CONTENTS

Guest Editorial

Novel HIV Prevention Strategies: The Case for Andhra Pradesh
JA Schneider ........ 1

Review Article

Chikungunya Fever: A Re-emerging Viral Infection
M Chhabra, V Mittal, D Bhattacharya, UVS Rana, S Lal ...... 5

Special Article

Fabrication and Evaluation of a Sequence-specific Oligonucleotide Miniarray for Molecular Genotyping
J Iqbal, F Hänel, A Ruryk, GV Limmon, A Tretiakov, M Dürst, HP Saluz ...... 13

Original Articles

A Comparison of PCR Detection of Meca with Oxacillin Disk Susceptibility Testing in Different Media and Sceptor Automated System for both Staphylococcus aureus and Coagulase-negative Staphylococci Isolates
S Ercis, B Sancak, G Hasçelik ...... 21

Effect of Exposure to Hydrogen Peroxide on the Virulence of Escherichia coli
A Hegde, GK Bhat, S Mallya ...... 25

A Low Molecular Weight Es-20 Protein Released In Vivo and In Vitro with Diagnostic Potential in Lymph Node Tuberculosis
N Shende, V Upadhye, S Kumar, BC Harinath ...... 29

Community-based Study on Seroprevalence of Herpes Simplex Virus Type 2 Infection in New Delhi
R Chawla, P Bhalla, K Bhalla, M Meghachandra Singh, S Garg ...... 34

Changing Patterns of Vibrio cholerae in Sevagram Between 1990 and 2005
P Narang, DK Mendiratta, VS Deotale, R Narang ...... 40

Rapid Serodiagnosis of Leptospirosis by Latex Agglutination Test and Flow-through Assay
TMA Senthilkumar, M Subathra, M Phil, P Ramadass, V Ramaswamy ...... 45

High Level Ciprofloxacin Resistance in Salmonella enterica Isolated from Blood
R Raveendran, C Wattal, A Sharma, JK Oberoi, KJ Prasad, S Datta ...... 50

Role of Enteric Fever in Ileal Perforations: An Overstated Problem in Tropics?
MR Capoor, D Nair, MS Chintamani, J Khanna, P Aggarwal, D Bhatnagar ...... 54
Brief Communications

Evaluation of a Modified Double-disc Synergy Test for Detection of Extended Spectrum β-lactamases in Ampc β-lactamase-producing Proteus mirabilis
MKR Khan, SS Thukral, R Gaind

Antimicrobial Susceptibility Profile of Neisseria gonorrhoeae at STI Clinic
C Shilpee, VG Ramachandran, S Das, SN Bhattacharya

Detection of Extra-cellular Enzymes of Anaerobic Gram-negative Bacteria from Clinically Diseased and Healthy Sites
JM Nagmoti, CS Patil, MB Nagmoti, MB Mutnal

Haemagglutination and Siderophore Production as the Urovirulence Markers of Uropathogenic Escherichia coli
MA Vagarali, SG Karadesai, CS Patil, SC Metgud, MB Mutnal

The use of Dried Blood Spots on Filter Paper for the Diagnosis of HIV-1 in Infants Born to HIV Seropositive Women
S Mini Jacob, D Anitha, R Vishwanath, S Parameshwari, NM Samuel

Evaluation of the Usefulness of Phage Amplification Technology in the Diagnosis of Patients with Paucibacillary Tuberculosis
D Biswas, A Deb, P Gupta, R Prasad, KS Negi

Case Reports

Cytomegalovirus Oesophagitis in a Patient with Non-hodgkin’s Lymphoma
SS Hingmire, G Biswas, A Bakshi, S Desai, S Dighe, R Nair, S Gupta, PM Parikh

Hydatid Cyst of Mediastinum
S Sehgal, B Mishra, A Thakur, V Dogra, PS Loomba, A Banerjee

Ochrobactrum anthropi Septicaemia
U Arora, S Kaur, P Devi

Intestinal Myiasis Caused by Muscina stabulans
S Shivekar, K Senthil, R Srinivasan, L Sureshbabu, P Chand, J Shanmugam, R Gopal

Pyopericardium Due To Group D Streptococcus
K Karthikeyan, KR Rajesh, H Poornima, R Bharathidasan, KN Brahmadathan, R Indra Priyadharsini

Pleural Effusion: A Rare Complication of Hepatitis A
A Bukulmez, R Koken, H Melek, O Dogru, F Ovali

Correspondence

Prevalence of Inducible AmpC β-lactamase-Producing Pseudomonas aeruginosa in a Tertiary Care Hospital in Northern India
A Battacharjee, S Anupurba, A Gaur, MR Sen

