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Multiple Drug Resistant (MDR) Strains of Escherichia coli Isolated from Urinary Tract Infection, a Predictor of Female Childhood Protein Deficiency in Southern Sindh, Pakistan

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Abstract: A cross-sectional study to examine a current phenomenon of great concern among the medical communities in developing countries is rising multi-drug resistant organisms and the challenges of curing the infections in children. In this study, we evaluated the multi drug resistant strains of Escherichia coli (E. coli) isolated from urinary tract infection in children in southern sindh, Pakistan. Multi-drugs strains of E. coli in Urinary Tract Infection (UTI) with protein malnutrition, Iron deficiency anemia and Vitamin B12 and Folic Acid deficiency anemia was conducted among female children in southern sindh, Pakistan. A total of 150 female children aged up to 5 years were studied. The data were collected using structured questionnaires, anthropometric measurements and laboratory analysis of blood and Urine samples. The results showed that 47.4% of the female children were infected with multi-drugs resistance Escherichia coli, while 18.3% had significantly underweight, stunting and wasting respectively. Urinary tract infection with multi-drugs resistance strains Escherichia coli was statistically identified as a strong predictor of significant wasting in this study population.

Key words: Urinary tract infection, Multiple drug resistant (MDR), protein deficiency

INTRODUCTION
Escherichia coli (E. coli) known as UPEC, is the most prevalent extra digestive pathogen causing UTI in children and accounts for about 80-90% of the community acquired cases (Ejrnæs et al., 2008; Johnson and Russo, 2005; Overturf, 2002). The exact prevalence rate of UTI is not clear yet and varies with the age and gender of the individuals. Eighty percent of the girls and 20% of the boys stand the chance of infections including pyelonephritis and cystitis at least once in their childhood (Mohkam et al., 2008; Jakobsson et al., 1999).
A remarkable increase in antibiotic resistance among the E. coli isolates has been observed during the last few years (Coulthard et al., 1997). Escherichia coli (E. coli) have been recognized as the most common resistance UTI pathogen worldwide. E. coli organisms form part of the normal microbial flora of intestinal tract of humans and animal. They can also be found in water, soil and vegetation. In tropical and temperate countries (Watson, 2003; Royal College of Physicians, 1991), children (in particular female) are more frequently infected than adults. In the developed countries, Escherichia coli have been implicated as a rising cause of resistance UTI among children. Such rising resistance is due to mechanisms of mutation and then resistance gene transfer by transport means. Since a plasmid or transposon can carry several resistance indexes, resistance to several antimicrobial agents may be acquired simultaneously and results in Multiple Drug Resistant (MDR) organisms (Smellie et al., 1995; Goonasekera et al., 1996). In developing countries, however, Escherichia coli is endemic and Escherichia coli infection as a predictor of childhood malnutrition commonly seen in children aged up to 5 years (Jacobsson et al., 1999; Alexander et al., 1999). The association of E. coli with Proteinenergy Malnutrition (PEM) and recently with deficiency of iron (IDA), Vitamin B12 and folic acid has been reported in few studies. In addition, a positive association between E. coli and PEM has been described before (Lewis et al., 1999).
Children with E. coli (MDR) had anaemia it may be lead to hematuria and treatment with antibiotic did not improve the condition (Farhat et al., 2004; Wolffish et al., 1993). The study also suggested that besides antibiotic agent, supplementary with iron, vitamin B12 and folic acid should be given to prevent mix deficiency anaemia that is microcytic hypochromic macrocytic anaemia. However, little is known regarding UTI (MDR) and its association with protein deficiency in southern sindh Pakistan. This study was carried out to examine the association between UTI, PEM, IDA and Macrocytic anaemia among female children up to 5 years.

MATERIALS AND METHODS
Study areas and study population: This is part of a cross-sectional study on the relationship between Multiple Drug Resistant (MDR) strains of Escherichia coli in urinary tract infections and female childhood protein

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deficiency in southern sindh Pakistan. The female child who belongs to remote area of southern sindh, most of the residents worked as laborers, farmers and some did odd jobs selling forest products. Houses were made of mud or bricks and cements without any cement plaster. Most of them do not have supplies water. The study population was aged upto 5 years and includes only females. All females child who agreed voluntarily through their parents to participate were included in this study. Of 150 female children studied and analyses for the association between UTI (MDR) and protein deficiency were based on these female Children. The study included only females children. Most of the child's mothers were not completely aware about the washing after pass stool by the children. Most of the Children included in this study do not have proper toilet. In addition, their personal hygienic practices were poor. The data were collected using a structured questionnaire, anthropometry and laboratory analysis of blood and urine samples.

