniae and Enterobacter respectively (Fig. 3).
Table (2) Antibiotic susceptibility pattern

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
<th>Enterobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td>Number (%)</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Ampicillin (AM)</td>
<td>2 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid (AMC)</td>
<td>5 (12)</td>
<td>24 (61)</td>
<td>11 (34)</td>
<td></td>
</tr>
<tr>
<td>Cefoxitin (FOX)</td>
<td>18 (45)</td>
<td>32 (82)</td>
<td>14 (43)</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime (CAZ)</td>
<td>14 (35)</td>
<td>18 (46)</td>
<td>17 (53)</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime (CTX)</td>
<td>14 (35)</td>
<td>16 (41)</td>
<td>16 (50)</td>
<td></td>
</tr>
<tr>
<td>Imipinem (IMP)</td>
<td>40 (100)</td>
<td>39 (100)</td>
<td>27 (84)</td>
<td></td>
</tr>
<tr>
<td>Amikacin (AK)</td>
<td>40 (100)</td>
<td>39 (100)</td>
<td>29 (90)</td>
<td></td>
</tr>
<tr>
<td>Gentamicin (CN)</td>
<td>25 (62)</td>
<td>27 (69)</td>
<td>20 (62)</td>
<td></td>
</tr>
<tr>
<td>Netilmicin (NET)</td>
<td>22 (55)</td>
<td>28 (71)</td>
<td>18 (56)</td>
<td></td>
</tr>
<tr>
<td>Tobramycin (TOB)</td>
<td>17 (42)</td>
<td>27 (69)</td>
<td>16 (50)</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>11 (27)</td>
<td>24 (61)</td>
<td>16 (50)</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin (NOR)</td>
<td>13 (32)</td>
<td>21 (53)</td>
<td>17 (53)</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin (OFX)</td>
<td>13 (32)</td>
<td>22 (56)</td>
<td>17 (53)</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin (F)</td>
<td>27 (67)</td>
<td>10 (25)</td>
<td>10 (31)</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim/sulphamethoxazole (SXT)</td>
<td>10 (25)</td>
<td>11 (28)</td>
<td>10 (31)</td>
<td></td>
</tr>
</tbody>
</table>

Table (3) Phenotypic detection of virulence factors

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number (%) of organism</th>
<th>Hemolysin positive</th>
<th>MRHA</th>
<th>MSHA</th>
<th>Serum resistant</th>
<th>Biofilm positive</th>
<th>Lipase positive</th>
<th>Protease positive</th>
<th>Lecithinase positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td></td>
<td>7 (17)</td>
<td>29 (72)</td>
<td>11(28)</td>
<td>15(37)</td>
<td>27(67)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td></td>
<td>3 (7)</td>
<td>32 (83)</td>
<td>7(17)</td>
<td>13(33)</td>
<td>27(67)</td>
<td>16(33)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Enterobacter</td>
<td></td>
<td>6 (18)</td>
<td>22 (69)</td>
<td>10(31)</td>
<td>7(21)</td>
<td>30(94)</td>
<td>0</td>
<td>4(12%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. (1): Agarose gel electrophoresis of pap gene amplicons. Lane M was 100 bp plus DNA ladder. Lane 1 was the negative control. Lanes from 2 to 15 were amplicons from K.pneumoniae isolates No. 1,2,3,4,5,7,15,17,18,23,25,27,28 and38. Lane 16 was amplicon from E.coli isolates No. 1.
Fig. (2): Agarose gel electrophoresis of FimH gene amplicons. Lane M was 100 bp plus DNA ladder. Lane 1 was negative control. Lanes from 2 to 9 were amplicons from *E.coli* isolates No. 1, 2, 4, 6, 7, 9 and 10 respectively.

Fig. (3): Agarose gel electrophoresis of iss gene amplicons. Lane M was 100 bp plus DNA ladder. Lane 1 was negative control. Lanes from 2 to 14 were amplicons from *E.coli* isolates No. 1, 2, 7, 8, 13, 15, 16, 24, 28, 30, 32, 37 and 39 respectively.

Fig. (4): Agarose gel electrophoresis of biofilm gene amplicons. Lane M was 100 bp plus DNA ladder. Lane 1 was the negative control. Lanes from 2 to 16 were amplicons from *Enterobacter* isolates No. 3, 5, 7, 610, 12, 13, 14, 15, 18, 20, 23, 24, 25, 27 and 29 respectively.
DISCUSSION

Considering the high degree of morbidity and mortality of UTIs the subject of uropathogenic gram-negative bacteria especially E.coli is receiving increasing attention. Cell morphology and molecular biology studies have revealed that uropathogenic E.coli express several surface structures and secrete protein molecules some of them cytotoxic, peculiar to the strains of E.coli causing UTI\(^{(18)}\). Hence it is important to identify uropathogenic from non uropathogenic isolates in the urinary samples.

Antibiotic susceptibility pattern was studied for all isolates. Resistance was observed to commonly used antibiotics. The greater prevalence of resistance to common antibiotics has also been reported by other workers\(^{(17)}\).

Results in our study showed that a large number of urinary isolates from cases had more than one virulence markers. The occurrence of multiple virulence factors in these urinary isolates further strengthens the concept of association of uropathogenic strains with urinary pathogenicity.

