INTRODUCTION

Urinary tract infections (UTI) are the most common microbial infections. UTI refer to the invasion of microbial pathogens into urinary tract and it is usually classified according to the site of infection i.e. bladder (cystitis), kidney (pyelonephritis), urine (bacteriuria). Children are relatively more prone to acquire UTI and its diagnosis is more significant in them as it is presented asymptptomatically, poor feeding, vomiting and only older children with UTI can be identified by foul smelling urine. Pyrexia is always associated with all pathologies presented in infancy. UTI presented with symptoms or without symptoms have more pronounced significance in children than in elder one’s because kidney problems occur after such etiologies during the first five years of human life.

It is predicted that not less than 1% of boys and 3% of girls suffer from urinary tract infection in first ten years of their life. During prematurity of one’s age (18–24 years), the yearly attack of UTI in males remain comparatively low at 0.83%; while, it increases significantly in females to 10.8%.

These facts make it important to properly diagnose the infection pattern, microbial identification and selection of antibiotic therapy along with determination of resistance pattern in order to design an effective therapeutic treatment against UTI in children. The disease causing microbes involved in urinary tract infections are originated from hospitals.

ABSTRACT... Objectives: The objectives of the study was to determine different microorganisms responsible for causing urinary tract infections UTI in children and to evaluate sensitivity and resistance pattern of different antibiotics used in UTIs. Setting: Study was conducted in Children Hospital Complex (CHCM), Multan, Pakistan. Methods: Total 125 children having UTI were taken to determine the antibiotic sensitivity and resistance pattern against microorganisms causing urinary tract infections in children. The urine samples were collected in urine bottles and smears were made within 2 hours of sample collection. Urine sample was inoculated on agar media and then incubated for 24 hours. A smear was prepared on a slide form culture of microorganisms and gram testing was conducted. The microorganisms were then characterized by use of API (Analytical Profile Index) MICROBACT TM 24E of Oxoid England. The antimicrobial susceptibility testing of various antibiotics was performed by disc diffusion method. Results and Conclusions: The percentage of Gram +ve bacteria causing urinary tract infections in children was 28%, Gram –ve 62.4% and Yeast 9.6%. The most prevailing species are S. aureus, S. epidermidis and E.coli. Results showed that female children are more prone to UTI than male children. Norfloxacin is effective in S. Aureus UTI while cefaclor is effective in S.epidermidis UTI. Amikacin, Norfloxacin and Cefuroxime are effective in UTI caused by E.coli.

Key words: Antibiotics, Children, Urinary tract infections, S. aureus, S. epidermidis and E.coli.
In current study microbial infection pattern regarding UTI was studied in 125 children in Children Hospital Complex, Multan, Pakistan irrespective of gender. Infection pattern is characterized with respect to hospitalized and non-hospitalized children. Microbes were characterized for gram staining and further bacterial characterization was carried by employing different culture media. Microbes were characterized for *Entrococcus facillus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Staphylococcus epidermidis* and yeasts. Antibiotic resistance pattern using respective discs of different antibiotics was determined and microbial susceptibility was classified as resistant, intermediate and sensitive.

**MATERIALS AND METHODS**

**Settings**

Study was conducted in Children Hospital Complex (CHCM), Multan, Pakistan from March 2012 to September 2012. It is an established Medical institution presently functioning with 300 beds, to provide medical facilities to Children. It is a tertiary care facility with subspecialties including Pediatrics, Oncology, Nephrology, Neurology, Gastroenterology, Neonatology, Psychiatry, Pulmonologist, Cardiology and Pediatric Rehabilitation centre.

**Collection of sample**

125 children were divided in two age groups i.e. <5 years (preschool) and >5 years (School age) and urine samples were collected in urine bottles. All the urine specimens were processed within 2 hours of collection or were kept refrigerated at 4 °C for no longer than 18 hours after collection. The techniques used to make smears from different specimens were as followed; 12

Liquid specimen of urine was inoculated on agar media and was incubated for 24 hours. Form culture of microorganisms on agar media, a smear was prepared on a slide and gram testing was performed.

**Characterization of microbes**

For identification of exact microorganism to specified level API (Analytical Profile Index) MICROBACT TM 24E of Oxoid England was used for aerobic and facultatively anaerobic Gram-negative bacteria (*Enterobacteriaceae* and miscellaneous Gram- negative bacteria). Microorganisms’ identification was based on pH change and substrate utilizations. The different codes of the biochemical reactions which were recorded after incubating the bacterial suspension in normal saline for 36 hours were entered in to the MICROBACT 2000 software of Oxoid by which the exact species of the microorganisms were identified 13. Microbes were characterized by Gram staining 14 and smears were evaluated at 40X for distribution of material and oil immersion objective. Cystine-Lactose-Electrolyte-Deficient (CLED) Agar was used as a medium for culturing microbes, isolation, enumeration and presumptive identification of microorganisms from the urine 15. Further identification of microbes was performed via catalase, oxidase, urease and DNAse tests 16,17.