Parental History of Ulcer and the Prevalence of Helicobacter pylori Infection in their Offspring
KS Ahmed, AA Khan, JD Ahi, CM Habibullah
<table>
<thead>
<tr>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin Breakpoints in Enteric Fever - Time to Revise our Susceptibility Criteria</td>
<td>91</td>
</tr>
<tr>
<td>C Rodrigues, N Jai Kumar, J Lalwani, A Mehta</td>
<td></td>
</tr>
<tr>
<td>West Nile Virus in the Blood Donors in UAE</td>
<td>92</td>
</tr>
<tr>
<td>M Alfaresi, A Elkoush</td>
<td></td>
</tr>
<tr>
<td>Estimation of Antibodies To HBsAg in Vaccinated Health Care Workers</td>
<td>93</td>
</tr>
<tr>
<td>TV Rao, IJ Suseela, KA Sathivathy</td>
<td></td>
</tr>
<tr>
<td>Seroprevalence of Rubella Among Urban and Rural Bangladeshi Women Emphasises the Need for Rubella Vaccination of Pre-pubertal Girls</td>
<td>94</td>
</tr>
<tr>
<td>A Nessa, MN Islam, S Tabassum, SU Munshi, M Ahmed, R Karim</td>
<td></td>
</tr>
<tr>
<td>Novel Digestion Patterns with Hepatitis B Virus Strains from the Indian Subcontinent Detected using Restriction Fragment Length Polymorphism</td>
<td>96</td>
</tr>
<tr>
<td>P Vivekanandan, HDJ Daniel, S Raghuraman, D Daniel, RV Shaji, G Sridharan, G Chandy, P Abraham</td>
<td></td>
</tr>
<tr>
<td>Acute Urticaria Associated with Dicrocoelium dendriticum Infestation</td>
<td>97</td>
</tr>
<tr>
<td>A Sing, K Tybus, I Fackler</td>
<td></td>
</tr>
<tr>
<td>Book Reviews</td>
<td>99</td>
</tr>
<tr>
<td>Guidelines to Authors</td>
<td>100</td>
</tr>
</tbody>
</table>
HAEMAGGLUTINATION AND SIDEROPHORE PRODUCTION AS THE UROVIRULENCE MARKERS OF UROPATHOGENIC ESCHERICHIA COLI

*MA Vagarali, SG Karadesai, CS Patil, SC Metgud, MB Mutnal

Abstract

A total of 160 strains of Escherichia coli isolated from urine of patients with clinically diagnosed urinary tract infection were included in the study and 50 faecal isolates of E. coli were studied. They were studied for virulence factors, namely mannose-resistant and mannose-sensitive haemagglutination (MRHA, MSHA) and siderophore production. Among 160 urinary isolates of E. coli, 40 (25%) showed MRHA, siderophore production was seen in 156 (97.5%). In 50 faecal isolates, two (4%) were MRHA, four (8%) MSHA and siderophore production in two (4%). The results suggest that MRHA and siderophore production positive strains can be considered as UPEC.

Key words: E. coli mannose-resistant haemagglutination, siderophore uropathogenic

Urinary tract is the second most common site of bacterial infection in humans and thus represents a major source of human discomfort. Escherichia coli is the most frequently isolated urinary pathogen, which accounts for 50 to 90% of all uncomplicated urinary tract infections. It is now recognized that there are subsets of faecal E. coli, which can colonize periurethral area, enter urinary tract and cause symptomatic disease. These are currently defined as uropathogenic E. coli.\(^1\) It has been traditionally described that certain serotypes of E. coli were consistently associated with uropathogenicity and were designated as uropathogenic E. coli. These isolates express chromosomally encoded virulence markers.

In the late 1970s, it was recognized for the first time that E. coli strains causing urinary tract infections typically agglutinate human erythrocytes despite the presence of mannose and this was mediated mainly by fimbriae.\(^2\) The virulence factors include different adhesins, hemolysin production and siderophore production. Fimbriae mediate the ability of E. coli to adhere to the uroepithelium, thereby resisting elimination by the flow of urine. Adhesion is therefore considered to be an important step in the pathogenesis of UTI.\(^3\)

Bacterial siderophores compete for iron with host iron binding proteins. When bound by siderophore, the iron is taken up by special bacterial surface receptors and can be utilized by the pathogen, many strains of E. coli associated with urinary tract infection produce siderophore.\(^4\)

The information on the characteristics of E. coli causing urinary tract infections is limited and less studied. So, the present study was designed to determine the urovirulence factors of E. coli isolated from the patients of UTI and to study their antimicrobial susceptibility pattern.

Materials and Methods

The study was conducted in the Department of Microbiology, J. N. Medical College, Belgaum, from October 2002 to September 2003. One hundred and sixty E. coli strains isolated from urine samples and 50 faecal isolates were studied for the detection of virulence markers of E. coli. Escherichia coli were identified as described by Bailey and Scott. The isolates were maintained by inoculating nutrient agar butts and stored at room temperature and tested for haemagglutination and siderophore production.