Structured questionnaire: The data were collected over a period of 6 months, beginning in July 2010. During many visits, the parents were read an Informed Consent Form and permission was obtained from parents whose children participated in this study. Each of the Children was given a code number accordingly and particulars were entered in the data sheet. The parents were interviewed directly on the personal particulars, as well as socio-economic status, using a standard questionnaire. Date of birth and birth weight were obtained from birth certificates, while immunization status was obtained from each child's health record.

Anthropometry: All female Children underwent anthropometrics measurement as follows: Children were weighed without shoes using Seca scales, which had intervals of 0.5 kg; height was measured to the nearest 0.1 cm using a calibrated scale consisting of a wooden platform with a scale and a sliding head piece. To reduce intra-individual error, weight and height were measured twice and the mean value was used for the analysis.

Blood examination: Approximately 6-7 ml venous blood was collected by disposable syringes through vein puncture technique from cubital vein. 3 ml blood were transferred in to an aliquot containing EDTA immediately after collection of blood sample for hematological analysis and remaining blood was transferred into a plain tube for biochemical analysis, sample were taken to the laboratory soon as possible. The plain tubes were centrifuged at 3000 rpm for 10 min to obtain the serum. Total protein and albumin were determined colorimetrically using the microlab 300 analyzer. Anaemic children (low hemoglobin concentration according to there age and sex) with increase red blood cells indices (Hct, MCV, MCH, MCHC) were considered to have Anemic (Mostly mix deficiency anemia). Serum Total protein and albumin was recorded in g/l and children with serum Total protein levels less than 3.5 g/l and albumin level 2.2 g/l were considered to have hypoalbuminaemia.

Urine culture examination: Urine samples were collected into wide mouth screw-cap sterile 100 ml containers. Mothers of the female children were instructed to cleanse the area around the urethral opening with clean water, dry the area and collect the urine with the labia held apart. Label the container with the date, number of the children. Sample was delivered to the lab as soon as possible. Possible pathogens of UTI in Gram positive are Enterococci, Staphylococcus saprophyticus, Hemolytic streptococci and in Gram negative E. coli, Proteus species, Pseudomonas aeruginosa, Klebsiella strain, Salmonella typhi, Salmonella paratyphi, Neisseria gonorrhoeae. Positive growth was analyzed by different drug including Ampicillin, Co trimoxazole, Tetracycline, Chloramphenicol, Nalidixic acid, Cefixim, Ceforoxime, Gentamicin, Ceftazidime, Ciprofloxacine, Norfloxacine, Nitrifurantoin, Amikacin, Imipenemand exclude those participant whom sample were sensitive to drug. Only two groups Amikacin and Imipenemand are sensitive to all most 98%.

RESULTS
One hundred and fifty female children aged up to 5 years with a mean age of 3.07±±2.02 years was analyzed. The overall significant underweight was 69.5% with significantly low values of Total protein and albumin. Table 2 shows that 47.4% were positive for E. coli (MDR) infection. Prevalence of UTI was slightly higher in female child in the age group 2-3 years but it does not have significant difference, Urine culture analysis identified with MDR E. coli infection as a predictor of significant underweight and significant wasting in this study population.

DISCUSSION
Antibiotic therapy is the first and the foremost for UTI, in which the invasive agents are controlled. Therefore, a correlation between the overuse of antimicrobials and increasing emergence of resistant bacteria seems natural (Watson, 2004; South Bedfordshire Practitioners' Group, 1990). Worldwide reports of antibiotic resistant E. coli isolates indicate the unwise and excessive consumption of antimicrobial drugs which in turn has brought about failure in treatment and consequently concerns about the related issues in all nations including the developed and developing ones (Pisacane
Table 1: Frequency and % of males and females children

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of children examined</th>
<th>No. of MDR E. coli infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>150</td>
<td>71</td>
</tr>
</tbody>
</table>

Table 2: Comparison of frequency and % of MDR E. coli infection with all others in different age groups