**Antimicrobial susceptibility testing**

The test was performed by disc diffusion method 18, placing filter paper disks impregnated with specific antimicrobial agents on agar plates pre seeded with the organism to be tested and judging the degree of sensitivity by the size of zone of inhibition resulting after overnight incubation. From an agar plate culture, at least three to five well-isolated colonies of the same morphological type were selected. The top of each colony was touched with a loop and the growth was transferred into a tube containing 4 to 5 ml of a suitable medium. The medium culture was incubated at 35 °C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (usually 2 to 6 hours).

Inoculation of test plates was performed within 5 minutes after adjusting the turbidity of the inocculum suspension by sterile, dry cotton swab. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface evenly.
Sealed inoculated rows were incubated at 35 ± 2 °C for 18-24 hours.

The sizes of the zones of inhibition were interpreted by referring to (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement and the organisms are reported as either susceptible, intermediate, or resistant to the agents that have been tested.

RESULTS
Out of 125 children having UTI, sixty six were hospitalized and fifty nine were non-hospitalized. The percentage of Gram +ve bacteria causing urinary tract infections in children was 28%, while Gram –ve and Yeast were found 62.4% and 9.6% respectively. The percentage of S. aureus and S. epidermidis were 26.4%. The other most prevailing species in UTI patients were E.coli (12%), Proteus mirabilis (16%) and E. faecalis (9.6%). 33.6% UTI infections were found in male children while 66.4% were observed in female children. It clearly indicates that female children are at a greater risk to accept UTI as compared to male children (Figure 1). In the present study, S. aureus had a sensitivity rate of 78.8% to Norfloxacin and the highest resistant rate to Fusidic acid as shown in figure 2.

Figure 3 presents the sensitivity and resistance pattern of S.epidermidis. S.epidermidis had a sensitivity rate of 81.81% to Cefaclor and showed resistance against Fusidic acid. E.coli had a sensitivity rate of 66.66% to Amikacin, Norfloxacin, Cefuroxime and the highest resistant rate of 20% to Norfloxacitin, Ciprofloxacin and Ampicillin. Proteus mirabilis had a sensitivity rate of 80% to Cefaclor and the highest resistant rate to Ampicillin. While E.faecalis had a sensitivity rate of 91% as compared to Cefaclor and the highest resistant rate to Doxycycline and Ciprofloxacin.

Resistance of different gram –ive and gram +ive bacteria develop through various means like receptors where drug binds may change their structure, the enzymes responsible for conversion of drug into its metabolites may change and inhibition of such enzymes results in resistance.
Here in this figure AUG = Augmentin, CFC = Cefaclor, CIP = Ciprofloxacin, DOX = Doxycycline, CEF = Cefuroxime, OXA = Oxacilin, AK = Amikacin, FA = Fusidic acid, NF = Norfloxacin, AMP = Ampicillin.

Thus Antibiograms of the isolated microorganisms of UTI patients collected from Children Hospital Complex Multan had resistance against various antibiotics such as penicillin, ampicillin, oxacilin, ciprofloxacin, amikacin, fusidic acid and cephalothin.

The pattern of sensitivity of microorganism to antibiotics varies over time. As with the development of resistance in microorganisms, their disease causing ability can’t be diminished. The antibiotic treatment should be based upon local experience of sensitivity and resistance pattern.

CONCLUSIONS

The most commonly found microorganisms causing UTIs in children were found to be S. aureus, S. epidermidis, E.coli, Proteus mirabilis and E. faecalis. 33.6% UTI infections were found more in female children than that were observed in male children. S. aureus and S.epidermidis had higher sensitivity to Norfloxacin and Cefaclor and showed resistance against Fusidic acid. Proteus mirabilis and E.faecalis showed more sensitivity to Cefaclor.

Here in this figure, AMP = Ampicillin.

AK = Amikacin, CFC = Cefaclor, CIP = Ciprofloxacin, AUG = Augmentin, CEF = Cefuroxime, NF = Norfloxacin, FA = Fusidic acid, DOX = Doxycycline.

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REFERENCES

Real leaders are ordinary people with extraordinary determination.

Unknown