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 per the direct bacterial haemagglutination test - slide method and mannose-sensitive and mannose-resistant haemagglutination tests.\(^5\) The strains of E. coli 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 guinea-pig, sheep and human (blood group ‘O’). The red blood cells were then washed thrice 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. The slide haemagglutination test was carried out on a multiple-concavity slide. One drop of the RBC suspension was added to a drop of the broth culture and slide was rocked to and fro 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 in the presence of 2% mannose.

**Siderophore production assay**

This test was carried out by using a method named ‘chrome azurol sulphonate (CAS) agar diffusion assay’. The chrome azurol sulphonate (CAS) assay detects colour change of CAS-Iron complex from blue to orange after chelation of the bound iron by siderophores. A strong ligand ‘L’ (e.g., a siderophore) is added to a highly coloured iron dye complex; when the iron ligand complex is formed, the release of the free dye is accompanied by a colour change.

The result was taken as positive if there was a colour change from blue to orange halo (Figure).

**Antibiotic sensitivity testing**

Antibiotic sensitivity testing was performed for all the isolates of *E. coli* by Kirby Bauer’s disc diffusion method like ampicillin, cotrimoxazole, gentamicin, nalidixic acid, norfloxacin, nitrofurantoin, ciprofloxacin, netilmicin to identify their resistance pattern to the commonly used antibiotics.

**Results**

A total of 40 (25%) among 160 isolates from cases and 2 (4%) out of 50 controls showed mannose-resistant haemagglutination (MRHA). There was a significant difference in MRHA between cases and controls.

Fifty-five (34.38%) from cases and four (8%) from controls showed mannose-sensitive haemagglutination (MSHA) (Table 1).

Siderophore production was seen in 156 (97.5%) among 160 cases and 2 (4%) among controls (Table 2).

Out of 160 isolates of *E. coli*, 150 (93.75%) were susceptible to nitrofurantoin followed by netilmicin 149 (93.13%), and 40 (25%) were sensitive to ampicillin.

**Discussion**

Considering the high degree of morbidity in urinary tract infections, the subject of uropathogenic *E. coli* (UPEC) is receiving increasing attention. Cell morphology and molecular biology studies have revealed that uropathogenic *E. coli* express fimbriae and siderophore production peculiar to the strains of *E. coli* causing urinary tract infection. Hence, it is important to identify UPEC isolates in the urinary samples.

The occurrence of virulence factors in UPEC strains strengthens the concept of association of UPEC with urinary pathogenicity. UPEC with virulence factors were significantly more in urinary isolates than in controls. Haemagglutination is mediated by fimbriae. MRHA can be mediated by P-fimbriae and X, FIC, DR fimbriae. Thus, MRHA-positive strains can be considered as UPEC most likely having P-fimbriae.

Virulence determinants such as P-fimbriae, siderophore production have been shown to be more frequent in *E. coli* from patients with UTI than in faecal isolates.

Type I fimbriae, which bind to a mannose-containing receptor, are found in most *E. coli* urinary isolates. The expression of type I fimbriae is indicated by MSHA.

In *E. coli*, the hydroxymate siderophore (aerobactin) is the most effective of the several iron chelation systems employed by the bacteria for iron acquisition. The siderophore (aerobactin) and P-fimbriae are commonly found together in isolates from patients with UTI.

### Table 1: Haemagglutination patterns of 160 *E. coli* strains

<table>
<thead>
<tr>
<th>Haemagglutination pattern</th>
<th>Cases</th>
<th>%</th>
<th>Control</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRHA</td>
<td>40</td>
<td>25.00</td>
<td>2</td>
<td>4.00</td>
</tr>
<tr>
<td>MSHA</td>
<td>55</td>
<td>34.38</td>
<td>4</td>
<td>8.00</td>
</tr>
<tr>
<td>NHT</td>
<td>65</td>
<td>40.62</td>
<td>44</td>
<td>88.00</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td></td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

\[ \chi^2 = 34.13, \text{ DF } = 2, \text{ P} < 0.001, \text{ MRHA - Mannose-resistant haemagglutination, MSHA - Mannose-sensitive haemagglutination, NHT - Non-haemagglutinating types} \]

### Table 2: Siderophore production

<table>
<thead>
<tr>
<th>Siderophore production</th>
<th>Cases</th>
<th>%</th>
<th>Control</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>156</td>
<td>97.50</td>
<td>2</td>
<td>4.00</td>
</tr>
<tr>
<td>(–)</td>
<td>4</td>
<td>2.50</td>
<td>48</td>
<td>96.00</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td></td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

\[ \chi^2 = 173.77, \text{ DF } = 1, \text{ P} < 0.001 \]
In view of the emerging drug resistance among UPEC, therapy should be advocated as far as possible after culture and sensitivity has been performed. This would not only help in the proper treatment of the patients, but would also discourage the indiscriminate use of the antibiotics and prevent further development of bacterial drug resistance.

References


Source of Support: Nil. Conflict of Interest: None declared.