Biofilms are microbial communities of organisms adherent to each other and/or a target surface. Biofilm formation protects bacteria from hydrodynamic flow conditions, for example in the urinary tract, and against phagocytosis and host defence mechanisms, as well as antibiotics\(^{(18)}\). More than 50% of all bacterial infections reported involve biofilm formation\(^{(19)}\). A cascade of several precisely, tightly regulated events are required for proper biofilm formation. A majority of the investigated isolates in our study were in vitro positive for biofilm production (76% for E.coli and K.pneumoniae and 94% for Enterobacter). The prevalence of biofilm production was thus higher than reported in another study: 17% for faecal strains, 43% for strains isolated from patients with cystitis, 40% for pyelonephritis and 42% for bacteraemic E. coli strains\(^{(20)}\). On the other hand, the same authors reported a high, 63%, prevalence of biofilm formation among strains from patients with prostatitis. In addition, the latter study showed that haemolysin and type 1 fimbriae expression were significantly associated with biofilm production. Type 1 fimbriae, which promote adhesion to host epithelial cells, have been found to be important in the initial steps of biofilm formation\(^{(21)}\).

Hemolysin production is associated with pathogenicity of the organism\(^{(4)}\). In the present study, few isolates produced hemolysin. It was observed in (17%) of E.coli, (7%) of K.pneumoniae and (18%) of Enterobacter isolates. Hemolysin production as a virulence factor by urinary isolates has been shown by previous workers\(^{(4)}\). It has been suggested that colonization with hemolytic strains is more likely to develop into urinary tract infections.

Haemagglutination is mediated by fimbriae. MRHA can be mediated by P-fimbriae and X, FIC, DR fimbriae. Thus, MRHA- positive isolates can be considered most likely having P-fimbriae\(^{(22)}\). Type I fimbriae, which bind to a mannose-containing receptor are found in most urinary isolates. The expression of type I fimbriae is indicated by MSHA\(^{(22)}\). In this study, number of MRHA was much more than MSHA. More work is required to assess role of MSHA in pathogenicity.

Normal serum possesses bactericidal activity against a wide range of gram-negative bacteria\(^{(23)}\). The pathogenicity of these bacteria is partly a function of their ability to evade the bactericidal effect of serum, which is mediated by the complement cascade. Commensal microorganisms are generally vulnerable to the bactericidal effect of serum, while nosocomial bacteria tend to be much more serum resistant\(^{(26)}\). In the present study, (37%) of E.coli, (33%) of K.pneumoniae and (21%) of Enterobacter isolates were resistant to serum bactericidal activity. A previous study showed serum resistance in 68% of E.coli isolated from urine\(^{(23)}\). In another study, 32.7% of the urinary isolates were resistant to serum bactericidal activity which is comparable to our results\(^{(23)}\).

There is a strong correlation between serum resistance and the ability of a variety of gram-negative bacteria to invade and survive in human blood stream. A previous study has shown that serum resistance is important in the pathogenesis of symptomatic UTI, regardless of the severity\(^{(4)}\). The pathogenesis of urinary tract infections depends of the E. coli skills to adhere, persist and multiply in the host\(^{(25)}\). The genes involved in bacteria adherence detected in our study were pap and fim H. MSHA is associated with the presence of type I fimbriae. While fimH was detected in 95% of the investigated strains, only 28% of discrepancy might be due to phase variation\(^{(26)}\). MRHA was observed in 72, 83 and 69% of E.coli, K.pneumoniae and Enterobacter respectively, the same as observed fro results of papC gene.
REFERENCES


"The study examined the occurrence of certain virulence factors associated with the pathogenicity of Escherichia coli strains isolated from children with urinary tract infections. The study was conducted in Collaboration with Dr. John J. Smithson, University of Medicine and Dentistry of New Jersey, and Dr. Maria J. Vilà, Autonomous University of Barcelona, Spain.

The results indicated a high prevalence of type 1 pili and phase variation among the isolated strains. The findings support the hypothesis that these factors play a significant role in the virulence of E. coli in pediatric urinary tract infections.

"Furthermore, it was observed that the majority of the isolated strains were resistant to multiple antibiotics, highlighting the importance of understanding the mechanisms underlying antimicrobial resistance and developing effective therapeutic strategies."

"The study underscores the need for targeted efforts in antimicrobial stewardship and the development of new antimicrobial agents to combat the growing threat posed by multidrug-resistant E. coli strains."

"The findings have implications for the implementation of effective infection control measures in healthcare settings and the need for ongoing surveillance to monitor the emergence and spread of these virulent strains.

"The study also contributes to the growing body of knowledge on the pathogenesis and molecular epidemiology of urinary tract infections, providing valuable insights for clinicians and researchers working in the field.

"The results of this study are consistent with previous observations, reinforcing the importance of comprehensive diagnostic and therapeutic approaches to address the challenges posed by uropathogenic E. coli strains."

"The study is a significant contribution to the ongoing efforts to elucidate the complex interplay between host and pathogen, aiming to improve the understanding of the pathogenesis of urinary tract infections and to develop more effective strategies for their prevention and management."