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. of female child examined</th>
<th>No. of MDR E. coli infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 1 years</td>
<td>38 (24.0%)</td>
<td>18 (10.6%)</td>
</tr>
<tr>
<td>1-2 years</td>
<td>31 (20.7%)</td>
<td>14 (8.4%)</td>
</tr>
<tr>
<td>2-3 years</td>
<td>34 (22.7%)</td>
<td>17 (11.4%)</td>
</tr>
<tr>
<td>3-4 years</td>
<td>20 (13.3%)</td>
<td>11 (6.7%)</td>
</tr>
<tr>
<td>4-5 years</td>
<td>23 (15.3%)</td>
<td>13 (8.8%)</td>
</tr>
<tr>
<td>Total No.</td>
<td>n = 150</td>
<td>71 (47.4%)</td>
</tr>
</tbody>
</table>

Table 3: Biochemical findings of patients

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean±SD (n = 150)</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>2.6±0.7</td>
<td>5.5-6.5 g/dl</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.4±0.5</td>
<td>3.5-5.5 g/dl</td>
</tr>
<tr>
<td>Blood glucose level (mg/dl)</td>
<td>71.03±21.56</td>
<td>80-180 mg/dl</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>38.1±9.52</td>
<td>10-50 mg/dl</td>
</tr>
<tr>
<td>Creatinine (mg %)</td>
<td>0.6±0.21</td>
<td>0.1-0.5 mg %</td>
</tr>
</tbody>
</table>

Table 4: Hematological finding of children

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean±SD</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>7.3±1.9</td>
<td>12.0±2.0</td>
</tr>
<tr>
<td>RBCs (C/mm)</td>
<td>4.6±1.7</td>
<td>4.7±0.7</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>30.9±10.7</td>
<td>41.0±4.0</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>98.2±16.9</td>
<td>84.0±9.0</td>
</tr>
<tr>
<td>MCH (dg)</td>
<td>31.1±7.3</td>
<td>29.5±2.0</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>34.7±5.9</td>
<td>33.0±2.0</td>
</tr>
</tbody>
</table>

Anemia, Weight loss, decreased muscle mass and weakness, Dry scaly skin, Edema (swelling due to lack of protein), Hair that has lost its pigment, Brittle and malformed (spooned) nails, Chronic diarrhea, Slow wound healing, Bone and joint pain, Growth retardation (in children), Mental changes such as confusion and irritability and Goiter, all are the symptoms of malnutrition (Craig et al., 1996). General malnutrition often develops slowly, over months or years. As the body’s stores of essential amino acid are depleted and the deficiency of different vitamins in general may take place, changes begin to happen at the cellular level, affecting biochemical processes and decreasing the body’s ability to fight infections. Over time, a variety of symptoms may begin to emerge (Kemper and Avner, 1992; Hansson et al., 2002).

The present study observed a high prevalence of UTI by the Multiple Drug Resistant (MDR) strains of *Escherichia coli* among the female child who participated in this study. Alan (2004) reported that *Escherichia coli* are responsible for at least 80% of UTI then other organisms include Proteus, *Enterococcus*, *Pseudomonas* and *Klebsiella* species. *Staphylococcal aureus* and *Staphylococcal epidermidis* are urinary pathogens in small children and young women. Any organism may cause sepsis in this young age group with the kidney and urinary tract becoming invaded by hemogenous spread from a generalized septicemia (Dohil et al., 1994; Kontiokari et al., 2001).

The prevalence of *E. coli* was slightly higher in children aged up to 1 years and this may be a cause of increase number of death in remote areas in children under 1 year. It spreads through poor hygienic condition, within households, perhaps from person to person, as young children usually play in the house and have very close contact with other members of the household (Lambert and Coulthard, 2003; Gorelick and Shaw, 1989). With positive results of UTI with MDR *E. coli* we also observed the significantly low values of Total protein (2.6±0.7 g/dl) and albumin (1.4±0.5 g/dl). Hemoglobin were reduce then the normal values according to the age and sex. All the indices include Hct, MCV, MCH, MCHC was show variation in results because of mix deficiency of different vitamins and minerals. Microbiological, biochemical and hematological results predicate the protein malnutrition and the presence of microcytic hypochromic/macrocytic normochromic anemia which is often iron deficiency/vitamin B12 and Folic acid deficiency in the female child with positive UTI with MDR *E. coli*.

Conclusion: In sum, the present finding, suggest that great percentage of *E. coli* isolates acts as the main risk for developing the resistance among the whole *E. coli* populations or closely related bacteria which become a case of anemia and protein deficiency as well.

Further suggestion: Further research on the characterization of resistant bacteria and prevalence rates of them may cause presently effective antibiotics to become ineffective in the treatment of bacterial infections and consequently bring about serious challenges in medical practices (Wolff and Maclennan, 2007; Dromigny et al., 2005).

REFERENCES


